1   **Effect of exogenous melatonin implants on the incidence and timing of puberty**

2   **in female red deer (*Cervus elaphus*)**

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1
2   **Abstract**

3   A study was conducted to test the hypotheses that exogenous melatonin treatment of

4   11-13 month-old red deer hinds (1) advances the timing of first ovulation, (2)

5   increases the proportion of individuals attaining puberty at ~16 months of age, and

6   (3) reduces the live-weight threshold for attainment of first pregnancy. A total of 3901

7   rising-2-year-old (R_2) hinds, representing young replacement hinds from two

8   commercial herds (Farms A and B) across two years either received single melatonin

9   implants on two occasions in summer (n = 1399) or were untreated controls (n =

10   2502). Hinds were joined with stags from mid January to mid May, and were

11   subjected to real-time rectal ultrasonography in early June to assess pregnancy

12   status (proxy for puberty attainment) and foetal age for conception date assignment.

13   Live-weights were recorded for each hind in January (12 months of age) as a proxy

14   for weight at puberty. Melatonin treatment of hinds was associated with a significant

15   advancement in mean conception dates on both farms in both years ($P < 0.05$), with

16   a cohort difference in mean dates between treated and control hinds ranging from 9

17   to 17 days. Analysis of the temporal distribution of conception dates for each cohort

18   revealed bi-modal or tri-modal patterns of conception indicative of conceptions to first

19   or subsequent ovulations (oestrous cycles). Across all cohorts, melatonin treatment

20   was associated with higher conception rates to first ovulation ($P < 0.05$) resulting in

21   greater overall synchrony of conceptions. Regression analysis demonstrated a

22   significant negative slope for conception date against live-weight ($P < 0.001$), but

23   there was no evidence that this slope varied with treatment, farm or year ($P > 0.05$);

24   for every 10 kg increase in live-weight conception date was advanced by an average

25   of 1.3 days.

1   On Farm A, melatonin treatment was associated with significantly higher pregnancy

2   rates in both years (90.3% vs 78.0% in Year 1 and 84.4% vs 57.1% in Year 2; P <

3   0.05). The principle effect of melatonin treatment was to increase the pregnancy rate

4   of hinds of low body-mass. In Year 1, at 60 kg live-weight a logit regression model

5   indicated a pregnancy rate of 52% for untreated hinds and 83% for treated hinds. At

6   105 kg the rate for both cohorts was 90%. On Farm B, melatonin treatment was

7   associated with higher conception rates in both years but these differences were not

8   significant following correction for slight differences in mean live-weight (P > 0.05). It

9   is notable that the pregnancy rate of 61% for control hinds in Year 1 was ~20

10   percentage points lower than that of previous years' cohorts of R2 hinds prior to this

11   experiment, despite generally higher mean 12-month live-weights. At 60 kg live-

12   weight the estimated pregnancy rate was only ~20% for both treated and control

13   cohorts, rising to 80-90% at 105 kg. This may relate to earlier drought conditions that

14   profoundly influenced the growth of this cohort of hinds within the first few months

15   from birth, indicating the possibility that early growth may influence puberty live-

16   weight thresholds. Year 2 conception rates on Farm B were better aligned with those

17   of previous years but exogenous melatonin treatment still did not influence

18   conception rates.

19   The study has demonstrated that factors influencing puberty attainment in R_2 red

20   deer hinds can vary between populations. On Farm A, in which body mass of hinds

21   immediately prior to their first potential breeding season may have been the principle

22   limiting factor, melatonin treatment appears to have instigated the pubertal process in

23   hinds that would otherwise be of insufficient body mass. This effect is unprecedented

24   in the available literature on puberty in seasonally-breeding ruminants, and may

25   relate to effects of exogenous melatonin on augmentation of photoperiodic

1   entrainment processes or through alteration of components of body-mass (e.g. lipid

2   acquisition) that can influence live-weight thresholds for entry into a pubertal state

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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 & 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 & 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 & Moore, 1977; Hamilton & 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 & Blaxter, 1980; Clutton-Brock et al., 1982).

High levels of reproductive failure amongst rising two-year-old (R_2) hinds have become a significant productivity issue for the New Zealand deer farming industry (Audige et al., 1998; Beatson et al., 2000). This is manifest as variable within herd pregnancy rates (assessed by ultrasonography at 18-20 months of age) ranging from >95% to <40% (Asher & Pearse, 2002). Barrenness in R_2 hinds largely appears to represent ovulation failure at 16 months of age, with these hinds generally delaying puberty by one year (Asher et al., 2005). While many factors have been postulated to contribute to puberty delay (Audige et al., 1998), it is likely that the most common cause is insufficient body mass acquisition (i.e. low live-weight) by 16 months of age (Asher et al., 2005).

The concept of body-mass thresholds for puberty induction is well engrained in the deer farming industry, 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 & Moore, 1977; Fisher & 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 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 & Short, 1980).

Early studies on reproductive seasonality of farmed 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. These studies aimed to advance conception dates to better align subsequent calving and lactation with spring pasture production occurring within the NZ pastoral environment (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. Anecdotal accounts from deer farmers who had used melatonin

1   implants to advance the time of conception in R_2 hinds have also indicated that there

2   appeared to be an additional benefit of increased pregnancy rates within treated

3   populations (B. Wellington, *pers.comm.*).

4   This introduces an intriguing concept that exogenous melatonin facilitates the

5   entry into a pubertal state in hinds that would otherwise by constrained by low body

6   mass. This was tested in a study involving two commercial herds that have

7   historically encountered issues with relatively high barrenness rates (~20%) amongst

8   cohorts of R_2 hinds. The hypotheses tested in the present study are that exogenous

9   melatonin treatment of 11-13 month-old red deer hinds (1) advances the timing of

10   first ovulation (i.e. conception date), (2) increases the proportion of individuals

11   attaining puberty at ~16 months of age (i.e. pregnant at ~18 months of age), and (3)

12   reduces the live-weight threshold for attainment of first pregnancy.

13   **2.   Materials and Methods**

14   *2.1. Animals and management*

15   A total of 3901 R_2 red deer hinds raised on two farms across two years,

16   representing the 2009 (Year 1) and 2010 (Year 2) cohorts of the "commercial" part of

17   their operations (i.e. breeding herds within the venison production units), were used

18   in the study between November (11 months of age) and June (18 months of age) in

19   each of the two years. Farm A (latitude 38° 49'S) was located within the Taupo

20   region of the North Island and Farm B (latitude 45° 33'S) within the Te Anau Basin of

21   the South Island. Where possible the study populations within each farm and year

22   were managed as a single cohort. However, the population of Farm B in Year 2 was

23   divided into three separate management groups throughout.

24   Both herds were of composite genotype representing crossbreeds between

25   Scottish red deer (*C.e.scoticus*) and Eastern European red deer (*C.e.hippelaphus*),

1   with minimal (<1%) introgression of North American Wapiti subspecies (*C.e.nelsoni,*

2   *roosevelti, manitobensis*). On both farms the hinds were joined with multiple R_2 sire

3   stags, at stag: hind ratios of 1:12 (Farm A) and 1:7 (Farm B) from mid-January to

4   mid-May in each of the study years. Data collected for each hind included 12-month

5   (January) live-weight (a proxy for 16-month weight), as well as 18-month (early June)

6   pregnancy status (a proxy for puberty attainment) and foetal age assessment (for

7   retrospective calculation of conception date).

8

9   *2.2   Treatment*

10   Across the two farms 1399 hinds (approximately 350 hinds per farm per year)

11   were randomly selected across all management groups to receive subcutaneous

12   melatonin implants (Regulin; CEVA Animal Health Ltd, Chesham, UK). A single

13   implant, containing 18mg melatonin, was delivered by subcutaneous injection

14   (Melovine Implanter; CEVA Animal Health Ltd.) at the base of the right ear on two

15   separate occasions, early December (11 months) and early January (12 months). All

16   hinds had unique ear tag identification and melatonin-treated hinds were additionally

17   tagged with a colour-coded sheep tag for rapid identification in the yards.

18

19   *2.3   Pregnancy and conception date assessment*

20   Pregnancy status was determined for all hinds between 3 and 17 June in both

21   years, at least 30 days following sire stag removal from the respective herds. While

22   restrained in a hydraulic crush, each hind was subjected to real-time rectal

23   ultrasonography using a linear array transducer (Aloka SSD210; MedTel Teletronics

24   Ltd, Auckland) to assess pregnancy status. Scanning was timed to ensure foetal

25   ages ranged between 30 and 90 days in order to visualise and assess foetal size.

1 When pregnancy was visualised, foetal age was assessed using foetal dimension

2 criteria described by White et al. (1989) to retrospectively calculate conception dates

3 to within 5 days (see Scott et al. 2008). Determination of non-pregnancy status was

4 based on visualisation of the non-gravid uterus.

5

6 *2.4 Statistical analyses*

7 Conception date was analysed using a linear model, fitting farm, year,

8 melatonin treatment, live-weight and their interactions. Inspection of the residuals

9 suggested that the distribution of conception rate was multi-modal, with evenly

10 spaced peaks corresponding to conception at the first, second and third cycles (see

11 Figure 1). A mixture model (McLachlan & Krishnan, 1997), consisting of a

12 probabilistic mixture of three normal distributions separately synchronised for each of

13 the eight experimental groups (corresponding to the factorial combination of farm,

14 year and treatment) was then fitted, with a common difference (*d*) in the mean of the

15 mixed distributions corresponding to cycle length, and common variance.

16 Mathematically, the log-likelihood for the data is given by

$$L = \sum_{t=1}^{8} \sum_{i=1}^{n_t} \log(\pi_{t1}\varphi(\mu_{ti};\ \sigma^2) + \pi_{t2}\varphi(\mu_t + d;\ \sigma^2) + \pi_{t3}\varphi(\mu_t + 2d;\ \sigma^2))$$

17 ,

18 where $\varphi(\mu;\ \sigma)$ is the probability density function of the normal distribution with mean μ

19 and variance σ^2, and π_{tj} is the probability that a hind in experimental group *t*

20 conceived at cycle *j*. The maximum log-likelihood was obtained using the EM

21 algorithm with standard errors estimated from an additional Newton-Raphson step,,

22 and sub-models were compared to the full model using the likelihood ratio test (LRT).

1 Pregnancy status was analysed using a Bernoulli-logit generalised linear model,

2 fitting farm, year, melatonin treatment, live-weight and their interactions.

3 **3.   Results**

4   Summary statistics on 12-month live-weight, conception rate and conception

5 date are presented in Table 1. It is noted that despite random allocation of hinds, in

6 all cases the mean live-weights of melatonin-treated hinds across cohorts were

7 slightly higher (generally by 0.7-1.3 kg) than those of controls. However, the

8 difference on Farm A in Year 2 was surprisingly large (5.7 kg; Table 1).

9

10 *3.1 Timing of conceptions*

11   Using a normal linear model, melatonin treatment of hinds was associated with

12 a significant advancement in mean conception dates on both farms in both years ($P <$

13 0.05; Figure 1). The difference in mean dates between control and treated hinds

14 ranged from 9 days (Farm A, Year 1) to 17 days (Farm A, Year 2) (Table 1). There

15 was a significant negative slope for conception date against live-weight (slope =

16 0.125; SE = 0.028; $P < 0.001$), but there was no evidence that this slope varied with

17 treatment, farm or year ($P > 0.05$). Essentially, a 10 kg increase in live-weight was

18 associated with a 1.3 day advance in conception date.

19   Residual analysis indicated that hinds were conceiving to different cycles and

20 it was apparent that additional insights into the underlying process could be gained

21 by analysis using a mixture of normal distributions by group. This analysis gave an

22 overall estimate for cycle length of 17.0 (SE 0.39) days, within the accepted range of

23 16-19 days (Guinness et al., 1971), with most cohorts spanning 3 cycles except for

24 Farm A in Year 1, which spanned 2 cycles (Table 2). In all cases the melatonin

25 treatment was associated with an earlier first cycle than the control treatment, with

1   differences ranging from 4 to 9 days, and the LRT was highly significant (x_1^2 = 603.2;

2   P<0.001) rejecting common first cycle mean parameters for treatment and control.

3   Further, in all cases a greater proportion of hinds conceived during the first cycle for

4   the melatonin treatment than for control, with a highly significant LRT (x_7^2 = 138.0;

5   P<0.001) rejecting common cycle proportion parameters for treatment and control

6   hinds within each case. Interestingly for control hinds on Farm B in Year 2 only 24%

7   conceived to first oestrus and 49% and 27% conceived to second and third oestrus,

8   respectively. This contrasted markedly with the pattern for treated hinds; 73%, 18%

9   and 1% conceiving to first, second and third cycles, This analysis demonstrated that

10   the melatonin treatment resulted in earlier conceptions and greater synchrony of

11   conceptions to first oestrus than for the control treatment.

12

13   *3.2   Pregnancy rate*

14   Farm A: Melatonin treatment resulted in a significantly greater number of

15   pregnancies in both years (90.3% vs 78.0% in Year 1 and 84.4% vs 57.1% in Year 2,

16   P < 0.01, Table 1). Logit regression analysis of pregnancy status on 12-month live-

17   weight demonstrated a positive relationship for all cohorts, but with significantly

18   different slopes between the treated and control hinds on Farm A in both years

19   (Figure 2). The probability prediction for pregnancy attainment (i.e. puberty) of the

20   control hinds in Year 1 showed a moderately steep slope in relation to live-weight,

21   with a 65% probability at 70 kg rising to 93% at 105 kg. However, melatonin

22   treatment resulted in a much higher probability at the low live-weight end, being 87%

23   at 70 kg rising to 94% at 105 kg. In contrast in Year 2 there was a shallow slope for

24   control hinds, with a probability of pregnancy at 70 kg of 55% rising to 70% at 90 kg,

1 while for melatonin-treated hinds there was a steeper slope, rising from 74% at 70 kg

2 to 94% at 90 kg.

3   Farm B: An apparent increase in conception rate associated with melatonin

4 treatment in Year 1 (68.5% vs 61.1%) was significant (P = 0.046) when analysed

5 without reference to live-weight but was not significant when adjusted for a slight

6 weight difference (0.7 kg) between the groups (P = 0.106). The difference favouring

7 melatonin treatment in Year 2 (80.5% vs 74.1%) was not significant (P > 0.05). The

8 logit regression model showed steep positive relationships between 12-month live-

9 weight and pregnancy attainment for both years, but the slopes were significantly

10 different between years (P < 0.05). In Year 1 the control and melatonin-treated hinds

11 both exhibited steep slopes, with only ~30% probability of pregnancy at 70 kg and

12 80-90% probability at 105 kg. These curves were essentially parallel for the treated

13 and control cohorts (Figure 2), showing no indication of a marked effect of melatonin

14 treatment on pregnancy attainment at any live-weight. In Year 2 the curves were not

15 as steep, with 59-67% probability of pregnancy at 60 kg, and ~90% probability of

16 pregnancy at 105 kg. Again, the curves in Year 2 were essentially similar for the

17 treated and control hinds (Figure 2).

18 **4.   Discussion**

19   A number of studies over the last 20 years have examined the use of

20 exogenous melatonin to advance the seasonal onset of ovulatory cycles in red deer

21 hinds, including advancing puberty in R_2 hinds (see Asher et al., 1993). In the

22 present study melatonin implants advanced the timing of conceptions in the R_2 hinds

23 in both populations. This was an entirely expected outcome consistent with the

24 results of previous studies on pubertal red deer hinds (Fisher et al., 1990; Asher

25 1990) and supports the first hypothesis. However, such advancement in the present

1   study was shown to be facilitated by two complimentary effects; first an advancement

2   of first oestrus/ovulation by between 7 and 9 days (Farm A) and between 4 and 7

3   days (Farm B), and second by increased conception rates to first oestrus, thus

4   reducing the proportion of hinds conceiving to subsequent ovulations. This has

5   resulted in a net advancement in conception dates of between 8 and 17 days for

6   Farm A and between 13 and 16 days for Farm B. The latter effect has not been

7   described previously following melatonin treatment of red deer hinds, probably

8   because of the smaller scale of earlier studies. While it is tempting to argue that

9   melatonin treatment has induced higher fertility to first oestrus/ovulation, the possibly

10 also exists that stag mating activity was more vigorous with hinds exhibiting their first

11 oestrus early in the rutting period.

12   The most remarkable outcome of the present study was a clear demonstration

13 of an effect of melatonin treatment on enhancement of the incidence of puberty on

14 Farm A. This effect on puberty appears to be unprecedented for any seasonally

15 breeding ruminant species, although the earlier study of Asher (1990) had indicated a

16 possible effect on conception rate of R_2 red deer hinds. Earlier studies on the use of

17 Regulin implants to advance seasonal reproductive activity of adult ewes have

18 indicated a possible effect of treatment on reducing the incidence of barren ewes and

19 have demonstrated improved fecundity through an increase in the proportion of twin-

20 bearing ewes (Williams et al., 1992). A similar effect of melatonin implant on

21 fecundity has been observed in goats (Chemineau et al., 1988). While the effects on

22 fecundity in adult sheep and goats may simply reflect the effects of early cycling

23 females conceiving to later oestrous cycles that are normally characterised by higher

24 ovulation rates (Thompson et al., 1985), the observed reduction in barrenness in

25 ewes may be analogous to the puberty enhancement in red deer hinds.

1   While it could be argued that advancement of first ovulation in pubertal hinds

2   could result in higher overall pregnancy rates in hinds due to increased mating

3   opportunities during the period of stag presence (i.e. higher incidences of repeat

4   oestrous cycles), the demonstration of higher conception rates to first oestrus

5   amongst treated hinds indicates that the enhancement effect was largely

6   independent of the advancement of oestrus/ovulation. The conception rate response

7   on Farm A provides support for the second hypothesis that exogenous melatonin

8   increases the proportion of individuals attaining puberty, but the lack of response on

9   Farm B indicates that this not a universal phenomenon across populations.

10   One of the more compelling aspects of puberty enhancement following

11   melatonin treatment on Farm A was the dramatic increase in conception rate of hinds

12   of lower body-mass in Year 1. In effect, exogenous melatonin appears to have

13   dramatically reduced the live-weight threshold for puberty attainment. Whereas the

14   control hinds showed a 65% probability of pregnancy at 70 kg (12-month weight), this

15   jumped to 87% probability of pregnancy for treated hinds. In contrast, at live-weights

16   in excess of 100 kg there was no difference in predicted conception rate between

17   treated and control hinds. While this provides support for the third hypothesis that

18   exogenous melatonin reduces the live-weight threshold for attainment of first

19   pregnancy, the relationship between body mass and pregnancy attainment was

20   somewhat different in Year 2. If anything, the relationship appears to be reversed,

21   with melatonin treatment favouring increased conception at higher live-weights.

22   However, recognition is given to the fact that the overall mean 12-month live-weight

23   of the R_2 hinds in Year 2 were substantially lower than for Year 1, and that despite

24   random allocation of hinds to treatment/control cohorts in Year 2, there was a marked

25   discrepancy in the mean live-weights between them, with treated hinds being 5.6 kg

heavier and exhibiting a wider live-weight range. What is clear in Year 2 is that the pregnancy rate of melatonin-treated hinds was substantially higher overall.

Thus, it appears that exogenous melatonin can, in some herds, influence the physiological processes that prevent hinds, particularly those of lower body mass, from initiating their first ovulation at ~16 months of age. This is a surprising observation that highlights a general paucity of knowledge on the exogenous and endogenous processes that are permissive to puberty attainment in seasonally breeding ruminants. As melatonin is the principle transducer of photoperiodic information to the brain in seasonal ruminants such as sheep (Robinson & Karsch, 1987; Deveson et al., 1992), the observed effect of exogenous melatonin on puberty enhancement in red deer is indicative of a profound interaction between photoperiodic signals and body mass (or components of body mass) on the seasonal attainment of puberty in this species. It is tempting to rationalise this observation as a rather simplistic phenomenon in which the exogenous melatonin augments the natural photoperiod cycle, perhaps allowing smaller hinds the capability of becoming photoperiodically responsive under circumstances in which they would normally remain in a photorefractory condition. However, it is acknowledged that there is no evidence that such refractoriness exists in small hinds at around the normal time of puberty. Furthermore, there is considerable evidence that young red deer are actually photoperiodically responsive, in terms of short-day effects on voluntary feed intake, early within their first year of life (Suttie et al., 1984).

The fact that photoperiod-melatonin signals mediate other profound seasonal changes in nutritional physiology of young red deer (Suttie et al., 1984; Yellon et al., 1992) suggests that the observed effect of exogenous melatonin on puberty enhancement may relate to subtle changes in body mass and/or body composition

during the pre-pubertal treatment phase. While body-mass thresholds for puberty attainment have been a widely held concept in mammalian biology, it has become clear that body composition, and more specifically, body fat are better predictors of puberty than body mass *per se*. Total body lipid content is correlated with puberty attainment across a range of mammalian species, including primates and rodents (Ebling, 2005). Although young red deer hinds appear to generally exhibit relatively low levels of carcass fatness, this does not preclude the possibility that subtle changes in total body fatness may be an important determinant of puberty attainment in female red deer. In white-tailed deer (*Odocoileus viginianus*), short days promote fat accretion in female fawns (Abbott et al., 1984) and it is possible that exogenous melatonin implants can likewise influence fat accretion in young red deer hinds, allowing low body-mass hinds to acquire sufficient body fat that is permissive to puberty attainment. A further consideration in relation to body mass vs. body composition is that while all cohorts of control hinds in the study exhibited a clear relationship between 12-month live-weight and pregnancy rate, there was substantial variation in slopes. This was manifest in considerable differences between cohorts in predicted pregnancy rate intercepts at specific live-weights, indicating that the threshold body-mass for entry into puberty varied between herds and years, despite similar genetic composition of the populations. It is possible that this also actually reflect population differences in average body composition at specific live-weights.

 While there appeared to be an effect of exogenous melatonin treatment on increased pregnancy rates on Farm B, this was not significant when small differences in live-weight were accounted for in the statistical model and pregnancy rates were compared at the same live-weight. While mean live-weights of the cohorts on Farm B were generally higher than for Farm A, there were certainly hinds of low body mass

1 represented in the population on Farm B, but there was no observable effect of

2 exogenous melatonin treatment on enhancing the incidence of pregnancy amongst

3 these individuals. Despite this, there were still profound relationships observed

4 between 12-month live-weight and pregnancy status in both years on Farm B.

5 Interestingly, the slopes differed significantly between Years 1 and 2. In Year 1 the

6 threshold body-mass for pregnancy attainment were substantially higher than for

7 Year 2, with the interpolated 50% probability threshold being >80 kg (cf. 60 kg for

8 Year 2). It is noted that in the years previous to the present study the pregnancy

9 rates of R_2 hinds on Farm B have been about 20 percentage points higher than

10 observed in Year 1 (I.Brown; pers.comm.). Thus, the results for the 2009 cohort of

11 hinds represent an anomalous event for the farm. One potential factor worthy of

12 consideration is the rearing environment of the 2009 cohort between birth and

13 weaning at three months of age (i.e. December 2007- March 2008). Along with many

14 regions of New Zealand, Farm B suffered its worst drought period in several decades

15 during this period. As a consequence of poor feed availability, the weaning live-

16 weights (i.e. at 3 months of age) of the cohort were considerably depressed

17 compared with those achieved by previous cohorts, and increased feeding levels

18 were instigated to compensate for low weaning weights in an attempt to ensure that

19 the hinds were of sufficient weight for optimal pubertal performance at 16 months of

20 age. The recorded live-weights at 12-months of age indicated that the feeding

21 strategy had been successful. However, despite this the overall pregnancy rate of the

22 R_2 hinds in 2009 was considerably lower than in previous years. According to the

23 anecdotal accounts of farmers, practitioner veterinarians and ultrasound scanning

24 operators, this scenario has occurred on numerous farms throughout New Zealand

25 for the 2009 R_2 hind cohorts, particularly on farms severely affected by the drought

1 on 2007/08. In support of this observation, the scan results from Year 2 on Farm B

2 were better aligned with the historical records of R_2 hind pregnancy rates, and the

3 relationship of 12-month live-weight to probability of pregnancy in Year 2 indicated a

4 lower threshold body mass for attainment of puberty.

5   This raises the possibility that irrespective of ultimate live-weight 9-12 months

6 later, growth within the first three months of life may be an important determinant of

7 the timing of puberty and the body-mass thresholds for entry into puberty at 16

8 months of age. Such a scenario would indicate that red deer may be able to cue

9 reproductive processes, such as entry into puberty, from events occurring well

10 beforehand. Again, it is possible that this reflects body compositional changes (e.g.

11 reduced body lipid composition at a given live-weight) as a result of prior nutritional

12 status. While the actual physiological mechanisms driving this are not apparent from

13 the current study, the concept of delaying the timing of first breeding of females when

14 there are early indications of a suboptimal nutritional environment is intuitively logical

15 from a species survival perspective, and even hints at epigenetic factors that

16 influence entry into puberty (e.g. rate of fat deposition). This concept remains

17 speculative at this stage but, given the potential implications for nutritional

18 management of young red deer hinds for optimal reproductive performance, warrants

19 further consideration.

20   While the pregnancy rates of R_2 hinds on Farm B in Year 1 may be considered

21 anomalous, the same cannot be stated for Year 2. However, the profound effects of

22 exogenous melatonin on pregnancy rate across both years on Farm A were not

23 replicated in Year 2 on Farm B. Hind genotype is probably not a factor, as both farms

24 have a common genetic base across their commercial breeding herds. A logical

25 possibility is latitude, and hence photoperiodic amplitude. The farms are separated

by $\sim 7^{\circ}$ of latitude, and this is reflected in a difference of ~ 2 hours in photoperiod amplitude at the solstices. It is unclear how exogenous melatonin may interact with the endogenous photoperiodic cycle in order to exert such differential effects over latitudinal distance. One possibility is that the lower amplitude photoperiodic cycle at higher latitudes is associated with a higher incidence of pubertal failure in R_2 red deer hinds due to diminished imprinting of the photoperiodic signal. In this case, exogenous melatonin would serve to reinforce the imprinting by amplifying the short-day sequence. However, we are not aware of any data demonstrating reduced reproductive performance of R_2 hinds at more northern latitudes in New Zealand, Furthermore, we cannot rule out the possibility that other environmental differences between the two farms are influencing the outcomes in relation to puberty enhancement despite the obvious effect common to both farms of advancing the onset of puberty.

The consequences of enhancing the incidence of pubertal pregnancy with exogenous melatonin, as observed on Farm A, need further evaluation. If indeed the effect is partly due to a reduction in the live-weight threshold for the instigation of pubertal processes, the resulting increase in pregnancy rates will largely be due to a greater proportion of lower live-weight hinds becoming reproductively capable at 16 months of age. It is a generally held concept across species that the first pregnancy and birth pose significant mortality risk to an individual female and it can be argued that the ability to delay puberty under adverse conditions and suboptimal somatic growth is, in itself, a species survival tactic. Accordingly, delaying puberty when confronted with an adverse environment is a logical adaptation to such an environment, allowing the female further time to acquire sufficient somatic growth before instigating the reproduction process. Enhancing the pregnancy rate of R_2

hinds by 'forcing' smaller individuals into a reproductive state could result in higher incidences of pregnancy failure (e.g. abortions), late-pregnancy complications (e.g. hind metabolic disorders) and birthing complications (e.g. dystocia). Thus, while exogenous melatonin presents an interesting model to further understand the relationship between body-mass and photoperiodic entrainment, it is clearly not a practical management tool to offset poor growth rates.

Conclusions

The study has shown that exogenous melatonin treatment to young red deer hinds not only advances the timing of pubertal ovulation but can also increase the incidence of puberty in some herds. This appears to be partly due to the instigation of ovulation in hinds of low body mass that would otherwise fail to enter puberty in their second year, and indicates that the exogenous melatonin may promote photoperiodic entrainment or alter the influence of body-mass on constraining the entry into puberty. The consequences of inducing pregnancy in small R_2 hinds have yet to be evaluated, but it is probable that it would incur ethical costs due to their general inability to cope with foetal demands and parturition. Other outcomes of the study hint at possible long-term consequences of poor early growth of hind calves on their subsequent entry into puberty, demonstrating a general complexity around processes associated with the timing of female puberty in red deer.

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Table 1: Summary statistics for animal numbers, live-weight, pregnancy rate and

conception date, by farm, treatment group and year.

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Herd	Treatment/Year	n.	Mean 12-month live-weight (Kg) (s.d.)	% pregnant at 18 months	Mean conception date (s.d.)
Farm A	Control 2009	1420	82.7 (6.9)	78.0	31 March (7.4 d)
	Melatonin 2009	340	83.5 (7.1)	90.3	22 March (6.1 d)
	Control 2010	396	72.1 (3.5)	57.1	1 April (16.9 d)
	Melatonin 2010	346	77.8 (6.3)	84.4	14 March (8.3 d)
Farm B	Control 2009	406	87.8 (8.1)	61.1	31 March (14.5 d)
	Melatonin 2009	346	88.5 (8.0)	68.5	18 March (11.4 d)
	Control 2010	324	86.0 (9.5)	74.1	1 April (13.3 d)
	Melatonin 2010	323	87.3 (10.3)	80.5	17 March (12.7 d)

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Table 2: Parameter estimates from the mixture model analysis of conception date for each treatment cohort, where π_j is the probability of conception at the jth cycle, μ is the mean conception day of the year (with corresponding date) for the first cycle, and estimates of $d^§$ and $\sigma^†$ are common across treatments. Standard errors are given in brackets.

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Herd	Treatment	π_1	π_2	π_3	μ	Date
Farm A	Control 2009	0.893 (0.019)	0.107 (0.019)	0.000 (0.000)	88.1 (0.33)	28 March
	Melatonin 2009	0.951 (0.021)	0.050 (0.021)	0.000 (0.000)	80.1 (0.50)	20 March
	Control 2010	0.662 (0.047)	0.062 (0.032)	0.276 (0.045)	81.0 (0.65)	21 March
	Melatonin 2010	0.932 (0.027)	0.050 (0.026)	0.018 (0.013)	71.6 (0.55)	12 March
Farm B	Control 2009	0.652 (0.047)	0.137 (0.039)	0.212 (0.039)	80.2 (0.68)	20 March
	Melatonin 2009	0.879 (0.035)	0.062 (0.029)	0.059 (0.023)	73.4 (0.60)	13 March
	Control 2010	0.244 (0.065)	0.491 (0.061)	0.265 (0.048)	73.4 (1.47)	13 March
	Melatonin 2010	0.725 (0.051)	0.177 (0.046)	0.099 (0.028)	69.4 (0.78)	9 March

8 $^§d$ = inter-cycle period = 17.0 (SE 0.39) d

9 $^†\sigma$ = 5.6 (SE 0.14) d (compared to 10.5 d from unmixed analysis)

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Figure 1: Histogram of percentage of herd by conception date for each farm and

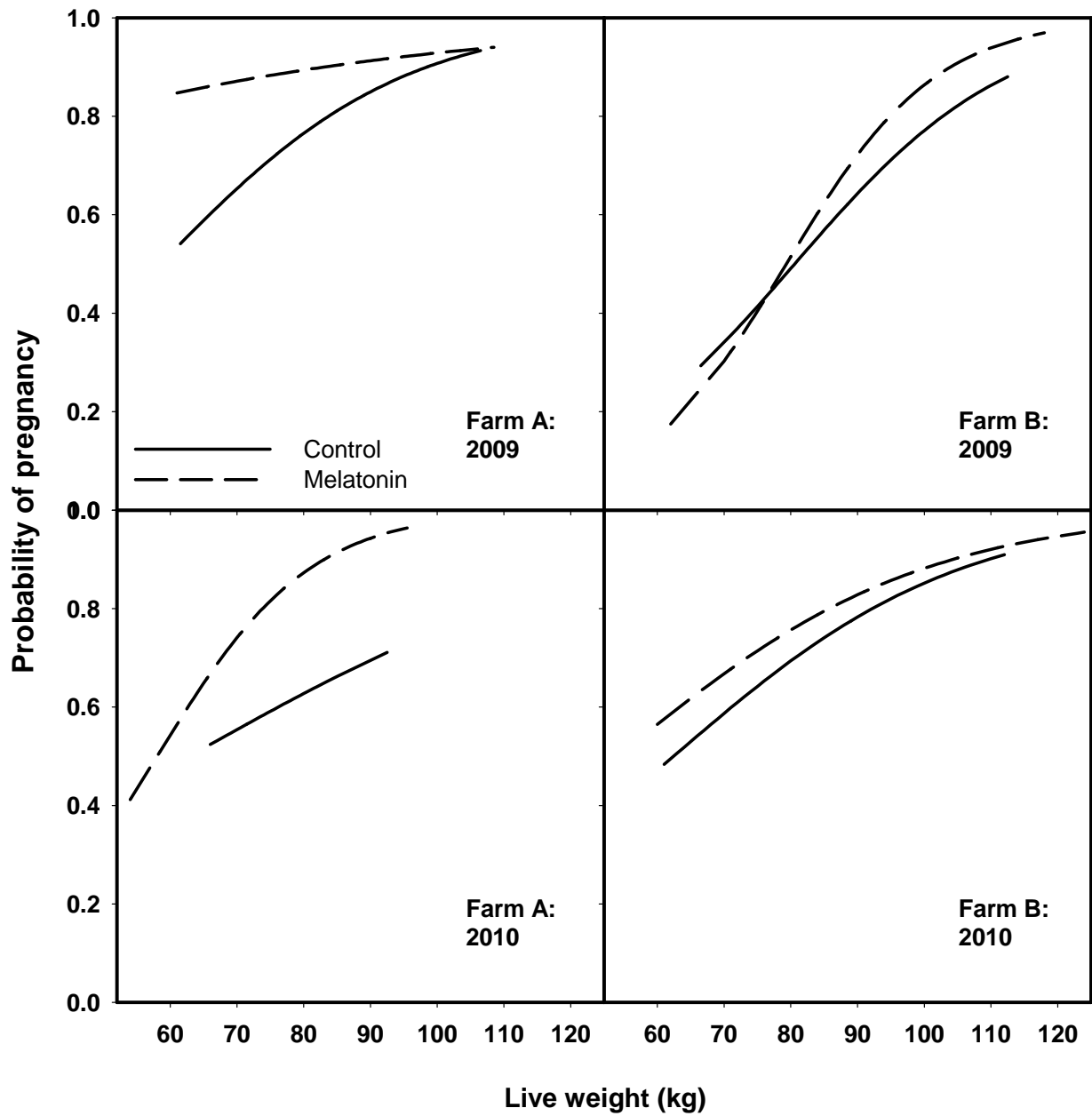melatonin treatment combination, pooled over both years of sampling.

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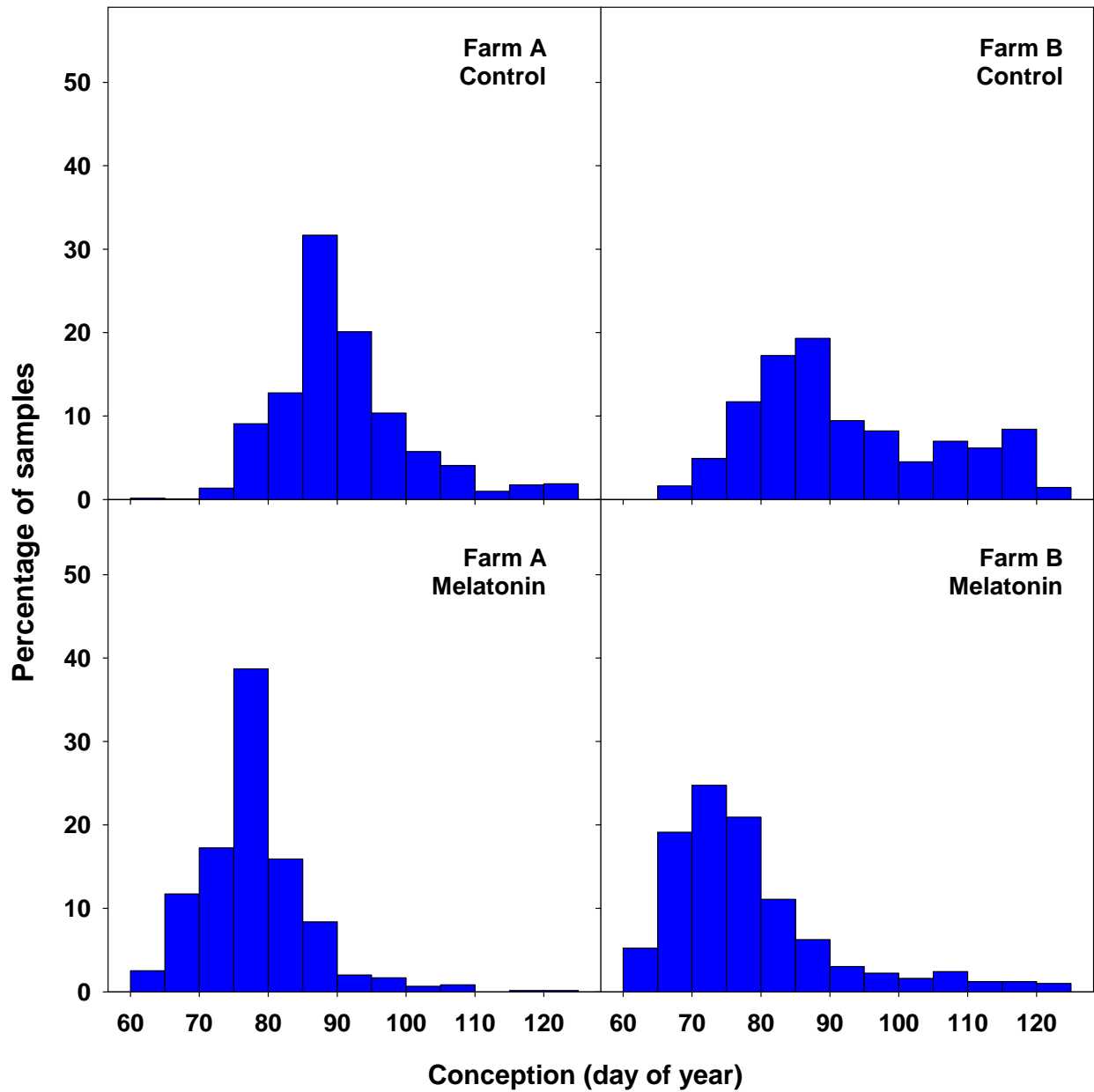
Figure 2: Predicted probability of pregnancy versus 12 month live-weight for each

farm and treatment combination.

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