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Short- and long-term effects of manipulation of the pineal/melatonin axis in ewes

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Summary. The experiments which have provided insight into the role of the pineal gland and melatonin in sheep reproduction are reviewed. There is now strong evidence that timed daily melatonin administration and continuous administration via implants are equally effective in promoting the same physiological consequences as short daylength. Thus melatonin treatments lasting six weeks can result in an advance of the breeding season and an advance in the seasonal peak in ovulation rate. Much longer periods of exposure to melatonin or long-term pinealectomy result in a different response. In the second part of this review the long-term effects of pineal manipulation in juvenile and pubertal ewes are compared and contrasted. Five years after pinealectomy (at 7.5 months) ewes displayed normal timed breeding seasons without exhibiting a normal annual pattern of LH sensitivity to oestradiol. Pinealectomy at 2.5 months of age resulted in delayed puberty and breeding seasons out of synchrony with normal ewes two years later. These animals maintained an LH sensitivity to oestradiol consistent with their own breeding season. Melatonin implants which delivered melatonin for over a year caused similar effects as pinealectomy at 2.5 months but had essentially opposite effects to pinealectomy in adults. These results generate many questions about the different perception of the melatonin signal in prepubertal versus adult ewes and the factors involved in the onset and offset of the breeding season.

Introduction.

The critical role of the pineal gland in controlling seasonal physiological changes in vertebrates is now well recognised. For many years the pineal and its hormone melatonin have been accepted as key intermediaries between changes in daylength and the endocrine system. On the basis of research on hamsters and rats the concept of an antigonadotrophic action of the pineal was proposed — indeed many reviewers give the impression that the pineal is only inhibitory. This is clearly not true because appropriate melatonin treatment of sheep, goats and deer promotes sexual activity, fertility and fecundity. There is great potential for the use of melatonin as a pharmaceutical agent in the agricultural industry following the success of laboratory and field tests. In this contribution to the colloquium we review the key studies on the short-term reproductive effects of short daylength, pinealectomy and melatonin treatment which have made large scale melatonin treatment of female domestic animals a viable agricultural practice. Recent studies of the long-term changes in reproductive function following pineal/melatonin manipulation in ewes will be discussed.

Short-term endocrine responses to pineal manipulation.

Yeates (1949) and Hafez (1952) were responsible for establishing the scientific base for experiments on the photoperiodic control of seasonal breeding. The elegant experiments of Ducker and his colleagues indicated the potential benefits and shortcomings of manipulating the sheep breeding season with artificial lighting. For instance, when anoestrus Clun Forest ewes were transferred from the field at the summer solstice into light-controlled rooms the onset of oestrus cyclicity was influenced by the degree of reduction of light each day. The greater the reduction in light the shorter the interval to oestrus (Natural daylength 66.4 $\pm$ 7.4 days ; 3.75 h reduction 59.5 $\pm$ 3.3 days ; 7.75 h reduction 44.8 $\pm$ 2.7 days ; 11.75 h reduction 33.6 $\pm$ 3.3 days (Ducker, Thwaites and Bowman, 1970). These authors also found that various patterns of increasing daylength affected the onset of anoestrus (Ducker and Bowman, 1970a). Finally, with respect to advancing the breeding season, the mean interval to oestrus was longer in spring treated compared to summer treated animals (Ducker and Bowman, 1970b). Full exploitation of these findings has been limited by the requirement for either darkened sheds or supplemental lighting. Nevertheless, the general concept that light manipulation could be used to produce three lambing periods in two years was proven in two studies (Ducker and Bowman, 1972 ; Vesely, 1975).

The relationship between daylength and pineal function was established during the period 1960-1970. It became clear that the pineal was active only during the night in most mammals. The development of melatonin radioimmunoassays revolutionised pineal research. Rollag and Niswender (1976) were the first to publish circulating levels of the pineal hormone melatonin in sheep. They showed that (1) melatonin levels in the circulation accurately reflected the duration of darkness, (2) that the melatonin rhythm persisted in constant darkness, (3) was suppressed by light, and (4) that there was no apparent change in peak melatonin levels during four different times of the year (Rollag, O'Callaghan and Niswender, 1978). These results were confirmed in ewes in various photoperiods by Kennaway *et al.* (1983). The next key element in the development of our understanding of light/pineal/melatonin interactions was the finding by Fiske and Huppert (1968) that the physiological response of the pineal gland to exogenous melatonin depended on the time of day it was administered. Tamarkin *et al.* (1976) exploited this concept and found that Syrian hamsters maintained in long daylength would only undergo gonadal regression if melatonin was injected in the late afternoon. This treatment regime was presumably interpreted physiologically as replicating the melatonin response to short days.

Although the pineal gland had always been thought to provide inhibitory influences upon the reproductive system, we were encouraged by results from Ducker's group to hypothesise that timed exogenous melatonin administration to anoestrous ruminants would promote an early onset of breeding activity. This hypothesis required the rejection of the notion of melatonin being anti- or pro-gonadotropic ; melatonin was proposed instead to provide a hormonal signal to the hypothalamic/pituitary axis which reflected the length of the dark period. Our studies were facilitated by the finding that melatonin could be administered orally to sheep and provide a sustained high level of melatonin in blood (Kennaway and Seamark, 1980). The first demonstration that exogenously administered melatonin could mimic the physiological effects of short daylength was in 1980 (Kennaway, Hooley and Seamark, 1980) (Fig. 1).

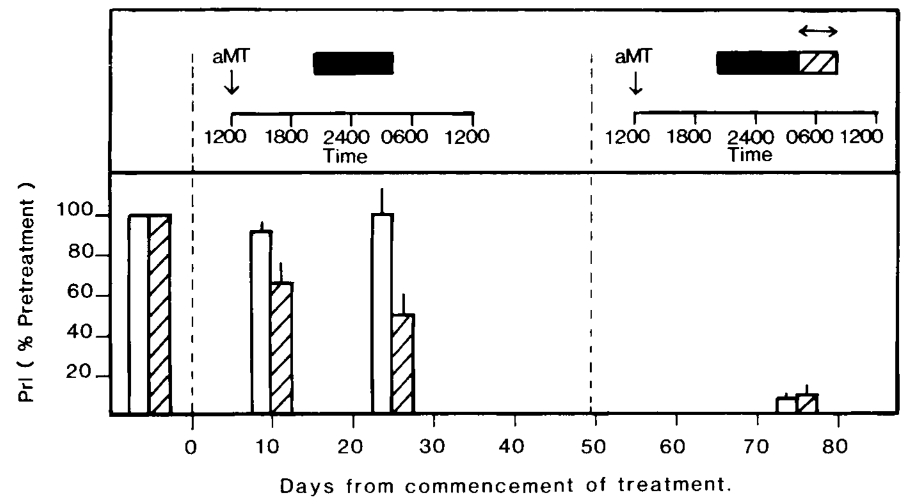

FIG. 1. — *Prolactin levels in female goats fed control pellets or pellets with 2 mg adsorbed melatonin daily at 1200 h.* The histograms represent the mean ± SEM of the percentage of pretreatment prolactin levels. The open histograms represent control treated and the hatched histograms the melatonin treated animals. In the top section the time of administration of melatonin is shown in relation to the period of darkness (black bar). After 49 days of treatment the daylength was reduced by four hours (hatched bar) for both groups. (Adapted from Kennaway, Hooley and Seamark, 1980).

Melatonin fed daily to anoestrus goats eight hours before darkness while maintained in a room with 16 hours light per day resulted in a 50 % decrease in prolactin levels after 23 days. Twenty-two days after a four hour reduction in light the prolactin levels in both control and melatonin treated ewes decreased to 5 % of the initial levels. These results confirmed in goats that one of the earliest physiological responses to short daylength was a precipitous drop in plasma prolactin (Walton *et al.,* 1980) and that showed that exogenous melatonin could replicate this response.

Confirmation of a gonadal response to melatonin treatment was provided in 1982 when it was shown that daily melatonin (8 h before darkness) feeding advanced the onset of ovarian cyclicity in ewes (Kennaway, Gilmore and Seamark, 1982a). Independent confirmation of the ability of melatonin treatments to advance the breeding season and decrease prolactin followed (Nett and Niswender, 1982 ; Arendt *et al.,* 1983 ; Symons, Arendt and Laud, 1983). Since that time there have been numerous studies showing the similarities between hormonal and gonadal responses following short daylength and timed daily melatonin treatments.

It was clear from research in Syrian hamsters that melatonin had to be administered in late afternoon in order to obtain the antigonadal effects (Tamarkin *et al.,* 1976). Furthermore, if implants of beeswax containing melatonin were placed subcutaneously in Syrian hamsters maintained in long daylength, there was no effect on the gonadal status of the hamster. Such a treatment, however, blocked the antigonadal effects of late afternoon injections of melatonin. Either continuous melatonin availability was having the same effect as long daylength or it interferred in some way with the physiological processing of the daily injected hormone (Reiter, 1980). The basis for this phenomenom has still not been elucidated and the prospects of *continuous* melatonin administration to sheep having the same effect as daily administration appeared poor.

Continuous melatonin administration to sheep does, however, result in the same physiological changes (decrease in prolactin) as short daylength and daily melatonin administration (Kennaway, Gilmore and Seamark, 1982b ; Kennaway *et al.,* 1982/1983 ; Poulton *et al.,* 1987). When anoestrus ewes were treated with silastic envelopes containing melatonin the breeding season was advanced by five to 10 weeks (English *et al.,* 1986 ; Poulton *et al.,* 1987). Lambs treated with similar melatonin implants from three weeks of age had delayed puberty onset (Kennaway and Gilmore, 1984 ; Kennaway *et al.,* 1986) whereas lambs treated from 19 weeks had puberty advanced by five weeks (Nowak and Rodway, 1985). Similar delays and advances in puberty had been obtained previously by Foster *et al.* (1986) using short daylength treatments instead of constant melatonin administration. Finally, the increased fecundity observed in ewes following short daylength treatment (Dunstan, 1977) is also found following daily feeding of melatonin (Kennaway *et al.,* 1984b) and following subcutaneous melatonin implant treatments (Kennaway, Dunstan and Staples, 1987).

How does continuous melatonin availability cause the same physiological responses as those occurring after extended pineal secretion of melatonin or timed daily administration of melatonin ? The early results of experiments with implants in Syrian hamsters led to the hypothesis that continuous melatonin availability « down regulated » melatonin receptors, Reiter (1980). In support of this notion was the finding that morning melatonin injections blocked the effects of daily afternoon injections. The failure of implants to act in a similar manner to short photoperiod in the Syrian hamster is apparently not universal ; the Djungarian and Turkish hamsters and *Peromyscus leucopus* each undergo gonadal collapse after treatment with melatonin implants (Stetson and Watson-Whitmyre, 1984). A simplistic alternative hypothesis is that circulating melatonin from the implants activates melatonin receptive mechanisms which have a rhythm themselves (a rhythm of melatonin sensitivity). The nature and biochemical consequences of this activation is not yet known.

Long-term consequences of pineal manipulation.

Appropriate treatment of domestic ruminants with melatonin can result in altered breeding patterns in the short-term (< 10 weeks). What are the consequences in the long-term of pineal manipulation ?

We have performed two studies on the effects of long-term pinealectomy and melatonin treatment. In the first study (Kennaway *et al.,* 1984a), 20 ewes were pinealectomised at 7.5 months of age and were studied between the ages of five to seven years. The aim of the study was to investigate, (1) the normal breeding season of the pinealectomized ewes and their controls, (2) the seasonal changes in the sensitivity of LH secretion to oestradiol feedback, and (3) the effect of continuous melatonin administration on the oestradiol sensitivity rhythm. All the animals were kept together and run continuously with rams. Under these conditions the sham and pinealectomised animals with intact ovaries had identically timed breeding seasons. By contrast pinealectomized/ovariectomized/oestradiol treated ewes had no synchronous rhythm in feedback sensitivity (Figure 2). When the additional treatment of continuous melatonin administration was applied both sham and pinealectomized ewes had sustained, elevated levels

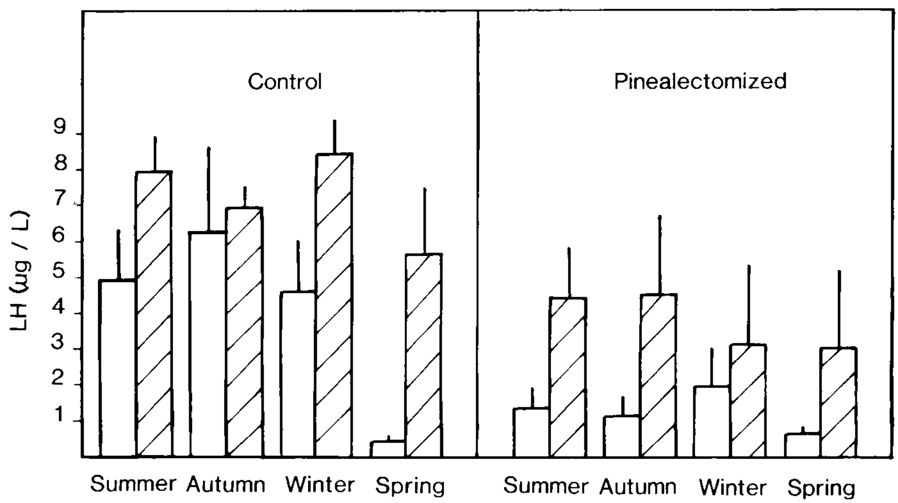

FIG. 2. — *Plasma LH levels in control and pinealectomized ovariectomized ewes treated with oestradiol* (open bars) *or oestradiol and melatonin implants* (hatched bars). Samples were taken weekly and averaged over 13 week periods to give summer, autumn, winter and spring levels of LH. Results are mean ± SEM for 3-5 animals. ANOVA results ; Control > Pinealectomy $P < 0.005$; Oestradiol < Oestradiol + Melatonin $P < 0.001$ (data adapted from Kennaway *et al.,* 1983).

of LH. Thus continuous melatonin availability reduced the sensitivity of LH secretion to oestradiol. Importantly the animals did not appear to be refractory to the melatonin.

The second study involved the monitoring of the breeding seasons of ewes treated with melatonin implants or pinealectomized during the first three months of life.

Melatonin implants placed subcutaneously in ewe lambs from three weeks of age resulted in delayed puberty (Kennaway and Gilmore, 1984 ; Kennaway *et al.,* 1986). Pinealectomy at 10 weeks of age similarly results in puberty delay (Kennaway, Gilmore and Dunstan, 1985). We have continuously monitored the breeding seasons of the ewes which were the subjects of the 1984 and 1985 studies (Fig. 3).

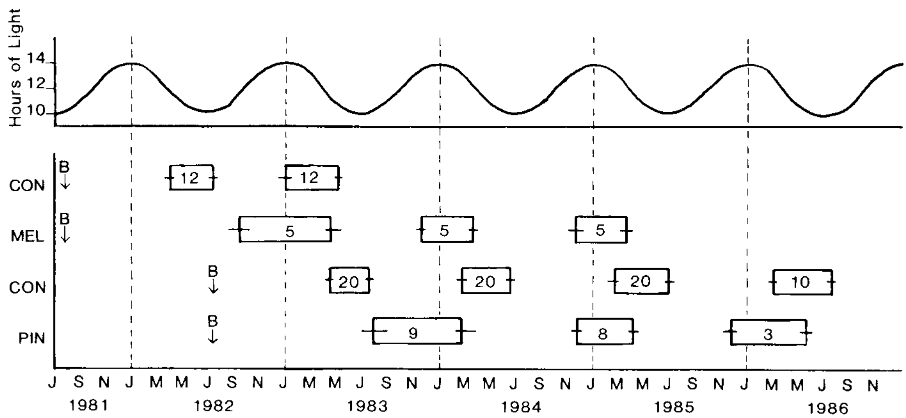

FIG. 3. — *The mean onset and offset of the breeding seasons* (determined by progesterone measurement and/or oestrous marks) *between 1981 and 1986 for the melatonin treated* (MEL) *and their controls* (CON) (Saxon Merino × Border Leicester) × Dorset ewes. The breeding seasons for ewes born to the same dams one year later and pinealectomized (PIN) at 10 weeks and their controls (CON) are also shown. The letter B represents the birth of the ewe lambs while the number in each box is the number of animals in each group.

Following a marked delay in the appearance of pubertal ovulatory cycles the two experimental groups of ewes had consistently earlier onset and offset of the breeding season. Clearly the manipulations performed during their early life permanently changed the seasonality of breeding. These results pose a number of interesting questions.

The first involves the pinealectomized ewes ; half of them had been born to pinealectomized dams and kept in constant light until they themselves were pinealectomized at 10 weeks of age. Presumably, they never received any photoperiodic input via the pineal system (unless they detected alternating light/dark *in utero* prior to moving into constant light three weeks before birth). Why then did the ewes start ovulating during the normal anoestrous period of the year ? The ewes were clearly not influenced by the control ewes in the flock —

they had ceased cycling some time earlier. One must presume that the innate drive to reproduce is very powerful and cannot be prevented in sheep with essentially normal hypothalamo/pituitary/gonadal axes. The next point of interest is the unusually long pubertal season shown by both the melatonin treated and pinealectomized lambs. This was the only time when long breeding seasons were observed ; thereafter, season lengths were similar in all groups. In examining this aspect one is faced with the question of why the ewes actually *stopped* cycling once they started ! What were the mechanisms by which the ewes shut down their ovaries at the time control ewes were activating theirs ? Again social interaction cannot be invoked as a cause. One possibility is that there is a finite time that ewes of this breed can sustain ovulations due to inadequate recruitment of follicles.

In an attempt to discover the endocrine basis for the unusual timing of the breeding season in control ewes ; thereafter LH levels rose to 10-20 μg/l as first 1985 we ovariectomized and oestradiol implanted seven control ewes, five pinealectomized ewes and the three remaining melatonin treated ewes (Fig. 4). The oestradiol implants caused LH to be suppressed until the time of the natural breeding season in control ewes ; thereafter LH levels rose to 10-20 μg/l as first shown by Legan, Karsch and Foster (1977). The pinealectomized/ovariectomized/oestradiol treated ewes were also released from the negative feedback effects of the oestrogen at the same time as their remaining intact peers began cyclic ovarian activity.

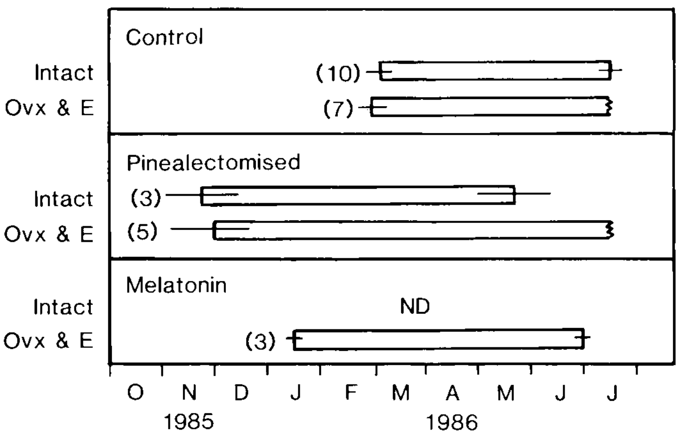

FIG. 4. — *Comparison of breeding seasons of control, pinealectomised* (2.5 months) *and melatonin implanted* (three weeks) *ewes determined either by plasma progesterone/oestrus records or by release from oestradiol negative feedback.* Numbers in brackets are the number of ewes in each group.

Thus whatever the cause of the premature seasonality it was reflected in an apparently normal feedback sensitivity change. This is in contrast to the observations previously made on ewes pinealectomized at 30 weeks of age ;

those ewes maintained normal breeding season timing several years later but displayed apparently aberrant oestradiol feedback sensitivity changes (Kennaway *et al.,* 1984a).

While the drastic operation of pineal removal might be expected to have major endocrine sequalae, the observation that continuous melatonin administration resulted in permanent seasonal disruption was not expected. We can only speculate on the possible mechanism of this phenomenon. Perhaps the continuous availability of melatonin during the critical ages of 10 -20 weeks when the pattern of photoperiod is important for timing of puberty (Foster *et al.,* 1986) permanently altered hypothalamic centres responsible for decoding normal circadian melatonin rhythms.

There have been few reports of the long-term effects of short-term melatonin treatments to adults. The increase in fecundity in ewes observed with short daylength exposure and the ram effect was repeatable over six years in the same flock (Kennaway, Dunstan and Staples, 1987) indicating there is no carryover effect. Similarly, treatment with Regulin melatonin implants to advance the breeding season and increase fecundity of Corriedale ewes had no deleterious effect the following year (Reeve *et al.,* 1987).

Conclusion.

Manipulation of the pineal system has remarkable effects upon the reproductive biology of sheep. In the short term treatments utilising the pineal gland hormone melatonin, procedures have been devised which will advance breeding behaviour and the seasonal rhythm of fecundity. Indeed such strategies are likely to be of economic importance for optimising production systems of sheep. From an endocrinologist's point of view there are still many gaps in our knowledge of the way the pineal gland and melatonin act upon the reproductive system. Major questions involve the mechanism of action of melatonin ; once this is determined pineal research will have entered another new era.

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Résumé. *Effets à court terme et à long terme de manipulations de l'axe pinéale/mélatonine chez la Brebis.*

Nous donnons une revue des expériences qui ont apporté un éclairage sur le rôle de la glande pinéale et de la mélatonine sur la reproduction du mouton. Il est maintenant évident que l'administration quotidienne contrôlée de mélatonine et son administration continue par des implants sont également efficaces pour obtenir des effets physiologiques de type « jours courts ». Ainsi des traitements par la mélatonine pendant 6 semaines provoquent une avance de la saison de reproduction et une avance dans le pic saisonnier du taux d'ovulation. Des périodes beaucoup plus longues d'exposition à la mélatonine ou l'effet à long terme de la pinéalectomie donnent des réponses différentes.

Dans la seconde partie de cette revue, les effets à long terme de l'ablation de la glande pinéale chez les agnelles ou les brebis pubères sont comparés et opposés. Cinq ans après la pinéalectomie (7,5 mois) les brebis montraient des saisons normales de reproduction mais sans montrer la cinétique annuelle normale de sensibilité de LH à l'oestradiol. La pinéalectomie à 2,5 mois d'âge provoquait un retard de la puberté et 2 ans plus tard des saisons de reproduction désynchronisées par rapport aux brebis normales. Ces animaux gardaient une sensibilité de la LH à l'oestradiol liée à leur propre saison de reproduction. Des implants, délivrant de la mélatonine pendant 1 an, ont provoqué des effets comparables à ceux de la pinéalectomie à 2,5 mois, mais avaient des effets complètement opposés à ceux de la pinéalectomie pratiquée chez les adultes. Ces résultats soulèvent beaucoup de questions sur les différentes perceptions du signal de la mélatonine par les brebis prépubères ou adultes et les facteurs responsables du début et de la fin de la saison de reproduction.

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