PLASMA THYROXINE AND TRIIODOTHYRONINE LEVELS AFTER SYNCHRONIZATION OF ESTRUS IN THE BREEDING SEASON IN EWES

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Twenty nonpregnant cycling Tsigai ewes in the breeding season were treated for synchronization of estrus using intravaginal progestagen sponges (medroxyprogesterone-acetate, MAP) followed by an intramuscular injection of 350-400 IU of pregnant mare serum gonadotrophin (PMSG). A control group of ewes was formed from 20 animals in the natural estrous cycle. Hormonal status was investigated by determination of plasma triiodothyronine (T₃) and thyroxine (T₄) concentrations using radioimmunoassay (RIA). Samples of jugular venous plasma were collected on the day of sponge insertion (P), the day of estrus (0. day), 7, 14 and 18 days after estrus. Plasma T₃ levels in the control group of ewes were significantly lower compared to the ewes after synchronization of estrus, except the level of T₃ on day 18 after estrus. Plasma T₄ level was not significantly different in the treated and control ewes, except on day 18 after estrus.

Key words: estrous season, ewes, progestagen, thyroxine, triiodothyronine.

INTRODUCTION

The thyroid gland plays an important role in the seasonality of reproduction in ewes. Many seasonally breeding birds and mammals require an intact thyroid gland for a normal end to the reproductive period (Jallageas et al., 1979; Follett et al., 1985; Weiselthier et al., 1972). The end of the breeding season in ewes seems to be the manifestation of an endogenous rhythm that is synchronized by environmental changes. At this time the rhythm expresses itself at the neuroendocrine level as an increased responsiveness of the hypothalamus to the negative feedback effect of estradiol on gonadotrophin secretion (Karsch et al., 1989).

The thyroid gland is necessary for the transition from the breeding season to anestrus in the ewe, but the importance of the seasonal changes in thyroid hormone secretion in this process remains to be investigated. In seasonal breeding sheep thyroidectomy does not alter the onset of the estrous cycle, but abolishes the transition to anestrus at the end of the breeding season (Nicholls et al., 1988). Thyroxine (T₄) replacement reverses the effect of thyroidectomy on transition to anestrus (Webster et al., 1991a), suggesting that this treatment
prevents the seasonal shift in potency of negative feedback of estradiol on luteinizing hormone (LH). Thyroxine (T4) treatment during the breeding season can advance anestrus and thus shorten the duration of the breeding season (O'Callaghan et al., 1993). Secretion of T4 after the onset of reproductive activity is required for the endogenously generated change in the neuroendocrine axis that leads to the intensified estradiol negative feedback and an end to the breeding season (Dahl et al., 1995). The aim of the present study was to investigate plasma thyroxine (T4) and triiodothyronine (T3) levels in ewes of the Tsigai breed after synchronization of estrus in the breeding season.

MATERIAL AND METHODS

The hormonal status of ewes after synchronization of estrus in the breeding season was investigated by determination of plasma T3 and T4 concentrations using commercial radioimmunoassay (RIA) kits (INEP-Zemun). During the breeding season (October) estrus was synchronized by progestagen treatment (intravaginal sponges impregnated with 60 mg of MAP/14 days) followed by 350-400 of PMSG/ewe (20 ewes). A control group of ewes during the breeding season (August/September) was formed with 20 cycling ewes. At the time of estrus all ewes were naturally mated to rams of known fertility. Samples of jugular venous plasma were collected on the day of sponge insertion (P), on the day of estrus (0. day), 7, 14 and 18 days after estrus. Differences in plasma hormone levels between treated and control ewes were tested using Students t-test.

RESULTS

Plasma T3 levels in the treated group of nonpregnant and pregnant ewes after the synchronization of estrus and the control group of cycling ewes are presented in Table 1.

Table 1. Plasma T3 levels (X ±SD nmol/L) in the treated group of nonpregnant and pregnant ewes after synchronization of estrus and in the control group of cycling ewes

<table>
<thead>
<tr>
<th>Time of investigation (days)</th>
<th>treated (I) (nonpreg.)</th>
<th>control (II) (nonpreg.)</th>
<th>treated (III) (preg.)</th>
<th>control (IV) (preg.)</th>
<th>statistically significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of ewes</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>P.</td>
<td>2.37 ±0.58</td>
<td>-</td>
<td>3.02 ±0.67</td>
<td>-</td>
<td>I:III*</td>
</tr>
<tr>
<td>0.</td>
<td>3.69 ±1.13</td>
<td>2.68 ±0.53</td>
<td>3.95 ±1.30</td>
<td>2.36 ±0.47</td>
<td>I:II*, III:IV**</td>
</tr>
<tr>
<td>7.</td>
<td>3.85 ±0.55</td>
<td>2.64 ±0.46</td>
<td>4.13 ±1.03</td>
<td>2.66 ±0.62</td>
<td>I:II**, III:IV**</td>
</tr>
<tr>
<td>14.</td>
<td>4.18 ±0.97</td>
<td>2.81 ±0.55</td>
<td>4.19 ±1.39</td>
<td>2.92 ±0.31</td>
<td>I:II**, III:IV*</td>
</tr>
<tr>
<td>18.</td>
<td>2.86 ±0.60</td>
<td>2.37 ±0.57</td>
<td>2.69 ±0.55</td>
<td>3.24 ±0.36</td>
<td>II:IV**, III:IV*</td>
</tr>
</tbody>
</table>

legend: * - p<0.05; ** - p<0.01; P. - day of sponge insertion in the treated ewes
The plasma T\textsubscript{3} levels presented in Table 1. show that there are no differences between pregnant and nonpregnant treated ewes, except on the day of sponge insertion (3.02 ± 0.67 : 2.37 ± 0.58 nmol/L, p<0.05). In the control group of ewes there were also no statistically significant differences between pregnant and nonpregnant ewes except on day 18, after estrus (3.02 ± 0.67 : 2.37 ± 0.58 nmol/L, p<0.05). Data presented in Table 1. also show that plasma levels of T\textsubscript{3} in the treated ewes are significantly higher on days 0, 7, and 14.

Plasma T\textsubscript{4} levels in the treated group of nonpregnant and pregnant ewes after the synchronization of estrus, and in the control group of cycling ewes are presented in Table 2.

Table 2. Plasma T\textsubscript{4} levels (X ±SD nmol/L) in the treated group of nonpregnant and pregnant ewes after synchronization of estrus and in the control group of cycling ewes.

<table>
<thead>
<tr>
<th>Time of investigation (days)</th>
<th>treated (I) (nonpreg.)</th>
<th>control (II) (nonpreg.)</th>
<th>treated (III) (preg.)</th>
<th>control (IV) (preg.)</th>
<th>statistically significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of ewes</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>P.</td>
<td>69.58 ±6.62</td>
<td>-</td>
<td>74.83 ±19.94</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>0.</td>
<td>86.93 ±19.28</td>
<td>75.91 ±10.04</td>
<td>88.57 ±15.71</td>
<td>84.79 ±6.30</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>101.30 ±12.54</td>
<td>91.5 ±12.32</td>
<td>103.54 ±10.02</td>
<td>104.28 ±5.03</td>
<td>II:IV*</td>
</tr>
<tr>
<td>14.</td>
<td>104.94 ±13.01</td>
<td>100.49 ±8.2</td>
<td>101.68 ±10.46</td>
<td>100.96 ±10.37</td>
<td>-</td>
</tr>
<tr>
<td>18.</td>
<td>89.26 ±6.15</td>
<td>87.9 ±15.79</td>
<td>103.94 ±10.69</td>
<td>90.71 ±6.27</td>
<td>I:III**, III:IV**</td>
</tr>
</tbody>
</table>

Legend: * - p<0.05; ** - p<0.01; P. day of sponge insertion in treated ewes

The results presented in Table 2. show that there were no significant differences in plasma T\textsubscript{4} levels between the treated and control group of ewes, except for the T\textsubscript{4} level on day 18, when it was significantly higher in the treated pregnant ewes compared to groups I and IV. Those results could be explained by a reversion of the possible inhibitory effect of progestagen treatment on thyroid hormone secretion in ewes during the anoestrous season (Gvozdić et al., 1997). Exact places where this effect is mediated are still to be identified. There were no significant differences between plasma levels of the thyroid hormones in pregnant and nonpregnant animals in both groups of ewes. Only the mean plasma T\textsubscript{4} level on day 18 in the treated pregnant ewes was higher compared to the control pregnant ewes (103.94±10.69: 90.71±8.27 nmol/L, p.05).

DISCUSSION

The exact mechanism of the involvement of the thyroid gland in the regulation of the seasonality of reproduction in ewes remains to be clarified, but the investigation of Webster et al., (1991a) revealed an annual cycle of serum T\textsubscript{4}
levels in ewes. Values of serum T4 (X±SEM) reached a peak in winter (late breeding season) and a nadir in summer (late anestrus). During the low stage of the cycle the level of T4 in the serum of the control group of ewes was 39 ± 1.6 ng/mL (50.19 ± 2.06 nmol/L), and the maximum level during the high stage was 52 ± 2.2 ng/mL (66.92 ± 2.83 nmol/L). OCallaghan et al., (1993) found even lower values for serum thyroxine in rams during the breeding season (33.4 ± 4 ng/mL: 42.47 ± 5.12 nmol/L). Bekeova et al. (1994) also observed lower serum T3 and T4 levels in control ewes during the postpartum period, but the experiment was conducted during the anestrous season. Our results show that the values of plasma T4 in Tsigai ewes after synchronization of estrus during the breeding season are comparable to the control group, while plasma T3 levels are higher on most days of investigation (Table 1).

The end of the breeding season in the ewe results from the expression of an endogenous rhythm that is synchronized by the annual photoperiodic cycle (Karsch et al., 1989). This seasonal change in reproduction in ewes is based on the sensitivity of the hypothalamo-hypophyseal axis to negative feedback of estradiol (Legan et al., 1977). Secretion of T4 after the onset of reproductive activity is required for the endogenously generated change in neuroendocrine axis that leads to an intensified negative feedback and an end of the breeding season (Webster et al., 1991a). Thyroidectomy prevented this endogenously driven transition to anestrus and allowed estrous cyclicity to continue all year-round (Nicholls et al., 1988). On the other hand, supplementary thyroxine during the breeding season can advance anestrus and thus shorten the duration of the breeding season (OCallaghan et al., 1993). One possible explanation of our results could be based on the hypothesis that progestagen treatment during the breeding season can have a stimulatory effect on thyroid hormone secretion, or at least it no longer has an inhibitory effect on thyroid gland function (Gvozdić et al., 1997).

It is necessary to point out that the levels of T3 in the jugular venous plasma in the control group of ewes of Tsigai breed were lower at most investigated times compared to the nonpregnant and pregnant synchronized ewes.

The proposed inhibitory effect of progestagen treatment on thyroid hormone secretion is possibly connected to the central inhibitory influence of GABA on TSH secretion, but further investigation is necessary to determine the significance of the registered phenomenon, as well as the exact sites of its realisation.

It is interesting to speculate that progestagen treatment may have an inhibitory effect on the thyroid hormone secretion during the subsequent estrous cycle. If we start from this speculation, another question is the exact site of the progestagen effect on thyroid hormone secretion? Possible places were the influence of progestagen could be mediated are the hypothalamus or the pituitary gland. In monkeys and rats progesterone probably acts in the mediobasal hypothalamus and somewhere in the brain to block LH secretion, while the stimulatory effects of progesterone on the LH surge may well occur at the hypothalamic level in both species (Goodman and Knobil, 1981). The investigation of Webster et al., (1991b) failed to connect high frequency pulses of GnRH and LH in thyroidectomized ewes with changes in total number, distribution and light microscopic morphology of GnRH neurons in the hypothalamus and preoptic area. The investigation of the role of central neurotransmitters in the regulation of thyrotropin (TSH) secretion revealed that the central dopaminergic system has an inhibitory influence on TSH secretion in humans and rats (Krulich, 1982). Vijayan and McCann (1978) reported that intraventricular administration of
gama-aminobutyric (GABA) lowered basal TSH levels possibly via activation of the dopaminergic system because the effect could be abolished by pretreatment of the rats with a dopamine receptor blocker. \(\gamma\)-Aminobutyric acid is generally recognized as the major inhibitory neurotransmitter in the mature mammalian brain. The investigation of Han et al., (1995) established that a progesterone metabolite in rat hippocampal neurons potentiated GABA-mediated chloride currents, but Hales et al., (1994) found that GABA has excitatory actions on GnRH-secreting immortalized hypothalamic neurons. Our present work as well as the previous one (Gvozdić, et al., 1997) suggest that the proposed inhibitory effect of progestagen treatment on thyroid hormone secretion may be connected to the central inhibitory influence of GABA on thyroid stimulating hormone.

REFERENCES

Ispitivanja hormonalnog statusa ovaca nakon sinhronizacije estrusa u sezoni parenja vršena su određivanjem nivoa T₃ (trijodtironina) i T₄ (tiroksina) u krvnoj plazmi radioimunološkom (RIA) metodom. Uzorci krvne plazme su prikupljeni na dan stavljanja sundera (P), dan estrusa (0. dan), 7, 14. i 18. dana od estrusa. Nivo T₃ u krvnoj plazmi kontrolne grupe ovaca u prirodnom estrusnom ciklusu je značajno niži u odnosu na tretirane životinje, osim nivoa T₃ 18. dana posle estrusa. Nivo T₄ nije se značajno razlikovao između tretiranih ovaca i kontrolne grupe, izuzev 18 dana ispitivanja, kada je ustanovljen značajno veći nivo kod tretiranih gravidnih ovaca.