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INDUCTION OF SEASONAL TESTICULAR REACTIVATION IN SOAY RAMS BY MELATONIN. I. HISTOLOGICAL AND MORPHOMETRICAL CHANGES IN THE SEMINAL GLAND

(With 1 Table & 10 Figs.)

By

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(Received at 8/5/1990)

حث النشاط الموسمي للخصية في خراف الصويا بواسطة الميلاتونين

ا- التغييرات النسيجية والمورفومترية في الغدة المنوية

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تم دراسة تأثير مادة الميلاتونين على التركيب الهستولوجي للغدة المنوية في خمسة عشر من خراف الصويا. وقسمت الخراف الى مجموعتين ، مجموعة ضابطه وأخرى تم زراعة مادة الميلاتونين فيها تحت الجلد لمدة أحدى عشر اسبوعا تبطن الغدد المنوية في خراف الصويا بنوعين من الخلايا ، خلايا إساسية عمادية وأخرى قاعدية وكان للميلاتونين تأثيرا ملحوظا في زيادة عدد وارتفاع الخلايا الاساسية العماديةكما ادى الى زيادة في النشاط الافرازى لهذه الخلايا. شوهد نشاطا انقساميا في الخلايا القاعدية. وأوضحت هذه الدراسة ايضا نقصا ملحوظا في كمية النسيج البيني والنسيج البين فصيصي . وقد استخلص من هذه الدراسة أنه يمكن استعمال الميلاتونين اقتصاديا للتحكم في أوقات مواسم التربية في الخراف .

SUMMARY

Soay rams of the semidomesticated sheep were used in this experiment. They were given a s.c. implants containing melatonin and after 11 weeks the rams were killed. In contrast to the control group of animals the melatonin-treated rams showed histomorphological and morphometrical changes as a result of reactivation of the glandular tissues of the seminal gland. Particularly, the secretory principal cells were increased in number and height and showed signs of increased seceretory activity; apical cytoplasmic protrutions became well-developed and covering the inner surface of the glandular end-pieces of the seminal vesicle. The basal cells were displayed mitotic divisions. The glandular portions were increased in size and the interstitial connective tissues were reduced. The overall results are consistent with the view that melatonin can be used commercially to modify the timing of th breeding season in sheep.

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INTRODUCTION

Studies with sheep and many other photoperiodic species have shown that the secretion of the melatonin from the pineal gland plays a key role in the physiological mechanism by which photoperiod affects the reproductive axis thus regulating the timing of the breeding season (KARSCH, et al. 1984; TAMARKIN, et al. 1985). Melatonin is secreted principally at night and the duration of the nocturnal period of the secretion changes with the annual cycle in daylength (ROLLAG, et al. 1978). Experimental studies involving infusing melatonin for different period each 24 h have clearly shown that the daily exposure to melatonin provides the index of night length and thus daylength; long duration exposure to melatonin each day induces short-day reproductive responses while short duratin exposure to melatonin produces long-day responses (BITTMAN, et al. 1983; KARSCH, et al. 1984). consistent with the duration hypothesis are the many recent observations that daily treatment of sheep or deer with melatonin during the non-breeding season will induce early onset of oestrous cyclicity (NETT and NISWENDER, 1982; KENNAWAY, et al. 1982; ARENDT, et al. 1983) and early reactivition of testicular activity (BUBENIK, 1983; LINCOLN, et al. 1984). Continuous administration of melatonin from a s.c. implant or a vaginal pessary is also effective at inducing these short-day reproductive resposes (LINCOLN and EBLING, 1985; NOWAK and RODWAY, 1985; ENGLISH, et al. 1986). These effects of melatonin are of commercial importance since the treatments can be used to modify the timing of the breeding season in domesticated animals (LINCOLN, 1983).

The aim of the present study was to induce premature seminal gland and testicular reactivation in rams using melatonin and to document the histological changes occuring in some of the reproductive tissues. Since the treatment results in an increase in testosterone secretion from the testes (LINCOLN, et al. 1984) it was predicted that there would be clear histological changes in the accessory sexual glands and testes.

MATERIAL and METHODS

Rams of the Soay breed of the semidomesticated sheep were used since they have a pronounced seasonal cycle in testicular activity; the testes regress to minimal size in March and April and redevelop during the summer and autumn to a peak in September and October at the onset of the rut (LINCOLN and SHORT, 1980). A group of 1.5 yearling Soay rams were kept in outdoor grass paddocks at the Dryden Field Station of Animal Breeding Research Organisation near Edinburgh. On 30 May 1985; 8 animals were given a s.c. implant containing melatonin and 7 animals were given empty implants to act as controls. The melatonin implants were made from Silastic sheeting (500-1 Dow Corning, Midland, MI, U.S.A.) sealed into an envelope with a surface area of 32 cm^2, containing 1g melatonin. (Sigma Chemicals, Poole, Dorset,

U.K.) and previously shown to produce a relatively constant daytime peripheral plasma concentration of melatonin of 200-500 pg/ml plasma (LINCOLN, et al. 1984). The implants were placed beneath the skin above the rib cage using local anaesthetic and left in place throughout the experiment.

From 25 May to 16 August, the reproductive changes occuring in the rams were monitored by measuring the diameter of the testes and intensity of the sexual skin flush every 2 weeks (LINCOLN and DAVIDSON, 1977). A blood sample was collected from jugular vein by vanutainer from each ram every week; these samples were heparinized, centrifuged within 1 h and the plasma frozen at -20°C until required for hormone assay.

On 16 August 1985, 11 weeks after the onset of the melatonin treatment, all the rams were killed with an overdose of pentobarbitone sodium (Euthatal, May and Baker, Dagenham, U.K.). The seminal glands were removed, and small tissue blocks fixed in formaldehyde and glutaraldehyde (KARNOVSKY, 1965). For paraffin sections, tissues were dehydrated in a graded series of ethanols, followed by methyle benzoate, embedded in paraffin and sections of about 5 um were cut and stained with PAS-Haematoxylin. For semithin sections, the tissues were postfixed for one hour in 1% Osmium tetraoxide in 0.1 M cocodylate buffer at pH 7.3, then dehydrated in a graded series of ethanols followed by propylene oxide, and embedded in Araldite. Semithin sections were stained with toluidine blue. For Scanning electron microscopy (SEM), the fiexed samples were washed in 0.1 M Sodium cocodylate containing 5% sucrose, processed through tanic acid, dehydrated in graded series of ethanols, critical-point dried, and then placed coated with gold palladium in a sputtering device. Specimens were then examined and photographed using SEM operated at 20 Kv.

RESULTS

Control animals:

The seminal gland of the control animals appeared as a collections of glandular lobules (Fig. 1). They were seperated from each other by a thick interlobular dense connective tissue. The surrounding connective tissue capsule consisted of a thick layer of collagenous fibers. Outside the capsule, there was a layer of loose connective tissue rich in small-sized blood vessels, nerve fibers and ganglia.

The secretory lobules were formed of tubuloalveolar glandular end-pieces and relatively thick interstitial connective tissue containing smooth muscle fibers. The diameter of these glandular end pieces measured about 115.9 Um. They were lined by one layer of two types of cells, the principal and the basal cells (Fig. 2).

The serial semithin and paraplast sections demonstrated that the principal cells were low columnar in shape, and their height was about 21.8 Um. The cells showed defined outlines and rested on a distinct basal lamina (Fig. 3). Very small number of fine PAS-positive granules in the apical portions have been distinguished. Their nuclei appeared vesicular and rounded or irregular in shape with distinct nucleoli.. They were found oftently in one level occuping the centres of the cells. They were about 5.36 Um in diameter. In some of the glandular end pieces, few of the lining principal cells possessed apical bleb-like cytoplasmic protrusions.

The basal cells were wedged between the principal cells and the basal lamina forming a basal discontinuous layer. They appeared oval or spherical in shape with distinguishable outlines. They were about 7.26 Um high and contained deeply-stained flattened or oval nuclei measuring about 4.13 Um in diameter (Fig. 3).

Treated animals:

In melatonin-treated animals, marked histomorphological and morphometrical changes have been observed in the glandular portions and the connective tissue of the seminal gland. Morphometrical analyses (table 1) showed that the glandular lobules became larger in size. The secretory end pieces increased significantly in width a diameter of about 199.7 Um. The height of the principal cells were significantly increased while the height of the basal cells as well as the nuclear diameter of both the principal and basal cells showed no significant variation in comparison with that of the control animals. In addition, the number of the principal cells were significantly increased ca. 25% increament of the secretory principal cells lining the glandular portions in melatonin-treated animals.

Morphologically, the interstitial fibromuscular connective tissue appeared as a thin layer of intensive PAS-positive reaction separating between the glandular end pieces in the seminal vesicles (Fig. 4). While the interlobular connective tissue decreased relatively in thickness in comparison with that in Melatonin-untreated animals.

The secretory principal cells (Fig. 5) were appeared closely packed and became tall columnar in shape. Sometimes, their nuclei were situated in two levels (Fig. 6). They were polymorphic in shape with irregular outline and displayed one or two distinct nucleoli attaching to the nuclear membrane (Fig. 7 & 8). They were found mostly in the middle region. The PAS-positive substance in the principal cells markedly increased, particularly in the apical cytoplasm. The apical cytoplasmic protrusions, that were considered as indication to the occurance of an apocrine activity were greatly increased and included most of the principal cells. Apocrine secretions (Fig.8) were also observed projecting within the lumen of the glandular portions of the seminal vesicles. Sometimes, in between the glandular cells, elongated columnar cells with dark-stained cytoplasm and oval or elongated, very condensed nuclei were seen. Basal cells (Fig. 6) showing mitotic divisions have been observed in the Melatonin-treated animals.

In addition, the SEM examinations demonstrated that, in control animals the inner surfaces of the glandular portions appeared mostly smooth, only very few number of cytoplasmic protrusions have been seen (Fig. 9). While the inner surfaces of the glandular end pieces in animals treated with Melatonin (Fig. 10) were covered with oval cytoplasmic structures that still attached to epithelial surface. They were represented the cytoplasmic bleb-like protrusions.

DISCUSSION

The present results indicated that constant administration of melatonin from a subcutanous implants induced a clear histological changes in the seminal glands in the treated rams. The main cytological alterations were a significant increase in number and height of the principal cells, and a marked increase in the apical cytoplasmic protrusions that were considered as a morphological evidence of apparent increase in the apocrine activity of the principal cells. Furthermore, the increased diameter of the glandular end-pieces together with visible increase in the apocrine secretions of the principal cells led to a significant increase in the seminal gland weight of the melatonin-treated rams. These histological changes in the seminal glands of melatonin-treated rams were correlated and also documented the previous results of LINCOLN and EBLING (1985) who demonstrated redevelopment of the testicular activity, increased blood plasma concentration of testosterone and FSH, and a decline of prolactin level. They also reported that melatonin implants during exposure to long days (summer) resulted in a rapid "switch on" of reproductive redevelopment similar to that produced by exposure to short day because these implants prevented the rams from showing the normal responses to changes in the prevailing photoperiod.

However, KENNAWAY, et al. (1982) and KENNAWAY and GILMORE (1984) performed experiments in which sheep have been given subcutanous implants of melatonin similar to the present experiment, were on Marino x Border Leicester ewes and ewe lambs treated in summer (long days). They reported a similar decline of blood plasma level of prolactin, but there were no clear effects on reproduction in the ewes and the onset of oestrous cyclicity was delayed in the ewe lamb. Moreover, in the experiment of KENNAWAY and GILMORE (1984), the lambs were treated at a very early age and might have not been exposed to long day as long as enough to render them responsive to the short days or the effect of constant melatonin implants.

In the present work, the increased glandular activity of the seminal glands in rams is due to increased level of plasma testosterone and in consistency with the view that melatonin is the hormone that functions physiologically to relay effects of changing photoperiod, and that exogenous melatonin induces its responses by interfering with this process. Also it can be stated that the constant supply of exogenous melatonin appears to be registered as a short day and to overcome any effects of

endogenous melatonin secretion. It is also interesting to mention that, melatonin could be of commercial importance since the treatment can be used to modify the timing of the breeding season of sheep, in order to reduce the period of reproductive inactivity or it can be used to achieve a better understanding the factors that regulate the reproduction in sheep.

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LEGEND OF FIGURES

Fig. (1): Small lobules of inactive seminal vesicle (D) and thick interlobular C.T. (C). Paraffin section of control animal, PAS-Haematoxylin stain x 40.

Fig. (2): The lining epithelium is composed of low columnar principal cells (➤) and basal cells (➡). The interstitial C.T. (C) is relatively thick. Seminal vesicle of control animal stained with PAS-Haematoxylin. x 400.

Fig. (3): Less active principal cells with smooth apical surface lining the end-piece (▷). Melatonin-untreated animal, semithin section, toluidine blue stain. x 1000.

Fig. (4): Reactive large lobules (D) of the seminal gland and relatively thin interlobular C.T. (C). Paraffin section of melatonin treated animal, PAS-Haematoxylin stain. x 40.

Fig. (5): The lining epithelium of the glandular portions of the seminal gland in melatonin-treated animal. The principal cells (*) are tall columnar and closaly packed. Paraffin section, PAS-Haematoxylin stain. x 400.

Fig. (6): The lining principal cells appear closaly packed and tall columnar in shape. Mitotic division (▽) in basal cell. Paraffin section in seminal gland of melatonin-treated animal, PAS-Haematoxylin stain. x 160.

Fig. (7): Inbetween the active principal cells dark cells (➡) with dark nuclei are located. Semithin section stained with toluidine blue, treated animal. x 1000.

Fig. (8): Well-developed apical cytoplasmic protrusions (➤). Semithin section stained with toluidine blue, treated animal. x 1000.

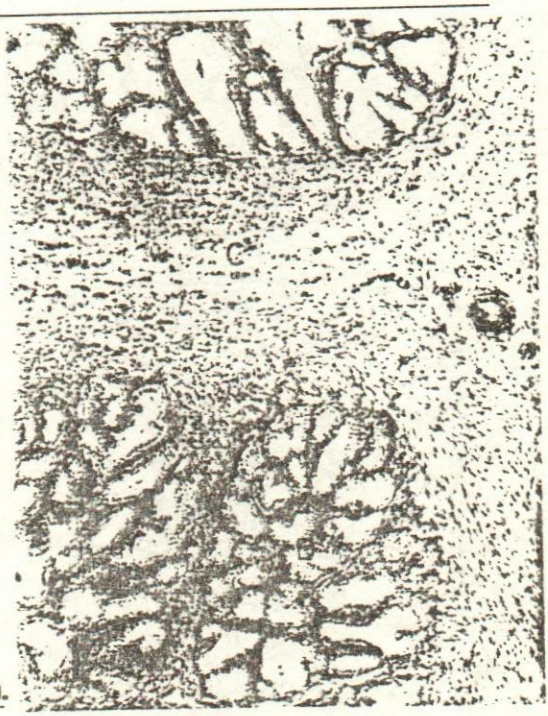
Fig. (9): Very few cytoplasmic protrusions (▼) are found over the inner surface of the end-piece. SEM picture, seminal gland of control animal. x 1300.

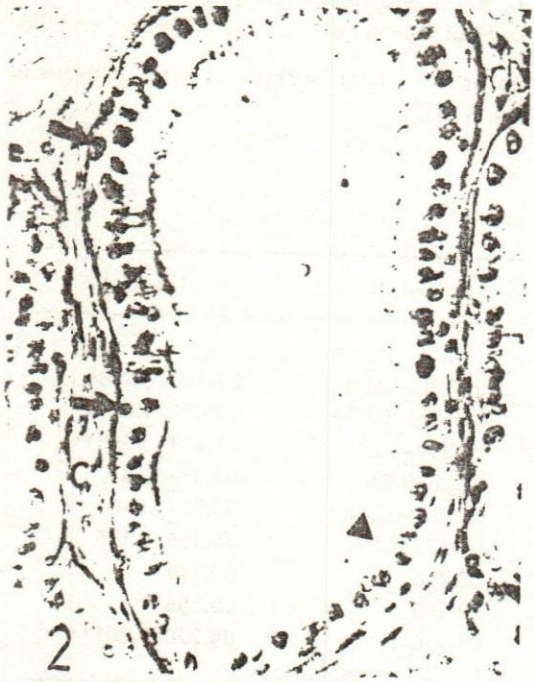
Fig. (10): Many cytoplasmic protrusions (*) cover the inner surface of the end-piece. SEM picture, melatonin-treated animal. x 8500.

Table (1):

Animals		Control	Treated
Mean ± SE Value of:			
Seminal gland (combined, gm)		03.700±0.600	07.710±0.440*
End-pieces ø (Um)		115.955±0.399	199.707±0.614*
C. height (Um)	PC	21.817±0.29	31.731±0.368**
	BC	07.26±0.23	07.83±0.183
Nuclear ø(Um)	PC	05.361±0.078	05.95±0.049
	BC	04.125±0.072	04.356±0.105
Nucl/cyto. ratio (Um)	PC	00.246	00.147
	BC	00.568	00.556
PC No/unit area		03.390±0.140	04.230±0.201**

PC = Principal cell,
BC = Basal cell.
C. height = Cell height.
No = number
Significance
* P = 0.05 (Student "t" test)
** P = 0.01 (Student "t" test)





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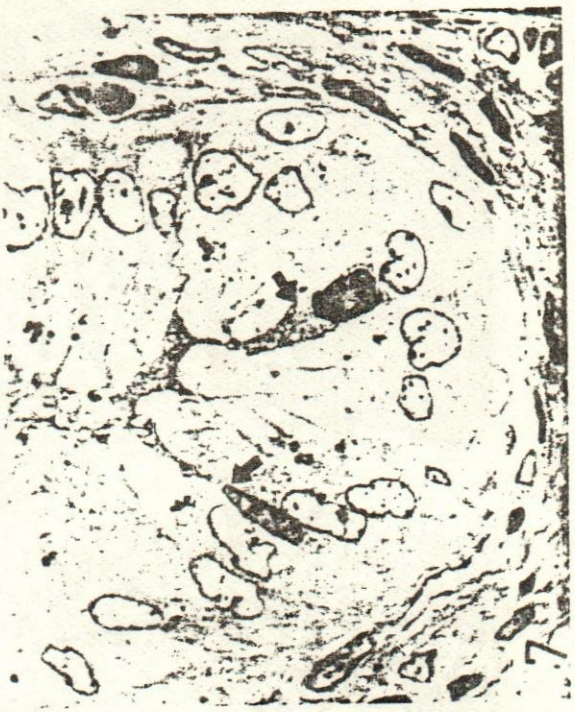

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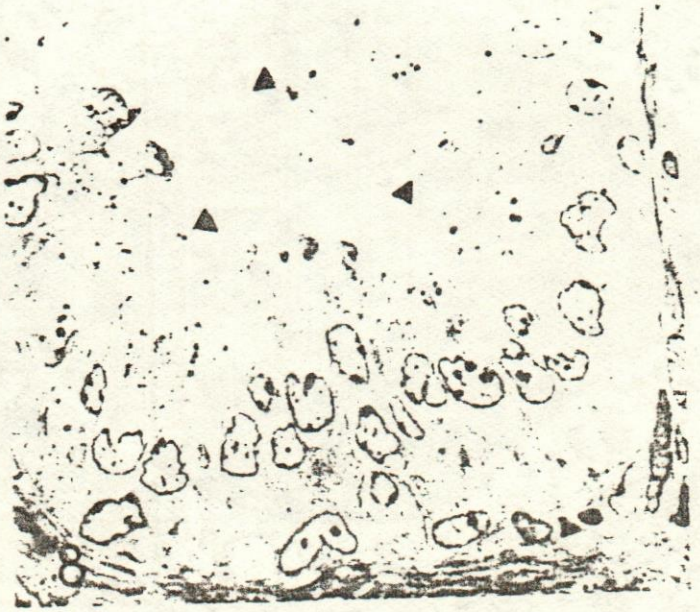

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