Lactational failure in red deer hinds treated with melatonin implants during late pregnancy

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Summary: Effects of administration of melatonin implants (Regulin) to pregnant red deer hinds on lactogenesis, prolactin secretion and reproductive seasonality were studied. Twenty three mature hinds, each expected to calve on or about 10 Dec, were allocated to one of four treatments: Treatment 1 (n=6) involved delivery of double s.c. implants at monthly intervals from ~80 days before parturition (ie. 2 Oct); Treatment 2 (n=6) received implants from ~40 days before parturition (ie. 2 Nov); Treatment 3 (n=5) received implants from the day of parturition; and Treatment 4 (n=6) served as controls, receiving no melatonin treatment. Final implants were delivered on 1 Feb, with overall treatment durations of 150, 120 and 90 days for Treatments 1-3 respectively. Blood samples were taken twice weekly from Sept to May, and plasma was analysed for concentrations of progesterone and prolactin by radioimmunoassay. Mammary development was assessed by palpation score (0-5) twice weekly from Oct to April inclusive, and liveweights were recorded at least fortnightly throughout the trial.

Calving occurred between 28 Nov and 24 Dec, with no significant treatment differences (P>0.10). Hinds in Treatment 1 exhibited significant retardation of mammary development and liveweight gain leading up to parturition (P<0.01). Furthermore, sex-adjusted calf birth weights were on average 3 kg lighter for Treatment 1 (P<0.05), with all calves either removed for bottle-rearing or having died within a few hours of birth. Failure of lactogenesis in Treatment 1 was characterized by the presence of under-developed, hard mammary tissue devoid of expressable milk. Hinds in Treatments 2-4 all exhibited full lactation and successfully reared their calves, with no significant differences in calf weaning weight and growth rates (P<0.10). Likewise, there were no significant differences in mean liveweight or lactation score profiles (P<0.10). Mean plasma prolactin concentrations varied between treatments, with control hinds exhibiting a marked seasonal pattern of secretion which peaked at calving. However, hinds in both Treatments 1 and 2 failed to show any discernable seasonal increase in mean prolactin concentrations, while hinds in Treatment 3 exhibited an increase in mean values up to parturition but thereafter declined rapidly relative to control hinds. Melatonin treatment significantly advanced the date of first oestrus and decreased the postpartum-oestrus interval (P<0.05). It is concluded that initiation of melatonin implant treatment about 80 days prior to parturition compromises mammary and fetal development in red deer hinds. However, the role of prolactin was not demonstated conclusively.

INTRODUCTION: Red deer (*Cervus elaphus scoticus*) reproduce seasonally, with the onset of breeding occurring in autumn and calving occuring in summer (1). The calving season and subsequent period of lactation of farmed red deer in New Zealand generally coincide with feed deficits due to the natural senescence of pasture and often effects of drought (2). Advancement of the calving season by up to two months would better align the high energy demands of lactation with peak pasture production and quality in spring, which could facilitate increased lactational yields and calf growth rates.

The breeding season of red deer and other temperate-zone cervids is under photoperiodic control (3, 4), which is mediated by circadian variations in the secretion of the pineal hormone, melatonin (5). Several recent studies on red deer have shown that advancement of seasonal reproductive activity can be achieved following delivery of exogenous melatonin in summer, either by oral administration (6, 7, 8, 9), daily injection (10) or subcutaneous implants (11, 12, 13, 14, 15). However, the degree of advancement elicited is strongly correlated with the seasonal timing of initiation of treatment in the previous summer (15), whereby oestrus advancements > 30 days necessitate the start of melatonin treatment 100-120 days after the winter solstice (i.e. Oct in the southern hemisphere). While this presents few management problems when treating prepubertal (< 16 months old) red deer hinds, similar treatment regimens for adult hinds superimpose the initiation of exogenous melatonin delivery with the later stages of pregnancy, which is normally terminated 150-180 days after the winter solstice.

Few studies have investigated the effects of exogenous melatonin treatment during late pregnancy on survival of neonates and lactogenesis. Recent studies on farmed fallow deer (*Dama dama*), involving the administration of subcutaneous melatonin implants to adult does during the last 40 days of gestation, indicated a high incidence (i.e. 4/6 does) of failure of lactogenesis at parturition, resulting in the death of fawns born to affected does (16). As prolactin has been shown to be essential for normal initiation of lactation in sheep (17, 18), cattle (19, 20) and goats (21), it was postulated that failure of lactogenesis in the fallow deer does may have been due to a reduction in endogenous prolactin secretion in response to the suppressive effects of exogenous melatonin (16). Certainly, the delivery of exogenous melatonin has been associated with a dramatic reduction in prolactin secretion in female sheep (22) and red deer (9). However, oral administration of melatonin to pregnant red deer hinds, initiated about 40 days before calving, was not associated with either an immediate reduction in prolactin secretion (as seen in non-pregnant hinds) or failure of lactogenesis at calving (23). Likewise, experimental reversal of photoperiod did not inhibit lactogenesis in red deer hinds (24).

The contrasting results on lactogenesis in the studies on fallow deer (16) and red deer (23, 24) may indicate either a significant species difference in response to exogenous melatonin or the differential effects of two differing forms of melatonin delivery (i.e. implantation verses oral). As commercial application of subcutaneous melatonin implants to alter red deer reproductive patterns presently exists in New Zealand (25), it is vitally important to determine any potential detrimental effects that treatment may have on overall reproductive performance. The present study was designed to investigate the effects of administration of exogenous melatonin, via subcutaneous implants, to pregnant red deer hinds on prolactin secretion, lactogenesis and reproductive seasonality. We also evaluated the effect of treatment on calf survival, calf growth and reproductive seasonality of hinds.

MATERIALS AND METHODS: A total of 23 mature (>4 years old), pregnant red deer hinds on the Ruakura Agricultural Centre (37° 46' S, 175° 20' E) were used in the study. The hinds were selected on the basis of a synchronised predicted calving pattern (10 Dec $\pm$ 10 days), assessed in June 1990 by rectal ultrasonography of fetal size (26, 27). The deer were grazed on ryegrass-white clover pastures as a single group with a mature melatonin-treated stag from 7 Sept 1990 until 30 May 1991. This study was performed following mandatory approval by the Animal Ethics Committee of the Ruakura Agricultural Centre, as per New Zealand law.

Each hind was allocated to one of four treatments on the basis of liveweight (liveweight range: 88-115 kg). Hinds in Treatment 1 (n=6) each received double subcutaneous melatonin implants (Regulin; Schering Agrochemicals, NSW, Australia) at monthly intervals from 2 Oct (i.e. ~ 80 days before predicted parturition) to 1 Feb (~ 150-day treatment). Hinds in Treatment 2 (n=6) each received double implants monthly from 2 Nov (i.e. ~ 40 days before predicted

parturition) to 1 Feb (~ 120-day treatment). Hinds in Treatment 3 (n=5) each received double implants monthly from parturition (early Dec) until 1 Feb (~ 90-day treatment). In all cases implants were placed at the base of the left ear. Hinds in Treatment 4 (n=6) served as controls, receiving no melatonin treatment. The stag received the same treatment protocol as hinds in Treatment 1. The Regulin implantation schedule was based on previous studies on red deer that demonstrated effective delivery of physiological (night time) concentrations of melatonin (50-200 pg ml^{-1} plasma) for periods of 30-40 days (13, 15).

Blood samples were taken from hinds by jugular venepuncture twice weekly from 4 Sept to 1 May. The deer were restrained individually in a pneumatically operated crush while samples were taken. Blood samples were centrifuged for 25 min at 1000 g within 30 min of collection, and the plasma stored at -10°C until required for assay. Plasma progesterone and prolactin concentrations were measured in duplicate by direct radioimmunoassay (15, 28). Mammary development was assessed by palpation twice weekly from 12 Oct until 12 April, two weeks after the last calf was weaned. The udders were scored on a scale from 0 to 5, representing the range of development from no palpable mammary tissue (0) to full mammary and teat extension (5). All scoring was performed by a single observer while the hinds were restrained in the crush. Liveweights were recorded at least every two weeks during the trial period, and weekly during the calving period (late Nov to mid-Jan). Hinds were monitored twice daily during the calving period and all calves were tagged within 12 h of birth. Calf birth date, birth weight, sex and dam were recorded. Calves were removed for bottle-rearing if their dams failed to initiate lactation within 12 h of birth. Surviving calves were weighed and weaned at exactly 12 weeks of age, necessitating successive calf removals from 2-19 March. From 1 Feb - 1 May the stag was fitted with a ram mating harness (Fergus; Merck, Sharp and Dohme NZ Ltd, Auckland) from 1 February to 10 May. Crayons were replaced at twice weekly intervals and daily observations were performed to record crayon mating marks on hinds (15). The date of first oestrus for each hind was determined either from direct evidence of mating (n=16) or estimated from plasma progesterone profiles as the sampling date that preceded a sustained increase (>1.0 ng ml^{-1}) in plasma progesterone concentrations (n=7).

Data were subjected to analysis of variance (Genstat V; Lowes Agricultural Trust, Rothamstead Experimental Station) following logarithmic transformation of plasma progesterone and prolaction concentrations and square-root transformation of lactation scores. Means are presented with either the standard error of difference (SED) or least significant difference (LSD), based on pooled variance.

RESULTS: All 23 hinds in the trial calved between 28 Nov and 24 Dec 1990, with no significant differences in mean calving date between treatments (P>0.1; Table 1). Sex-adjusted calf birth weights were significantly lighter, by an average of ~ 3 kg, for hinds in Treatment 1 (P<0.05; Table 1). While all calves born to hinds in Treatments 2-4 survived to weaning at 12 weeks of age (Table 1), the 6 calves born to hinds in Treatment 1 were either found dead within 3-4 h of birth (n=2) or were removed from their dams within 12 h of birth (n=4) because of lactational failure. Only one of these calves ultimately survived bottle-rearing; the other three died within 2-3 days and were assessed as being non-viable (i.e. unable to walk or adequately suckle). The mean sex-adjusted weaning (12-week) weights and growth rates of surviving calves (Treatments 2-4) were not significantly different between treatments (P>0.1; Table 1).

The mean date of first oestrus varied significantly between treatments, with all melatonin-treated hinds (Treatments 1-3) exhibiting first oestrus of the 1991 season significantly earlier than control hinds (Table 1). Differences between Treatments 1, 2 and 3 were apparent but not significant (P>0.10). However, the mean postpartum-oestrus interval showed significant differences both between control hinds and melatonin-treated hinds (P<0.05) and between

melatonin treatment groups ($P<0.05$), ranging from 104 days for control hinds to 72 days for hinds in Treatment 1 (Table 1).

Table 1 Mean parameters and SED for parturition, calf weights, onset of oestrus and postpartum intervals

Treatment	Calving day 1990	Calf birth weight (kg)*	Calf survival	Calf weaning weight (kg)*	Calf growth rate (g/day)*	Day of first oestrus 1991	Postpartum-oestrus interval (days)
1	345.8	6 07	0/6	-	-	53.0	72 2
2	347 7	9 04	6/6	35 6	316	63.7	80.8
3	337.4	8.94	5/5	39.0	358	67 8	95.4
Controls	342.0	8 65	6/6	37.3	341	81 2	104.2
SED	4.7	0.72	-	2 7	25	9 9	10 1

* Data adjusted by co-variance for calf sex

Analysis of hind liveweight changes (difference in liveweight from that recorded on 7 Sept 1990) indicates significant differences for hinds in Treatment 1 ($P<0.01$). This was manifest as a reduction in the rate of liveweight increase over the 8 weeks before parturition, such that the differential between hinds in Treatment 1 and all other treatments averaged ~ 8 kg just prior to calving (Fig. 1). All subsequent changes in liveweight did not significantly differ between treatments. In general, a substantial decrease in mean liveweights, of between 15-20 kg, was observed at calving. Thereafter, mean liveweights declined by 8-10 kg over the subsequent 12 weeks when most hinds were lactating, resulting in mean values 3-4 kg below initial pre-trial (Sept) weights by the end of the lactational period. For all treatments, first oestrus of the 1991 season occurred during a period of declining mean liveweights, with no significant differences occurring between treatments (P>0.10; Fig. 1).

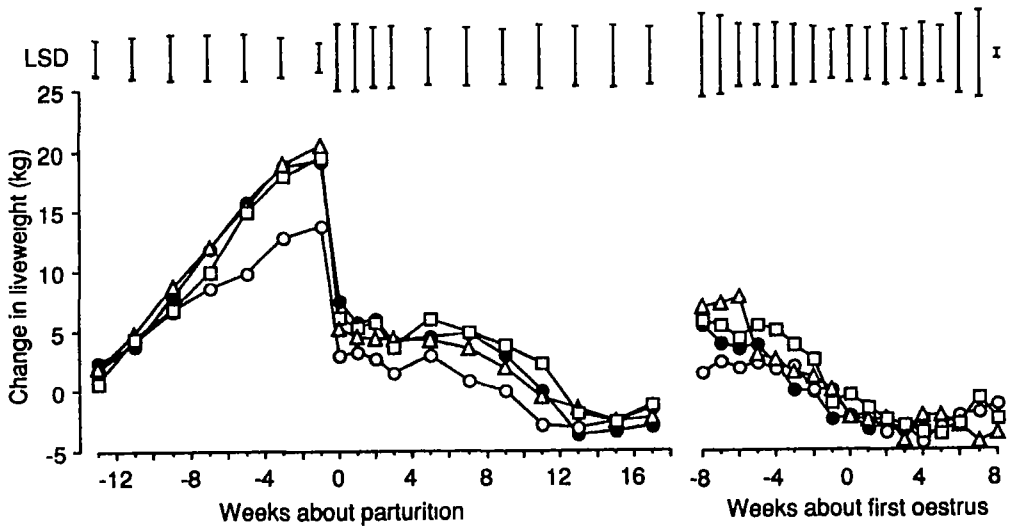

Fig. 1 Profiles of mean liveweight change, normalized about parturition and first oestrus, for red deer hinds in Treatment 1 (o), 2 (Δ), 3 (□) and 4 (●) Vertical bars denote the LSD

Profiles of mean lactation scores over the period from -6 and +17 weeks about parturition (Fig. 2) did not show any significant differences for hinds in Treatments 2-4; with initial mean scores 6 weeks before calving being <1.0 and increasing progressively to attain peak mean values of 4.8 at calving. Thereafter, mean lactation scores declined to ~ 3.0 by the eighth week from calving. Calf removal at 12 weeks was associated with a subsequent increase in mean scores to ~ 4.0 over a two-week period, followed by a rapid and progressive decline to attain low mean scores of <0.5 by 17 weeks from calving (Fig. 2).

Hinds in Treatment 1 exhibited significantly different mammary development ($P<0.01$) that was ultimately interpreted as failure of lactogenesis. While mean lactation scores increased during the pre-parturient period, the maximum mean score within one week of calving was only 2.4 (Fig. 2). At this point, palpable mammary tissue was described as being "hard" and no milk was able to be expressed. The subsequent decline in mean lactation scores, which occurred in the absence of any suckling stimulus (all calves had died or were removed), was rapid, with no mammary tissue being palpable 8 weeks after calving (Fig. 2).

When the data were normalized about first oestrus of the 1991 breeding season (Fig. 2), it was evident that mean lactation scores leading up to oestrus were significantly lower for hinds in Treatment 1 ($P<0.05$). However, mean scores declined for all hinds in Treatments 2-4 within 4-6 weeks after oestrus, at which point there were no differences between treatments ($P<0.10$).

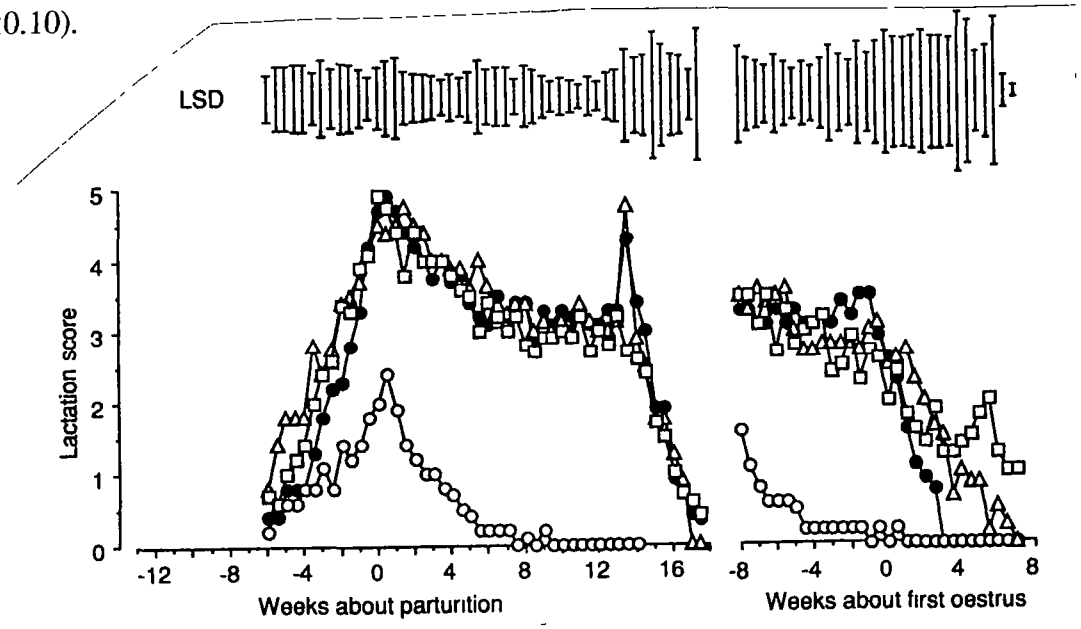

Fig. 2. Profiles of mean lactation scores, normalized about parturition and first oestrus, for red deer hinds in Treatment 1 (o), 2 (Δ), 3 (□) and 4 (●) Vertical bars denote the LSD.

Profiles of mean plasma prolactin concentrations are presented in Fig. 3. The control hinds exhibited a profile characterised by an increase in mean concentrations from ~ 10 ng ml^{-1} 12 weeks before calving to ~200 ng ml^{-1} at calving. Mean concentrations fluctuated about a plateau of ~120 ng ml^{-1} for 8 weeks after calving, then declined progressively to <20 ng ml^{-1} 16 weeks postpartum.

Hinds in Treatments 1 and 2 had mean plasma prolactin profiles that significantly differed from that of the control hinds ($P<0.01$). In both cases, mean values were significantly lower from 4 weeks before calving to 12.5 weeks postpartum. The profiles of these two groups were essentially similar, with significantly different means occurring on only 4 occasions up to 4 weeks from calving; at all other times there were no significant differences ($P>0.05$). Mean profiles for Treatments 1 and 2 were characterised by relatively low concentrations (<50 ng ml^{-1}) throughout the study, although occasional fluctuations up to 90 ng ml^{-1} were observed for Treatment 1 between 12 and 8 weeks before calving.

Hinds in Treatment 3 exhibited a mean profile that was initially similar to that observed for control hinds. However, mean plasma prolactin concentrations declined 2-3 weeks after

parturition/melatonin implantation, and remained below 30 ng ml^{-1} from 8 weeks postpartum.

Mean plasma prolactin concentrations were not discernably different for hinds in Treatment 1-3 by 8 weeks postpartum ($P<0.01$), although those of control hinds were significantly elevated at this stage ($P<0.01$). By 16 weeks postpartum, mean values for all treatments were similar ($P>0.1$). Likewise, mean concentrations at first oestrus (Fig. 3) were not significantly different for any treatment ($P>0.1$) and were uniformly low (<20 ng ml^{-1}).

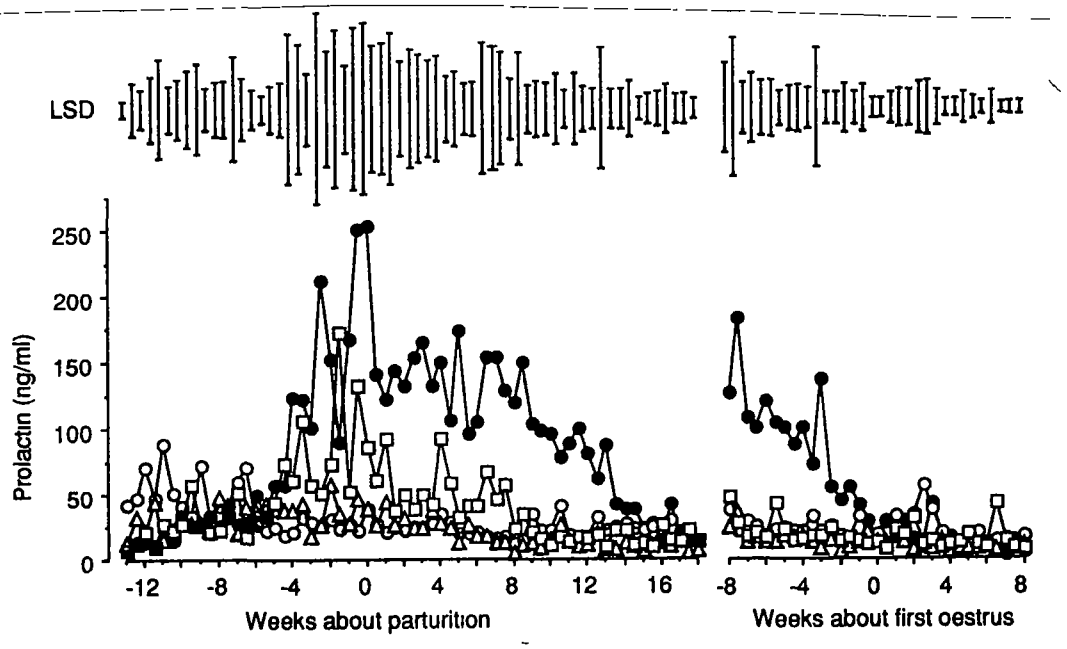

Fig. 3. Profiles of mean plasma prolactin concentrations, normalized about parturition and first oestrus, for red deer hinds in Treatment 1 (o), 2 (Δ), 3 (□) and 4 (●) Vertical bars denote the LSD.

Mean plasma concentrations of progesterone (Fig. 4) during the preparturient period were elevated between 2 and 6 ng ml^{-1} for all treatments. Parturition was associated with a precipitous decline in plasma progesterone concentrations in all hinds, with mean values immediately after calving being < 0.3 ng ml^{-1} for all treatments. The anoestrous period was characterized by low mean plasma progesterone concentrations (< 0.6 ng ml^{-1}) for all treatments. However, the duration of the anoestrous period (i.e. postpartum - oestrus interval; Table 1) differed significantly between treatments ($P<0.05$), being shortest for Treatment 1 and longest for Treatment 4 (controls). This trend was manifest as differential timing of increased progesterone secretion as a consequence of reinstatement of ovulatory (luteal) activity. Postpartum mean plasma progesterone concentrations exceeded 1.0 ng ml^{-1} at weeks 10, 12, 15 and 16 for Treatments 1-4 respectively. Plasma progesterone data normalized about first oestrus (Fig. 4) demonstrate overall similarity between treatments in progesterone secretion associated with the onset of ovulatory activity.

DISCUSSION: The attainment of a significant degree of seasonal reproductive advancement (i.e. > 30 days) in mature red deer hinds requires the initiation of exogenous melatonin treatment during the latter phase of gestation in spring and early summer. This is due to the relatively long gestation (234 days) and short postpartum-oestrus interval (120 days), whereby latency of "cause and effect" between initiation of simulated "short days" and the ovarian response (90-150 days; 15) necessitates the imposition of treatment on pregnancy. The present study has demonstrated that certain aspects of reproductive performance can be compromised by the initiation of melatonin implant treatment ~ 80 days before parturition, raising questions

about the effect of melatonin treatment on fetal and mammary development.

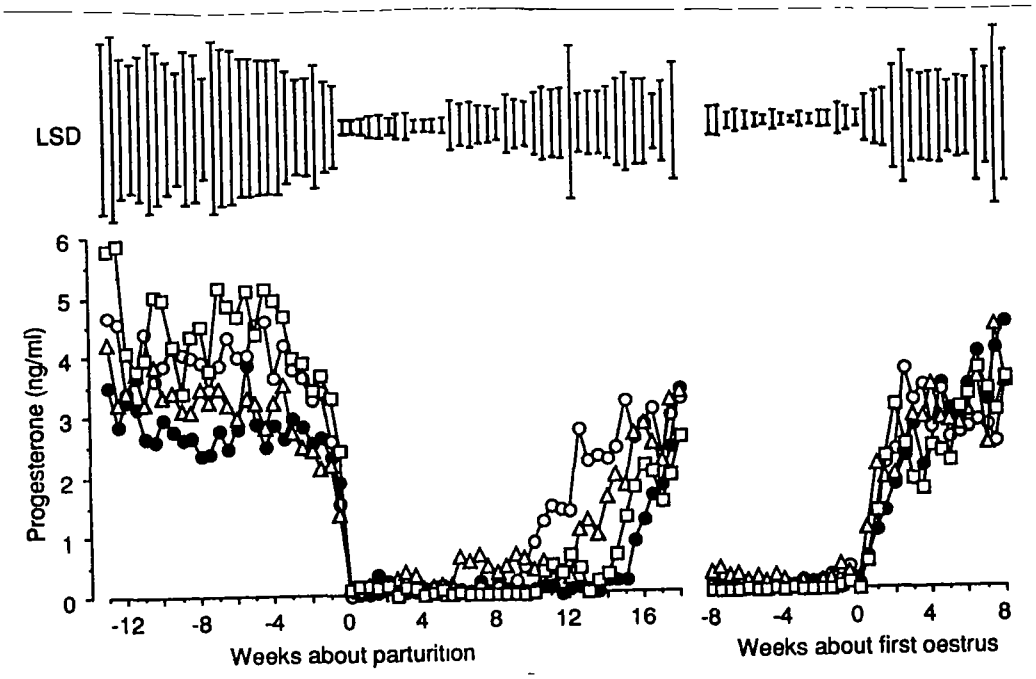

Fig. 4. Profiles of mean plasma progesterone concentrations, normalized about parturition and first oestrus, for red deer hinds in Treatments 1 (o), (Δ), 3 (□) and 4 (●). Vertical bars denote the LSD

The conclusion drawn from the present study is that there appears to be a critical phase during late gestation whereby continuous infusion of exogenous melatonin interferes with normal development of both the fetus and developing mammary tissue. This derives from the fact that initiation of melatonin implant treatment about 80 days before parturition was associated with significantly reduced hind liveweight gains and calf birth weights (and calf survival), as well as apparent failure of lactogenesis; whereas treatment initiations both ~ 40 days before parturition and on the actual day of calving did not elicit any apparent effects on hind weights, birth weights or lactation relative to control hinds. It should be noted at this point that even though the Regulin implantation regimen was designed to elevate continuously plasma melatonin concentrations to those approaching normal night-time levels in this species (13, 15), continuous infusion of exogenous melatonin does not mimic short photoperiods, and the treatment must therefore be considered pharmocological rather than physiological. In this respect, it was recently shown that pregnant red deer hinds subjected to short photoperiods from about 19 weeks of gestation (ie. 14 weeks from parturition) exhibited normal lactational response (24), this being in marked contrast to the results of the present study.

In the present study, hinds in Treatment 1 exhibited a marked reduction in prepartum liveweight gain relative to all other hinds. This culminated in an overall mean liveweight difference of ~ 8 kg just prior to calving. While exogenous melatonin treatments have previously been observed to reduce liveweight gains in young red deer (14, 15), the present data indicate that in late pregnancy the effect may be due to a reduction in fetal growth, as evidenced by reduced calf birth weights. This indicates that conceptus development is sensitive to pharmocological levels of melatonin, although the precise mechanisms cannot be determined from the present data. Irrespective of the mode of action, the overall effect on calf viability and survival appears quite dramatic, with all but one of the calves born to hinds in Treatment 1 failing to survive beyond a few days of age. This alone strongly mitigates against such early

treatment regimens in pregnant hinds.

Perhaps the most conclusively demonstrated effect of initiating implant treatment ~ 80 days before parturition is the failure of lactogenesis in all hinds in Treatment 1. This contrasts with full lactational development in all hinds in Treatments 2-4. Profiles of mean lactation scores indicate that mammary development occurred from ~ 6 weeks before parturition, culminating in peak development within a few days of calving. Thus, imposition of melatonin implant treatment on pregnancy in Treatment 1 probably occurred prior to the the natural initiation of mammary tissue growth, whereas all other treatment regimens were initiated at various intervals after tissue growth had commenced. Again, this is suggestive of a critical phase, between -80 and -40 days from parturition, during which development of mammary tissue is normally initiated. The fact that prior treatment with exogenous melatonin can strongly affect development, whereas later treatment initiation appears to have no effect at all, raises questions about the endogenous cues responsible for growth of mammary cells. In particular, early initiation of implant treatment may block normal cellular development in the mammary gland either by the direct inhibition of cellular processes, indirectly by perturbation of maternal endocrine events (e.g. prolactin secretion), or by retardation of fetal and placental growth (and hence suppression of such factors as placental lactogen). It is important to note that failure of lactogenesis in Treatment 1 was not associated with complete inhibition of mammary development. At the time of calving, expanded mammary tissue was palpable as a hard mass within the udder, with no milk able to be expressed. This contrasted with hinds in other treatments, in which mammary tissue was spongy and milk was easily expressed.

The removal/death of all calves born to hinds in Treatment 1 removed any potential suckling stimulus amongst these hinds, and subsequent rapid regression of mammary tissue was predictable. However, given the degree of under-development at calving, it would seem highly unlikely that an appropriate lactation would have been instigated if a suckling stimulus had occurred. The failure of later Treatments (i.e. Treatments 2 and 3) to affect mammary development and subsequent lactation is consistant with previous studies on red deer, in which exogenous melatonin was delivered orally starting 37 days before parturition (23) or by implant after parturition (29). Thus, the maintenance of established lactation in this species is not noticeably affected by exogenous melatonin. This also appears to be the case for fallow deer (16). The increase in mean lactation score immediately after calf removal at 12 weeks of age probably represents increased udder volume from unharvested milk. However, subsequent regression of entire mammary tissue was complete 5 weeks after weaning and no complications were observed.

The pituitary hormone, prolactin, is secreted in a manner that reflects the photoperiodic cycle, with high and low concentrations of plasma prolactin being concomitant with long and short daylengths respectively, as demonstrated in the ewe (30). As expected for a seasonal species such as red deer, the control hinds in the present study exhibited a marked increase in plasma prolactin concentrations concomitant with increasing daylength, followed by a decline with decreasing daylength. These data are consistent with previous studies (23, 24). It is interesting to note that in all of these studies marked increases in apparent prolactin secretion were not demonstrable until about 6-8 weeks before parturition, at about the same time initial mammary development was observed in the present study. It is also noteworthy that the studies demonstrated the occurence of peak mean plasma prolactin concentrations within a few days of parturition that may relate to a transient stimulatory effect of parturient oestrogens on prolactin secretion (31).

Of particular interest in the present study, however, is the consequence of a reduction in prolactin secretion, mediated by exogenous melatonin delivery, on lactation. Prolactin stimulates mammary parenchymal gland growth, either directly or synergistically with steroid

hormones, in several laboratory species (32, 33). In this respect, an adequate level of prolactin secretion has been shown to be essential for normal initiation of lactation in sheep (17, 18), cattle (19, 20) and goats (21). Suppression of prolactin secretion by exogenous melatonin has been well demonstrated for sheep (22, 34, 35) and red deer (7, 9, 10, 23, 36). Therefore, it would be reasonable to assume that exogenous melatonin treatment may inhibit lactogenesis in red deer by suppressing prolactin secretion. While the present study demonstrated that Regulin implants resulted in a decrease in plasma prolactin concentrations, it was not possible to distinguish statistically between mean concentrations for hinds in Treatments 1 and 2; both groups exhibiting relatively low mean plasma values throughout the study period, yet contrasting markedly in lactational development. Furthermore, Adam *et al.* (24) demonstrated a marked reduction in mean plasma prolactin concentrations in red deer hinds subjected to short photoperiods from ~14 weeks before parturition but observed no apparent detrimental effects on lactogenesis. This focusses some doubt on a possible mediatory effect of melatonin-induced (or photoperiod-induced) reductions in prolactin secretion on failure of lactogenesis in red deer hinds. Given the markedly circadian pattern of prolactin secretion in red deer hinds (23), it is possible that the sampling regimen employed in the present study was inadequate to assess the true secretory pattern of prolactin, particularly with respect to detecting differences between Treatments 1 and 2.

There were other aspects of plasma prolactin profiles in the present study that contrasted with previous studies. Adam *et al.* (23) showed that reductions in plasma prolactin concentrations following initiation of oral melatonin treatment 37 days before parturition were delayed by about 50 days (ie. 14 days after calving), which contrasted with more rapid reductions within 21 days in non-pregnant hinds, indicating that pregnancy could in some way overide the suppressive effects of exogenous melatonin. In the present study, melatonin delivery via Regulin implants was associated with a more rapid reduction in prolactin secretion in pregnant hinds, with minimum mean plasma concentrations apparent within 14 days of implantation. Furthermore, previous studies involving the treatment of pregnant hinds with either orally-administered melatonin (23) or short photoperiods (24) demonstrated the characteristic parturient increase in prolactin secretion in both treated and control hinds. This feature was notably absent from the mean plasma prolactin profiles of hinds in Treatments 1 and 2 in the present study. Such contrasting results indicate fundemental differences in the effects of various forms of melatonin delivery on prolactin secretion, perhaps further stressing the implications of pharmocological delivery via subcutaneous implants. It would appear that the Regulin implantation regimen used in the present study elicited more rigorous control over prolactin secretion than observed following oral delivery or photoperiod manipulations.

While evidence from the present study that suppression of prolactin secretion is causative of failure of lactogenesis is equivocal, there is no doubt that reduced plasma concentrations of prolactin had no effect on the maintainence of established lactation. This is consistent with other studies on red deer (23, 29) and other ruminants in general (37, 38), and was evidenced in the present study by profiles of mean lactation scores and growth rates of suckled calves for Treatments 2 and 3.

In the present study, melatonin treatment resulted in a significant advancement of first oestrus of the 1991 breeding season and an overall reduction in the postpartum-oestrus interval. The maximum advancement obtained, measured relative to control hinds, was 32 days for hinds in Treatment 1. While this is less than the 54 days recorded for pubertal hinds under a similar treatment regimen (15), hinds in the two trials were managed differently. In the former study, pubertal hinds were physically separated into treatment groups to avoid possible social facilitation effects on the seasonality of first oestrus, as demonstrated by (14). In the present trial, there was the potentially confounding effect of social facilitation from maintaining all

hinds in a single group in the presence of a treated stag, irrespective of treatment. Thus, control hinds exhibited oestrus around late March, about 2 weeks earlier than expected within the overall population. There is also the further consideration that hinds in Treatment 1 were not lactating, having lost their calves. This may have been a contributing factor to their early return to oestrous activity.

In summary, while treatment of mature red deer hinds with subcutaneous melatonin implants during the later stages of pregnancy is efficacious in advancing the onset of seasonal ovarian activity, treatment initiation ~ 80 days before parturition has been shown to be associated with retardation of fetal development and failure of lactogenesis, with consequences on hind reproductive productivity.

REFERENCES

(1) **Guinness, F.E., Lincoln, G.A. and Short, R.V. (1971)** The reproductive cycle of the female red deer, *Cervus elaphus*. *Journal of Reproduction and Fertility* 27, 427-438.

(2) **Asher, G.W. and Adam, J.L. (1985)** Reproduction of farmed red and fallow deer in northern New Zealand. In *Biology of Deer Production*, pp 217-224. Eds P.F. Fennessy and K.R. Drew. The Royal Society of New Zealand, Wellington, NZ.

(3) **Marshall, F.H.A. (1937)** On the change over in the oestrous cycle in animals after transference across the equator, with further observations on the incidence of the breeding seasons and the factors controlling periodicity. *Proceedings of the Royal Society, London.* Series B122, 413-428.

(4) **Jaczewski, Z. (1954)** The effect of changes in length of daylight on the growth of antlers in deer (*Cervus elaphus* L.) *Folia Biol., Praha*, 2, 133-143.

(5) **Bittman, E.L., Dempsey, R.J. and Karsch, F.J. (1983)** Pineal melatonin secretion drives the reproductive response to daylength in the ewe. *Endocrinology* 113, 2276-2783.

(6) **Adam, C.L. and Atkinson, T. (1984)** Effect of feeding melatonin to red deer (*Cervus elaphus*) on the onset of the breeding season. *Journal of Reproduction and Fertility* 72, 463-466.

(7) **Nowak, R., Elmhirst, R.N. and Rodway, R.G. (1985)** A note on the effect of melatonin feeding on the initiation of ovarian activity and on plasma prolactin levels in lactating and non-lactating red deer hinds. *Animal Production* 40, 515-518.

(8) **Adam, C.L., Moir, C.E. and Atkinson, T. (1986)** Induction of early breeding in red deer (*Cervus elaphus*) by melatonin. *Journal of Reproduction and Fertility* 76, 569-573.

(9) **Adam, C.L., Atkinson, T. and Moir, C.E. (1987)** Melatonin lowers plasma prolactin levels in female red deer (Cervus elaphus). *Journal of Pineal Research* 4, 13-20.

(10) **Webster, J.R. and Barrell, G.K. (1985)** Advancement of reproductive activity, seasonal reduction in prolactin secretion and seasonal pelage changes in pubertal red deer hinds (*Cervus elaphus*) subjected to artificially shortened daily photoperiod or daily melatonin treatments. *Journal of Reproduction and Fertility* 73, 255-260.

(11) **Lincoln, G.A., Fraser, H.M. and Fletcher, T.J. (1984)** Induction of early rutting in male red deer (*Cervus elaphus*) by melatonin and its dependence on LHRH. *Journal of Reproduction and Fertility* 72, 339-343.

(12) **Webster, J.R., Mackintosh, C.G., Suttie, J.M., Fennessy, P.F., Davis, G.H. and Staples, L.D. (1986)** Advancing the breeding season of yearling red deer hinds with melatonin. *Proceedings of the Endocrine Society of Australia* 29 (Suppl. 2): abstract E5.

(13) **Webster, J.R., Suttie, J.M. and Corson, I.D. (1991)** Effects of melatonin implants on reproductive seasonality of male red deer (*Cervus elaphus*). *Journal of Reproduction and Fertility* 92, 1-11.

(14) **Fisher, M.W., Fennessy, P.F. and Milne, J.D. (1988)** Effects of melatonin on seasonal physiology of red deer. *Proceedings of the New Zealand Society of Animal Production* 48, 113-116.

(15) **Asher, G.W. (1990)** Effect of subcutaneous melatonin implnts on the seasonal attainment of puberty in female red deer (*Cervus elaphus*). *Animal Reproduction Science* 22, 145-159.

(16) **Asher, G.W., Barrell, G.K., Adam, J.L. and Staples, L.D. (1988)** Effects of subcutaneous melatonin implants on reproductive seasonality of farmed fallow deer (*Dama dama*). *Journal of Reproduction and Fertility* 84, 679-691.

(17) **Fulkerson, W.J., McDowell, G.H. and Fell, L.R. (1975)** Artificial induction of lactation in ewes: the role of prolactin. *Australian Journal of Biological Science* 28, 525-530.

(18) **Gow, C.B., McDowell, G.H. and Jenkin, G. (1983)** The importance of prolactin for initiation of lactation in the pregnant ewe. *Australian Journal of Biological Science* 36, 357-367.

(19) **Karg, H. and Schams, D. (1974)** Prolactin release in cattle. *Journal of Reproduction and Fertility* 39, 463-472.

(20) **Akers, R.M., Bauman, D.E., Capuco, A.V., Goodman, G.T. and Tucker, H.A. (1981)** Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. *Endocrinology* 109, 23-30.

(21) **Hart, I.C. and Morant, S.V. (1980)** Roles of prolactin, growth hormone, insulin and thyroxine in steroid-induced lactation in goats. *Journal of Endocrinology* 84, 343-351.

(22) **Poulton, A.L., English, J., Symons, A.M. and Arendt, J. (1986)** Effects of various melatonin treatments on plasma prolactin concentations in the ewe. *Journal of Endocrinology* 108, 287-292.

(23) **Adam, C.L., Moir, C.E. and Shiach, P. (1989)** Plasma prolactin concentrations in barren, pregnant and lactating red deer (*Cervus elaphus*) given melatonin to advance the next breeding season. *Animal Reproduction Science* 18, 77-86.

(24) **Adam, C.L., Kyle, C.E. and Young, P. (1992)** Influence of prenatal photoperiod on postnatal prolactin secretion in red deer (*Cervus elaphus*). *Journal of Reproduction and Fertility* 95, 959-964.

(25) **Barry, T.N. and Wilson, P.R. (1991)** The role and usage of melatonin for deer production. *Advances in Pineal Research* 6, 249-258.

(26) **White, I.R., McKelvey, A.C., Busby, S., Snedon, W.J. and Hamilton, W.J. (1989)** Diagnosis of pregnancy and prediction of foetal age in red deer by real-time ultrasonic scanning. *Veterinary Record* 124, 395-397.

(27) **Wilson, P.R. and Bingham, C.M. (1990)** Accuracy of pregnancy diagnosis and prediction of calving date in red deer using real-time ultrasound scanning. *Veterinary Record* 126, 133-135.

(28) **Asher, G.W., Veldhuizen, F.A., Morrow, C.J. and Duganzich, D.M. (1993)** Effects of exogenous melatonin on prolactin secretion, lactogenesis and reproductive seasonality of adult female red deer (*Cervus elaphus*). *Journal of Reproduction and Fertility* (in press).

(29) **Fisher, M.W. and Fennessy, P.F. (1990)** A note on melatonin-treated red deer stags advancing the onset of the calving season in hinds *Animal Production* 51, 213-216.

(30) **Thimonier, J., Ravault, J.P. and Ortavant, R. (1978)** Plasma prolactin variations and cyclic ovarian activity in ewes submitted to different light regimens. *Annales de Biologie Animale Biochimie et Biophysique* 17, 459-473.

(31) **Fell, L.R., Beck, C., Brown, J.M., Cumming, I.A. and Goding, J.R. (1972)**

Radioimmunoassay for ovine prolactin. The secretion of prolactin as affected by milking, oestradiol administration and onset of parturition. *Journal of Reproduction and Fertility* 28, 133-134.

(32) **Topper, Y.J. and Freeman, C.S. (1980)** Multiple hormone interactions in the developmental biology of the mammary gland. *Physiology Reviews* 60, 1049-1106.

(33) **Muldoon, T.G. (1987)** Prolactin mediation of estrogen-induced changes in mammary tissue estrogen and progesterone receptors. *Endocrinology* 121, 141-149.

(34) **Kennaway, D.J., Gilmore, T.A. and Seamark, R.F. (1982)** Effect of melatonin feeding on serum prolactin and gonadotropin levels and the onset of seasonal estrous cyclicity in sheep. *Endocrinology* 110, 1766-1772.

(35) **Symons, A.M., Arendt, J. and Laud, C.A. (1983)** Melatonin feeding decreases prolactin levels in the ewe. *Journal of Endocrinology* 99, 41-46.

(36) **Milne, J.A., Loudon, A.S.I., Sibbald, A.M., Curlewis, J.D. and McNeilly, A.S. (1990)** Effects of melatonin and a dopamine agonist and antagonist on seasonal changes in voluntary intake, reproductive activity and plasma concentrations of prolactin and tri-iodothyronine in red deer hinds. *Journal of Endocrinology* 125, 241-249.

(37) **Karg, H., Schams, D. and Reinhardt, V. (1972)** Effects of 2-Br-&-ergocryptine on plasma prolactin level and milk yields in cows. *Experientia* 28, 574-576.

(38) **Hart, I.C. (1973)** Effect of 2-bromo-&-ergocryptine on milk yield and the level of prolactin and growth hormone in the blood of the goat at milking. *Journal of Endocrinology* 57, 179-180.