THE EFFECTS OF VARIOUS MELATONIN TREATMENTS ON THE SUMMER SEMEN CHARACTERISTICS OF ANGORA RABBITS

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Abstract - Angora bucks (n = 15) of 1 to 1.5 years of age were divided into 3 groups and shorn every other week. Their semen was collected twice a week, two ejaculations taken per day. The rabbits of the first group (A) were treated twice with melatonin, on July 19th and 6 weeks later, administering 18 - 18 mg each time. The second group (B) was treated only once, on July 19th with 36 mg of melatonin, while the third group was left untreated. The treatments were carried out by inserting melatonin implants subcutaneously.
Our results suggest that the favourable effect of the melatonin treatment becomes obvious after 6 weeks in most cases. During the second experimental period (weeks 7 to 12) the results of groups A, B and C were as follows, respectively: body weight (12th week): 3.71, 3.41 and 3.31 kg (NS); daily feed intake: 175, 176 and 164 g/day (NS); time interval between introduction of the doe and ejaculation by the buck: first ejaculation: 11.3, 11.5 and 18.0 sec. ($P<0.001$); second ejaculation: 8.8, 9.1 and 14.3 sec. ($P<0.01$); ratio of bucks not ejaculating: 8.2, 9.1 and 11.7 % ($P<0.05$); volume of ejaculate: 0.77, 0.72 and 0.45 ml ($P<0.001$); sperm concentration: 14.2, 11.5 and 11.9 x 10^6/ml (NS); ratio of live sperm: 52.5, 56.7 and 34.2 % ($P<0.01$), ratio of normal sperm: 89.5, 88.1 and 85.5 ($P<0.001$).

INTRODUCTION

Under moderate climate fleece forming and fertility traits show certain seasonality. Fleece forming is poorest in summer (ROUGEOT and THÉBAULT, 1983; ALLAIN and THÉBAULT, 1988), and the fecundity of bucks is most unfavourable at the end of the summer and at the beginning of autumn (HU *et al.*, 1983; HSU *et al.*, 1988; BATTAGLINI *et al.*, 1992; BATTAGLINI and COSTANTINI, 1983).
Treated with melatonin, angora rabbits can be prevented from reducing their fleece growth activity in summer (ALLAIN and THÉBAULT, 1988). In fur animals (mink, foxes) not only can the moult be brought forward to an earlier time (ALLAIN and ROUGEOT, 1980; VALTONEN *et al.*, 1987; VALTONEN *et al.*, 1990), but their reproductive rhythm, strictly attached to the natural photoperiod, can also be altered (SMITH *et al.*, 1987; FORSBERG *et al.*, 1990). In the male silver and arctic foxes the testicles can be induced to develop earlier (earlier sperm production) in the silver fox, or maintained longer with melatonin treatment in the arctic fox, than in control. So, in the experiments the males were able to produce semen of normal density and motility, proper for fertilization 2 months before (silver fox) and 2 months after (arctic fox) the natural mating period. Melatonin treatment has an impact on the reproductive activity, and this impact is similar to that of short days (FORSBERG *et al.*, 1990).
The favourable experience obtained with fur animals and other species of animal raise the possibility of influencing the reproductive characteristics of angora bucks by exogenous melatonin treatment in order to reduce the seasonal fluctuations. The objective of our experiment was to examine the effects of melatonin treatment given at different times and with different doses on the libido of angora bucks and on the quantitative and qualitative characteristics of the semen.

MATERIAL AND METHOD

The experiment was carried out on the rabbit research farm of the university, using 1 to 1.5 year- old male angora rabbits of German type. The animals were accommodated in a building with 4 walls but fitted with windows, in a one-floor wire cage system, in individual cages. As there was no air conditioning installed in the building, the temperature could become elevated in summer. Also, the natural photoperiod was effective. The

rabbits had free access to the feed mix (with contents of CP: 17.1 %, CF: 13.2 %) - ad libitum feeding -, and water was also available continuously, from a weight-valved drinker.
The experiment started on July 19th, 1995 (after the summer solstice) and continued for 12 weeks. The animals were treated with melatonin by administering subcutaneously Regulin implants (Hoechst UK Ltd., No.: 0086/4176, 18 mg eff. mat./pc. implant).
Based on the date and dose of the treatment 3 groups of animals were formed as follows:
A = melatonin implants placed in the flank subcutaneously, as a single dose (18 mg/ind., Regulin) on two occasions, i.e. at the beginning of the experiment (July 19th) and 6 weeks later (August 30th) (n = 5).
B = melatonin implants placed subcutaneously as a double dose (36 mg/ind., Regulin) on one occasion, at the beginning of the experiment (July 19th) (n = 5).
C = untreated control (n = 5).

The rabbits were shorn at the beginning of the experiment and every other week later on. Measurements of their body weight and feed intake were taken every week. Semen was collected from the bucks twice a week - possibly twice within one hour - using an artificial vagina.
The libido of the bucks was measured by the time required from the doe's being placed in the buck's cage for the buck to ejaculate for the first and second time.
The semen was evaluated based on the second, or lacking this, the first - ejaculate by determining the following parameters:
Quantity: volume of the first and second ejaculate in millilitres.
Motility: based on the ratio of sperm cells with progressive movement 0 = 0 %, 1 = 1-20 %, 2 = 21-40 %, 3 = 41-60 %, 4 = 61-80 %, 5 = above 80 %.
Concentration: number of sperm cells/ml (with Buerker-chamber).
Total number of sperm cells: number of sperm cells in ejaculate.
Ratio of live sperm (with Eosin-Nigrosin stain).
Ratio of normal sperm (with Christal violet-Congo red stain).
Number of live sperm = number of sperm cells x ratio of live sperm/100.

The data measured during the first (Period 1) and second (Period 2) 6 weeks of the experiment were evaluated and statistically processed by ANOVA, T-and χ^2 - tests (MS Office, 1994). In addition, the effects of the treatments were considered.

RESULTS AND DISCUSSION

Body weight and feed intake

The body weight of bucks developed according to different patterns in the experimental and control groups during the experimental period (Figure 1). The greatest change was observed in Group A, the lowest in Group C. In some cases, there were considerable - although statistically not verified - differences between the groups, so it can be suspected that the changes were - in part - caused by the melatonin treatments. Body weight increased continuously in Group A throughout the whole experimental period. In contrast to this, it increased only during the first 4 weeks in Group B. It is to be mentioned, however, that shearing of the bucks every other week may have had an impact on the rate of growth, since it is known that feed intake becomes increasing after shearing (SCHLOLAUT, 1987; FINZI *et al.*, 1992). Our findings also support this idea: feed intake was significantly higher (A: $P<0.01$; B and C: $P<0.05$) during the second 6 weeks of the experiment as compared to the first 6 weeks (Figure 2). Although the differences were not significant between the groups, it is worth noting that the treated rabbits ate by 13-18 % more feed during the first period and 8 % more during the second. An increase in the feed intake through melatonin treatment was observed in mink by VALTONEN *et al.* (1990).

Sex drive / readiness for ejaculation

There were not any significant verifiable differences between the groups concerning the time necessary for the buck to ejaculate after meeting the doe (Table 1). The first mounting took 11.9 to 12.6 sec., the second took 8.8 to 9.4 sec.
During the second part of the experimental period (weeks 7 to 12) similar ejaculation times were observed in the case of the bucks treated (first mounting: 11.3 to 11.5 sec., second mounting: 8.8 to 9.1 sec.) On the

contrary, the ejaculation times increased significantly (by approx. 50 %) in the case of the control bucks (*first* mounting 18 sec., second mounting: 14.3 sec.).
These results suggest that - irrespective of the dose and number of treatments - the bucks treated with melatonin also maintain up their activity under continuous use, whereas the activity of untreated animals becomes reduced. Not only ejaculation time support the above statement, but also the observation that in the control group there were more bucks who were not ready to ejaculate at all or for the second time (Table 1).

Table 1 : Mounting readiness and volume of semen (average ± SD) of melatonin-treated and control angora bucks

Group	Time interval for ejaculation (sec.)						Ratio of bucks not ejaculating (%)			Volume of ejaculate (ml)		
	1st ejaculate			2nd ejaculate								
	1.	2.	p<	1.	2.	P<	1.	2.	p<	1.	2.	p<
	period			period			period			period		
A	12.6 ± 4.3	11.3^{a} ±3.0	NS	9.3 ± 1.5	8.8^{a} ±3.0	NS	1.7	8.2^{ab}	0.05	0.85^{a} ± 0.04	0.77^{a} ± 0.05	NS
B	11.9 ± 2.5	11.5^{a} ±1.5	NS	8.8 ± 1.8	9.1^{a} ±1.3	NS	2.5	4.5^{a}	NS	0.66^{b} ± 0.05	0.72^{a} ± 0.05	NS
C	12.2 ± 2.5	18.0^{b} ±1.4	0.01	9.4 ± 2.0	14.3^{b} ±3.9	0.05	6.7	11.7^{b}	NS	0.65^{b} ± 0.06	0.45^{b} ± 0.05	0.05
P<	NS	0.001	-	NS	0.01	-	NS	0.05	-	0.05	0.001	-

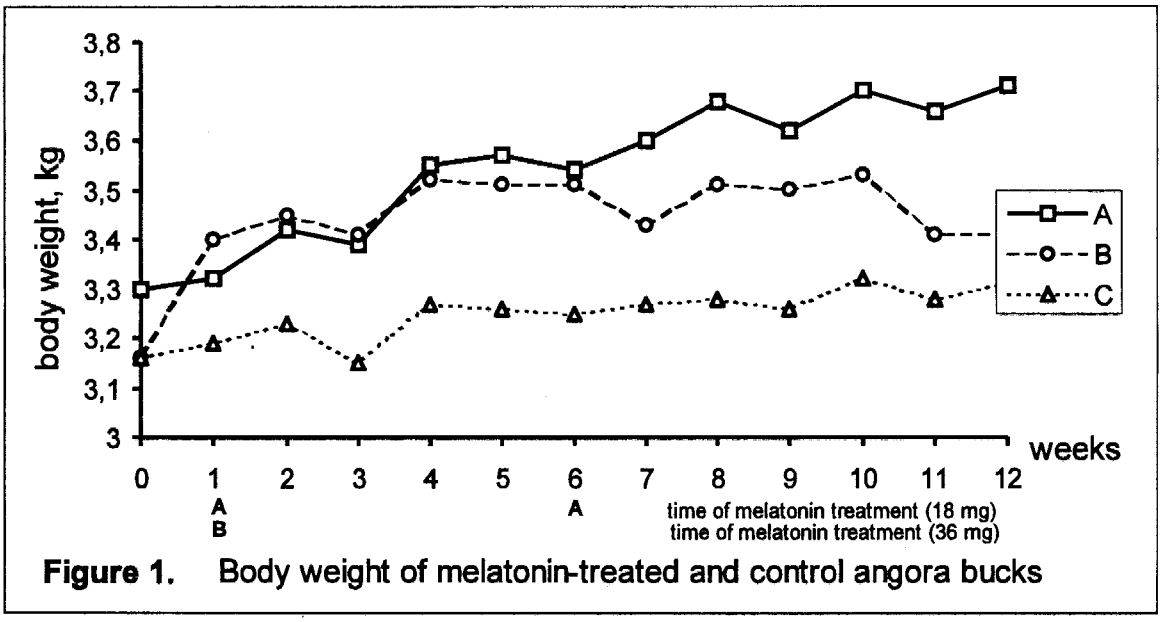

Figure 1. Body weight of melatonin-treated and control angora bucks

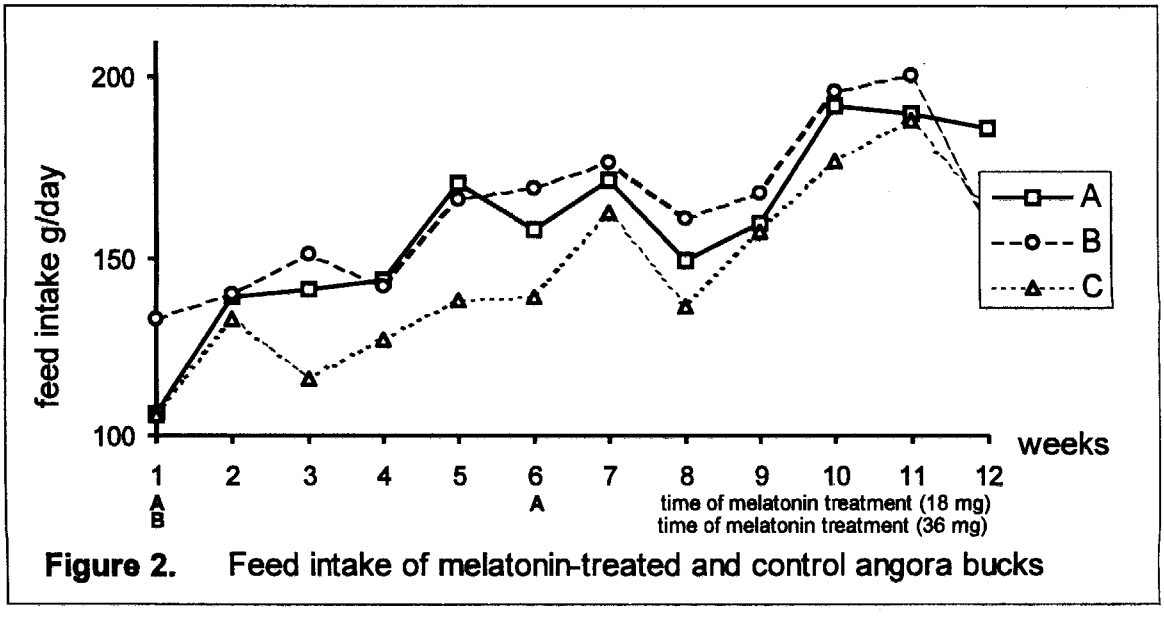

Figure 2. Feed intake of melatonin-treated and control angora bucks

Volume of ejaculate

During the first 6 weeks the bucks of Group A produced significantly more semen than the other two groups (Table 1). During the second part of the experimental period the treated bucks produced approx. the same volumes as before (the volume decreased slightly in Group A and increased in Group B), but the control bucks produced significantly less ($P<0.05$). Normally, the volume of the semen decreases at the end of summer and at the beginning of autumn as reported by HU *et al.*, 1983; HSU *et al.*, 1988; BATTAGLINI *et al.*, 1992; BATTAGLINI and COSTANTINI, 1983. Our experiences indicate that the reduction in the volume of semen in August and September can be eliminated by melatonin treatment. Also, the semen production of angora bucks can be maintained at a high level even in the case of the continuous use of these.

Motility

During the first 6 weeks the ratio of sperm cells with progressive movement was highest in Group A and lowest in Group B ($P<0.05$) (Figure 3). In this latter Group (B) the ratio of ejaculate with mark 0 was particularly high (30.5 %). During the second period the degree of motility improved in group B but declined considerably in Group C. So, the melatonin-treated rabbits achieved significantly better results than the control group ($P<0.05$) between the 7th and 12th weeks. As spermatogenesis takes approx. 42-47 days (HARASZTI and ZÖLDÁG, 1993) the effect of the treatment became obvious after the 6th week. Looking at the data the favourable influence of melatonin on motility can be suspected.

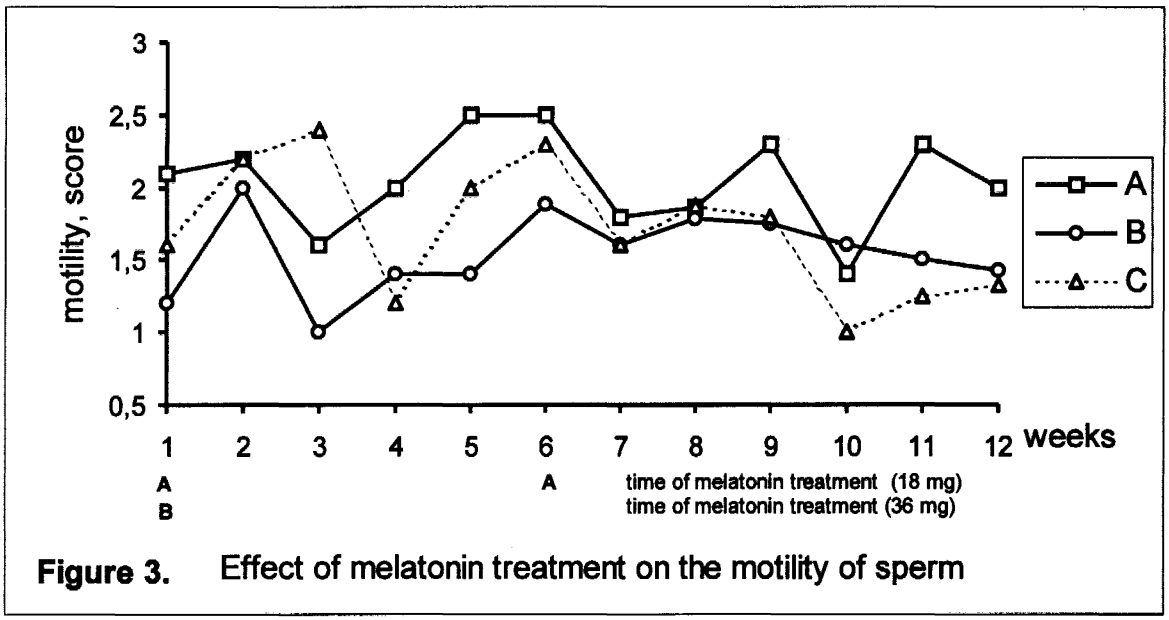

Figure 3. Effect of melatonin treatment on the motility of sperm

Sperm concentration

During the first 6-week period the sperm concentration of Group B was behind that of Group A and C significantly ($P<0.01$) (Table 2). This finding is probably in connection with motility, since BATTAGLINI *et al.*, (1992) detected positive correlation between these two characteristics. During the second period the sperm concentration did not change much in the melatonin - treated groups (A and B). On the contrary, it declined significantly ($P<0.001$) in the control group. Our results emphasize that in melatonin-treated bucks the sperm concentration does not decline even with intensive use of the buck. At the same time, the untreated bucks perform unfavourably in this respect.

Ratio of live sperm

There were not any statistically differences between the groups during the first period (Table 2). The ratio of live sperm varied between 23.5 and 34.7 %. During the second period the ratio of live sperm increased significantly ($P<0.001$) in the groups treated (A and B), while it actually remained unchanged in the control group. As a result of the above changes the ratio of live sperm in the melatonin-treated groups (52.5 and 56.7 %) exceeded that of the control group (34.2 %) significantly ($P<0.01$) during the second period. Consequently, treatment with melatonin affects the ratio of live sperm favourably.

Table 2 : Semen quality (average ± SD) of melatonin-treated and control angora bucks

Group	Sperm concentration (no./ml x10^6)			Ratio of live sperm (%)			Ratio of normal sperm (%)			Number of sperm cells per ejaculate (no. x 10^6) total			live		
	1	2	P<	1	2	P<	1	2	P<	1	2	P<	1	2	P<
	period			period			period			period			period		
A	19,31^a ± 2,59	14,16 ± 6,19	NS	29.6	52.5^a	0.001	86.6	89.5	0.05	7.44^a ± 0.69	6.49^a ± 2.66	NS	2.22 ± 0.59	2.60^a ± 1.32	NS
B	1,45^b ± 2,63	11,54 ± 4,21	NS	34.7	56.7^a	0.001	85.4	88.1	0.05	4.05^b ± 1.22	4.59^{ab} ± 1.70	NS	1.42 ± 0.64	3.38^a ± 1.01	0.05
C	20,30^a ± 3,56	11,93 ± 2,65	0.001	23.5	34.2^b	NS	85.5	85.5	NS	6.60^a ± 1.44	3.32^b ± 1.17	0.01	1.63 ± 0.73	1.20^b ± 0.66	NS
P<	0.001	NS	-	NS	0.01	-	NS	0.001	-	0.001	0.05	-	NS	0.01	-

Notes to Table 1 and 2: P = level of significancy; NS = non-significant difference; a-b = in the vertical colomns the difference between the groups marked with different letters is significant

Ratio of normal sperm

There was no difference among the ratio of normal sperm of the groups in the first period (Table 2). During the second period in the melatonin-treated groups (A and B) the ratio of normal sperm was significantly higher, than in the control group (89.5 and 88.1 % with respect 85.5 %, P<0.001). The most frequent abnormal forms were the deformations of the tail of the spermium.

Number of sperm cells in the ejaculate

During the first period, total sperm number was lowest in Group B (P<0.001) because of the pour sperm concentration (Table 2). During the second period, the number of sperm decreased significantly (from 6.60x10^6 to 3.32x10^6; P<0.01) in the ejaculate of the control bucks. Therefore, this group showed the poorest performance in this feature, and the lag behind Group A proved to be significant (P<0.05).
Regarding the number of live sperm no differences could be detected between the groups during the first period. During the second 6-week period, however, both treated groups (A and B) proved superior to the control bucks significantly (2.60 and 3.33x10^6 vs. 1.20x10^6; P<0.01).

CONCLUSION

Our preliminary results show that an administration of melatonin implants during summer in male angora rabbits, raised under natural photoperiod, improves both libido and semen characteristics as compared to control males. Usually, the fecondity of the angora rabbit buck is most unfavourable during summer (HU *et al.*, 1983, HSU *et al.*, 1988, BATTAGLINI *et al.*, 1992, BATTAGLINI and COSTANTINI, 1983) although it is known that long days have a desirable effects on rabbit semen production (THEAU-CLEMENT *et al.*, 1995). But we do not yet if this decrease in fertility during summer is due to inhibitory effects of elevated temperature or if the male angora rabbit is becoming refractory to long days after a long term exposition. Therefore, it is well known that administration of melatonin mimics the effects of short days. Thus our results would confirm the hypothesis that a long term exposition to long days would have undesirable effects on fertility as an administration of melatonin during summer which is equivalent to a short day treatment abolishes this photorefractory effect and allow a significant recrudescence of semen production in the male angora rabbit. However, more information is required to confirm such results as.

Acknowledgement - This project was supported by the National Scientific Research Fund (OTKA) (No. of contract: F 17671).

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Effet de différents traitements à la mélatonine en été sur les caractéristiques du sperme chez le lapin angora - Des lapins Angora âgés de 1-1.5 ans sont divisés en trois groupes. Les animaux ont été tondus toutes les deux semaines. Un prélèvement de sperme a été effectué deux fois par semaine et chaque fois deux fois par jour.
Les animaux du premier groupe (A) ont recu le 19 juillet, puis 6 semaines après, un implant sous-cutané de 18 mg de mélatonine. Les animaux du deuxième groupe (B) ont reçu le 19 juillet, une dose de 36 mg de mélatonine, sous forme d'implants sous-cutanés. Le troisième groupe (C) n'a pas été traité.
Selon nos résultats, il apparaît qu'une administration de mélatonine a des effets favorables après la 6ème semaine de traitement . Entre la 7ème et la 12ème semaine après le début du traitement, nous avons observé les résultats suivants : respectivement pour les groupes A, B et C, poids du corps la 12ème semaine: 3.71, 3.41 et 3.31 kg (NS); consommation journalière de fourrage: 175, 176 et 164 g/jour (NS); intervalle de temps entre présentation de la femelle et l'éjaculation: 11.3, 11.5 et 18.0 sec ($P < 0.001$); pour la première éjaculation; 8.8, 9.1 et 14.3 sec ($P < 0.01$) pour la deuxième éjaculation; proportion de lapins n'ayant pas éjaculé 8.2, 9.1 et 11.7 % ($P < 0.05$); volume du sperme 0.77, 0.72 et 0.45 ml ($P < 0.001$); concentration du sperme 14.2, 11.5 et 11.9 x 106 /ml (NS); proportion des spermatozoides vivants 52.5, 56.7 et 34.2 % ($P < 0.01$).