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EFFECTS OF A SHORTENED DAILY PHOTOPERIOD OR MELATONIN
TREATMENT ON PUBERTAL RED DEER HINDS (<u>CERVUS ELAPHUS</u>)

A thesis
submitted in partial fulfilment
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EFFECTS OF A SHORTENED DAILY PHOTOPERIOD OR MELATONIN
TREATMENT ON PUBERTAL RED DEER HINDS (CERVUS ELAPHUS)

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J.R.Webster

The effects of a shortened daily photoperiod or daily, afternoon injections of melatonin on the the onset of the breeding season and other seasonal changes associated with the transition from summer to winter were examined in pubertal red deer hinds. Eleven pubertal hinds and four non-lactating adult hinds were randomly allocated to three treatment groups. Control hinds (Group 1) were maintained under natural photoperiodic conditions. From 8 January until 31 March one group of hinds (Group 2) was subjected to a daily photoperiodic regime of eight hours light to sixteen hours dark (8L:16D) by keeping them in a darkened room from 1600h until 0800h the following day and another group of hinds (Group 3) was given an intramuscular injection of 3.75 mg melatonin in safflower oil daily at 1600h.

Both shortened photoperiod and melatonin treatments advanced the time of moulting of summer pelage and the autumnal decline in serum prolactin levels in comparison

with the control hinds. In addition these treatments advanced the onset of puberty (judged by the date of first oestrus) which was significantly earlier ($P < 0.005$) for Groups 2 ($n=3$) and 3 ($n=4$) than for Group 1 ($n=3$). The earlier date of first oestrus in pubertal hinds subjected to shortened photoperiod and melatonin treatments led to a significant ($P < 0.001$) advancement of calving dates by about 30 days. [Group 2 (mean 12 Nov ± 1.67 s.e.m.), Group 3 (mean 11 Nov ± 3.24 s.e.m.) and Group 1 (mean 13 Dec ± 7.90 s.e.m.)]. Gestation length recorded from eleven hinds was 230.5 ± 1.11 (mean \pm s.e.m.). Serum progesterone concentrations indicative of luteal activity were recorded prior to the first oestrus in many (8/13) of the hinds and were attributed to silent ovulations. Earlier calving of the treated hinds led to the body weight of female calves from these hinds being 8 kg heavier than calves from control hinds between January and May although the difference was not significant. Female calves ($n=10$) grew at a rate of 310 ± 8.33 g/day (mean \pm s.e.m) until five to six months of age compared with 380 ± 20.83 g/day (mean \pm s.e.m.) for male calves ($n=3$).

These results indicate that autumnal events in red deer hinds such as the onset of puberty, moulting of summer pelage and decline in prolactin secretion are under photoperiodic control and are caused by changes in the daily rhythm of melatonin secretion from the pineal gland. Daily administration of melatonin provided an effective method for simulating the effects of an artificially shortened photoperiod on the timing of puberty in red deer hinds and

as a result this procedure caused earlier calving. Silent ovulations seemed to occur in many of the hinds, but this study did not establish whether the treatments utilised here had any effects on these silent ovulations. Earlier calving may give rise to calves with a body weight advantage over those born later, at least until their first winter. Mating and calving data from the adult hinds was too limited to indicate whether the advancement of these events achieved in the yearlings could be readily translated to older animals.

KEYWORDS: red deer hinds; Cervus elaphus; puberty; melatonin; photoperiod; breeding season; ovulation; oestrus; calving; pelage; prolactin; progesterone.

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CHAPTER 1

INTRODUCTION

Red deer (Cervus elaphus) which evolved in Europe and Asia were first introduced to N.Z. from Great Britain in the late 19th Century for sporting purposes. N.Z. proved to be an ideal habitat and they multiplied to such an extent that eventually they were considered pests and government control operations had to be instigated in 1931 to limit their numbers. Recently red deer have been recognised for their potential as a farmed animal in N.Z. and so have regained some favour.

In the animal farming industry one of the most important periods of the year is when the birth and subsequent rearing of young occurs. Rearing the newborn places a lactational demand on the mother which means that her food requirements are maximised throughout this period. In turn the ability of the mother to provide an adequate supply of milk has an important influence on her calf's survival and rate of growth. Where pasture is the main source of nutrients, the food supply available to the lactating mother and thus the survival of young are both very dependent on the prevailing climate. In red deer the time of parturition has evolved so that it occurs in mid-summer, presumably at a time which in their original habitat was most favourable for survival of offspring. Summer in N.Z. generally has warm settled weather, which is ideal for calf survival, but it can also

be a time of drought and poor grass growth, especially in drier regions such as Canterbury. Because of this, the normal time of calving for red deer may not be compatible with the high production and efficiency demands of modern agricultural systems. If the time of calving could be advanced by one to two months this may synchronise the period of high feed demand of hinds with peak pasture production in late spring. This would result in more efficient utilisation of pasture and could ultimately lead to increased stocking rates and higher calf weights at weaning. Higher calf weights in turn could mean increased venison production, and even lead to the earlier onset of puberty.

Gestation period is relatively constant for most species therefore the time of parturition is governed by the date of conception. In sheep, mating activity is initiated by the reduction in the duration of daylight (or photoperiod) during autumn. The length of the photoperiod is converted into an endocrine signal by the pineal gland which receives this information about the external lighting from the eyes and responds by changing its pattern of secretion of melatonin. In sheep it is possible to utilise this information to advance the onset of seasonal breeding; either by artificially reducing the daily photoperiod or by giving appropriately timed doses of melatonin. The onset of puberty in juvenile sheep also can be altered by manipulating the photoperiod because this event appears to be controlled by the same mechanisms as the onset of seasonal breeding in adults.

Red deer have a gestational period of around 7.5 months, so to calve in summer mating must occur during autumn, at the same time as sheep (although their shorter gestation means that sheep give birth in spring). It seems reasonable to presume that the onset of the breeding season and puberty in red deer are controlled by a similar mechanism to that in sheep and that these events can thus be advanced by decreasing the daily photoperiod or by administration of melatonin. The present study examined the effects of an artificially shortened photoperiod or melatonin treatment on pubertal red deer hinds in order to establish whether the date of first calving could be advanced by these treatments and to provide basic information on the mechanisms controlling seasonality in this species.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 SEASONAL BREEDING

2.1.1 INTRODUCTION

Most mammals have evolved so that they give birth at the optimum time of year for survival of the new-born. Usually this means parturition at a time of increasing food availability and/or favourable climatic conditions. A major influence on food availability and climate is the annual change in length of daylight. Changes in the length of daylight (or photoperiod) are highly predictable from year to year, so it is not surprising that the mechanism that mammals have evolved to time correctly the birth of young uses photoperiod as the proximate stimulus. This means that each animal must respond to the appropriate photoperiod and commence breeding activity at a time of year that will produce young at the correct time, given the gestation length of the species.

2.1.2 PHOTOPERIODIC CONTROL OF SEASONAL BREEDING

An account of photoperiodic control of seasonal breeding was provided by Marshall (1937) who, from observations on sheep and red deer which reversed their breeding seasons after transfer across the equator, suggested that if these animals react to light it must be to its diminution and not to its increase. Yeates (1949) used artificially reversed

photoperiods to change the timing of the breeding season of ewes and showed that decreases in photoperiod stimulate the onset of breeding, while increasing photoperiods cause the cessation of oestrous activity. Further experimentation with ewes showed that the time from a decrease in photoperiod until the onset of oestrous cyclicity is reduced if the decrease in photoperiod is large (Ducker et al., 1970) and abrupt (Newton & Betts, 1972). The response of an animal to reduced photoperiods is also faster the closer a decrease is to the natural breeding season (Newton & Betts, 1972). Artificially shortened photoperiods have been used to; advance the breeding season of ewes (Hafez, 1952; Fraser & Laing, 1969; Ducker & Boyd, 1974; Walton et al., 1977; Walton et al., 1980; Arendt et al., 1983), decrease wool growth of sheep (Morris, 1961; Ryder & Lincoln, 1976), and stimulate reproductive activity of rams (Lincoln & Davidson, 1977).

Studies with deer using experimental manipulations of the photoperiod have indicated that light also has a role in timing seasonal events for these species. Artificial photoperiods have induced a variety of seasonal changes such as; antler growth of sika deer (Goss, 1969a), coat moulting of roe deer (Lincoln & Guinness, 1972), pelage change and puberty of white-tailed deer (Budde, 1983), and coat growth, moulting (Kay & Ryder, 1978), antler cycles, rutting behaviour (Blaxter et al., 1974; Pollock, 1975), testicular size and testosterone levels (Blaxter et al., 1974) of red deer. Yearly antler growth cycles of sika deer persisted under constant long or short daily

photoperiods (Goss & Rosen, 1973) indicating that these deer may possess an endogenous circannual rhythm, yet under a constant twelve hours light to twelve hours dark (12L:12D) photoperiod, this rhythm disappeared (Goss, 1969b). This latter finding contrasts with sheep which can maintain a seasonal reproductive rhythm under equatorial lighting (Radford, 1961b; Wodzicka-Tomazewska et al., 1967).

A contributory role of temperature in the control of breeding seasons was suggested by Marshall (1937), who stated that in Scottish red deer "the rut does not occur properly until there has been a sharp frost". Evidence for the role of temperature includes an advancement of the breeding season of sheep by exposure to low temperatures (Dutt & Bush, 1955) and the failure of the breeding season of ewes to follow a reversed photoperiod completely when the temperature cycle had not been reversed as well (Thwaites, 1965). If temperature does have a role in timing breeding seasons in sheep it is only a minor one, as annual cycles of temperature could not alter a seasonal reproductive rhythm in ewes housed under a constant equatorial photoperiod (Wodzicka-Tomaszewska et al., 1967).

Severinghaus & Cheatum (1956) suggested that in North America the climate may influence the timing of the breeding season of white-tailed deer. Peak calving time of white-tailed deer in Virginia varied by up to eight days during a ten year study and it was found that high rainfall in spring (which by stimulating plant growth could result in improved body condition of hinds at oestrus) was positively correlated with early calving the following year (McGinnes &

Downing, 1977). Therefore while the control of seasonal breeding is undoubtedly under photoperiodic control, other factors such as temperature and rainfall may cause minor changes in the timing of the breeding season and perhaps further enhance survival of offspring.

Although photoperiod controls the timing of the breeding season in sheep it does not drive the annual rhythm, as annual breeding seasons still persist under constant daily photoperiods (Radford, 1961a; Wodzicka-Tomazewska et al., 1967; Ducker et al., 1973; Speedy & Owen, 1975; Kennaway et al., 1983). It has been suggested that such ewes remain seasonal either in response to other environmental cues or from the expression of an endogenous circannual rhythm of reproduction (Bittman et al., 1983b). Instead photoperiod acts as an entraining agent, synchronising breeding to the appropriate season each year.

2.1.3 NEUROENDOCRINE CONTROL OF SEASONAL BREEDING

With photoperiod established as the environmental signal responsible for the timing of the breeding season in certain species, a physiological mechanism must exist which is capable of switching oestrous cycles on or off in response to gradual changes in the photoperiod. This mechanism appears to involve both steroid dependent and steroid independent control of LH secretion (Turek & Campbell, 1979); the absence of a sustained increase in tonic LH secretion being the endocrine basis of seasonal anoestrus (Legan & Karsch, 1979). Seasonal changes in tonic LH concentrations are caused by changes in the episodic release

of the hormone from the pituitary gland (Lincoln, 1976; Lincoln et al., 1977; Karsch et al., 1980; Lincoln & Short, 1980; McNatty et al., 1981) which reflect changes in the pulsatile release of gonadotrophin releasing hormone (GnRH) from the hypothalamus (Carmel et al., 1976; Lincoln & Short, 1980). Supporting this, pulsatile infusion of either LH (McNatty et al., 1982b; McNeilly et al., 1982) or GnRH (McNatty et al., 1982a; McNatty et al., 1982b; McCleod et al., 1983) was capable of inducing an ovulation in seasonally anoestrous ewes. A study by Goodman et al. (1982) indicated that the inhibitory photoperiod which causes anoestrus exerts its effect on tonic LH secretion by reducing LH pulse frequency.

Roche et al. (1974) proposed that an oestrogen may be the steroid which inhibits LH secretion during anoestrus and evidence for this was provided by Legan et al. (1977). These latter workers found that the seasonal change in LH secretion from low levels during anoestrus to high levels throughout the breeding season required the presence of oestradiol, and they proposed an hypothesis whereby the occurrence of breeding or non-breeding seasons is governed, at least in part, by the capacity of oestradiol to function as a negative feedback hormone. According to this hypothesis the negative feedback inhibits LH secretion during anoestrus then diminishes and allows LH levels to rise at the onset of the breeding season. Subsequent investigation has shown that these changes in the negative feedback effect of oestradiol followed artificial changes in

the photoperiod (Legan & Karsch, 1978; Legan & Karsch, 1980). Artificial short day photoperiods were accompanied by a decrease in this negative feedback whereas artificial long days were associated with an increase in negative feedback. This provides a mechanism whereby photoperiod controls seasonal breeding primarily by altering the negative feedback of oestradiol on LH secretion. This theory has since been tested experimentally (Karsch et al., 1980) and described in review articles (Legan & Karsch, 1979; Goodman & Karsch, 1981; Legan & Winans, 1981). Further studies on ewes by Karsch's group have provided evidence that during the breeding season oestradiol may suppress LH pulse amplitude, while during anoestrus oestradiol gains the capacity to reduce the frequency of hypothalamic GnRH discharges, thereby reducing LH pulse frequency (Goodman et al., 1982). These workers proposed that seasonal shifts in the negative feedback effect of oestradiol represent shifts in hypothalamic responsiveness to this steroid. In the breeding season oestradiol acts on the pituitary decreasing the response to GnRH while during anoestrus oestradiol acts primarily on the brain to decrease the frequency of episodes of GnRH secretion (Goodman et al., 1982).

Oestradiol-sensitive neurons inhibiting the hypothalamic pulse generator (which is responsible for GnRH pulses) have been demonstrated in the ewe (Goodman & Meyer, 1982).

Photoperiodic alteration of serum levels of LH in castrated rams (Pelletier & Ortavant, 1975) and of LH pulse frequency in ovariectomised ewes (Karsch et al., 1980;

Goodman et al., 1982) indicate that a steroid independent mechanism also may play a part in regulating seasonal changes in LH secretion in sheep. In contrast, no seasonal changes in tonic LH levels were found in castrated red deer stags (Lincoln & Kay, 1979). Steroid independent changes in LH secretion in sheep may signify a direct photoperiodic drive acting on the centres governing tonic LH levels (Karsch et al., 1980) and therefore seasonal anoestrus may be caused simply by reduced hypothalamic activity (McNatty et al., 1982a). From a series of experiments on rams, Lincoln and Short (1980) proposed that the primary effect of long photoperiods is to inhibit GnRH secretion directly and as a consequence any inhibition from the gonads becomes more effective. During short photoperiods there is no such central inhibition and negative feedback from the gonads is less effective. Thus the variations in the negative feedback of oestradiol may be accounted for by changes in direct photoperiodic drive on the hypothalamus. The demonstration that progesterone as well as oestradiol become potent in suppressing LH pulse frequency during anoestrus supports this possibility (Goodman et al., 1982). Work by Robinson et al. (1982), has indicated that there is a tight coupling of both steroid-dependent and steroid-independent photoperiodic effects on gonadotrophin secretion in ewes making it difficult to separate these two mechanisms.

2.1.4 THE ROLE OF THE PINEAL GLAND

In the ewe retinal photoreceptors are required for

photoperiodic control of seasonal breeding (Karsch et al., 1981; Legan & Karsch, 1983). From the eyes information about the external lighting conditions reaches the non-photoreceptive mammalian pineal gland by a pathway involving the accessory optic tracts and the pineal's unusual sympathetic innervation (Wurtman et al., 1964; Wurtman & Moskowitz, 1977). The mammalian pineal can therefore be considered as an indirect photosensory organ (Ariens Kappers, 1969).

An early report of the effect of pinealectomy on ruminant reproduction failed to demonstrate a role for this organ in ewes (Roche et al., 1970), however these authors did not study the effects of imposed changes in lighting. Subsequent examination of testosterone and prolactin secretion with rams (Barrell & Lapwood, 1979) and LH secretion in ewes (Karsch et al., 1981) found that pinealectomy reduced the effects of changing photoperiod on the secretion of these hormones.

Pinealectomy of white-tailed deer (Mazur, 1973; Brown et al., 1978; Schulte et al., 1981; Snyder et al., 1983) altered the timing of photoperiod linked seasonal cycles such as antler development, testicular size and testosterone and prolactin concentrations, but did not abolish these cycles. Thus in sheep and some deer species the pineal seems to be responsible for synchronising biological cycles to the season. This role appears clear when sheep are exposed to out-of-season photoperiodic challenges to which they could not respond without a functional pineal gland (Barrell & Lapwood, 1979; Lincoln,

1979; Karsch et al., 1981; Lincoln & Almeida, 1981).

From experiments on ewes, Bittman et al. (1983b) indicated that the pineal mediates the photoperiodic control of seasonal breeding, in part via its influence on the negative feedback effect of oestradiol on LH secretion.

2.1.5 THE ROLE OF MELATONIN

The pineal responds to light or dark by synthesising and releasing the indoleamine melatonin as a result of stimulation from sympathetic nerves which are inhibited when light activates retinal photoreceptors (Wurtman & Moskowitz, 1977). In the ewe the pineal releases melatonin predominantly into the blood (Rollag et al., 1978a) producing serum melatonin levels during darkness which are up to ten times the levels present during daylight (Rollag & Niswender, 1976). Melatonin levels accurately reflect the light and dark pattern to which the animal is exposed, (Rollag et al., 1978b) except under constant light when the rhythm of melatonin secretion is abolished and under constant dark when a circadian rhythm persists (Rollag & Niswender, 1976). The nocturnal rise in melatonin is also abolished by pinealectomy (Kennaway et al., 1977; Arendt et al., 1980; Bittman et al., 1983a; Bittman et al., 1983b; Kennaway et al., 1982/1983) or disruption of the pineal's sympathetic innervation (Lincoln & Almeida, 1981; Lincoln et al., 1981) confirming the role of the pineal in melatonin secretion. However, it is worth noting that the pineal gland is not the only source of melatonin in the body since melatonin could be detected in

plasma from ewes up to three months after pinealectomy (Kennaway et al., 1977).

Studies by Bittman et al. (1983a) in which melatonin was infused into pinealectomised, ovariectomised ewes implanted with oestradiol indicated that melatonin controlled the capacity of oestradiol to inhibit LH secretion. An increase in the duration of melatonin infusion produced a reduction in the negative feedback effects of oestradiol on LH secretion. This provided a mechanism which would account for pineal mediation of seasonal breeding and supported the hypothesis of Legan et al. (1977) that seasonal breeding is due to the changing potency of oestradiol inhibition of gonadotrophin secretion (see Section 2.1.3).

In terms of the daily melatonin secretion profile the major difference between long and short days arises from the fact that melatonin levels in the blood are elevated during darkness (Rollag et al., 1978b). Arendt et al. (1979) have reported also that the pattern of melatonin secretion differs between long and short days. A single peak of melatonin occurs during short nights and a bimodal pattern during long nights. Studies with Djungarian hamsters (Carter & Goldman, 1983) and ewes (Kennaway et al., 1983; Bittman & Karsch, 1984) have suggested that the duration of elevated melatonin is important in determining the reproductive response to photoperiod. Other studies with the various species of hamsters have suggested however that it is the precise timing of melatonin release, relative to an endogenous circadian rhythm which is important in determining the reproductive response to

photoperiod (Elliot et al., 1972; Tamarkin et al., 1976; Tamarkin et al., 1979; Watson-Whitmyre & Stetson, 1983). Experimentation with artificial photoperiods on sheep has indicated that if a light pulse of one hour is given seventeen hours after dawn during a short day (8L:16D) photoperiod, the photoperiod is perceived as a long day (Ravault & Ortavant, 1977; Schanbacher & Crouse, 1981; Thimonier, 1981). The existence of this light sensitive period in sheep indicates that timing of changes in melatonin secretion also may be important in this species.

Several theories have been proposed to explain the actions of melatonin in sheep. It has been suggested that during winter elevated melatonin concentrations overlap a sensitive portion of an endogenous circadian rhythm (Rollag et al., 1978b). A mechanism incorporating both the differing patterns and duration of melatonin secretion (described in the previous paragraph) has been proposed (Arendt et al., 1981a; Arendt et al., 1981b).

This hypothesis assumes that a single short duration peak of melatonin is inhibitory to reproductive function, and that melatonin receptors in the sheep exhibit reduced sensitivity after exposure to high levels of melatonin (down regulation) as has been postulated (Reiter, 1980) and tested experimentally (Chen et al., 1980) in Syrian hamsters. During long nights a second peak of melatonin levels in the blood or simply extended secretion of melatonin may down regulate its own receptors, decrease the sensitivity of the neuroendocrine axis to the anti-gonadotrophic effect of a

single peak or short duration secretion, and allow reproductive resurgence.

2.1.6 CONTROL OF SEASONAL BREEDING WITH EXOGENOUS MELATONIN

As the pineal may exert its effects on reproduction by secreting melatonin and since one of the major differences between stimulatory and inhibitory photoperiods is in the duration of elevated melatonin levels in the blood, then administration of exogenous melatonin could be used to overcome the effects of an inhibitory photoperiod on reproduction.

Administration of melatonin either by subcutaneous injection (dissolved in saline solution or peanut oil) or orally (dissolved in water/ethanol and added to feed pellets) was examined in sheep and goats by Kennaway and Seamark (1980). They found that injection of melatonin produced a rapid increase in melatonin concentration in the blood, reaching a peak at fifteen minutes, although melatonin levels then declined quickly due to a short half-life of about thirty minutes. Oral administration produced a longer period of elevated melatonin owing to its slow absorption from the rumen. This latter method of administration was used by Arendt et al. (1981b) in sheep under a 16L:8D photoperiod to produce a melatonin pattern characteristic of short days and it caused a reduction in plasma prolactin levels. Since then daily oral administration of melatonin has been used to advance the onset of the breeding season in ewes (Kennaway et al.,

1982a; Arendt et al., 1983; Knight, 1983a).

Constant administration of melatonin to sika deer by using subcutaneous beeswax implants replaced weekly (Goss & Rosen, 1973) produced no effect, but daily oral dosing advanced mineralisation of antlers, shedding of velvet and change of pelage in white-tailed deer (Bubenik, 1983) and advanced the onset of oestrous cyclicity in red deer (Nowak et al., 1983).

Intramuscular injection of sheep with melatonin in safflower oil was found to maintain melatonin levels in blood at or above normal night time levels for at least six hours and this method of administration was used to advance the onset of the breeding season in ewes (Nett & Niswender, 1982).

In ewes melatonin implants have decreased prolactin secretion and thereby apparently simulated short daily photoperiods (Kennaway et al., 1982b; Kennaway et al., 1982/1983).

2.1.7 THE ROLE OF PROLACTIN

Prolactin secretion follows a seasonal rhythm, with highest levels in summer and lowest levels in winter in cattle (Karg & Schams, 1974; Schams & Reinhardt, 1974), sheep (Ravault & Ortavant, 1977; Barrell & Lapwood, 1978/79a; Munro et al., 1980; Thimonier, 1981), goats (Buttle, 1974) and deer (Mirarchi et al., 1977; Kelly et al., 1982). The seasonal variation in prolactin level is closely linked with changes in; photoperiod (Karg & Schams, 1974; Schams & Reinhardt, 1974; Snyder et al.,

1983; Webster & Haresign, 1983), temperature (Schams & Reinhardt, 1974; Peters & Tucker, 1978; Snyder et al., 1983) and level of feeding (Forbes et al., 1975).

Prolactin secretion appears to be mainly under photoperiodic control because blood levels will respond to artificially imposed changes in photoperiod in sheep (Pelletier, 1973; Ravault & Ortavant, 1977; Lincoln et al., 1978; Kennaway et al., 1983; Worthy & Haresign, 1983) and red deer (Brown et al., 1979).

Disruption of pineal function in goats (Buttle, 1977), white-tailed deer (Schulte et al., 1981; Snyder et al., 1983) and sheep (Barrell & Lapwood, 1978/79b; Munro et al., 1980; Kennaway et al., 1981) changes the normal seasonal rhythm of prolactin secretion in these species and prevents prolactin secretion in sheep from responding to artificial photoperiods (Barrell & Lapwood, 1979; Kennaway et al., 1982/1983). Administration of melatonin to sheep on long daily photoperiods in order to mimic the short day pattern of melatonin secretion also produces the short day pattern of prolactin secretion (Arendt, 1981b; Kennaway et al., 1982a; Kennaway et al., 1982/1983) indicating that the pineal, via its secretion of melatonin, is responsible for synchronising the circannual rhythm of prolactin secretion to the annual pattern of changes in daily photoperiod.

Hyperprolactinaemia associated with lactation can be linked to the delay in return to oestrous cyclicity in sheep. Lamb removal (Restall, 1971) or suppression of

prolactin in suckling ewes with the dopamine agonist bromocriptine (Kann & Martinet, 1975) results in an early return to cyclic ovarian activity. Likewise conception in wild Scottish red deer hinds, is delayed (Mitchell & Lincoln, 1973) or even prevented by lactation (Mitchell et al., 1976; Guinness et al., 1978a), and if the calf is lost near to birth the time taken for hinds to return to oestrus can be markedly reduced (Guinness et al., 1971). High prolactin levels induced by frequent suckling due to poor milk production of the hind have been proposed as a cause of delayed conception in red deer (Loudon et al., 1983). High prolactin levels may affect gonadotrophin secretion either by impairing the positive (Kann et al., 1976), or by enhancing the negative (McNeilly, 1980) feedback effects of steroids. Alternatively the hyperprolactinaemia may act directly on the ovary by inhibiting oestradiol secretion (McNeilly & Baird, 1983)

It has been postulated that a seasonal elevation in prolactin level may contribute to the occurrence of seasonal anoestrus in sheep (Walton et al., 1977). In both sheep and deer the time of lactation coincides with that of seasonal anoestrus making it difficult to determine the extent prolactin contributes to each. While some evidence (described in the previous paragraph) indicates a possible role for prolactin in lactational anoestrus, similar evidence is lacking for a role in seasonal anoestrus. Although suppression of prolactin secretion in ewes by treating them with bromocriptine during lactational

anoestrous resulted in an early return to oestrous cyclicity (Kann & Martinet, 1975), reducing prolactin secretion by this method during seasonal anoestrus failed to initiate an early return to oestrous cyclicity (Land et al., 1980; Schanbacher, 1980) and failed to prevent ewes from returning to anoestrus after an induced oestrous cycle (Rodway et al., 1983). Increased prolactin secretion does not inhibit reproductive activity in sheep (Kennaway et al., 1983) and seasonal anoestrus can occur in the absence of high blood prolactin levels (Worthy & Haresign, 1983). A study by Jackson and Davis (1979) concluded that high serum prolactin levels are a symptom of seasonal anoestrus rather than a cause.

2.2 PUBERTY

2.2.1 INTRODUCTION

Puberty has been defined as the time at which reproduction first becomes possible (Asdell, 1946). The process of puberty is complicated in many species by recurring seasonal anoestrus and though individuals may be physiologically capable of producing young, they may be prevented from doing so by seasonal factors, thus making it difficult to determine the initial onset of puberty (Sadleir, 1969a). The role that photoperiod plays in timing the onset of puberty and other constraints that may exist on the age at which puberty can occur are important considerations if puberty is to be advanced.

2.2.2 TRANSITION TO PUBERTY

The first outward sign of puberty in the female is oestrus, a sign that she is ready to mate and that fertilisation is possible. Prior to this event in the lamb one or two silent ovulations (ovulations without behavioural oestrus) can occur (Foote et al., 1970; Foster & Karsch, 1975; Hare & Bryant, 1982). Therefore the transition to puberty in lambs resembles the transition at the beginning of each breeding season in adult ewes, as the first ovulation of a new season also is usually silent (Thorburn et al., 1969; Robertson, 1977; Walton et al., 1977). Studies with ewes have shown that exposure to alternate progesterone and oestrogen is a prerequisite for expression of oestrous behaviour and this pattern of steroid secretion does not occur before the first ovulation of the season (Robinson, 1954).

It has been suggested that the marked increase in physical activity of white-tailed deer at oestrus (Ozoga & Verme, 1975) is induced by a progressive rise in oestrogen that follows a fall in progesterone and since the first ovulation of the season is not preceded by high progesterone levels, oestrus can not occur at this ovulation (Plotka et al., 1980). Silent ovulations at the start of the breeding season appear to be common in several deer species such as; white-tailed deer (Plotka et al., 1977; Harder & Moorhead, 1980; Plotka et al., 1980), black tailed deer (Thomas & McCowan, 1975) and moose (Simkin, 1965).

2.2.3 NEUROENDOCRINE CONTROL OF PUBERTY

Studies with sheep indicate that although the hypothalamic pre-ovulatory mechanism does not develop fully until puberty, it is functional from shortly after birth, as indicated by the finding that exogenous oestradiol could cause surges of LH release in immature lambs (Foster & Karsch, 1975). These pre-ovulatory LH surges do not occur naturally in pre-pubertal lambs but appear to be suppressed until puberty. The onset of puberty is associated with a sustained increase in tonic LH secretion reflecting an increase in the rate of pulsatile LH discharges and this sustained increase eventually leads to a pre-ovulatory LH surge (Foster & Ryan, 1979b). Ovariectomy of pre-pubertal lambs resulted in an increase in frequency of LH pulses (Foster et al., 1975) and when the LH pulse rate was increased artificially in entire lambs ovulation occurred (Foster & Ryan, 1979b). Treatment of ovariectomised lambs with oestradiol implants inhibited LH secretion until the normal time of puberty (Foster & Ryan, 1979a) indicating that puberty is associated with a marked decrease in oestradiol inhibition of tonic LH secretion. It has been proposed therefore that the inhibition of LH secretion in pre-pubertal lambs is controlled by the same mechanism as that which inhibits LH secretion during anoestrus in adults (i.e. via the negative feedback effect of oestradiol (see Section 2.1.3)), and the beginning of both puberty and the breeding season is associated with a decrease in this negative feedback (Foster & Ryan, 1979b). This agrees with the 'gonadostat' hypothesis of Ramirez and McCann (1963) who

postulated from their studies on rats that part of the mechanism of puberty was a resetting of hypothalamic sensitivity to gonadal steroids.

2.2.4 FACTORS GOVERNING THE ONSET OF PUBERTY

In a review on puberty in sheep Dyrmondsson (1973) stated that there is neither a fixed age, body weight nor time of year at which ewe lambs experience their first oestrus, owing to the complexity of the interaction between these factors and the time of birth.

Size appears to be more important than age in influencing the onset of puberty in sheep and cattle (Rattray, 1977) and a minimum threshold body weight, first proposed by Hafez (1952) or a critical metabolic rate (Foster & Ryan, 1981) below which puberty cannot occur, needs to be reached. Age at first oestrus seems to be controlled largely by the rate of development during the first few months of a lamb's life (Dyrmondsson & Lees, 1972) and puberty can be delayed or advanced by varying the plane of nutrition during rearing (Allen & Lamming, 1961). Lambs on a high plane of nutrition tend to attain puberty at a lower age and higher body weight than those reared on a low plane of nutrition (Dyrmondsson, 1973).

Sadleir (1969b) stated that the attainment of puberty in deer is related to the level of nutrition available to them during their pre-pubertal life. This is supported by a higher incidence of ovulation and puberty reported in white-tailed deer raised on high energy diets (Abler et al., 1976) and also appears to be true for red deer (see

Section 2.3.1).

Time of puberty in seasonal breeders is complicated by the constraints that seasonal breeding imposes. Most spring born ewe lambs experience oestrus in their first autumn and winter months (Dyrmondsson, 1973). Lambs born early tend to attain puberty at a greater age and body weight than those born later in the season, and lambs born very late may fail to reach puberty during the first breeding season (Dyrmondsson & Lees, 1972).

If, as was proposed by Foster and Ryan (1979b) the mechanism controlling the onset of puberty is identical to that proposed to control seasonal breeding in adults (described in Section 2.2.3) then it is probable that puberty is under photoperiodic control. However some experiments in which lambs were reared under artificial photoperiods (Radford, 1961a; Smith, 1967; Ducker et al., 1973; Fitzgerald et al., 1982) have failed to demonstrate a pronounced effect of photoperiod on puberty and it has been suggested that inherent rhythms may be more important. In contrast, evidence that photoperiod is involved in the control of puberty comes from studies on autumn born lambs which have puberty delayed until the following autumn, at the same time as much younger spring born lambs (Foster, 1981a). Manipulation of photoperiod to which these autumn born lambs were subjected, so that it was identical to that which they would have experienced if they had been born in spring, advanced both the onset of the decrease in negative feedback of oestradiol and puberty to a similar age as those lambs born in spring. This finding led

to the hypothesis that the delay in onset of ovulation of lambs born in autumn is due to a photoperiod-induced prolongation of inhibition of LH secretion by oestradiol. Two explanations for delayed puberty in autumn born lambs have been proposed. Firstly that sexual development in such lambs is retarded by photoperiodic stimuli and the delayed onset of oestrous cyclicity reflects late sexual maturity, and secondly that such development is not retarded but photoperiod induces seasonal anoestrus at sexual maturity and the eventual onset of cyclicity simply reflects the onset of a breeding season. Further experiments by Foster (1981b) indicated that photoperiod can variously advance, delay or even prevent the onset of ovulation in the lamb depending upon the age at which the photoperiod is reduced, although at least 11 weeks exposure to long photoperiods is required before lambs can respond to stimulatory short photoperiods.

There has been only one report indicating a role for photoperiod in the control of puberty in deer species. This was by Budde (1983) who advanced the onset of puberty in white-tailed deer by early reduction of the photoperiod.

The precise role of the pineal gland and melatonin in the control of puberty has not been established. Treatment of lambs with melatonin implants, which mimic the effect of short days on prolactin secretion in ewes (Kennaway et al., 1982b; Kennaway et al., 1983), delayed the onset of puberty (Kennaway & Gilmore, 1984).

2.3 REPRODUCTION IN RED DEER

2.3.1 PUBERTY

The majority of wild red deer hinds living on Scottish hill land reach puberty as two-year-olds, calving for the first time at the age of three (Lowe, 1969; Mitchell, 1973), except in areas of unusually low population density where they become sexually mature as yearlings (Mitchell *et al.*, 1981). Mitchell (1973) concluded that the age at which a wild Scottish red deer hind reaches puberty is clearly affected by its growth rate, which in turn is primarily affected by nutrition. Therefore while the majority of hinds on the island of Rhum (off the west coast of Scotland) attain puberty at two years of age, puberty can occur as yearlings if these hinds are hand reared (Lincoln *et al.*, 1970; Youngson, 1970).

Wild red deer hinds in the indigenous Nothofagus and Podocarp forests of N.Z., where food is relatively abundant, have their first oestrus and are successfully mated as yearlings of about 16 months of age and calve at about 24 months of age (Daniel, 1963). On N.Z. deer farms puberty as yearlings is expected for hinds (Coop & Lamming, 1977; Kelly & Moore, 1977; Bray & Kelly, 1979). Evidence of sexual maturity in calves as young as three to five months has been reported (Daniel, 1963). These calves were grazing on high protein lucerne and white-clover pastures.

2.3.2 THE OESTROUS CYCLE

Red deer hinds are polyoestrous and may have as many as

eight oestrous cycles during the course of a mating season if pregnancy is prevented (Guinness *et al.*, 1971). Each oestrous cycle lasts about 18 days (see Table 2.1), although short cycles of seven to nine days can occur at the start of the breeding season and long cycles of 34 to 59 days have been reported in yearlings (Guinness *et al.*, 1971). The presence of both short and long oestrous cycles is consistent with studies on sheep (Land, 1971; Hare & Bryant, 1982), black-tailed deer (Thomas & McCowan, 1975), white-tailed deer (Harder & Moorhead, 1980) and reindeer and caribou (McEwan & Whitehead, 1972).

Table 2.1: Length of oestrous cycle in red deer hinds.

MEAN LENGTH OF OESTROUS CYCLE (DAYS \pm s.d.)	REFERENCE
18.3 \pm 1.7	Guinness <i>et al.</i> (1971)
17.5 \pm 1.9	Lincoln <i>et al.</i> (1970)
18.2 \pm 1.6	Kelly & Moore (1977)
18.8 \pm 1.8	Krzywinski & Jaczewski (1978)

The duration of oestrus in red deer hinds has not been accurately defined. Clutton-Brock *et al.* (1982a) mentioned that hinds are in oestrus for a period of 12 to 24 hours although the observations of Guinness *et al.* (1971) indicated that oestrus can last longer than 24 hours. The latter authors also noted that the duration of oestrus appeared to be extended if the hind was not allowed to be served by a stag.

Although silent ovulations occur in sheep and some

species of deer (see Section 2.2.2) it has been stated that they do not occur in red deer (Lincoln et al., 1970; Guinness et al., 1971), yet later studies have reported that 10% (Lincoln & Guinness, 1973) and 16.3% (Kelly & Challies, 1978) of red deer hinds ovulate at the start of the breeding season but do not conceive which presumably could be attributed to the occurrence of silent ovulations.

2.3.3 CONCEPTION AND CALVING

Red deer hinds conceive during the autumn rutting season and calve in early summer. The breeding season is short with about 70% of hinds conceiving within two weeks (Lincoln & Guinness, 1973; Clutton-Brock et al., 1982a). Consequently the majority of calving occurs within a similarly short period (Guinness et al., 1978b; Hamilton & Blaxter, 1980) although the calving pattern is skewed with a greater proportion of hinds calving later than would be expected from a normal distribution pattern (Hamilton & Blaxter, 1980; Asher et al., 1981).

Red deer hinds are generally monotocous. Incidences of twins in various populations of red deer have been reported as 1.4% (including one set of triplets) in Germany and Austria (Kroning & Vorreyer, 1957), 0.1 to 0.2% in Scotland (Mitchell, 1969; Mitchell, 1973), 0.5% in southern England (Chapman, 1974) and 0.7% in north west England (Mitchell et al., 1981).

Calving percentages reported for various populations of red deer are shown in Table 2.2. Reproductive performance

Table 2.2: Calving percentages of red deer hinds.

The method by which the calving percentages were calculated are abbreviated as follows:

- CS/HA - calves in spring/hinds in previous autumn;
- CH/HS - calves in spring/hinds;
- CB/HIH - calves born/hinds in herd;
- CA/HA - calves in autumn/hinds in autumn;
- CB/HES - calves born/hinds exposed to stag;
- CB/HM - calves born/hinds mated.

Country	Calving Percentage	Method	Reference
<u>WILD</u>			
Scotland	37	CS/HA	Mitchell (1969)
Scotland	37.7	CS/HS	Lowe (1969)
Scotland	72-92	CB/HIH	Guinness et al. (1978a)
Scotland	40	CB/HIH	Mitchell (1973)
England	65	CA/HA	Mitchell et al. (1981)
N.Z.(North Island)	28	CA/HA	Riney (1956)
N.Z.(Nelson/ Marlborough)	70	CA/HA	Riney (1956)
N.Z.(Canterbury)	39	CA/HA	Riney (1956)
N.Z.(Southern Lakes)	47	CA/HA	Riney (1956)
N.Z.(South Westland)	35	CA/HA	Riney (1956)
<u>FARMED</u>			
Scotland	72	CB/HES	Hamilton & Blaxter (1980)
N.Z.(Invermay)	76	CB/HIH	Kelly & Drew (1977)
N.Z.(Invermay)	87.8	CB/HM	Asher et al. (1981)
N.Z.(Papamoa) mature	91	CB/HIH	Bray & Kelly (1979)
N.Z.(Papamoa) 2-year old	74	CB/HIH	Bray & Kelly (1979)
N.Z.(Invermay) mature	85	CB/HIH	Bray & Kelly (1979)
N.Z.(Invermay) 2-year old	70	CB/HIH	Bray & Kelly (1979)
N.Z.(Lincoln College)	90	CB/HM	Coop & Lamming (1977)
N.Z.(Invermay)	74	CB/HIH	Kelly & Whateley (1975)

varies from being poor for wild deer in Scotland (Mitchell, 1973) to good in N.Z. farming conditions where calving percentages in excess of 90% can be expected (Kelly & Moore, 1977).

2.3.4 GESTATION LENGTH

Gestation lengths reported for red deer are listed in Table 2.3 and it can be seen that a gestation length of about 233 days is normal. A shorter gestation length (by four days) for first pregnancies than for subsequent pregnancies was reported by Lincoln and Guinness (1973). While a later study showed no such difference (Guinness *et al.*, 1978b), it did record a shorter (by five days) gestation length in captive hinds than in wild hinds and the authors concluded that although nutrition may affect gestation length, variations in gestation length are small and therefore variations in calving date are most likely to be due to factors affecting the time of conception.

Table 2.3: Gestation length of red deer hinds.

MEAN GESTATION LENGTH (DAYS \pm s.d.)			REFERENCE
231.0	4.5		Guinness <i>et al.</i> (1971)
230.2	4.4	1st pregnancy	Lincoln and Guinness (1973)
234.3	0.95		Lincoln and Guinness (1973)
231.5	4.4	enclosure	Guinness <i>et al.</i> (1978b)
236.6	6.3	study area	Guinness <i>et al.</i> (1978b)
236.1	4.75	male	Clutton-Brock <i>et al.</i> (1982b)
234.2	5.04	female	Clutton-Brock <i>et al.</i> (1982b)
233.1	3.7		Kelly and Moore (1977)

2.3.5 NORMAL DATES OF CONCEPTION AND CALVING

There are various accounts of mating and calving dates for red deer in N.Z. and some of these are listed in Table 2.4. It can be seen that mating generally occurs in mid to late April and calving occurs throughout December.

It was noted by Marshall (1937) that the seasons of oestrus and of parturition for red deer in N.Z. are the same as those in Europe, and this was supported by Fletcher (1974) who found that the calving date differed by precisely six months between the Southern and the Northern Hemispheres. Adding six months on to the mating and calving dates reported in British studies gives corresponding N.Z. dates for peak conceptions of mid to late April (Lincoln *et al.*, 1970; Guinness *et al.*, 1971; Lincoln & Guinness, 1973; Mitchell & Lincoln, 1973; Mitchell *et al.*, 1981) and for calving of early to mid December (Guinness *et al.*, 1978b; Hamilton & Blaxter, 1980), which agree well with the N.Z. studies.

2.3.6 FACTORS AFFECTING CONCEPTION AND CALVING

2.3.6.1 Nutrition and lactation

In a study of calving dates of red deer in various locations around the world, Fletcher (1974) found that red deer in the Northern Hemisphere calve at a similar time irrespective of latitude, although different populations at similar latitudes showed variations of up to two weeks between median calving dates. One such case is between the

Table 2.4: Mating and calving dates for red deer hinds in New Zealand.
 Dates underlined are calculated by counting back 233 days (see section 2.3.4) from the reported calving date.

Locality		Mating Date	Calving Date	References
Invermay (1973)		<u>10 April-13 Jul</u>	range 29 Nov-1 Feb	Kelly & Drew (1977)
Invermay (1975)	mature	<u>20 April</u>	mean 9 Dec \pm 7.0 days (sd)	Bray & Kelly (1979)
Invermay (1975)	2-year old	<u>26 April</u>	mean 15 Dec \pm 8.1 days (sd)	Bray & Kelly (1979)
Invermay (1976)	mature	<u>24 April</u>	mean 13 Dec \pm 8.1 days (sd)	Bray & Kelly (1979)
Invermay (1976)	2-year old	<u>6 May</u>	mean 25 Dec \pm 11.5 days (sd)	Bray & Kelly (1979)
Invermay (4 yrs data)		13 April	mean 2 Dec	Kelly & Moore (1977)
Lincoln College		<u>Apr-May</u>	Dec	Coop & Lamming (1977)
Fiordland		<u>20 April</u>	median 9 Dec \pm 16.7 days (sd)	Caughley (1971)
Northern North Island		<u>Mid April</u>	early Dec	Asher et al. (1981)

island of Rhum (west Scotland) and Glen Feshie (in central Scotland) where mean conception date is seven to ten days later (Mitchell & Lincoln, 1973). It has been suggested that the later conception dates of hinds at Glen Feshie are due to their poorer nutritional status, which is reflected in their lower body weights and poorer body condition (Mitchell & Lincoln, 1973). Furthermore it has been proposed that body condition could account for year to year fluctuations in the time of the breeding season within a population, with earlier conception and calving in years when hinds are in better body condition (Mitchell & Lincoln, 1973; Guinness et al., 1978a).

Studies on Scottish deer populations have indicated that 'milk' hinds (lactating) conceive and calve up to a week later than 'yeld' hinds (non-lactating) (Guinness et al., 1978b; Mitchell & Lincoln, 1973; Clutton-Brock et al., 1983). Many of these 'milk' hinds fail to conceive at all, with the result that they breed only every other year (Mitchell, 1969; Mitchell, 1973; Mitchell & Brown, 1973; Chapman, 1974; Mitchell et al., 1976; Guinness et al., 1978a; Clutton-Brock et al., 1983). The delayed conception and lower fertility of 'milk' hinds has been attributed to their poorer live weight and body condition (Mitchell, 1973; Mitchell & Lincoln 1973) arising from the nutritional stresses of pregnancy and lactation (Mitchell, 1973; Mitchell et al., 1976).

Hinds which reared a male calf had lower fertility (Guinness et al., 1978a) and later subsequent calving dates (Clutton-Brock et al., 1982c) than those which had

reared a female calf. This may be due to a high energy cost of producing and rearing male calves which results in hinds with poor body condition when they have raised a male calf (Clutton-Brock et al., 1982c).

The importance of body weight for good hind fertility is indicated by a higher calving percentage for hinds in English woodland than on Scottish hill range; the English hinds being 34 to 38% heavier than their Scottish counterparts (Mitchell et al., 1981). Body weight at the time of the rut has an important influence on fertility (Kelly & Moore, 1977; Hamilton & Blaxter, 1980; Fennessy, 1982) and a body weight of 65 to 70 kg for yearling hinds is required for them to achieve high calving percentages in N.Z. (Kelly & Moore, 1977; Fennessy, 1982).

While body weight was found to be a useful predictor of fertility in Scottish red deer, a depression of fertility in 'milk' hinds which was independent of their body weight was described by Mitchell and Brown (1973). It has been proposed that the lower fertility of 'milk' hinds is due to suckling stimuli and associated raised blood prolactin levels (see Section 2.1.7.) rather than just to maternal body condition and that later conception of hinds rearing a male calf may be due to the higher suckling frequency of male calves (Loudon et al., 1983).

2.3.6.2 Age

Studies on red deer in English woodland (Mitchell et al., 1981) and under Scottish hill farm conditions (Hamilton & Blaxter, 1980) have indicated that yearlings are mated slightly later in the breeding season than older

hinds. On Rhum, Mitchell and Lincoln (1973) reported that calving occurs earlier as a hind gets older until it reaches seven years of age after which the date of calving gets later again. However, another study on Rhum failed to show any difference in calving dates between first calvers and experienced breeders (Guinness et al., 1978b). Research at Invermay (Otago, N.Z.) showed that rising two-year-old hinds calved six to twelve days later than mature hinds (Bray & Kelly 1979, see Table 2.4).

As well as influencing calving date, the age of a hind also may influence its fertility. Guinness et al. (1978a) reported an increase in fertility as hind age increased until five years of age; first calvers having a 22% lower calving percentage than mature hinds. From studies on Invermay and Papamoa (Canterbury, N.Z.) herds, a 15 to 19% lower calving percentage for yearlings than for mature hinds has been obtained (Bray & Kelly, 1979, see Table 2.2). Increases in fertility with increasing age may be due to body weight increasing with age, and under Scottish hill farm conditions Hamilton and Blaxter (1980) found no effect of age on fertility when weight was taken into account. This contrasts with a study on wild Scottish deer (Mitchell & Brown, 1973) in which age was positively correlated with fertility even when weight was allowed for, with maximum fertility being reached at seven years of age.

2.3.7 STAG FERTILITY

If the dates of conception and calving in red deer hinds are to be advanced then it is clear that stags must be

capable of mating and achieving fertilisation in hinds at an earlier date than is usual.

The rut, which is a period of intense sexual activity for both sexes, lasts for six to eight weeks (Lincoln, 1971; Lincoln & Guinness, 1973) during which time most hinds conceive (Lincoln & Guinness, 1973). Although the breeding season of red deer is normally short they are capable of breeding for a much longer period, the stags being in complete reproductive quiescence for only three months of the year (Lincoln et al., 1970) and hinds may continue to have oestrous cycles for up to five months (Guinness et al., 1971). Spermatogenic activity increases from late summer, reaches a maximum just prior to the rut and lasts for up to five months after the rut (Lincoln, 1971). The rut starts three to four weeks prior to the first hind coming into oestrus (Lincoln et al., 1970; Lincoln & Guinness, 1973) and is associated with increasing testosterone levels in the blood (Lincoln et al., 1970). It has been suggested therefore that stags initiate the rut and are ready to mate before the first hinds come into oestrus (Lincoln et al., 1970). This earlier activity of the stags may even induce oestrus in the hinds and thus synchronise conceptions (Lincoln & Guinness, 1973).

Marshall (1937) reported that hinds imported from N.Z. to Warnham Park in Britain came on heat in April, which is the normal time for N.Z., and yet were mated by Warnham Park stags. Although no calves resulted from these matings it indicated that hinds may play a role in initiating the rut. Lincoln and Guinness (1973) proposed that stags and hinds

are both involved in initiating the rut. Supporting this indication that pheromonal factors may be involved, Bakke and Figenshou (1983) identified volatile compounds from the tail gland of red deer and found that these secretions differed between males and females, both in the quantity produced and in their constituents. These workers also demonstrated some evidence of pheromonal activity for these secretions since flehmen behaviour could be induced in a stag by exposing it to a mixture of the compounds.

To ensure that a stag is both willing and able to fertilise a hind earlier than the normal time of the rut it may be necessary to advance reproductive activity in the stag. Testosterone secretion and rutting activity in red deer have been manipulated by subjecting stags to artificial photoperiods (Blaxter et al., 1974), showing this to be a feasible method for achieving advancement of the rut.

2.4 CONCLUSION

Red deer are seasonal breeders with mating in N.Z. occurring at the time of decreasing photoperiod during mid to late April. Hinds reach puberty at 15 to 16 months of age and ovulate at 18 day intervals with the majority conceiving to the first or second cycles of the breeding season. After a gestation period of about 233 days a single calf is born in December. There have been many studies on wild populations of red deer which indicate that poor nutrition may delay puberty and impair reproductive performance. However on deer farms in N.Z. it appears that nutritional stress is rare and fertility is high, although

there have been few detailed studies on reproduction in deer under these conditions.

While most aspects of seasonal breeding are well documented for sheep this is not the case for deer, although from the reports that do exist it seems that the physiology of seasonal breeding in many deer species is similar to that of sheep. Therefore the time of the onset of puberty and each subsequent breeding season in red deer is likely to be under photoperiodic control and to be mediated by the pineal gland via its daily rhythm of melatonin secretion. Administration of exogenous melatonin to mimic the effect of a shortened daily photoperiod could thus be expected advance the onset of breeding activity in both pubertal and adult red deer.

Prolactin does not appear to have a major role in seasonal breeding but its secretion is under photoperiodic control and therefore may serve as a useful physiological monitor of the seasonal status of an animal.

CHAPTER 3

MATERIALS AND METHODS

3.1 TRIAL OUTLINE

The trial was conducted on the Lincoln College Research Farm (latitude $43^{\circ}39'S$, longitude $172^{\circ}26'E$, altitude 11 m) Canterbury, New Zealand. It examined the effects of both an artificially shortened photoperiod and of melatonin treatment on the onset of the breeding season and other photoperiodically entrained seasonal changes in pubertal red deer hinds. The trial began on 7 January (Day 0) 1983 when all hinds were weighed and had blood samples taken, and ended when the last hind calved in December of the same year. In addition the growth of calves born in the trial was monitored until May 1984.

3.2 CLIMATE

A long dry spell in Canterbury beginning during November 1981 did not end until April 1983. Consequently the first three months of 1983 were extremely dry with rainfall only a third of average. Irrigation was used during this period to improve pasture growth and quality. In contrast the rest of the year was wetter and cooler than normal. Air temperature variations during the treatment period are shown in Fig.

3.1.

3.3 ANIMALS

Red deer hinds on the College Research Farm include

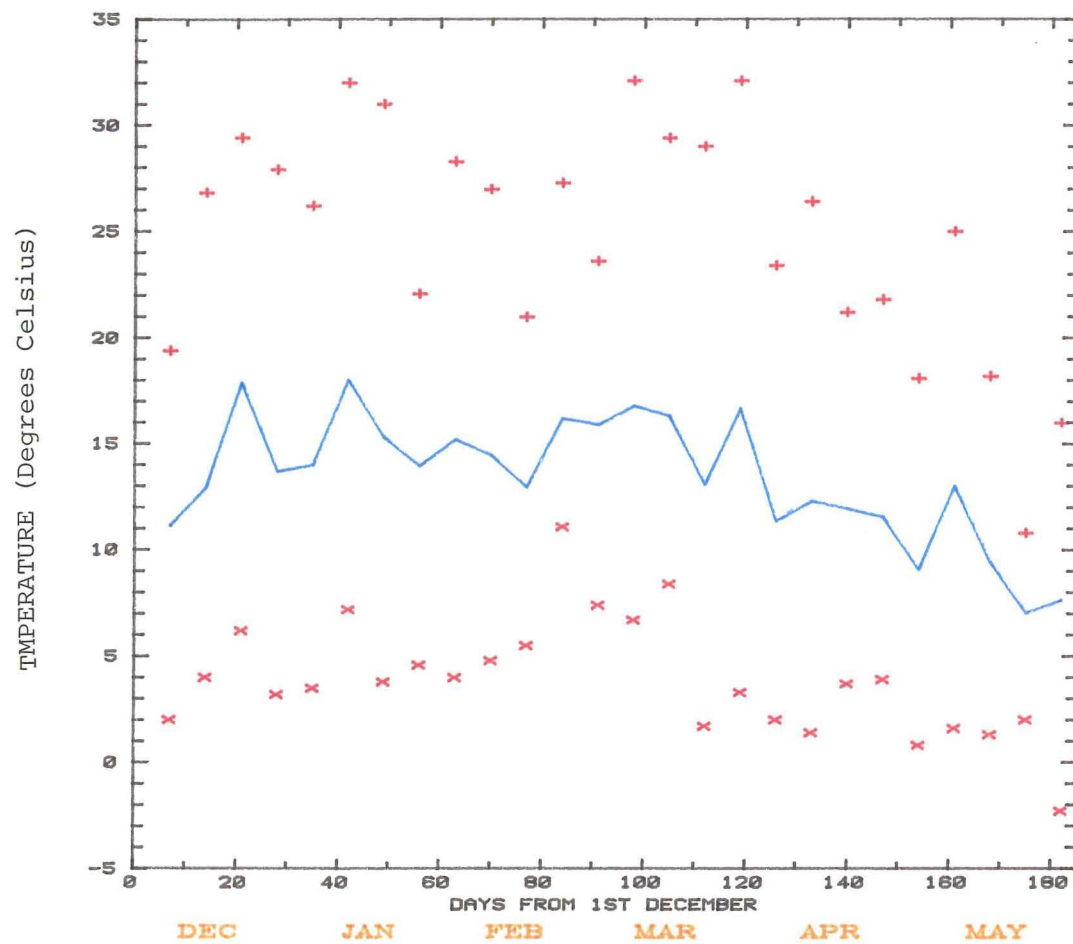


Fig. 3.1: Mean weekly air temperature recorded at Lincoln College from December 1982 until May 1983. (Graph shows mean temperature as solid line and symbols represent maxima (+) and minima (x).)

feral animals captured from the Rakaiia River catchment in the Canterbury high country during 1980, and their offspring born on the farm.

At the time treatments were to begin most adult hinds on the research farm had recently calved and were lactating. To avoid disrupting early growth of calves, yearling hinds were used in the trial.

Eleven yearling hinds (born December, 1981) were available. These were ranked according to weight and sequentially allocated to three treatment groups. To bring the number in each group up to five four non-lactating adult hinds, two of which were barren and two which had calves that had died from natural causes shortly after birth, were randomly added to each of the three treatment groups.

Two two-year-old stags were used in the trial to aid oestrous detection and to mate the hinds.

3.4 TREATMENTS

Treatments began soon after the summer solstice on 8 January (Day 1) and ended on 31 March (Day 83, see Fig. 3.2).

At 1500h each day the hinds were moved from pasture to yards for the treatments to be applied.

Control hinds (Group 1) were maintained under natural photoperiodic conditions.

Shortened photoperiod hinds (Group 2) were placed in a well ventilated darkened room at 1600h each afternoon and released at 0800h the next day. This produced a daily photoperiodic regime of eight hours light to sixteen hours

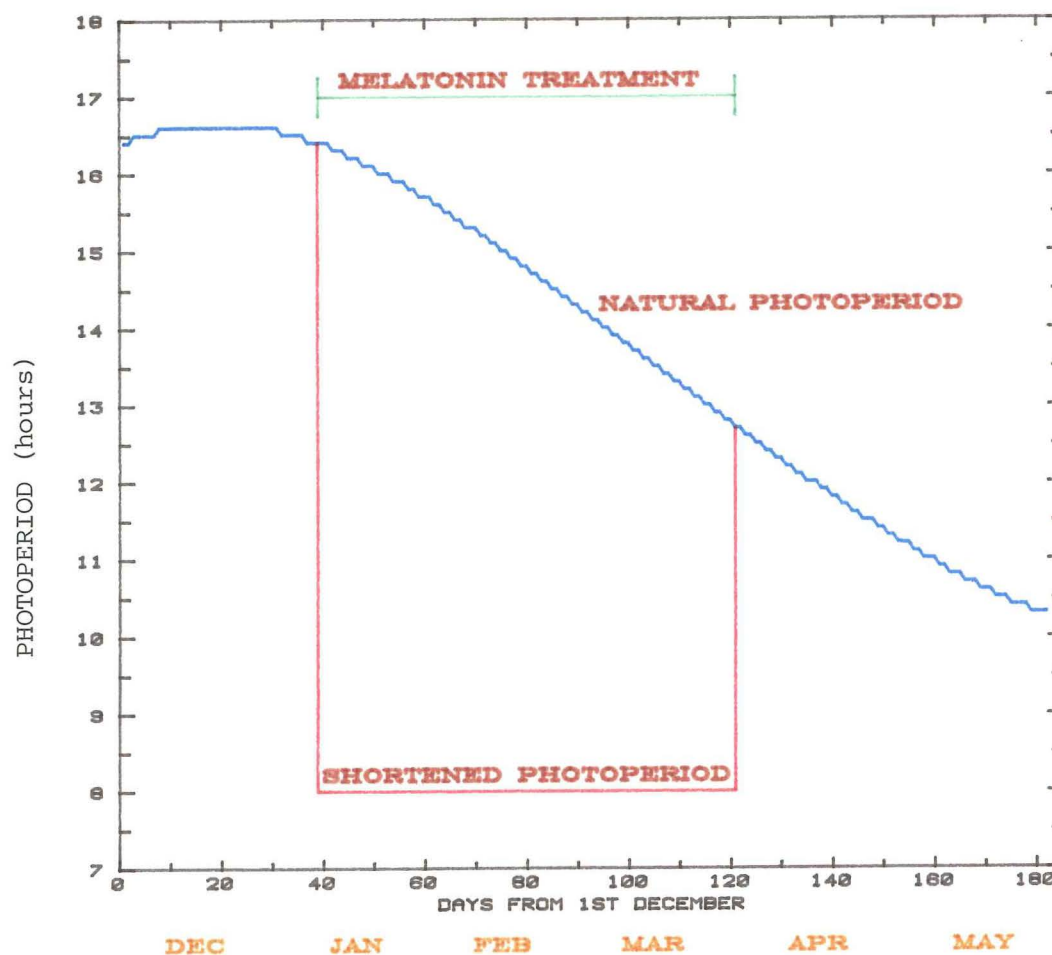


Fig. 3.2: Hours of effective daylight (hours of daylight plus 60 min for Civil Twilight, at 43°S) from December to May. Hinds in control and melatonin treated groups (Groups 1 and 3) experienced the natural photoperiod and hinds in Group 2 were subjected to the shortened photoperiod. The duration of melatonin treatment is indicated by the horizontal bar.

darkness (8L:16D).

Melatonin treated hinds (Group 3) were given an intramuscular injection in the neck of 3.75 mg crystalline melatonin (Sigma, U.S.A.) in 1 ml safflower oil. The injection was made up by boiling safflower oil to sterilise it, adding crystalline melatonin when it had cooled, then shaking the mixture. A week's supply was made up at a time and stored at -20°C . Prior to injection the solution was shaken again to disperse any undissolved melatonin crystals. Injections were given by 2 ml disposable plastic syringes with 18G x 38 mm needles.

Hinds in all three groups were injected together in a darkened room; the hinds in Groups 1 and 2 being given an intramuscular injection of 1 ml safflower oil daily at the same time that the melatonin was administered to Group 3.

While Group 2 hinds were kept in the dark, the hinds in Groups 1 and 3 stayed in an adjacent enclosure until 0800h when the hinds were reunited into one mob and returned to pasture for the day. Hinds in all three groups thus had the same amount of access to pasture each day.

3.5 ANIMAL HUSBANDRY

Routine animal husbandry practices were carried out and all the animals were in good health throughout the trial. During the trial the animals grazed a 1 hectare field of predominantly tall fescue (Festuca arundinacea), ryegrass (Lolium perennae) and white clover (Trifolium repens) pasture.

All hinds were weighed at intervals of 2 to 4 weeks

during the treatment period.

Although some compensatory grazing of pasture during the day was expected to make up for the time that hinds did not have access to pasture there were losses in body weight during the first two weeks of the trial. To overcome this a supplementary ration of 1 kg Deer Nuts (86.9% DM, 11.5 MJME/kg DM; NRM Feeds Ltd., Christchurch) was fed to each hind daily from 21 January (Day 14) to 31 March (Day 83). This was equivalent to 50% of a hind's energy requirement at that time (Fennessy et al., 1981).

The hinds were vaccinated against clostridial diseases before calving on 31 October (2 ml 'Tasvax' I.C.I. Tasman, Upper Hutt).

3.6 BLOOD SAMPLING

Blood was collected weekly (every Thursday morning) in a darkened room before the hinds were released to pasture for the day. Hinds were restrained manually while the blood was taken from an external jugular vein using a 10 ml evacuated glass test tube and 20G x 25 mm 'Venoject' needle (Terumo, Australia). The sample was centrifuged and the serum aspirated off to be stored at -20°C until assay.

In the case of one hind (no. 2/8, Group 3) blood sampling caused considerable stress and became progressively difficult so that after 17 March (Day 69) no further blood samples were taken from this hind.

3.7 HORMONE ASSAYS

3.7.1 PROLACTIN ASSAY

Serum prolactin was determined by the double antibody radioimmunoassay described by Barrell and Lapwood (1978/79a) except that in this case the serum samples were from deer and separation of antibody-bound from free tracer was carried out with goat anti-rabbit gamma globulin serum (Calbiochem). This assay utilises a radioiodinated preparation of highly purified ovine prolactin (NIH-I-AFP-4328C, provided by Dr A.F. Parlow, Harbor General Hospital, California) for tracer and rabbit antiserum raised against ovine prolactin (provided by Dr D.F.M. van de Wiel, Research Institute for Animal Husbandry 'Schoonoord', The Netherlands) was diluted to 1:50 000. There was no detectable cross reactivity with up to 1 μ g/ml of ovine GH, LH, FSH, TSH or ACTH in the assay system. Hormones or extracts of deer pituitary origin were not available for testing, but serial dilutions of serum and plasma samples from stags or hinds produced inhibition curves which were parallel to those of the ovine standard (NIH-P-S12) in buffer. This assay therefore measures a prolactin-like immunoreactive material in deer serum and the results were expressed as ng of NIH-P-S12 for convenience. Assay sensitivity was 1 to 2 ng/ml serum and the within- and between-assay coefficients of variation were 9.2% and 13.7% respectively for a serum sample containing 72 ng/ml. Recovery of ovine prolactin added to deer serum (one occasion) and plasma (three occasions) was always within the 95% confidence limits of 100%.

3.7.2 PROGESTERONE ASSAY

3.7.2.1 Reagents

Acetate buffer consisted of 13.61 g sodium acetate trihydrate and 1.0 g sodium azide dissolved in 1 l water (Solution A) plus glacial acetic acid (5.7 ml) which had been diluted to 1 l with water (Solution B). Solution A (678 ml) was made up to 1 l with Solution B and the pH corrected to 5.0.

Phosphate buffer was a solution of 13.61 g potassium orthophosphate, 1.0 g bovine gamma globulin (BGG, Cohn fraction II) and 0.1 g thiomersal, made up to 1000 ml with 0.9% saline; pH was adjusted to 7.0.

Dextran coated charcoal solution consisted of 10% (w/v) dextran T70 (Pharmacia, Sweden) and 0.5% (w/v) activated neutralised charcoal (Sigma, U.S.A.) in phosphate buffer. The solution was constantly stirred while in use.

Toluene based scintillant consisted of 15g 'Omnifluor' (98% 2,5-diphenyloxazole and 2% p-bis-o-methylstyrylbenzene, New England Nuclear, U.S.A.) and 3.75 l toluene (J.T.Baker, U.S.A.).

3.7.2.2 Radioimmunoassay procedure

(a) Preparation of progesterone antiserum

Antiserum (provided by Mr K.H.J. Yeo, Steroids Unit, Christchurch Public Hospital) was raised in rabbits by immunising them to progesterone-3-carboxymethyloxime-bovine serum albumin (Q2606; Steraloids, U.S.A.).

(b) Extraction of progesterone from serum

Frozen deer serum was thawed at room temperature and 300 μ l of each sample was pipetted into 30 ml glass tubes.

Seven hundred μ l of acetate buffer was then added to each tube, followed by 4 ml of redistilled hexane. The tubes were then rotated for 10 minutes. Extraction efficiency, judged by extracting samples which had a small amount of tritiated progesterone added, was 100%.

(c) Assay procedure

Standard solutions of progesterone (K. Light, England) were prepared in 100 μ l phosphate buffer to provide a standard curve corresponding to a range of serum concentrations from 0 to 63.7 nmol/l.

Duplicate 1 ml aliquots of the hexane extract of each sample were placed into small glass tubes and dried down under air in a waterbath at 45°C, then redissolved in 100 μ l of phosphate buffer. One hundred μ l of antiserum at a dilution of 1/2500 in phosphate buffer was added to each tube followed by 100 μ l tritiated progesterone tracer, [1,2,6,7-3H]-progesterone (The Radiochemical Centre, Amersham) containing 21 000 dpm. The tubes were then mixed by vortexing and incubated at 4°C overnight.

(d) Separation of antibody-bound and free tracer

Free steroid was precipitated by the addition of 500 μ l of dextran-coated charcoal solution to each tube which was then centrifuged (1720G) at 4°C for 20 minutes. Five hundred μ l aliquots of supernatant were then added to glass scintillation vials containing 300 μ l 0.1 M HCl. Following the addition of 10 ml toluene based scintillation fluid, and holding for 4 hours at 4°C, the radioactivity of the bound progesterone was counted on a Packard TriCarb Liquid Scintillation Spectrometer, Model 3375.

Calculation of the progesterone present in the sample was performed by computer (program courtesy Dr W.A. Sadler, Dept. of Nuclear Medicine, Christchurch Public Hospital) using a four parameter logistic function, first proposed by Healy (1972).

3.7.2.3 Validation of progesterone radioimmunoassay

Assay sensitivity, defined as the smallest amount of unlabelled hormone which can be distinguished from no hormone (Midgely *et al.*, 1970) averaged 0.99 nmol/l.

Mean within-assay coefficient of variation (C.V.) was 4.05% and the between-assay C.V. was 17.4% and 19.8% for samples with means of 17.69 nmol/l and 10.71 nmol/l respectively, over nine assays.

Major cross reactivities (compared with progesterone=100%) at an antibody titre of 1 in 500 were produced by 5 α -pregnandione, (117%); pregnenolone, (41%); pregnandiol, (168%); deoxycorticosterone, (5.1%); 17 α -OH-progesterone, (1%); testosterone, (0.5%) and aldosterone (0.2%). Cross-reactivities from cortisol, oestradiol, androstenediol, aldosterone and dexamethasone were all less than 0.2%.

Studies of the non-pregnant ewe (Baird *et al.*, 1971) have shown that with the exception of progesterone and 20 α -dihydroprogesterone the amount of steroids secreted by the ovary and the adrenal glands into the peripheral plasma is extremely low in this species. On this basis it was assumed that the steroids listed above which had significant levels of cross reaction in the present assay were unlikely to interfere with progesterone determination in deer serum.

3.8 PELAGE CHANGE

On 9 March (Day 61) each hind's pelage was examined visually and assessed as to whether it had a full summer, a partially moulted summer or a full winter coat.

3.9 STAG FERTILITY

To ensure that the stags would be capable of successfully mating a hind that came into oestrus before the natural breeding season they were subjected to the same shortened photoperiod as Group 2 hinds but in a separate room. On 11 March (Day 63), shortly before the predicted early onset of oestrous cyclicity, the stags' semen was examined for the presence of spermatozoa. To do this, two lactating adult hinds were given two weeks' pre-treatment with progesterone, using an intravaginal controlled internal drug releaser (C.I.D.R., Alex Harvey Industries, Plastics Moulding Co., Hamilton) impregnated with progesterone (0.5 g per device, Upjohn, New Zealand) implanted while the hinds were lightly sedated (2 ml 2% xylazine HCl, 'Rompun', Bayer, Germany) on 24 February. On 10 March (Day 62) the C.I.D.R. devices were removed and the hinds injected intramuscularly with oestradiol benzoate in oil (0.05 to 0.1 ml, 5 mg/ml, Intervet, The Netherlands). The next day the two hinds aroused mating behaviour in the stags but would not allow them to mount. Each hind was given 1 ml of 'Rompun' which caused them to stand quietly. One of the stags successfully mated a hind and microscopic examination of cervical mucus showed it to be rich in

spermatozoa although motility was poor. As a result it was decided that the stags were probably capable of fertilizing a hind by this date and shortened photoperiod treatments for the stags ended on 11 March (Day 63).

3.10 OESTROUS DETECTION

At the beginning of the trial hinds and stags were together during the day and from 17 February (Day 41) were observed using a spotting telescope (Bushnell, 40 mm x 20), from an observation position in a small building at the corner of the field. Animals had numbered ear tags ('Allflex', Delta Plastics Ltd., Palmerston North) and were also sprayed with coloured stock marker ('Spray Line', Stafix Ltd., Invercargill) to help identify individuals. On 11 March (Day 63), as the predicted mating season approached, stag contact with the hinds was limited to approximately half an hour each morning and evening to increase the chance of observing all mating activity. During the mating season visual examination of the vulva and a ram harness with crayon (Mannings, Auckland) fitted to a stag were also used to help in detecting oestrus. From 21 April (Day 104), after the peak of mating, a harnessed stag was run with the hinds constantly and the hinds were checked daily and any crayon marks recorded. This continued until the last hind was mated in late May.

The date of first oestrus was taken as the first date at which a hind showed behavioural or physical signs of oestrus (Guinness et al., 1971). Actual mating was usually observed (9 out of 12 times) on these dates. Mating was

defined as when a stag ejaculated into the vagina of a hind. An upward bodily thrust by the mounting stag characterises ejaculation in this species (Clutton-Brock et al., 1982d). Where neither mating nor oestrus was observed the date of first oestrus was calculated by subtracting a gestation period of 233 days (see section 2.3.4) from the calving date (2 hinds). One hind (no. 9/1, Group 2) showed no signs of oestrus at all and did not calve (see Discussion).

Changes in serum progesterone during the trial were used also to detect luteal activity and confirm that oestrus and pregnancy had occurred. Studies on red deer hinds have shown that progesterone levels greater than 9.5 nmol/l are indicative of luteal activity (Guinness et al., 1971) and pregnancy (Kelly et al., 1982). A level of 2 nmol/l was taken as evidence that a hind was cycling (Nowak et al., 1983). In this study (and for this assay) no such critical value was adopted but instead the progesterone profile of each hind was examined subjectively and the date, size and duration of elevations in progesterone above baseline were taken into account when determining evidence for luteal activity.

3.11 CALVING DATES

The approximate time of first calving was calculated as the first mating date plus a gestation period of 233 days, and from this date the field where the hinds calved was checked for new-born calves twice daily. When a calf was born it was left for at least twelve hours then tagged,

weighed and the mother's identity unequivocally established. The identity of a calf's mother was determined by combining evidence from mating date and observation of behaviour patterns. These included separation of a hind from the herd just before calving, loss in abdomen size after calving, response of the mother to her calf's squeal when ear tagged and the mother licking her calf's anus.

3.12 GESTATION LENGTH

Gestation length was calculated by subtracting the date when oestrous activity was observed and which resulted in a maintained conception, from the calving date.

3.13 CALF GROWTH

Of the fourteen calves born, ten were female and four were male. All the calves were weighed at birth and then at one to two month intervals until May, 1984 when they had reached five to six months of age. The body weight of the female calves born from control (Group 1) hinds (n=4) was compared with that of the female calves from shortened photoperiod (Group 2) and melatonin (Group 3) treated hinds (n=6). Male calves were not included in this comparison (see Discussion). Growth rates of the calves were calculated by dividing the difference between final weight and birth weight by the time interval. The mean growth rate of calves from control hinds was compared with that of calves from treated hinds and the mean growth rates for all female (n=10) and male (n=3, one male calf died) calves were calculated.

3.14 STATISTICAL ANALYSIS

The effects of shortened photoperiod and melatonin treatment on hind body weight and on serum prolactin results were analysed using data from all fifteen hinds. Reproductive parameters (time of first oestrus and calving) were analysed both for pubertal hinds (n=10, one pubertal hind which showed no sign of oestrus and did not calve was omitted from this part of the analysis) and for all fifteen hinds. For serum prolactin and animal body weight data, one-way analysis of variance was performed for each sampling occasion. Mating and calving dates were also analysed by one-way analysis of variance. Following a significant F ratio, treatment means (weighted for unequal animal numbers) were compared using the following orthogonal contrasts (Steel & Torrie, 1960); Groups 2 and 3 vs Group 1 (treated vs control) and Group 2 vs Group 3 (shortened photoperiod vs melatonin).

CHAPTER 4

RESULTS

4.1 HIND BODY WEIGHT

Changes in body weight of the hinds during the study are shown in Fig. 4.1. A loss of around 6.5 kg body weight per animal occurred during the first two weeks of the trial. As a result two hinds went below the target mating weight of 65 kg which has been recommended for optimum fertility in yearling hinds (Kelly & Moore, 1977; Fennessy, 1982). Body weights increased after supplementary feeding with deer nuts had commenced and all hinds were above 65 kg by 10 February. From late January to late April there was an average weight gain of around 10 kg per animal.

No significant difference in mean body weight between treatments was detected at any of the weighing dates during the trial.

4.2 PELAGE CHANGE

Both shortened photoperiod and melatonin treatments advanced the time of moulting the red/brown summer coat to reveal the new grey/brown winter coat. On 9 March (Day 61) all Group 2 hinds and three of the Group 3 hinds had fully moulted their summer coats while the remaining two hinds in Group 3 had partially moulted their summer coats. Three of the Group 1 hinds still had full summer coats and two had partially moulted coats.

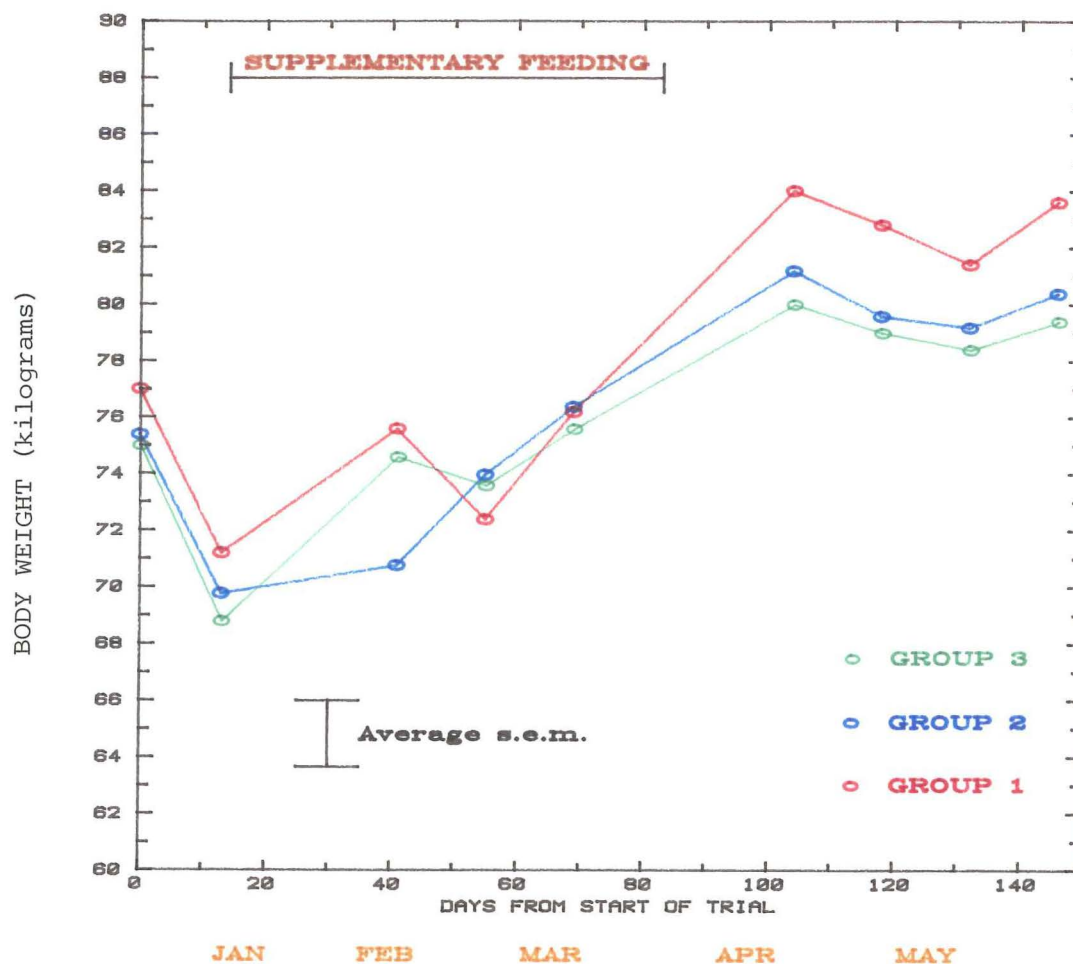


Fig. 4.1: Mean body weights of hinds in control (Group 1, $n = 5$), shortened photoperiod (Group 2, $n = 5$) and melatonin (Group 3, $n = 5$) treatment groups recorded from January until May 1983. The horizontal bar represents the period during which a supplementary ration of deer nuts was fed to all hinds. The average s.e.m. was calculated from the s.e.m. for each point.

4.3 SERUM PROLACTIN

Mean serum prolactin concentrations for all treatment groups were high but variable during January before declining to lower levels and finally approaching the lower detection limit of the assay by April (see Fig 4.2). [N.B. In Group 3 there were no blood samples from one hind (no. 2/8) after Day 69 (see Materials and Methods, Section 3.6).] The decrease in prolactin levels was more rapid in hinds of the shortened photoperiod and melatonin treated groups (Groups 2 and 3) which had basal levels of serum prolactin around 35 days earlier than hinds in the control group (Group 1). This is supported by significantly lower ($P < 0.05$) means for Groups 2 and 3 on most (5/6) of the sampling dates during February and early March (Day 20 to 55).

4.4 DATE OF FIRST OESTRUS

The date of first oestrus for each hind was gained from: observed matings (9 hinds), behavioural or physical changes associated with oestrus (3 hinds) and by subtracting a gestation period of 233 days from calving date (2 hinds). One hind in Group 2 (no. 9/1) showed no sign of oestrus and did not calve and so was omitted from mating and calving results (see Discussion).

Mean date of first oestrus in pubertal hinds (see Table 4.1) was earlier ($P < 0.005$) in both shortened photoperiod and melatonin treated hinds than in control hinds (32.0 and 26.5 days earlier for Groups 2 and 3 respectively than for Group 1).

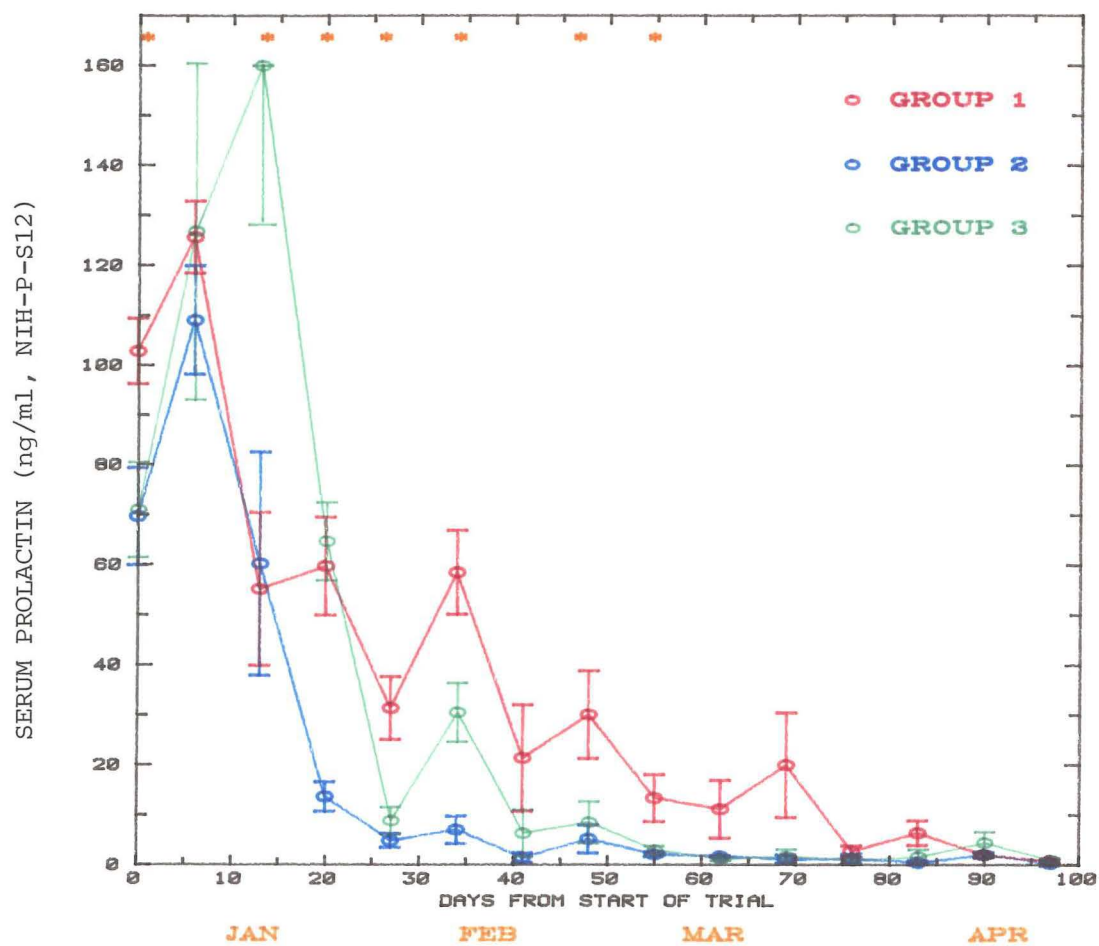


Fig. 4.2: Mean (\pm s.e.m.) serum prolactin (ovine, NIH-P-S12) concentrations of hinds in control (Group 1, $n = 5$), shortened photoperiod (Group 2, $n = 5$) and melatonin (Group 3, $n = 5$ until Day 69 after which $n = 4$) treatment groups recorded weekly from January until April 1983. Significant differences ($P < 0.05$) between treatments are indicated by asterisks.

Table 4.1: Mean Day of first oestrus and subsequent calving of yearling hinds in control (Group 1), shortened photoperiod (Group 2) and melatonin (Group 3) treatment groups.

Treatment	Number of hinds	Mean Day of first oestrus \pm s.e.m.	Mean Day of calving \pm s.e.m.
Control	3	107.3 \pm 7.90	340.3 \pm 7.90
Shortened photoperiod	3	75.3 \pm 2.33	308.7 \pm 1.67
Melatonin	4	80.8 \pm 3.38	308.0 \pm 3.24

Table 4.2: Mean Day of first oestrus and subsequent calving of hinds in control (Group 1), shortened photoperiod (Group 2) and melatonin (Group 3) treatment groups.

Treatment	Number of hinds	Mean Day of first oestrus \pm s.e.m.	Mean Day of calving \pm s.e.m.
Control	5	99.6 \pm 6.6	336.2 \pm 7.3
Shortened photoperiod	4	75.0 \pm 1.7	314.2 \pm 5.7
Melatonin	5	84.6 \pm 4.7	313.4 \pm 6.0

With the four adult hinds included in the analysis (see Table 4.2) the advancement of mating in shortened photoperiod and melatonin treated groups was less pronounced ($P<0.01$), being 24.6 and 15.0 days earlier for Group 2 and 3 respectively, than for Group 1.

4.5 DATE OF CALVING

The advancement of the date of first oestrus which had occurred in shortened photoperiod and melatonin treated groups resulted in earlier ($P<0.001$) mean calving dates for the yearling hinds in these two treatment groups (see Table 4.1). Mean calving dates were 31.6 and 32.3 days earlier for Groups 2 and 3 respectively, than for Group 1.

With the four adult hinds included in the analysis (see Table 4.2) advancement of calving was earlier ($P<0.025$) in shortened photoperiod and melatonin treated hinds although the size of the advancement was less pronounced than for the yearling hinds alone. Mean calving dates were 22.0 and 22.8 days earlier ($P<0.025$) for Groups 2 and 3 respectively, than for Group 1.

4.6 SERUM PROGESTERONE

Serum progesterone profiles for all five hinds in Groups 1 and 2 and for four hinds in Group 3 are shown in Figs. 4.3, 4.4 and 4.5 respectively. In Group 3 the progesterone profile of hind no. 2/8 was not obtained (see Materials and Methods, Section 3.6).

Oestrus and mating were generally followed by an increase in serum progesterone levels. Serum progesterone

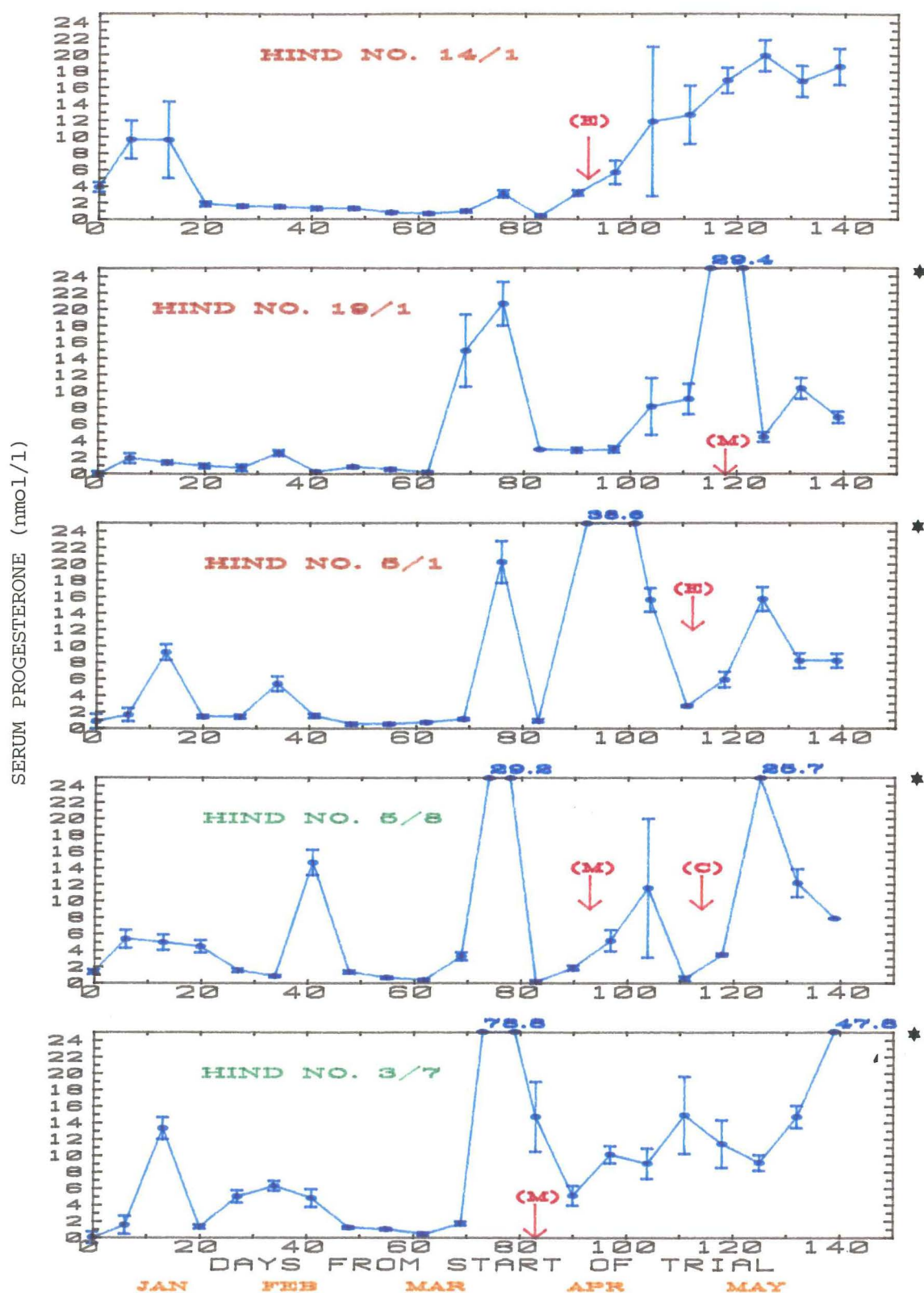


Fig. 4.3: Serum progesterone concentrations of control (Group 1) hinds recorded weekly from January until May 1983 (Green labels indicate adult hinds). For each hind the day of first oestrus and any subsequent mating activity is indicated by an arrow. The letter above each arrow denotes the method by which mating date was determined: (M) = observed mating, (E) = estimated from calving date and (C) = crayon mark. The profiles which were considered to show evidence of luteal function prior to the first oestrus are indicated by an asterisk.

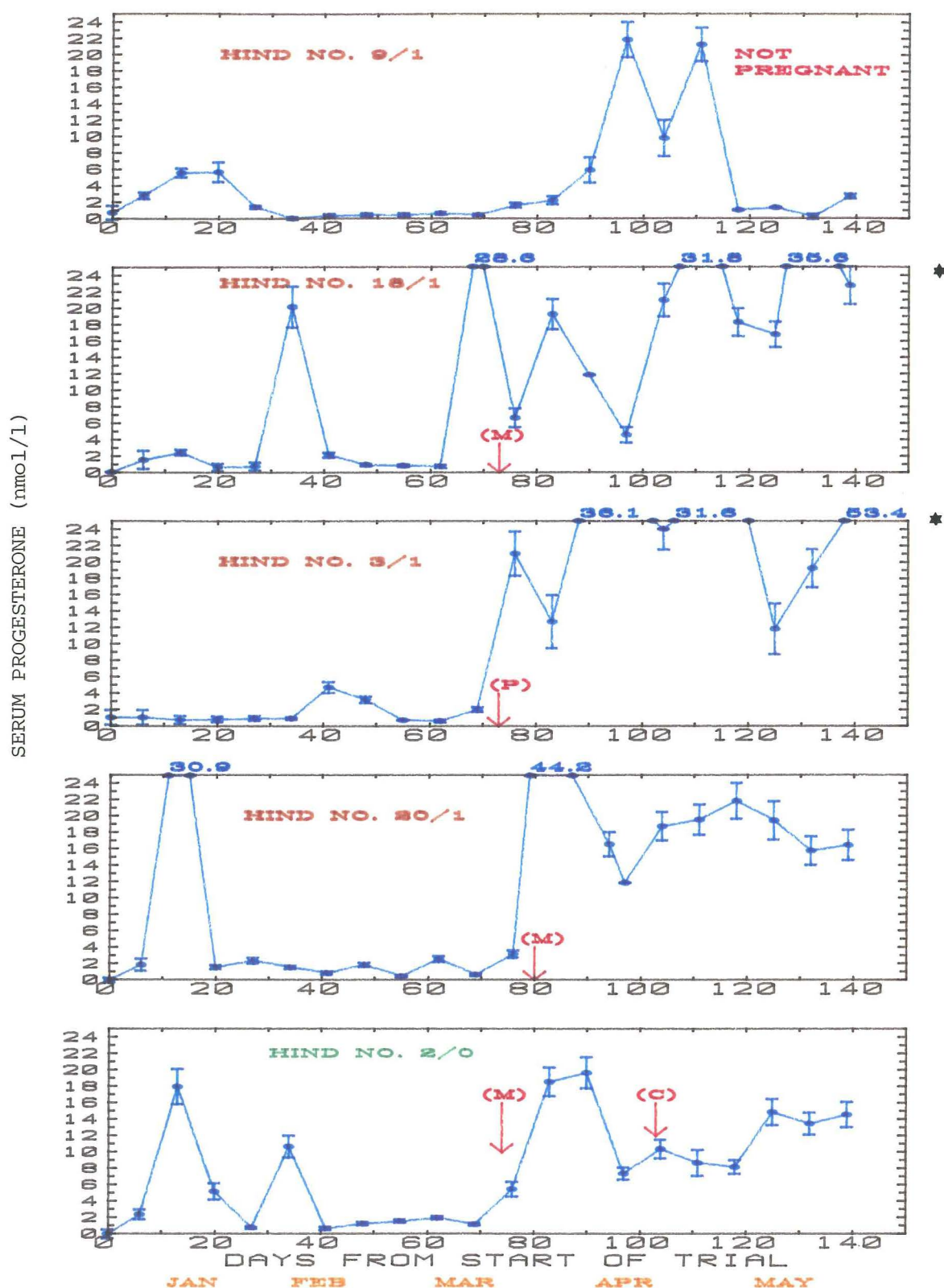


Fig. 4.4: Serum progesterone concentrations of shortened photoperiod (Group 2) hinds recorded weekly from January until May 1983 (Green labels indicate adult hinds). For each hind the day of first oestrus and any subsequent mating activity is indicated by an arrow. The letter above each arrow denotes the method by which mating date was determined: (M) = observed mating, (P) = physical state of vulva, and (C) = crayon mark. The profiles which were considered to show evidence of luteal function prior to the first oestrus are indicated by an asterisk.

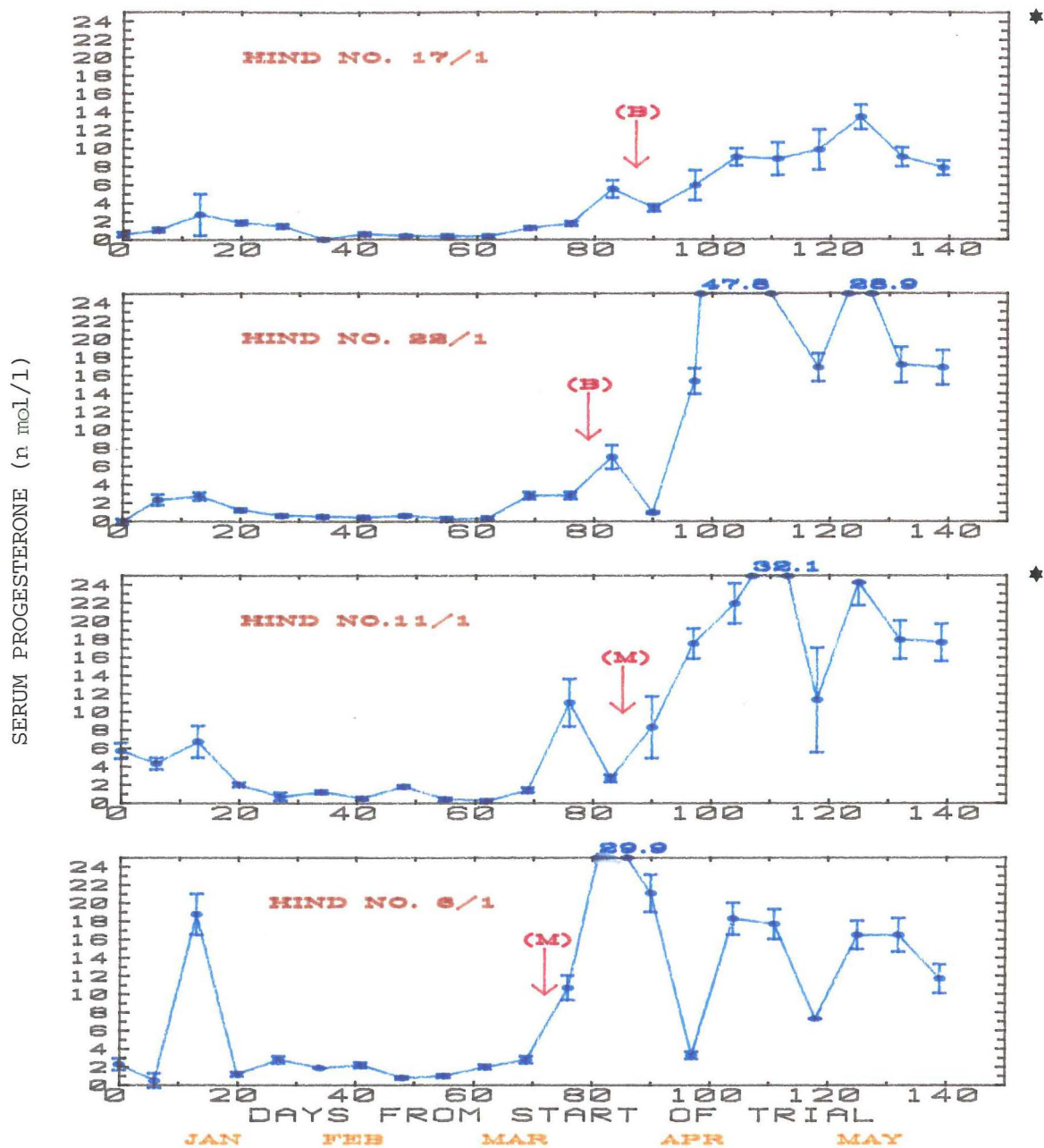


Fig. 4.5: Serum progesterone concentration of melatonin (Group 3) hinds recorded weekly from January until May 1983. For each hind the day of first oestrus is indicated by an arrow and above this the method by which mating date determined: (M) = observed mating and (B) = behaviour associated with oestrus. The profiles which were considered to show evidence of luteal function prior to the first oestrus are indicated by an asterisk.

levels remained high confirming pregnancy in all hinds except hind no. 9/1 (Fig 4.4) which did not calve.

Elevated serum progesterone levels, indicative of luteal activity (see Materials and Methods, Section 3.10) were recorded prior to the day of first oestrus in eight of the hinds (four hinds in Group 1 and two hinds each in Groups 2 and 3). The data were too few to determine whether there was any effect of the treatments on the dates of these elevations in progesterone.

4.7 GESTATION LENGTH

The mean gestation length of eleven hinds for which the day of mating or oestrus was observed was 230.5 ± 1.11 (s.e.m.) days. Gestation length was not significantly affected by the treatments.

4.8 CALF GROWTH

The mean birth date of female calves was 21.8 days earlier ($P < 0.05$) from shortened photoperiod and melatonin treated hinds ($n=6$) than from control hinds ($n=4$). Mean birth weight of female calves from the treated hinds (8.0 ± 0.26 kg s.e.m.) was not significantly different to that of female calves from control hinds (7.37 ± 0.52 kg s.e.m.). At all subsequent weighing dates the mean body weight of female calves from the treated hinds was higher than that of female calves from control hinds (see Fig 4.6) although the differences were not significant. Growth rate for female calves ($n=10$) from birth until the final sampling date at five to six months of age was 310 ± 8.33 g/day

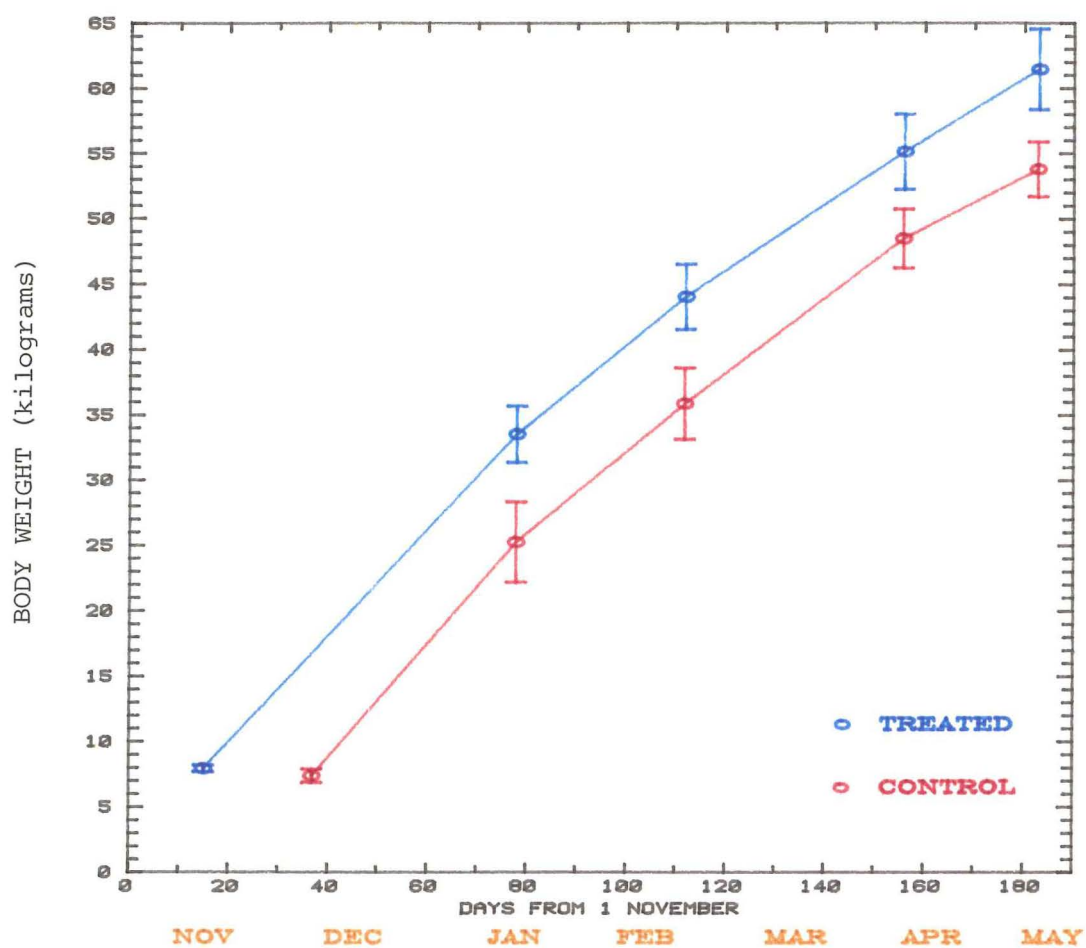


Fig. 4.6: Mean (\pm s.e.m.) body weights of female calves born from treated hinds (Groups 2 and 3, $n = 6$) and from control hinds (Group 1, $n = 4$) recorded from birth until May 1984.

(mean \pm s.e.m.) while the growth rate for male calves (n=3) over the same period was 380 \pm 20.83 g/day (mean \pm s.e.m.).

CHAPTER 5

DISCUSSION

The present study was designed to determine whether the onset of oestrous cycles and mating could be advanced in pubertal red deer hinds by treating them with shortened daily photoperiods or daily melatonin administration during late anoestrus. Earlier calving would establish that such prematurely induced oestrous cycles are fertile. Effects of these two treatments on other seasonally linked events such as moulting of summer pelage and decline in prolactin secretion during autumn also were examined. It was assumed that any response influenced by melatonin treatment would constitute evidence for a role of the pineal gland in seasonality of red deer.

Loss of body weight which occurred in the hinds at the start of the trial was probably due to the decrease in grazing time combined with stress on the hinds arising from additional handling. However, supplementary feeding was instituted which allowed the hinds to regain weight and so removed any possibility of nutritional restrictions on fertility.

Advancement of the calving date to spring by use of artificially reduced daily photoperiods could be of major benefit in the farming situation because it would allow more efficient matching of pasture growth to the nutritional demands of the hinds. Shortening of the daily photoperiod

can be achieved by shutting hinds in a darkened room but they then require supplementary feeding. This could defeat any advantage that the earlier calving would have on pasture utilisation. In contrast melatonin administration need not interfere with grazing nor create any need for supplementary feeding.

This study has shown that the time of autumnal moulting of pelage in red deer hinds can be advanced by an artificially shortened photoperiod confirming similar findings of Kay and Ryder (1978). The ability of melatonin administration to mimic the effects of a shortened photoperiod on moulting of the summer coat indicates that in this species the photoperiodic control of pelage change is mediated by the pineal gland. This is consistent with the similar shift in timing of pelage moulting in male white-tailed deer which was achieved by Bubenik (1983) using daily oral dosing with melatonin.

The autumnal decline in mean serum prolactin levels recorded from all hinds in this trial is similar to that also recorded from red deer hinds in N.Z. by Kelly et al. (1982). Variable prolactin levels such as were recorded during the first few weeks of this trial were possibly due to the stressful nature of the initial blood sampling sessions. Stress such as that caused by venepuncture has been shown to raise blood prolactin levels in cattle (Raud et al., 1971) and goats (Hart, 1973). It was noted during the course of the study that the hinds soon became more tolerant of blood sampling but it is probably unwise to attempt to interpret the variable prolactin results obtained

before the animals had become accustomed to handling stresses.

The early decline in serum prolactin levels of hinds exposed to a shortened photoperiod in this study confirms the work of Brown et al. (1979) showing that the secretion of this hormone in red deer is under photoperiodic control. That daily melatonin administration was as effective as a shortened photoperiod for decreasing serum prolactin levels strongly suggests that photoperiodic control of prolactin secretion in red deer is mediated by the pineal gland. This is also consistent with studies on sheep in which melatonin administration lowered blood prolactin concentrations (Arendt et al., 1981b; Kennaway et al., 1982a; Kennaway et al., 1982/1983).

The present study has shown that the onset of the breeding season in red deer hinds is associated with low serum prolactin levels as is the case with sheep (Walton et al., 1977). However, studies with ewes have established that artificially decreased prolactin levels during anoestrous do not alter the onset of oestrous cyclicity (Land et al., 1980; Schanbacher, 1980). This could also be the case for red deer, but experimental suppression of prolactin secretion in red deer hinds, such as by treatment with bromocriptine during anoestrous, would be necessary to determine whether the early reduction in prolactin levels recorded from hinds in the shortened photoperiod and melatonin treated groups was a causal factor in their earlier onset of mating activity.

One hind (9/1) which was exposed to a shortened

photoperiod showed no sign of oestrus and did not calve. This might have been due to her being trapped, possibly for some days, in a narrow gap between a shed and a fence prior to the trial. Although she had regained much condition by the time treatments began the trauma and associated loss of weight may have been sufficient to upset her reproductive system. Consequently no significance can be attributed to her failure to produce a calf during this study.

While the age at puberty has been influenced by artificial photoperiods in sheep (Foster, 1981a; Foster, 1981b) and white-tailed deer (Budde, 1983), studies in which melatonin treatment was used to advance the breeding season of sheep (Kennaway et al., 1982a; Arendt et al., 1983; Knight, 1983a) and red deer (Nowak et al., 1983) have been confined to adult animals. The advancement of the date of first oestrus in pubertal red deer hinds in this study achieved by using shortened photoperiod and melatonin treatments suggests that the onset of puberty in this species is under photoperiodic control, mediated by the pineal gland. Also, together with the findings of Nowak et al. (1983) it could mean that the onset of breeding in red deer at puberty and the onset of the breeding season in adults are both controlled by a similar mechanism.

In this study the use of melatonin and the method used to administer this compound were based on two assumptions. One assumption was that deer have a diurnal secretion pattern of melatonin similar to that of sheep (Rollag & Niswender, 1976) and cattle (Hedlund et al., 1977), i.e. with low blood levels during the day and elevated levels

during the night. The other assumption was that the afternoon injections of melatonin (intramuscularly in safflower oil) would result in elevated melatonin levels in blood, similar to those normally present during darkness, and this elevation would persist from shortly after the time of administration until the rise in endogenous melatonin secretion occurred at dusk; thus extending the daily duration of elevated melatonin levels in the blood and thereby mimicking the effects of a shortened daily photoperiod. In sheep this same procedure has maintained blood melatonin levels at or above normal night time levels for at least six hours (Nett & Niswender, 1982). Based on the first assumption, placing hinds in a darkened room for sixteen hours also would extend the daily duration of elevated melatonin levels, although in these hinds this would be achieved by stimulating endogenous secretion. Advancement of the breeding season in deer achieved by both of these treatments provided some confirmation that the assumptions made above were justified and is consistent with results from a study with pinealectomised ewes in which an increase in the daily duration of elevated melatonin levels in blood (produced by infusion) led to the onset of reproductive activity (Bittman & Karsch, 1984). These authors proposed that the duration of the night time melatonin rise normally determines the reproductive response of the ewe, i.e. long duration stimulating reproductive activity and short duration inhibiting reproductive activity, and is the basis of the mechanism by which different daily photoperiods alter other physiological

events. The results of the present study indicate that this may well be true for pubertal red deer hinds.

In the pubertal hinds the early onset of oestrus induced by shortened photoperiod or melatonin treatments led to early calving. This confirmed that the early oestrous cycles were fertile which is in agreement with studies where adult sheep exposed to shortened photoperiods (Fraser & Laing, 1969; Newton & Betts, 1972) or treated with melatonin (Knight, 1983a) had normal fertility at the induced oestruses.

The degree of advancement of the onset of oestrus and calving achieved by the two treatments used in this study was more marked when comparisons were made between pubertal hinds only. This is because two of the hinds in the control group were adults and had earlier dates of first oestrus (mean 5 Apr, Day 88 ± 5.0 s.e.m.) than the three yearling hinds (mean 24 Apr, Day 107.3 ± 7.9 s.e.m.) in this group. This result would be expected as the tendency for yearling hinds to mate later than adults has been described in the literature (Mitchell *et al.*, 1981; Hamilton & Blaxter, 1980). However the adult hinds in the control group also had an earlier date of first oestrus than that calculated (from calving dates) for the main herd of adult hinds on the College Research Farm (mean 18 Apr, Day 100.9 ± 3.40 s.e.m.). While this may suggest some stimulation, possibly pheromonal, of control adult hinds by the stags or the treated hinds in the study, it is unlikely because the pubertal hinds in the control group did not calve any earlier than would be expected for yearlings. [The control

yearlings calved on average seven days later than adults in the main College herd, a result which is consistent with the calving date difference (twelve days) between yearling and adult hinds reported by Bray and Kelly (1979).] A more likely explanation for the earlier date of first oestrus for adult hinds in the control group than adults in the main herd is that the resumption of oestrous cyclicity in the case of hinds in the main herd was delayed by lactation. Earlier conception and calving in non-lactating hinds compared with lactating hinds has been reported previously (Mitchell et al., 1973; Guinness et al., 1978b; Mitchell & Lincoln, 1973; Clutton-Brock et al., 1983). Non-lactating adult hinds were included in this study mainly to make up numbers. Since there was only one adult hind in each of the two treated groups, it is impossible to make any valid statement regarding the effect of these treatments on the date of first oestrus in the adult animals.

It is conceded that the advancement of mating and calving in pubertal hinds achieved here may not be directly applicable to adults. Adult hinds usually would be lactating at the time of the treatment period in this trial and lactation may impair fertility of red deer hinds (Loudon et al., 1983). Also melatonin treatment of adult hinds (Nowak et al., 1983) failed to advance the onset of ovarian cycles in the case of lactating animals. Although the adult hinds in this trial were not lactating, they were not ideal animals to include in the trial because they had failed to rear a calf the previous year thus making them dubious subjects for a reproductive study. Another factor

is involution of the uterus following parturition, a process which must be completed before conception is possible in ewes (Hafez, 1962), and which likewise may provide a finite limit to advancement of the breeding season in adult red deer hinds. However, the fact that calving can be advanced in pubertal hinds means that if the interval between parturition and mating as adults the following year is of normal length, there is time for the effects of lactation to diminish before the onset of induced oestrous cyclicity. This may eliminate lactation as an inhibitory factor to advancement of the breeding season in adult hinds although further studies would be required to establish this point.

Previous studies have reported a low incidence (Lincoln & Guinness, 1973; Kelly & Challies, 1978) or even tended to rule out (Lincoln et al., 1970; Guinness et al., 1971) the occurrence of silent ovulations in red deer hinds at the start of the breeding season. However, serum progesterone levels recorded during this trial provide evidence of luteal function prior to the first oestrus in many of the hinds (8/13; not out of 15 because one hind showed no signs of oestrus and one hind did not have blood samples taken during the mating period) which must be attributed to silent ovulation. In this trial progesterone was measured to detect if an ovulation had occurred and weekly sampling was considered to be sufficient for this purpose. Further research is required to determine the precise effects of shortened photoperiod and melatonin treatment on the pattern of ovarian activity and occurrence of silent ovulations at the start of the breeding season.

This would mean more animals for statistical reasons and would require tame, e.g. hand reared, hinds to enable frequent blood sampling to be carried out without stressing the animals or interfering with mating activity. It is possible that stress may interfere with determination of luteal progesterone secretion since studies on white-tailed deer have indicated that the adrenal gland may be a significant source of progesterone (Wesson et al., 1977; Plotka et al., 1983). In this trial high serum levels of progesterone occurred in some animals during January when no luteal activity would be expected and, as there was a long period of near baseline progesterone levels following these peaks, it seems possible that stress at the start of the trial may have been the cause of these early peaks. This would indicate that caution should be taken when interpreting luteal activity from blood progesterone levels and that adoption of a critical progesterone value as the sole indicator of luteal activity (e.g. 2nmol/l, as used by Nowak et al., 1983) may lead to erroneous conclusions.

In general serum progesterone levels recorded during the present trial which were associated with luteal activity and pregnancy were similar to values reported by other workers (Guinness et al., 1971; Kelly et al., 1982). During pregnancy serum progesterone concentrations were more variable and higher in some animals than in others, as was also recorded by Kelly et al. (1982) who suggested that this was due to the variable number of corpora lutea present.

The gestation length for red deer hinds of 230.5 ± 1.11 days reported in this study is consistent

with earlier reports (Guinness et al., 1971; Lincoln & Guinness, 1973; Guinness et al., 1978b; Clutton-Brock et al., 1982b; Kelly & Moore, 1977) and was not affected by the treatments used here. In addition this confirms that the mating dates recorded here actually correspond to conception dates.

Due to the low number of male calves born ($n=4$) and the higher birth weight and growth rate of male calves compared with females (Blaxter & Hamilton, 1980), only the weights of female calves born in this study were used to compare the effects of early calving on the subsequent weight of calves. Earlier calving gave the calves from treated hinds about 22 days of extra growth before calves from control hinds were born. This translated into a higher mean body weight (although not significant) of around 8 kg above the calves from control hinds, and this difference was maintained until the end of the sampling period due to identical growth rates in both groups of calves. Growth rates recorded between birth and five to six months of age (310 g/day for female calves and 380 g/day for male calves) are slightly higher than those reported by Kelly and Drew (1977) (287 g/day and 318 g/day for female and male calves respectively).

The tendency for earlier calving to lead to heavier calves at weaning suggests that advancement of the breeding season of red deer hinds may be of importance to the deer farming industry, although further work is needed in this area. Heavier stag calves may mean increased venison production and in female calves heavier body weights could mean that puberty may be sufficiently advanced to occur in

their first autumn. Puberty in red deer hinds in N.Z., at about sixteen months of age, is earlier than in Scotland where it occurs at two years of age (Lowe, 1969; Mitchell, 1973) and this is mainly a result of the better nutrition of deer in N.Z.. However, evidence of puberty occurring at three to five months of age in calves grazing on high protein lucerne and white-clover pastures (Daniel, 1963) suggests that there may be scope for heavy calves to reach puberty even earlier than in the present experiment.

Although this study has shown that melatonin treatment may have potential for advancing the calving date of red deer hinds, several other methods for achieving early calving should be considered. Techniques which induce reproductive activity in anoestrous ewes may be applicable to red deer hinds. These methods include introduction of a ram to ewes during late anoestrus (the so-called "ram-effect" which has been reviewed by Knight, 1983b; Martin, 1984), administration of a progestagen followed by pregnant mare's serum gonadotrophin (PMSG) or other FSH-like material (reviewed by Thimonier, 1979; Thimonier, 1981) and treatment with LH (McNeilly et al., 1980) or gonadotrophin-releasing hormone (GnRH) (Haresign & Lamming, 1978; McNatty et al., 1981; McNatty et al., 1982a; McNatty et al., 1982b; McCleod et al., 1983; Wright et al., 1983). Treatment with a progestagen followed by PMSG has been used in red deer hinds although its success for advancement of mating is difficult to ascertain because few early calves have resulted from its use. Reports of results obtained so far (C. Adam, personal communication,

reported also in Rowett Research Institute Annual Report, 1982; Moore, 1984) have not been published in the scientific literature. Other methods for advancing breeding activity, such as early introduction of the stag or administration of either a gonadotrophin or GnRH, have not been reported for red deer but deserve consideration.

Cross-breeding with other species of deer which have earlier or less pronounced seasonal breeding patterns may provide an alternative means for achieving early calving in red deer. Pere David's deer (Elaphurus davidianus) which mate considerably earlier than red deer have been imported into N.Z., reputedly with this aim in mind (Yerex, 1984). Hybridisation between these species is possible and the resulting offspring apparently are fertile (Gray, 1972). However, although Pere David's deer do experience early mating this trait may not necessarily lead to early calving in hybrid offspring if the 280 day gestation period of this species (Chaplin, 1977) is also inherited. Tropical species of deer which are closely related to red deer, for example sambar (Cervus unicolor) or rusa (Cervus timorensis), also may be used in cross-breeding programmes to produce offspring capable of calving at different times of the year. (This possibility has been discussed by Short, 1984.) In addition it should be noted that these two species also are present in N.Z..

The present study has indicated that melatonin administration may provide a practical method for advancing the calving date of red deer hinds and has highlighted the need for further research to investigate methods of

administration of melatonin and other techniques for modifying reproduction in red deer. Further experimentation also is necessary to determine whether the advancement of calving date by about 30 days achieved in this study represents the limit that can be attained in pubertal red deer hinds and whether this advancement can be achieved by adult hinds.

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