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Effect of melatonin implants on the incidence and timing of puberty in female red deer (*Cervus elaphus*)

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ABSTRACT

A study was conducted to test the hypotheses that exogenous melatonin treatment of 11–13 month-old red deer hinds: (1) advances the timing of first ovulation, (2) increases the proportion of individuals attaining puberty at ~16 months of age, and (3) reduces the live-weight threshold for attainment of first pregnancy. A total of 3901 rising-2-year-old (R_2) hinds within two herds (A and B) across two years either received single melatonin implants on two occasions in summer ($n = 1399$) or were untreated controls ($n = 2502$). Hinds were joined with stags from mid January to mid May, and were subjected to real-time rectal ultrasonography in early June to assess pregnancy status (proxy for puberty attainment) and foetal age for conception date assignment. Live-weights were recorded for each hind in January (12 months of age) as a proxy for weight at puberty. Melatonin treatment of hinds was associated with a significant advancement in mean conception dates in both herds in both years ($P < 0.05$), with a cohort difference in mean dates between treated and control hinds ranging from 9 to 17 days. Analysis of the temporal distribution of conception dates for each cohort revealed bi-modal or tri-modal patterns of conception indicative of conceptions to first or subsequent ovulations (oestrous cycles). Across all cohorts, melatonin treatment was associated with higher conception rates to first ovulation ($P < 0.05$) resulting in greater overall synchrony of conceptions. Regression analysis demonstrated a significant negative slope for conception date against live-weight ($P < 0.001$), but there was no evidence that this slope varied with treatment, herd or year ($P > 0.05$); for every 10 kg increase in live-weight conception date was advanced by an average of 1.3 days. In Herd A, melatonin treatment was associated with significantly higher pregnancy rates in both years (90.3% vs. 78.0% in Year 1 and 84.4% vs. 57.1% in Year 2; $P < 0.05$). The principle effect of melatonin treatment was to increase the pregnancy rate of hinds of low body-mass. In Year 1, at 60 kg live-weight a logit regression model indicated a pregnancy rate of 52% for untreated hinds and 83% for treated hinds. At 105 kg the rate for both cohorts was 90%. In Herd B, melatonin treatment was associated with higher conception rates in both years but these differences were not significant following correction for slight differences in mean live-weight ($P > 0.05$). The study has demonstrated that factors influencing puberty attainment in R_2 red deer hinds can vary between populations. In Herd A, in which body mass of hinds immediately prior to their first potential breeding season may have been the principle limiting factor, melatonin treatment appears to have instigated the pubertal process in hinds that would otherwise be of insufficient body mass.

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1. Introduction

The European red deer (*Cervus elaphus*) is a seasonal annual breeder, with conceptions occurring in autumn, and calving occurring in summer following an average gestation length of 230–240 days (Lincoln and Short, 1980; Guinness et al., 1971). The role of photoperiod in seasonal regulation of reproductive cycles, and other aspects of their highly seasonal physiology, is well documented for this species (Lincoln, 1985; Suttie and Simpson, 1985). Puberty in female red deer, defined as the age of first ovulation, normally occurs during the second autumn of life at 16 months of age (Kelly and Moore, 1977; Hamilton and Blaxter, 1980). However, entry into puberty is constrained by photoperiod and body mass, with young hinds in nutritionally deprived environments often delaying first ovulation until their third or fourth autumn (Hamilton and Blaxter, 1980; Clutton-Brock et al., 1982).

The concept of body-mass thresholds for puberty induction is well established, and it is a commonly held maxim that red deer hinds generally need to attain 65–70% of their ultimate mature body mass in order to reach puberty (Kelly and Moore, 1977; Fisher and Fennessy, 1985). However, hind genotype influences mature body size, particularly with crossbreeding between subspecies, thus influencing genotype-specific live-weight thresholds for puberty (Asher et al., 2005). The precise timing is also constrained by photoperiod, and rising-2-year-old (R_2) hinds must be within the correct photoperiodic milieu (short-days) and state of photoperiodic responsiveness (non-refractory) in order to enter puberty, irrespective of body mass (Lincoln and Short, 1980).

Early studies on reproductive seasonality of red deer have investigated the effects of exogenous melatonin delivery in summer on the precise seasonal onset of ovulation, with particular emphasis on pubertal hinds (Asher et al., 1993). Evidence of a possible link between photoperiodic entrainment and body mass in the induction of puberty in red deer hinds emerged from a study by Asher (1990) in which melatonin implants were administered to prepubertal hinds (i.e. 11–12 months of age) to advance the timing of pubertal ovulation. In this particular experiment, in which the average live-weight of the hinds (mainly *scoticus* subspecies) was only 70 kg at 16 months of age (i.e. many hinds fell below the theoretical live-weight threshold for the subspecies), it was notable that within the control (untreated) group of 9 hinds, the 4 hinds of lowest body mass remained anovulatory. By contrast, of 28 hinds treated with melatonin only 2 remained anovulatory despite an equivalent range in live-weights. This indicated that the exogenous melatonin may have facilitated the transition into a pubertal state in the smaller hinds. This introduces an intriguing concept that exogenous melatonin facilitates the entry into a pubertal state in hinds that would otherwise by constrained by low body mass and indicates a profound interaction between photoperiod and body mass in puberty attainment in this species. This was tested in a study involving two herds that have historically encountered issues with relatively high barrenness rates (~20%) amongst cohorts of R_2 hinds. The hypotheses tested in the present study are that exogenous melatonin treatment of 11–13 month-old red deer hinds (1) advances the timing of first ovulation (i.e. conception date), (2) increases the proportion of individuals attaining puberty at ~16 months of age (i.e. pregnant at ~18 months of age), and (3) reduces the live-weight threshold for attainment of first pregnancy. We emphasize that this study used exogenous melatonin implants only to investigate physiological mechanisms pertaining to puberty in red deer hinds and we do not seek to promote implants as a management tool for improving reproductive productivity of R_2 hinds within managed populations.

2. Materials and methods

2.1. Animals and management

A total of 3901 R_2 red deer hinds from two herds across two years, representing the 2009 (Year 1) and 2010 (Year 2) cohorts, were used in the study between November (11 months of age) and June (18 months of age) in each of the two years. Herd A (latitude 38° 49′S) was located within the Taupo region of the North Island and Herd B (latitude 45° 33′S) within the Te Anau Basin of the South Island. Where possible the study populations within each herd and year were managed as a single cohort. The population of Herd B in Year 2 was divided into three separate groups but managed in the same environment throughout the study period. As no significant differences in any parameters were observed ($P > 0.05$), data for the three groups were pooled for the overall analysis.

Both herds were of composite genotype representing crossbreeds between Scottish red deer (*C.e. scoticus*) and Eastern European red deer (*C.e. hippelaphus*), with minimal (<1%) introgression of North American Wapiti subspecies (*C.e. nelsoni, roosevelti, manitobensis*). In both herds the hinds were joined with multiple R_2 sire stags, at stag: hind ratios of 1:12 (Herd A) and 1:7 (Herd B) from mid-January to mid-May in each of the study years. Data collected for each hind included 12-month (January) live-weight (a proxy for 16-month weight), as well as 18-month (early June) pregnancy status (a proxy for puberty attainment) and foetal age assessment (for retrospective calculation of conception date).

2.2. Treatment

Across the two herds 1399 hinds (approximately 350 hinds per herd per year) were randomly selected across all management groups to receive subcutaneous melatonin implants (Regulin; CEVA Animal Health Ltd., Chesham, UK). A single implant, containing 18 mg melatonin, was delivered by subcutaneous injection (Melovine Implanter; CEVA Animal Health Ltd.) at the base of the right ear on two separate occasions, early December (11 months) and early January (12 months). All hinds had unique ear tag identification and melatonin-treated hinds were additionally tagged with a colour-coded sheep tag for rapid identification.