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The Proposed Use of Melatonin in Controlled Sheep Breeding

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Abstract
The regulation by melatonin of hypothalamic–pituitary events in the ewe to advance seasonal oestrous activity, with no undesirable effects upon fertility, and its induction of those seasonal responses associated with short days indicates an essential role for melatonin in controlled-breeding programs in major sheep-producing countries. The development of suitable controlled-release systems to provide a choice of practical methods of melatonin delivery under field conditions is discussed as also are geographical and breed factors in controlled breeding with melatonin.

Introduction

The annual change in day length (photoperiod) regulates the reproductive activity of most domestic farmed animals to ensure that the young are born at a time of the year when climate and feed availability are most adequate for survival and subsequent growth.

Although the sheep is defined as a 'short-day breeder', because of a tendency towards autumn mating for spring lambing, there are many geographical and breed variations within this pattern. British breeds demonstrate a distinct seasonal reproductive activity with those strains from extreme latitudes (Scottish Blackflace) or altitudes (Herdwick) displaying a shorter season than those from more southerly (Dorset) or lowland (Romney) areas. At the other end of the spectrum are breeds such as the Merino that have evolved in more temperate climates and display a more extended season. To generalize, the greater the latitude, the more restricted the period of breeding activity.

The seasonal nature of this breeding process imposes a similarly restricted pattern upon the supply of prime quality lamb with premium market prices consequently paid for 'out of season' produce. By overriding these seasonal constraints, this situation can be commercially exploited to produce (i) 'early season' (born in winter) or (ii) 'reverse season' (born in autumn) lamb.

Photoperiod, Seasonality and Pineal Function

'The sheep has been, of all domestic animals, the subject of the most intense studies in the physiology of reproduction' (Hammond 1944).

The sheep has indeed proved an historic subject of reproductive interest. Heape (1900–cited by Yeates 1949) commented that the British breeds of sheep had a defined breeding season which commenced around the early winter months. Marshall (1903 — cited by Yeates 1949) observed that ovaries from ewes killed in July or August contained no corpora lutea or protruding Graafian follicles whereas ovaries removed from sheep in October contained mature follicles. Marshall (1937) further proposed that decreasing day length initiated reproductive activity in sheep and Yeates (1949) conducted an elegant experiment that confirmed this. Hafez (1952) considered that the period of breeding activity centered around the winter solstice but this view was modified by Robinson (1959) who concluded that late autumn proved a more accurate mid-point. While formative studies concentrated on the ewe, it was apparent that the ram also displayed a seasonality in reproductive activity. Unlike the ewe of the corresponding breed, the ram appears capable of mating throughout the year (Pepelko and Clegg 1965), although

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a definite seasonal cycle linked to changing photoperiod has been formally demonstrated in spermatogenesis, semen quality and libido (Ortavant *et al.* 1964; Lees 1965; Ortavant 1977; Lincoln and Davidson 1977; Lincoln 1979). When rams and ewes were maintained together under conditions controlled for photoperiod, nutrition and temperature, a time lag between maximum testicular and ovarian activity was evident (Evans and Robinson 1980) with peak sexual behaviour in rams coincident with the mid-point of breeding activity in ewes (Poulton and Robinson 1987).

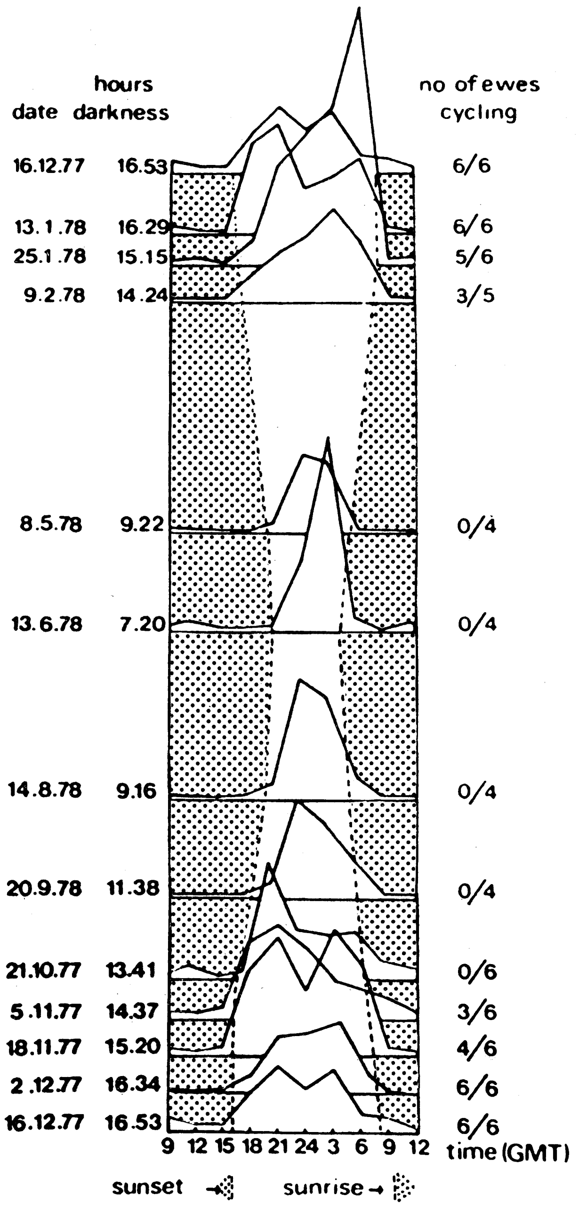

Fig. 1. Plasma melatonin at 3-h intervals in four to six ewes sampled at 2-week to 2-month intervals for 1 year. Mean values are shown, without variance, for clarity. Analysis of variance indicates significant effects of time of day, time of year, number of hours after sunset, and number of hours after sunrise (P < 0·01–0·001). From Arendt (1985) with permission.

During the last 20 years the mechanistic relationship between the perception of changing photoperiod and the expression of sexual rhythms in mammals has been the subject of major investigations. It is now assumed that seasonal changes in photoperiod are effectively monitored by the eye (retina) transmitting a neural signal to the pineal gland which reacts by secreting an indole amine, melatonin, mainly during the hours of darkness (see review by Arendt 1985). It has been further demonstrated that secretion of melatonin mediates the effect of changing photoperiod upon seasonal reproductive activity in many mammals including sheep (Fig. 1, see also reviews by Bittman 1984, Kennaway 1984). Melatonin secretion cannot simply be regarded as either an anti- or progonadotrophic process but rather an entraining signal for inherent rhythms of biological activity (Herbert 1981; Hoffman 1981).

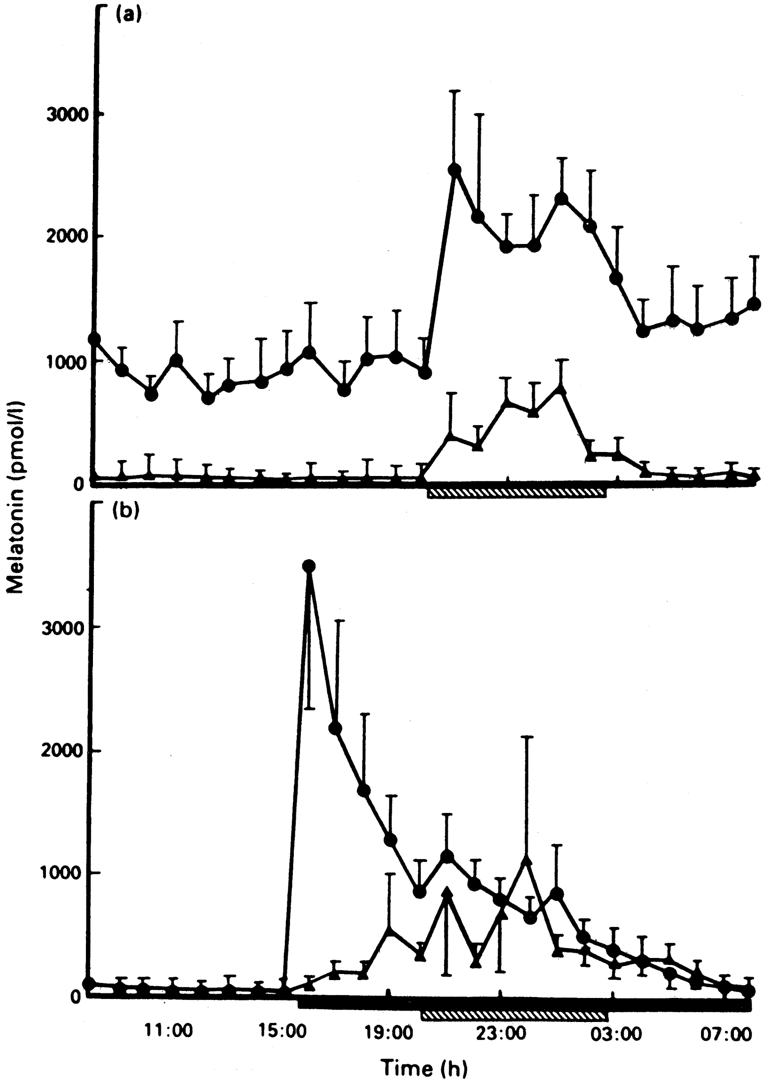

Fig. 2. Hourly plasma melatonin concentrations throughout a 24-h period in ewes (*a*) receiving no treatment (▲); given a subcutaneous melatonin (1 g) Silastic implant 7 days previously (●); (*b*) fed melatonin (3 mg) daily at 1530 h for 7 days previously, these three groups having been kept under natural photoperiod since birth (●); maintained under an artificial short (8 L:16 D) photoperiod (▲). The natural photoperiod (sunrise to sunset) is shown as a hatched bar on the time axis and the artificial photoperiod as a solid bar. Each point is the mean ± s.e.m. (n = 4). From English *et al.* (1986) with permission.

Supplementary Melatonin mimics Short Photoperiod

Following confirmation of the link between pineal function and seasonality, coupled with the availability of sufficient quantities of melatonin for experimental procedures, melatonin was routinely administered to many mammalian species with a view to manipulating seasonal rhythms.

Research groups from Australia, America and Britain simultaneously demonstrated that daily (late afternoon) administration of supplementary melatonin to ewes in mid-summer substituted for short photoperiodic conditions and advanced the onset of breeding activity (Kennaway *et al.* 1982*a*; Nett and Niswender 1982; Arendt *et al.* 1983). It was shown that continuous melatonin treatment by implant methods also simulated short day length (Kennaway *et al.* 1982*b*) to induce early reproductive activity in both ewes (Nowak and Rodway 1985; English *et al.* 1986) and rams (Lincoln and Ebling 1985). Such sustained elevations of plasma melatonin (Fig. 2) might be physiologically perceived as a 'super short' day.

Timed and continuous melatonin delivery and artificial short photoperiod similarly advance onset of oestrus and reduce plasma prolactin levels (Fig. 3), this hormone providing a convenient

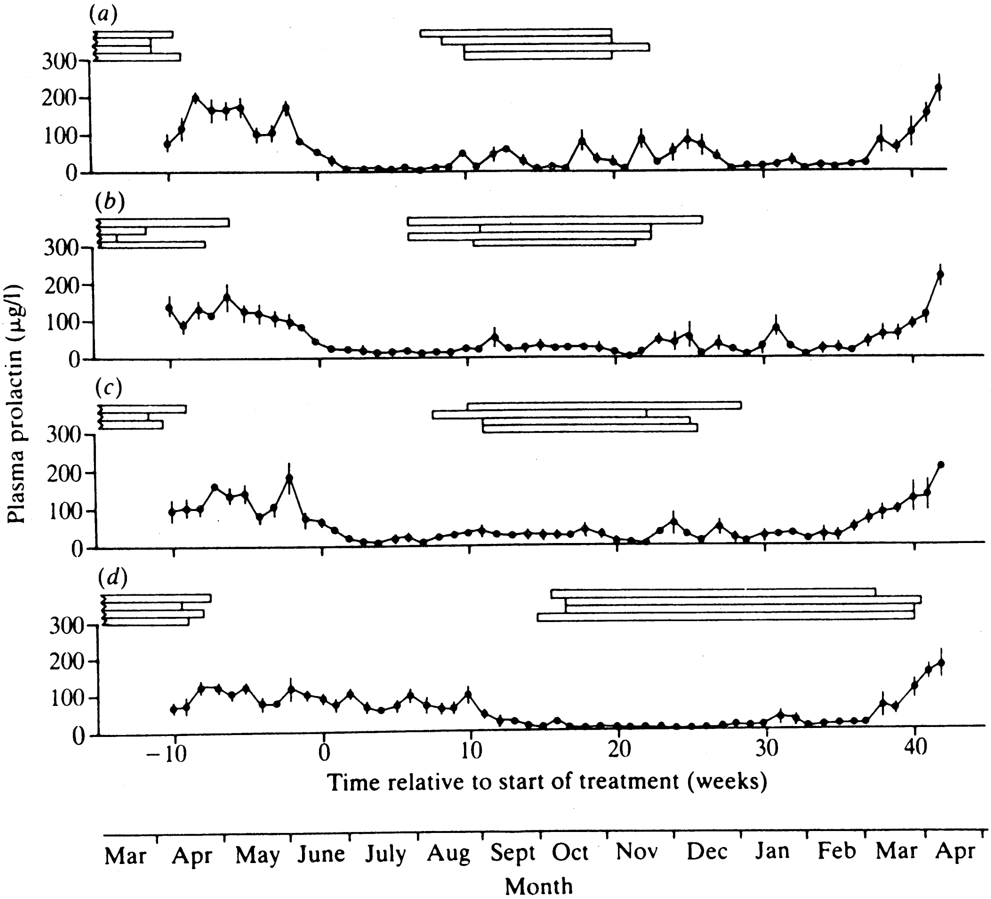

Fig. 3. Weekly plasma prolactin concentrations in ewes (*a*) given a subcutaneous melatonin (1 g) Silastic implant, (*b*) given a daily (at 1530 h) oral dose of 3 mg melatonin, (*c*) maintained under an artificial short (8 L:16 D) photoperiod, and (*d*) receiving no treatment. All treatments commenced on 18 June 1984 (week 0) and were terminated on 22 November (week 22). Each point is the mean ± s.e.m. ($n = 4$). Individual periods of breeding activity, as determined from plasma progesterone concentrations, are presented as horizontal bars for each treatment group. Onset of breeding activity was significantly advanced in the melatonin-fed ($P < 0{\cdot}01$), melatonin-implant ($P < 0{\cdot}001$) and (8 L:16 D) ($P < 0{\cdot}005$) groups. From Poulton *et al.* (1987*a*) with permission.

physiological index of the prevailing photoperiod (Poulton *et al.* 1986). Although inducing early onset of breeding activity, melatonin has no immediate effect upon plasma LH and FSH release (Fig. 4), rather changes in these hormones are detected immediately prior to the first LH surge of the breeding season (Ryan and Foster 1980; Walton *et al.* 1980).

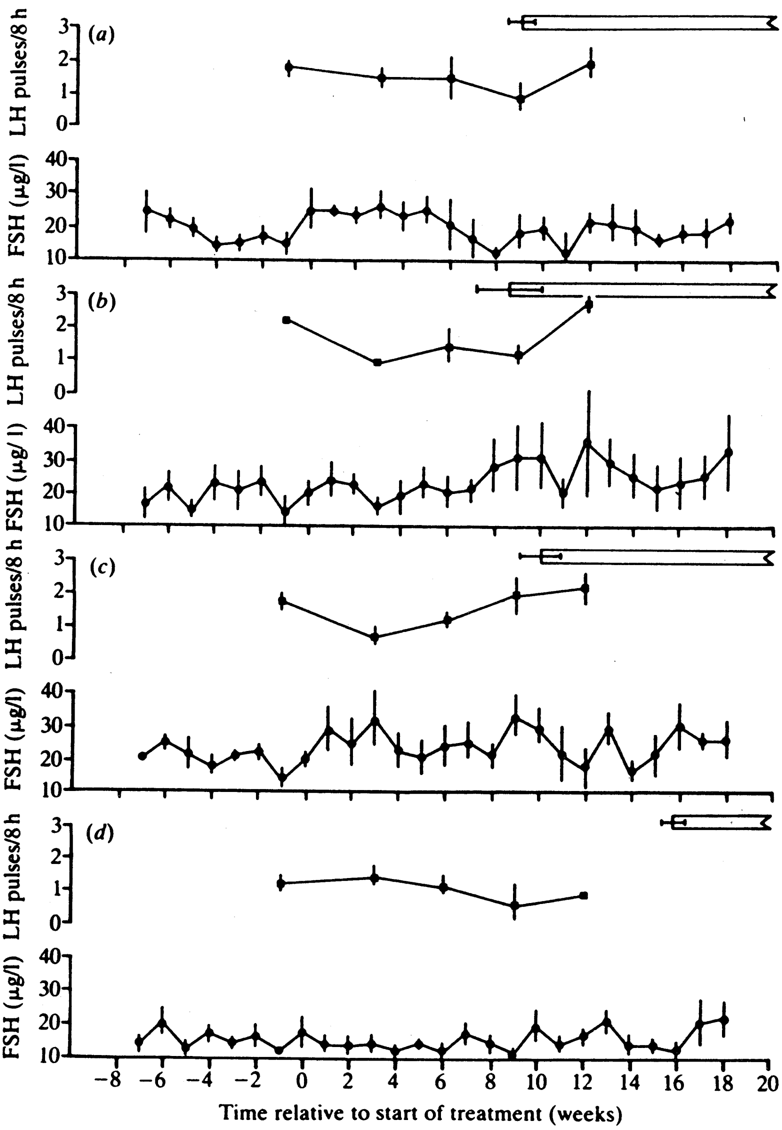

Fig. 4. Weekly plasma FSH concentrations and LH pulse frequency in ewes (*a*) given a melatonin implant, (*b*) given a daily (at 1530 h) oral dose of 3 mg melatonin, (*c*) maintained under an artificial short (8 L:16 D) photoperiod, and (*d*) receiving no treatment. All treatments commenced on 18 June 1984 (week 0). Each point is the mean ± s.e.m. ($n = 4$). Following melatonin or artificial photoperiod treatments, neither plasma FSH concentrations nor LH pulse frequency changed prior to onset of breeding activity. The onset (mean ± s.e.m.) of breeding activity as determined from plasma progesterone concentrations is presented for each treatment group (bar). From Poulton *et al.* (1987*a*) with permission.

In 'seasonal' breeds such as the Suffolk Cross and Swaledale × Bluefaced Leicester (Mule), melatonin treatment is only effective around mid-summer (Nowak and Rodway 1985; English *et al.* 1986), probably following exposure to a critical duration of long photoperiod during which the ewe might acquire sensitivity to melatonin (or short photoperiod). However, Suffolk-cross ewes induced to early (late summer) breeding activity by mid-summer melatonin administration were responsive to spring melatonin treatment in the following year in that onset of breeding activity was further advanced (to mid-summer) by the same margin. Untreated animals in the following year reverted to a normal (autumn) breeding pattern (Symons *et al.* 1987). This observation underlines the possibility of maintaining melatonin-treated flocks that exhibit oestrus at different times of the year for a year-round breeding program. In the less seasonal Merino crossbreeds, melatonin treatment can be effective prior to the summer solstice (Kennaway *et al.* 1982*a*).

Peak ovarian activity, as indicated by maximum ovulation rate, occurs around the mid-point of the breeding season (late autumn) in both British breeds (Hammond 1952) and the Australian Merino (Dun *et al.* 1960) and would appear to be linked to decreasing photoperiod (Dunstan 1977). Kennaway *et al.* (1984) demonstrated that artificial short photoperiod and daily melatonin treatment similarly increased the lambing percentage, compared with control ewes maintained under natural photoperiod, reflecting an increase in ovulation rate at the time of mating. With pressure upon successful conception during the initial period of the breeding season for subsequent early lambing, it would appear that maximum flock fertility is rarely realized in such systems.

If melatonin treatment can effectively induce those seasonal responses associated with short photoperiod, it has an obvious commercial application in all sheep-producing countries, whether its primary intention is for early lambing, improved fecundity or a combination of both.

Methods of Melatonin Delivery

Injection (Nett and Niswender 1982) or feeding (Kennaway *et al.* 1982*a*; Arendt *et al.* 1983) of small quantities of melatonin during late afternoon on a daily basis provided an effective experimental method for mimicking an interval of short photoperiod to induce early breeding activity. However, such methods were considered impractical to implement on a large-scale commercial basis. The observation that melatonin delivery from subcutaneous Silastic sachets also produced an effective short-day signal has precipitated the search for other controlled-release systems for this hormone.

Subcutaneous melatonin implants employing either silicone elastomer (Hsieh and Chien 1985), biocompatible polymer (Staples *et al.* 1986) and polyethyleneglycol-based hydrogel (Rodway *et al.* 1987) as the delivery vehicle have been developed to achieve elevated (daytime) concentrations of plasma melatonin for several weeks. These offer improvement on the large Silastic-based devices that require minor surgery for implantation and removal.

The ruminant stomach has proved an ideal site for controlled delivery preparations (Telfer 1984; Cardinal 1985). An intraruminal bolus of a special polymer-bonded soluble phosphate glass has been developed that allows controlled release of melatonin into the ruminal system and consequently to the peripheral circulation (Fig. 5). The bolus system of melatonin delivery affords a convenient non-invasive (oral) application of a biodegradable (non-residual) device (Poulton *et al.* 1987*b*, 1987*c*).

The critical (daytime) plasma melatonin concentrations and minimum duration of such required to substitute for a short-day response has yet to be accurately defined. It has been

Fig. 5. Twice-weekly plasma melatonin (●) concentrations (means ± s.e.m., $n = 4$) in Suffolk-cross ewes orally dosed with polymer-bonded soluble glass boluses designed to release melatonin within the rumen (reticulum) at a 'fast' (group A), 'medium' (group B) or 'slow' (group C) rate. Group D received a sham (no melatonin) control bolus. (*$P < 0{\cdot}05$, **$P < 0{\cdot}001$, groups A and B *v.* group C). The boluses were given on 4 July 1985 (day 0). Occurrence of cyclic ovarian (breeding) activity is indicated by consecutive oestrous cycles as defined by individual plasma progesterone profiles. The onset of breeding activity in the ewes treated with the 'slow' release bolus (group C) was significantly ($P < 0{\cdot}05$) advanced compared with the control group. From Poulton *et al.* (1987*b*) with permission.

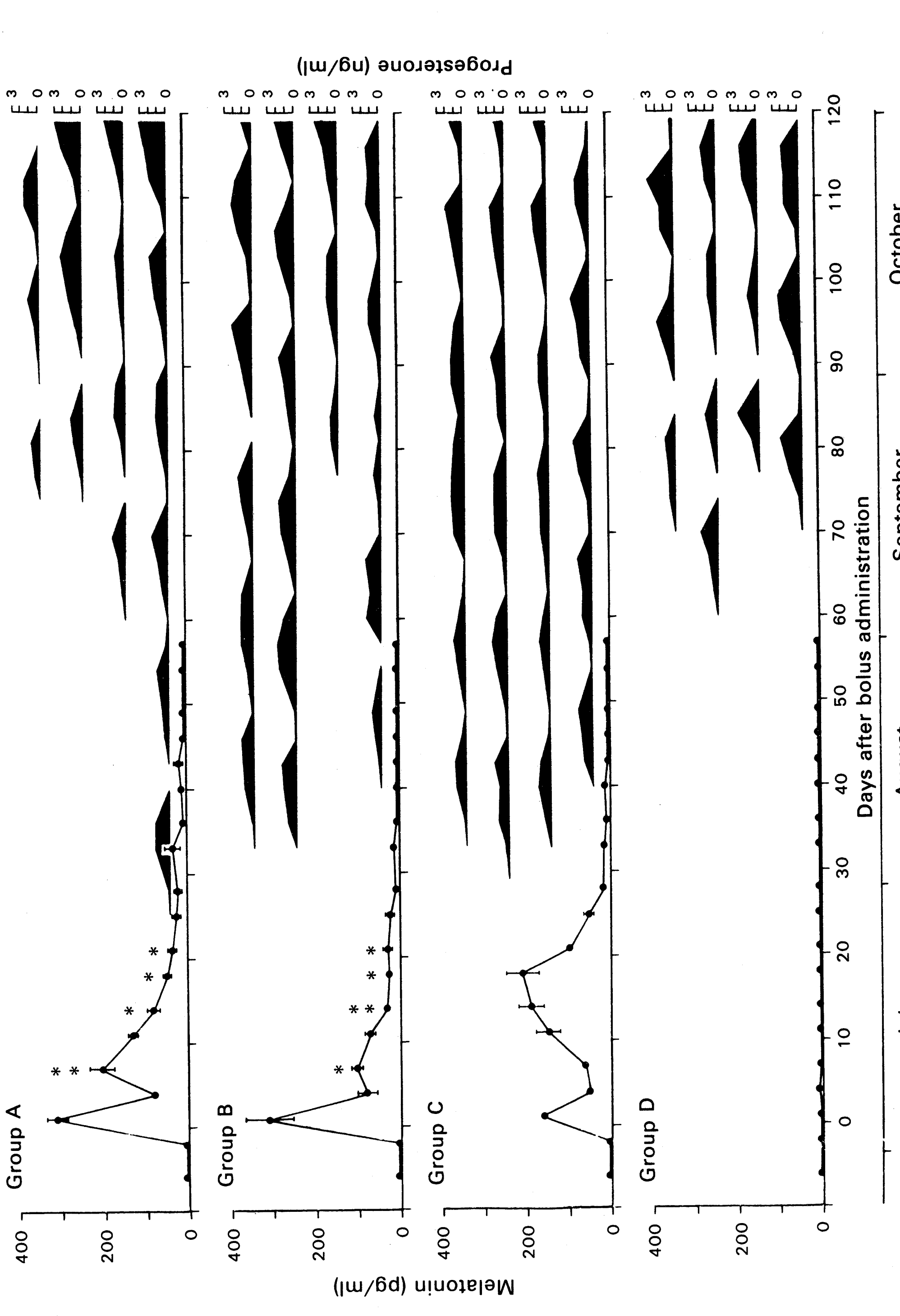

Fig. 5. For legend see opposite page.

suggested that a minimum of 5 weeks exposure to elevated melatonin levels are needed to advance the onset of breeding activity in ewes reliably (Nowak and Rodway 1987) and that at least nocturnal melatonin concentrations (i.e. 30–200 pg/ml) are required (Poulton *et al.* 1987*b*) although supraphysiological levels over a longer period are also effective (Hsieh and Chien 1985).

Geographical and Breed Factors in Controlled Breeding with Melatonin

The ability of melatonin to adjust the timing of the breeding season lies with the assumption that sheep can, in fact, 'respond' to an artificial short-day signal. Whereas most British breeds do not display behavioural oestrus until autumn, the Australian Merino is commonly bred before the summer solstice under increasing day length. Other factors such as nutrition ('flushing') and the presence or absence of rams ('ram effect') can be employed to manipulate breeding activity in this breed (see review by Martin 1984). While influenced by other environmental cues, it is considered that the Merino has retained a basic inherent seasonality (Yeates 1956) that, under more intensive investigation, appears to be less closely entrained to photoperiod than in British breeds (Poulton and Robinson 1987). Also, melatonin treatment can interact synergistically with the pheromonal stimulus of the 'ram effect' to achieve a degree of synchronization of oestrus and/or conception (Robinson *et al.* 1985) to enable a similar pattern of lambing for management purposes (A. L. Poulton, unpublished data).

Economic pressures within the major sheep-producing countries for controlled-breeding husbandry will ultimately determine the graduation of melatonin from the laboratory into the market place. Ongoing work in this laboratory is evaluating the use of melatonin in other farmed ruminants — the deer and goat.

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