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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 (T_3) and thyroxine (T_4) 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 T_3 levels in the control group of ewes were significantly lower compared to the ewes after synchronization of estrus, except the level of T_3 on day 18 after estrus. Plasma T_4 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 gonadotropin 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 (T_4) 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 (T_4) treatment during the breeding season can advance anestrus and thus shorten the duration of the breeding season (OCallaghan *et al.*, 1993). Secretion of T_4 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 (T_4) and triiodthyronine (T_3) 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 T_3 and T_4 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 T_3 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 T_3 levels ($\bar{X} \pm SD$ nmol/L) in the treated group of nonpregnant and pregnant ewes after synchronization of estrus and in the control group of cycling ewes

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

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

The plasma T_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_3 in the treated ewes are significantly higher on days 0, 7, and 14.

Plasma T_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_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.

Time of investigation (days)	treated (I) (nonpreg.)	control (II) (nonpreg.)	treated (III) (preg.)	control (IV) (preg.)	statistically significant difference
number of ewes	9	13	11	7	
P.	69.58 ±6.62	-	74.83 ±19.94	-	-
0.	86.93 ±19.28	75.91 ±10.04	88.57 ±15.71	84.79 ±8.30	-
7.	101.30 ±12.54	91.5 ±12.32	103.54 ±10.02	104.28 ±5.03	II:IV*
14.	104.94 ±13.01	100.49 ±82	101.68 ±10.46	100.96 ±10.37	-
18.	89.26 ±8.15	87.9 ±15.79	103.94 ±10.69	90.71 ±8.27	I:III**, III:IV*

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_4 levels between the treated and control group of ewes, except for the T_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_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_4

levels in ewes. Values of serum T_4 ($\bar{X}\pm SEM$) 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 T_4 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 T_3 and T_4 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 T_4 in Tsigai ewes after synchronization of estrus during the breeding season are comparable to the control group, while plasma T_3 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 T_4 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 T_3 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 hypophyseal 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 neurotransmiters 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. γ-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 imortalized hypothalamic neurons. Our present work as well as the previous one (Gvozdić, *et al.*, 1997) suggest that the proposed inhibitory effect of progestagen tretment on thyroid hormone secretion may be connected to the central inhibitory influence of GABA on thyroid stimulating hormone.

REFERENCES

1. *Dahl GE, Evans NP, Thrun LA, Karsch FJ*, 1995, Thyroxine is permisive to seasonal transitions in reproductive neuroendocrine activity in the ewe, *Biol Reprod*, 52(3), 690-96
2. *Follett BK, Nicholls TJ* 1985, Influences of thyroidectomy and thyroxine replacement on phoperiodically controled reproduction in quail, *J Endocrinol*, 107, 211-22.
3. *Gvozdić D, Stojić V* 1997, Plasma thyroxine and triiodthyronine levels after induction and synchronization of estrus in the anoestrus season in ewes, *Acta Veterinaria*, 5-6, 271-76.
4. *Goodmann RL, Knobil E* 1981, The sites of action of ovarian steroids in the regulation of LH secretion. *Neuroendocrinol*, 32, 57-63.
5. *Hales GT, Sanderson MJ, Charles AC* 1994. GABA has excitatory actions on GnRH-secreting imortalized hypothalamic (GT1-7) neurons, *Neuroendocrinol*, 59, 297-308.
6. *Han MC, Hu YF, Zorumski CF, Covey DF* 1995, Neurosteroid analogues.3. The synthesis and electrophysiological evaluation of benz(e)indene congeners of neuroactive steroids having the 5 beta configuration, *J Med Chem*, 38, 22, 4548-56.
7. *Jallageas M, Assenmacher I* 1979, Further evidence for reciprocal interactions between the the annual sexual and thyroid cycles in male Peking ducks, *Gen Comp Endocrinol*, 37: 44-51.
8. *Karsch FJ, Robinson JE, Woodfill CJI, Brown MB* 1989, Circannual cycles of luteinizing hormone and prolactin secretion in ewes during prolonged exposure to a fixed photoperiod: evidence for an endogenous reproductive rhythm, *Biol Reprod*, 41, 1034-46.
9. *Krulich L* 1982, Neurotransmiter control of thyrotropin secretion. *Neuroendocrinol*, 35, 139-47.
10. *Legan SJ, Karsch FJ, and Foster DL* 1977, The endocrine control of seasonal reproductive function in the ewe: a marked change in response to the negative feeback action of estradiol on luteinizing hormone secretion, *Endocrinol*, 101, 818-24.
11. *Nicholls TJ, Goldsmith AR, Dawson A* 1988, Photorefractoriness in birds and comparison with mammals, *Physiol Rev*, 68, 133-76.
12. *OCallaghan D, Wendling A, Karsch FJ, Roche JF* 1993, Effect of exogenous thyroxine on timing of seasonal reproductive transmision in ewes, *Biol Reprod*, 49,2, 311-15.
13. *Vijayan E, McCann SM* 1978, Effects of intraventricular injection of γ-aminobutyric acid (GABA) on plasma growth hormone and thyrotropin in conscious ovariectomized rats, *Endocrinol*, 103, 1888-93.
14. *Webster RJ, Moenter SM, Woodfill CJI, Karsch FJ* 1991a, Role of the thyroid gland in seasonal reproduction. II. Thyroxine allows a season-specific suppression of gonadotropin secretion in sheep, *Endocrinol*, 129, 176-183.
15. *Webster JR, Moenter SM, Barrell CJ, Lehman MN, Karsch FJ* 1991b, Role of the thyroid gland in seasonal reproduction. III. Thyreoidectomy blocks seasonal suppression of gonadotrophin-releasing hormone secretion in sheep, *Endocrinol*, 129,3, 1635-43.
16. *Weiselthier AA, Van Tienhoven A* 1972, The effect of thyroidectomy on testicular size and on photorefractory period in the starling (*Sturnus vulgaris L.*), *J Exp Zool*, 179, 331-38.

KONCENTRACIJA TIROKSINA I TRIJODTIRONINA U KRVNOJ PLAZMI OVACA POSLE SINHRONIZACIJE ESTRUSA U SEZONI PARENJA

GVOZDIĆ D i STOJIĆ V

SADRŽAJ

Ispitivanja hormonalnog statusa ovaca nakon sinhronizacije estrusa u sezoni parenja vršena su određivanjem nivoa T_3 (trijodtironina) i T_4 (tiroksina) u krvnoj plazmi radioimunološkom (RIA) metodom. Uzorci krvne plazme su prikupljani na dan stavljanja sunđera (P), dan estrusa (0. dan), 7, 14. i 18. dana od estrusa. Nivo T_3 u krvnoj plazmi kontrolne grupe ovaca u prirodnom estrusnom ciklusu je značajno niži u odnosu na tretirane životinje, osim nivoa T_3 18. dana posle estrusa. Nivo T_4 nije se značajno razlikovao između tretiranih ovaca i kontrolne grupe, izuzev 18 dana ispitivanja, kada je ustanovljen značajno veći nivo kod tretiranih gravidnih ovaca.