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LANSZKI J., THÉBAULT R-G., ALLAIN D., SZENDRŐ ZS., EIBEN CS.

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THE EFFECT OF MELATONIN TREATMENT ON HAIR FOLLICLE CYCLE IN ANGORA RABBITS

J. LANSZKI¹, R-G. THÉBAULT², D. ALLAIN³, Zs. SZENDRÕ¹, Cs. EIBEN⁴

¹University of Kaposvár, Faculty of Animal Sciences, P.O. Box 16, 7401 Kaposvár, Hungary, lanszki@atk.kaposvar.pate.hu

²INRA, Génétique Animale Phanères, Le Magneraud, B.P. 52, 17700 Surgères, France
³INRA, SAGA, B.P. 27, 31326 Castanet-Tolosan Cedex, France
⁴Institute for Small Animal Research, P.O.Box 417, 2101 Gödöllö, Hungary

ABSTRACT

Male and female angora rabbits, aged between 200 and 210 days at the beginning of the experiment and kept according to a natural photoperiod, were either treated with melatonin in May (group M: 36 mg per animal), or remained as an untreated control (group C). Two methods of wool harvesting were used: defleccing or shearing, the duration of the experimental period being 98 days. Skin samples were taken from 36 animals (plucked, n= 16; shorn, n= 20) six times during the experimental period.

Melatonin treatment resulted in a 32 % increase in the number of active follicles per hair follicle group (*lateral primaries* and *secondaries*) (M: 45, C: 34, P<0.001). At the end of the experimental period the number of wool fibres per follicle group in the melatonin-treated plucked angora rabbits had reached that of the shorn control group. The males surpassed the females in this respect by 24 % (male 45, female 37, P<0.01).

By the effect of the melatonin treatment the proportion of hair fibres still in the process of growth began to decrease sooner. On the 98th day hair follicles in the telogen phase were found only in the treated plucked angora rabbits (13%, P<0.001); within the shorn groups no substantial difference between groups M and C was ascertained. The proportion of follicles in the anagen phase decreased more rapidly in the males.

INTRODUCTION

In temperate climates the wool production of angora rabbits fluctuates with the seasons, summer production levels having found to be the lowest (ROCHAMBEAU and THÉBAULT, 1985; ROUGEOT and THÉBAULT, 1983; THÉBAULT and VRILLON, 1994; SCHLOLAUT, 1987) in both French and German strains and in the case of both defleecing and shearing as methods of wool removal (THÉBAULT and VRILLON, 1994). The weight of the shorn wool produced is influenced by the number of wool fibres, their length and their diameter (ROUGEOT and THÉBAULT, 1983). The number of active wool fibres per follicle group is determined by the season, via the photoperiod, but the total number of hair follicles is not influenced by this (THÉBAULT and VRILLON, 1994). The relationship between wool production and photoperiodism has been verified by histological examinations; the ratio of inactive hair follicles has been measured at 12% in spring and 28 to 30% in summer (ROCHAMBEAU and THÉBAULT, 1985). In summer the adverse effects of both increased day length and hot conditions are simultaneously present. In defleeced angora does a decrease in wool production can be prevented by means of melatonin treatment in summer (ROUGEOT et al., 1986; ALLAIN and THÉBAULT, 1988), which provides evidence that photoperiod may, in itself, also exert a substantial influence.

This study examined the effect of melatonin treatment on the number of hair follicles (lateral primaries and secondaries) in a stock of deflected or shorn male and female angora rabbits.

MATERIAL AND METHOD

The German type angora rabbits used were 200-210 days old (after the 3^{rd} shearing or defleccing) at the beginning of the experiment. The animals were kept individually in a closed building, fitted with windows, according to a natural photoperiod (46°40' N), in flat-deck cages (LANSZKI *et al.*, 2000).

The interval between two harvests (shearing or defleccing) was 98 days. Before defleccing (at the beginning and end of the experiment) Lagodendron-R mix (trade mark, Proval S.A., Paris, France) was fed.

The animals were allocated to two groups at random: one group treated with melatonin (M: 36 mg per animal) and a control group (C) without melatonin treatment. Melatonin treatment was performed in spring (on May 6th and 13th) by Regulin implant, containing 18 mg melatonin (Hoechst UK Ltd., catalogue no. 0086/4176).

For the purpose of the histological examination 33 mm² skin samples were taken, by means of a skin biopsy procedure, at the beginning of the experiment, and subsequently after 2, 4, 8, 12 and 14 weeks, from the dorsal region of the pelvis, from 16 defleeced females, 10 shorn females and 10 shorn males. The skin sampling procedure was performed with local anaesthetic (by the use of Lidocain spray) and use of disinfectant thereafter. The skin samples were stored in 10 % formalin solution, then in Bouin solution for the 24 hours immediately prior to processing. Sectioning of skin samples at 7 μ m thickness was performed by means of paraffin section Mikrotom apparatus at the level of the sebaceous gland. The ROAN procedure (ROUGEOT and THÉBAULT, 1983) was used for the staining of the sections.

A light microscope was used for the histological evaluation procedure, which was based on 10-20 hair follicle groups per sample, with respect to the following parameters:

(1) The number of hair follicles in anagen and telogen phases (lateral primaries and secondaries) in the central hair follicle group;

(2) The proportion of hair fibres per follicle group which were in the anagen phase (i.e., in the process of growth), in relation to the total number of hair fibres in the follicle group (THÉBAULT and VRILLON, 1994).

The data were analysed by means of the SAS GLM procedure (SAS, 1993). The variables studied were the ratio of anagen, the number of anagen, telogen and total hairs per group, using a fixed effect model of variance analysis, such as:

$$Y_{ijkl} = \mu + MELT_i + HM_j + S_k + e_{ijkl}$$

Where: Y_{ijk} is the *l*th observation on the *i*th experimental group, the *j*th method of fleece harvest, the *k*th sex; μ the overall mean; MELT_i the treatment effect (i=2, melatonin or control); HM_j the harvest method effect (j= 2, defleecing or shearing); S_k the sex effect (k= 2, male or female) and e_{ijkl} the random error. An additional effect due to an interaction between melatonin treatment and harvesting method was introduced into the model for the number of follicle types.

RESULTS AND DISCUSSION

The melatonin treatment was found to exert a substantial effect on the hair follicle cycle (<u>table</u> <u>1</u>). In treated angora rabbits the number of follicles (lateral primaries and secondaries) per hair follicle group was greater than that in the control group as early as the 2nd week. By the 14th week the difference between the groups had risen to 32 %. Thus, by the use of melatonin

treatment in May one third more hair follicles had become active by the end of the experimental period.

The method used for wool harvesting influenced significantly (P<0.001) hair follicle activity throughout the experimental period. From the beginning of the experiment the number of wool fibres per hair follicle group in the shorn angora rabbits was higher than that in those defleeced (table 1). However, by the end of the experimental period the number of wool fibres per hair follicle group in treated defleeced rabbits had reached that recorded in the control shorn animals (figure 1). No significant interaction between treatment and wool harvesting method was found in any case. However, although with respect to the number of hair follicles in the telogen phase a statistically verifiable (P<0.001) interaction was ascertained in the 4th, 8th and also the 12^{th} week (table 1).





***P<0.001

A significant sex effect was observed in most cases in the second half of the experimental period (table 1). At the end of the experiment the number of wool fibres per follicle group in the males surpassed that recorded in the females by 24 %. This may have been why the significantly (p<0.001) lighter males did not produce significantly less wool (Lanszki *et al.*, 2000).

Due to the melatonin treatment hair follicle activity increased rapidly, reached a maximum in the 4th week and decreased slowly thereafter (table 1). The number of telogen hair follicles was higher in treated animals than in controls while in controls the number of anagen hair follicles remained nearly constant throughout the experimental period.

The wool harvesting method also influenced hair follicle activity (table 1). Where rabbits were defleeced there were no hair follicles in the telogen phase even in the 14th week in the control group, while in melatonin-treated rabbits 13% of hair follicles were in the telogen phase at that time (figure 2). No significant difference due to the treatment was observed in the shorn groups with respect to the proportion of hair fibres in the process of growth (figure 2). Irrespective of which method was used for removing the wool, a small proportion of hair fibres reached the resting phase within 98 days. Sex exerted a significant influence on hair follicle activity between the 2^{nd} and the 10^{th} week: the number of hair follicles in the telogen phase increased more rapidly in the males by the effect of melatonin treatment.



Figure 2 - Hair follicle activity (proportion of hair follicles in the anagen phase) in melatonintreated (M) and control (C) angora rabbits, with respect to method used for wool removal

CONCLUSIONS

The findings of the histological examinations of the skin supported the results obtained by the authors in a study on wool production (LANSZKI *et al.*, 2000). By the effect of the melatonin treatment wool production increased by one third, and the number of hair fibres (lateral primaries and secondaries) per follicle group was also found to rise by a similar proportion.

Our results corroborate the hypothesis according to which the seasonal period of increased day length leads to a decrease in wool production via decreasing hair follicle activity (ROCHAMBEAU and THÉBAULT, 1985). The melatonin treatment administered in May exerted an effect opposite to the increase in day length; therefore, the number of wool fibres per follicle group increased in relation to that recorded in the control group.

These findings demonstrate that, when shearing or defleccing is performed at the end of spring, hair follicles do not reach maturation in 98 days, even with melatonin treatment (as most of the hair follicles were still active at that time). In fact the duration of hair growth required before maturation is reached between 110 and 120 days, according to ROCHAMBEAU (1988). Proportions of hair follicles in the anagen or telogen phase remained nearly constant throughout the entire experimental period in the shorn groups; this is attributable to an asynchronous activity of hair follicles caused by shearing.

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								Tin	ne of sa	mpling	L. C.							
	Begim	ning o	f expt.	21	nd wee	k	4	th week		81	h wee	×	12	th wee	k	17	th wee	sk
						Nui	nber of	fibres 1	per hair	follicl	e grou	p (LSN	1)					
	Α	Τ	S	A	Η	S	A	Н	S	A	Τ	S	A	Н	S	A	Н	S
Overall mean	25.4	3.1	29.6	27.1	1.7	28.8	32.8	1.9	35.5	32.0	3.4	35.0	31.7	4.6	36.7	31.2	7.8	39.0
RSD	11.1	4.6	10.8	11.3	3.1	11.4	11.9	3.0	13.3	11.2	4.9	12.0	11.8	5.4	12.9	11.9	7.8	12.0
Melatonin treatm. effect	ns	ns	SU	* * *	ns	* *	* * *	ns	* * *	* *	* * *							
M (n= 15) - C (n= 15)	-2.7	-1.1	-0.7	3.7	-0.2	3.4	8.8	0.7	9.5	7.8	3.0	10.9	10.5	0.8	10.4	8.4	2.4	10.9
Harvesting method effect	* * *	* * *	* * *	* * *	* * *	* * *	NS	* * *	* * *	* * *	* * *	* * *	*	* * *	* * *	*	* * *	* * *
defleeced - sheared	-29.4	-4.5	-33.4	-17.3	-3.0	-19.8	-9.3	-11.0	-13.1	-4.0	-5.3	-8.3	-4.5	-7.8	-12.7	0.9	-9.5	-8.7
Melatonin x harvest method effect	*	us	ns	su	us	ns	* * *	* * *	ns	su	* * *	ns	ns	* * *	ns	ns	su	ns
Sex effect	*	us	ns	ns	* *	ns	* * *	* * *	*	ns	* * *	ns	ns	ns	*	* * *	ns	*
male - female	16.8	2.8	16.0	12.8	1.3	13.8	10.7	1.3	10.3	1.1	5.6	6.9	6.4	4.9	12.2	4.1	4.4	8.7

Table 1. Number of fibres per hair follicle group (estimates within effect) in melatonin-treated and control angora rabbits

LSM= least square mean, RSD= residual standard deviation, n= number of animals, A= anagen, T= telogen, S= total number of hair fibres, M= melatonin-treated, C= control, ns: P>0.05, *P<0.05, **P<0.01, ***P<0.001