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

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The thyroid hormonal status of ewes after induction and synchronization of estrus in the anoestrous season was investigated by determination of plasma triiodorthyronine (T_3) and thyroxine (T_4) concentrations using radioimmunoassay (RIA). Samples of jugular venous plasma were collected on the day of sponge insertion (P), at estrus (day 0.) as well as 7, 14 and 18 days after estrus. Plasma T_3 and T_4 levels in the control group of anoestrous ewes were significantly higher than those in the ewes after synchronization of estrus, except, for the level of T_3 on days 14. and 18. after estrus, and T_4 on the day of sponge insertion.

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

INTRODUCTION

The thyroid gland plays a fundamental role in seasonal reproduction in the ewe. Thus, it is necessary for the transition from the breeding season to anoestrus, but the importance of the seasonal changes in the thyroid hormone secretion in this process remains to be investigated. Thyroxine (T_4) treatment during the breeding season can advance anoestrus and thus shorten the duration of the breeding season *(O'Callaghan et al., 1993)*. Secretion of T_4 after the onset of reproductive activity is required for endogenously generated changes in the neuroendocrine axis that lead to 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 anoestrous ewes of the Tsigai breed.

MATERIAL AND METHODS

After induction and synchronization of estrus in the anoestrous season plasma T_3 and T_4 concentrations of ewes were determined using commercial radioimmunoassay (RIA) kits (INEP-Zemun). Induction and synchronization of estrus during the anoestrous period (May/June) was made by progestagen treatment (intravaginal sponges impregnated with 60 mg of MPA/12 days) followed by 600-700 IU of PMSG/ewe (20 ewes). The control group during the anoestrous season consisted of 10 untreated ewes. Samples of jugular venous plasma were collected on the day of sponge insertion (P), on the day of estrus (day 0). and 7, 14 and 18 days after the estrus. Differences in mean plasma hormone levels between treated and control ewes were tested using Student's t-test.

RESULTS

Plasma T_3 levels in the experimental groups of nonpregnant and pregnant ewes after the induction and synchronization of estrus and in the control group of anoestrous ewes are shown in Table 1.

Table 1. Plasma T_3 levels ($\bar{X} \pm SD$ nmol/L) in the experimental groups of nonpregnant and pregnant ewes after induction and synchronization of estrus and in the control group of anoestrous ewes.

Day of investigation	experimental group - (I) nonpregnant	experimental group- (II) pregnant	Control group - (III)	statistical significance of difference
number of ewes	13	7	10	
P.	1.68 ± 0.32	1.66 ± 0.46	2.31 ± 0.64	I : III*, II : III*
0.	2.06 ± 0.51	2.06 ± 0.72	2.87 ± 0.91	I : III*
7	1.62 ± 0.21	1.63 ± 0.41	2.22 ± 0.41	I : III**, II : III*
14.	1.83 ± 0.58	1.83 ± 0.30	2.11 ± 0.20	–
18.	2.01 ± 0.39	2.03 ± 0.74	1.98 ± 0.36	–

legend: * - $p<0.05$; ** - $p<0,01$

Plasma T_4 levels in the experimental groups of nonpregnant and pregnant ewes after the induction and synchronization of estrus, and in the control group of anoestrous ewes are shown in Table 2.

Table 2. Plasma T_4 levels ($\bar{X} \pm SD$ nmol/L) in the experimental group of nonpregnant and pregnant ewes after the induction and synchronization of estrus, and in the control group of anoestrous ewes.

Day of investigation	experimental group - (I) nonpregnant	experimental group- (II) pregnant	Control group - (III)	statistical significance of difference
number of ewes	13	7	10	
P.	58.30 ± 13.01	52.99 ± 7.18	57.06 ± 15.37	–
0.	48.72 ± 5.40	47.72 ± 3.76	60.63 ± 3.06	I : III**, II : III**
7.	54.40 ± 7.90	55.67 ± 7.02	67.65 ± 18.43	I : III**
14.	43.24 ± 11.50	50.13 ± 16.71	80.25 ± 24.33	I : III**, II : III*
18.	63.24 ± 7.11	61.06 ± 3.66	91.71 ± 15.08	I : III**, II : III**

legend: * - $p<0.05$; ** - $p<0.01$

Plasma T_3 and T_4 levels in the control group of anoestrous ewes were significantly higher then in the ewes after synchronization of estrus,except for the level of triiodthyronine on day 14. and 18. after estrus, and thyroxine on the day of sponge insertion. Those results could be explained by a possible inhibitory effect of progestagen treatment on thyroid hormone secretion in ewes during the anoestrous season. The exact site of this effect cannot yet be defined. There were no significant differences in plasma levels of the thyroid hormones between pregnant and nonpregnant ewes after the synchronization of estrus.

DISCUSSION

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 anoestrus). During the low stage of the cycle the level of T_4 in the serum of the control group of ewes was 39$\pm$1.6 ng/mL (50. 19$\pm$2.06 nmol/L), and the maximum level during the high stage was 52$\pm$2.2 ng/mL (66.92$\pm$2.83 nmol/L). *O'Callaghan et al., (1993)* found even lower values of the serum thyroxine in rams during the breeding season (33.4$\pm$4 ng/mL: 42.47$\pm$5.12 nmol/L). Our results show that the values of plasma T_4 and T_3 in Tsigai ewes after the induction and synchronization of estrus during the anoestrous season are lower than in the control group.

It is well established that the seasonal changes in reproduction in ewes depend 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., 1991s).* In the ewe, the end of the breeding season results from the expression of an endogenous rhythm that is synchronized by the anual photoperiodic cycle *(Karsch et al., 1989).* Thyroidectomy prevented this endogenously driven transition to anoestrus and allowed estrous cyclicity to continue year-round *(Nicholls et al., 1988).* On the other hand, supplementary thyroxine during the breeding season can advance anoestrus and thus shorten the duration of the breeding season *(O'Callaghan et al., 1993).* It is interesting to speculate that progestagen treatment may have an inhibitory effect on thyroid hormone secretion during the subsequent estrous cycle. If we start from this speculation, another question is the exact place of progestagen effect on thyroid hormone secretion. Possible places of progestagen influence could be 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 neurotransmitters in the regulation of thyrotropin (TSH) secretion showed that the central dopaminergic system inhibited TSH secretion in humans and rats *(Krulich, 1982). Vijayan and McCann (1978)* reported that intraventricular administration of γ-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 potentiates GABA-mediated chloride currents,but *Hales et al., (1994)* found that GABA has excitatory actions on GnRH-secreting immortalized hypothalamic heurons.

However, it is neccessary to point out that the levels of thyroid hormones in the jugular venous plasma in almost all investigated intervals in the control group of anoestrous ewes of Tsigai breed were higher compared to both the nonpregnant and pregnant synchronized ewes. The proposed inhibitory effect of progestagen treatment on thryoid hormone secretion may be connected to the central inhibitory influence of GABA on TSH secretion, but further investigation is necessary to determine the significance of the registred phenomenon, as well as the exact place of its occurrence.

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KONCENTRACIJA TIROKSINA I TRIJODTIRONINA U KRVNOJ PLAZMI OVACA POSLE INDUKCIJE I SINHRONIZACIJE ESTRUSA VAN SEZONE PARENJA.

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SADRŽAJ

Ispitivanja hormonalnog statusa ovaca nakon indukcije i sinhronizacije estrusa van sezone 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 i T_4 u krvnoj plazmi kontrolne grupe ovaca je značajno viši u svim ispitivanim periodima od nivoa hormona kod ogledne grupe nakon indukcije i sinhronizacije estrusa, osim nivoa T_3, 14. i 18. dana od estrusa, i nivoa T_4,na dan stavljanja sunđera.