

## Evolution of the Thyroid Hormones (T<sub>3</sub>, T<sub>4</sub>) and Progesterone Levels in Ewes and Gimmers after the Insertion of Melatonin Implants

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### Abstract

Investigations were carried on in a private farm from Cenad, Timis County, on 14-15 months of age gimmers from the indigenous breeds Transylvanian Merino (9) and Turcana (6). The gimmers were inserted in 14 May 2009 with Melatonin implants of 18 mg. Blood samples were collected immediately after the insertion of the implants and after 15 and 30 days. Hormones analysis triiodothyronine (T<sub>3</sub>), triiodothyronine (T<sub>4</sub>) and progesterone level from the blood samples were assayed using ELISA method. Data shown a decrease of the progesterone levels after 30 days from the insertion of the implants, but the difference of 4.69 mg/ml is not statistically significant ( $p>0.05$ ), and the triiodothyronine (T<sub>3</sub>, T<sub>4</sub>) levels have registered small and insignificant variations ( $p>0.05$ ). Results may suggest that melatonin implants do not influence significant ( $p>0.05$ ) the thyroid hormones (T<sub>3</sub>, T<sub>4</sub>) and the progesterone levels.

**Keywords:** ewe, progesteron, thyroid hormones

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### 1. Introduction

Sheep breeders from Romania are starting to show interest in practicing some low input reproduction biotechnologies that are easy to carry out and are not time consuming. By witch to register fall lambing in September and October months, or early lambing (in December-January) in order to produce milk-lambs for the southern European market (Italy, Croatia, Greece etc) at a moment when the request for this product is high. [1, 2]

The best reproduction season of ovine in our country is autumn, which accords with the gradual decreasing of long day-light (over 14 hours) to an ratio of 12 hours of light and 12 hours of dark (1:1). There have been specified some important

differences between breeds: sheep with fine and semifine have a normal reproduction season near to the light-dark ratio 1:1 (September), while latish sheep (with thick wool) answer to the days with a long period of dark (October). The seasonality of the reproduction function on sheep is more striking, as the change between seasons is higher. [3] The photoperiodism effect is used in spring-summer period, when the light-dark ratio is more in the favor of light (14-16 hours of light). In this way, there are used some reducing of the day-light procedures (dark chambers) and of its intensity, or there are used melatonin implants for significant increase of the frequency of the sheep who develop estrus and reproduction indexes. [4, 5, 6] The continuous administration of melatonin through the help of some hypodermic implants (Melovine R, Requilin R, types) can permit the minimization of the short days (specific to the principal autumn season), even if retina accepts long spring –summer days. [7, 8, 9] The ovine

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breeders that use natural mating, will insert melatonin implants, with 30-40 days before rams are inserted. On rams, these implants are inserted 10 days sooner than the ewes are. [10]

Aim of this research was to highlight if the utilization of one 18 mg implant of melatonin/ewe at the end of spring season influences significant the level of three very important hormones (triiodothyronine, tetraodothyronine and progesterone) for the ewes organism.

## **2. Materials and methods**

Investigations were carried on in May-June 2009, in a private farm from Cenad, Timis County.

On 14-15 month of age young sheep from Transylvanian Merino (15 subjects) breeds, one implant (Melovin) was inserted subcutaneous in the ear, on 15.05.2009, implant were impregnated with 18 mg melatonin. Immediately after the insertion of the implants, 5 ml of blood was collected from each gimmer from the jugular vein, on heparin. The blood was sampled again, from the same animals after 15 and 30 days from the melatonin implants insertion. Entire batch of blood samples were frozen at  $-18^{\circ}\text{C}$ .

In order to assay triiodothyronine, tetraodothyronine and progesterone hormones, blood samples have been defrosted at the room temperature ( $20-30^{\circ}\text{C}$ ), and then the steps from the ELISA method were carried out. The results riding has been made with the ELISA reader at a length of 450 nm, in maximum 30 minutes after adding the stopping solution.

Raw values obtained in mg/ml have been bio-statistic analyzed.

The significance of differences between the analyzed hormones (triiodothyronine, tetraodothyronine and progesterone) from the three blood samples were calculated by using the nonparametric Mann Whitney test, that is recommended for experiments on relatively low number of subjects.

## **3. Results and discussion**

Sustentation of the health status of the ewes and the intensification of the reproduction and also the increase in milk, meat and fleece production depends directly on the normal function of the endocrine glands.

Stimulation of productions and breeding activity by using endocrinological methods, like the usage of melatonin, must not significant modify activity of other hormones that have important roles in the general metabolism [11].

In that direction, the evolution of three important hormones was studied (triiodothyronine, tetraodothyronine and progesterone), after the insertion of a melatonin implant that was impregnated with 18 mg melatonin.

Results registered are presented in table 1.

From the analysis of the information from this table it can be observed that triiodothyronine in the moment of the insertion of the melatonin implants has varied at the first blood sampling between 1.611 and 8.0000 mg/ml serum, with an mean of  $4.33\pm 0.620$  ng/ml. After 15 days, variation limits remained almost unchanged (1.732 ng/ml and 8.000 ng/ml), and the average value is slightly lower (3.867 ng/ml/serum) compared to the first blood sampling.

At the third blood sampling (30 days) variation of the triiodothyronine is sensitively higher from 2.091 ng/ml to 10.516 ng/ml/serum, with an average of 5.366 mg/ml/serum.

Tetraodothyronine (tyroxin) has evaluated slightly different. At the first blood sampling this hormone has varied in wider limits, between 3.331 mg/ml and 14.342 ng/ml/serum, with a mean per entire blood samples analyzed  $7.172\pm 0.720$  ng/ml/serum. Variation amplitude has grown at second sampling, being between 5.260 and 30.000 ng/ml/serum and an average of  $9.376\pm 2.19$  ng/ml/serum.

At the third blood sampling, variation amplitude (4.929 ng/ml – 30.000 ng/ml/serum) has been reduced compared to the results registered during the second blood sampling, but the average rised at 9.807 ng/ml/serum.

Our study's results are in concordance with results presented by Constantin et al, [11], emphasizing that tetraodothyronine is less active but is produced in higher level compared to triiodothyronine.

Comparative to the tyroidian hormones ( $T_3$ ,  $T_4$ ) the serum level of progesterone has varied in much wider limits in all three blood samplings.

During first blood sampling, progesterone has varied between 0.273 ng/ml and 40.000 ng/ml/serum with an average of 11.2588 ng/ml/serum. A similar situation was registered during second blood sampling (0.273 ng/ml –

40.000 ng/ml/serum) with an average of 11.324 ng/ml serum.

At the third blood sampling, variation limit was almost the same (0.1000 ng/ml – 40.000 ng/ml/serum) but the average has dropped to 6.598±3.53 ng/ml/serum, very probably because of the regression of the luteal body, at the great majority of the analyzed subjects.

By carrying out the significance testing between the blood samplings for the analyzed hormones levels (table 2) results shown that differences regarding average levels between the three blood samplings, although have high values, especially in the case of the progesterone, are not significant (p<0.05)

Analyzing the variance of hormones levels by the variability coefficient emphasize that this value is very great (38.91-206.98%), especially in the case of the progesterone. [12] The high variability could be the explanation for the large differences between the averages, like in the case of progesterone, differences between the levels of this hormone at the three blood samplings being insignificant.

Based on this study we can conclude that implants with 18 mg melatonin are not influencing significantly the levels of triiodothyronine, tetraiodothyronine and progesterone.

**Table 1.** Evolution of the tyroidian hormones (T<sub>3</sub>, T<sub>4</sub>) and of progesterone (P<sub>4</sub>) after the insertion of the melatonin implants (18 mg/implant)

Cod	Triiodothyronine (T <sub>3</sub> ) ng/ml/serum			Tetraiodothyronine (T <sub>4</sub> ) ng/ml serum			Progesterone (P <sub>4</sub> ) ng/ml serum		
	1	2	3	1	2	3	1	2	3
1	2.392	1.890	7.608	7.126	7.624	6.732	40.000	0.747	2.117
2	1.683	1.978	10.516	6.346	8.251	7.058	0.529	40.000	1.865
3	8.000	2.897	2.587	3.331	8.251	6.674	40.000	1.329	0.100
4	2.101	8.000	2.410	6.065	30.000	5.985	0.273	40.000	0.812
5	8.000	2.282	8.000	5.731	5.234	30.000	1.036	0.438	40.000
6	3.517	8.000	7.663	12.483	6.090	7.504	0.969	1.565	2.685
7	3.497	2.388	8.000	6.217	4.514	5.262	0.845	0.903	0.731
8	3.624	2.185	2.745	5.329	5.260	7.671	0.901	0.920	0.932
9	1.611	1.732	2.749	5.870	6.115	5.923	40.000	0.273	0.368
10	4.010	1.887	2.475	5.573	5.501	7.322	0.100	40.000	0.554
11	8.000	3.194	7.294	14.342	3.760	4.960	40.000	1.005	40.000
12	3.182	8.000	2.091	7.959	30.000	4.929	0.915	40.000	0.770
13	8.000	2.315	8.000	7.745	7.327	30.000	2.899	0.733	0.400
14	3.981	8.000	5.380	6.107	6.570	9.900	0.365	0.751	6.700
15	3.437	3.263	2.975	7.362	6.136	7.184	0.488	1.192	0.939
x	4.336	3.867	5.366	7.172	9.376	9.807	11.288	11.324	6.598
sx	0.62	0.68	0.74	0.72	2.19	2.14	4.63	4.63	3.53
s	2.408	2.618	2.883	2.791	8.471	8.293	17.933	17.902	13.657
CV%	55.54	67.70	53.72	38.91	90.35	84.57	158.87	158.09	206.98

Note: 1 – blood samples immediately after the implants insertion

2 – blood samples after 15 days from the insertion and 3 blood samples after 30 days from insertion

x – mean, sx- mean error, s – standard deviation, CV% - coefficient of variation

**Table 2.** Difference significance between the tyroidian hormones (T<sub>3</sub>, T<sub>4</sub>) and progesterone (P<sub>4</sub>) at the three blood samplings

Sampling	T <sub>3</sub>				T <sub>4</sub>				P <sub>4</sub>			
	1		2		1		2		1		2	
	d	p	d	d	d	p	d	p	d	p	d	p
3	1.000 <sup>NS</sup>	0.63	1.469 <sup>NS</sup>	0.110	2.635 <sup>NS</sup>	0.60	0.431 <sup>NS</sup>	0.60	4.69 <sup>NS</sup>	0.90	4.726 <sup>NS</sup>	0.60
2	0.469 <sup>NS</sup>	0.27	-	-	2.204 <sup>NS</sup>	0.98	-	-	0.036 <sup>NS</sup>	0.58	-	-

Note: d – difference; p – error value threshold; NS – insignificant difference (p>0,05)

#### 4. Conclusions

From the thyroidian hormones, the lowest level in all three serum samples (right after the insertion of the melatonin implants and after 15 and 30 days) was registered at triiodothyronine ( $4.336 \pm 0.62$ ;  $3.867 \pm 0.68$ ;  $5.366 \pm 0.74$  ng/ml/serum) and a more moderate level was registered in tetraiodothyronine ( $7.172 \pm 0.72$ ;  $9.376 \pm 2.19$ ;  $9.807 \pm 2.14$  ng/ml/serum).

Progesterone registered average values very similar at the first two samplings ( $11.288 \pm 4.63$  ng/ml/serum;  $11.324 \pm 4.63$  ng/ml/serum) and a very high variability, but with similar CV % (158.87%; 158.09%). At 30 days from the implant insertion, progesterone level has dropped significantly, to 6.589 ng/ml/serum, representing 41.6%.

Differences between average levels of the hormones during the three blood samplings are not significant ( $p > 0.05$ ) and the results suggest that melatonin (18mg) from the implants is not influencing the levels of triiodothyronine, tetraiodothyronine and progesterone.

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