# Seasonal luteal cyclicity of pubertal and adult red deer (*Cervus elaphus*)

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**Abstract**

Reproductive failure of rising-two-year-old ($R_2$) hinds and seasonal misalignment between calving and pastoral feed production are two factors limiting reproductive productivity of farmed red deer hinds in New Zealand. This study aimed to better understand processes around female puberty and breeding seasonality by describing the potential breeding season (i.e. oestrous cyclicity) of 3 red deer genotypes. A total of 27 hinds born in December 2005, representing Eastern European (*Cervus elaphus hippelaphus*), Western European (*C.e.scoticus*) and F1 crossbred (*C.e.hippelaphus x scoticus*) red deer, were blood sampled thrice-weekly for 7-8 months (February-September/October) across two years spanning the potential breeding seasons as $R_2$'s in 2007 (i.e. puberty) and as adults in 2008 . Plasma progesterone profiles were used to construct breeding cycle histories for each hind. Four $R_2$ hinds failed to initiate oestrous cycles (i.e. puberty failure). The remaining $R_2$ hinds, including all F1 hinds, exhibited between 2 and 7 oestrous cycles. F1 hinds were significantly earlier to initiate, and later to terminate, cyclic activity, resulting in a longer mean pubertal breeding season (139 days) than for Eastern (86 days) and Western hinds (86 days). However, the data for $R_2$ hinds are confounded by live-weight, with the F1 hinds being significantly heavier than other genotypes. There were significant correlations between live-weight and seasonality parameters in 2007. All hinds were cyclic as adults in 2008, exhibiting between 4 and 9 oestrous cycles, and a mean breeding season duration of between 132 (Western) and 137 (F1) days. For adult hinds there were no significant genotype differences in cyclic onset and cessation timing, and no observable relationships between live-weight and any reproductive parameter. However, the mean dates for the onset of the breeding season for all genotypes in 2008 were 2-3 weeks later than normally expected for adult hinds in New Zealand. The reasons for this are unclear but may relate to chronic stress of frequent animal handling. The study has demonstrated that puberty in red deer hinds is associated with a shorter potential breeding season than for adult hinds, and that perturbation of breeding activity appears to be quite common, leading to incidences of puberty failure and possibly other aberrant cyclic events. Live-weight x genotype interactions may influence puberty but do not appear to be strongly expressed in adults. However, the relatively late onset of oestrous cyclicity in the adult hinds may be an artefact of the study that has masked genetic influences on seasonal breeding patterns.

**Keywords**: red deer, *Cervus elaphus*, reproduction, puberty, ovulation, oestrous cycle

## 1. Introduction

Red deer (*Cervus elaphus*) form the basis of the New Zealand (NZ) deer farming industry. Reproductive productivity of hinds is a core measure of overall farm productivity. Two issues of considerable relevance to improving the reproductive performance are (i) increasing the pregnancy rates of rising-two-year-old ($R_2$) hinds (ie. increasing the proportion of hinds entering puberty at 16 months of age), and (ii) improving the lactational performance of hinds (and hence the growth rate of their calves) by better seasonal alignment of feed production with the high energy demands of lactation (Asher & Pearse 2002).

Most red deer hinds enter puberty during their second autumn at around 16 months of age. While this is dependent upon the attainment of sufficient body mass at this age (i.e. critical live-weight threshold of 65-70% of ultimate mature body weight; Kelly and Moore 1977, Asher et al. 2005), timing of pubertal oestrus is seasonally constrained by the prevailing photoperiod (Lincoln and Short 1980), with hinds generally becoming responsive to photoperiodic cues sometime prior to 16-months of age. There has been an apparent trend over the last 20 years for the pregnancy rate of $R_2$ hinds on NZ farms to decline by 10-20 percentage units (ie. from >90% to <80%), as measured by early pregnancy (first trimester) ultrasonography (Beatson et al. 2000). This is despite the fact that average hind live-weights at 16 months of age have increased by about 10-15 kg over this period, and are generally well above the putative critical live-weight threshold of 65-70 kg for Scottish red deer (*C.e. scoticus*) (Kelly and Moore 1977). The introgression of genotypes of larger mature body mass (e.g. North American wapiti; *C.e.nelsoni, roosevelti, manitobensis*) has increased the critical live-weight threshold within some populations . While this has been shown to influence the pregnancy rates within those populations (Asher et al. 2005a), it only accounts for a relatively small proportion of the reproductive wastage across the entire industry. It is considered, therefore, that factors other than introgression of large genotypes also contribute to reproductive wastage in young red deer hinds.

The potential breeding season (i.e. luteal cyclicity in the absence of pregnancy) of $R_2$ red deer hinds in NZ has received little scrutiny. Duckworth and Barrell (1992) and Asher et al. (1997), investigating luteal cyclicity in a total of 4 and 7 individuals respectively, demonstrated that pubertal

hinds exhibited later onset and earlier cessation of luteal/oestrous cycles (i.e. shorter breeding season) relative to adult hinds, with luteal/oestrous cycle lengths of 19-20 days duration being comparable to those of adult hinds. All $R_2$ hinds in these studies exhibited between 3 and 6 normal cycles over a period of 52-102 days. Interestingly, none of the hinds in these two studies failed to enter puberty, nor showed any evidence of abnormal cyclic activity. However, the numerous accounts within the last few years of high non-pregnancy rates of $R_2$ at early pregnancy scanning indicates failure to establish a viable pregnancy despite stag joining dates that span at least 2 potential oestrous cycles. Plasma progesterone data presented by Asher (1990) for $R_2$ hinds joined with fertile stags under normal management practices indicated a high level of ovulation failure, rather than early embryonic mortality. Again, this represents a very small data set of 9 animals only.

Temperate-zone pastoral production systems are highly seasonal, as are the deer themselves. However, there is a notable misalignment between annual pasture production cycles throughout much of NZ and the annual feed demand cycles of reproducing females, particularly evident during lactation over summer. While early summer calving is clearly adaptive to the northern boreal environment to align the high energy demands of lactation with the seasonal peak of forage production, within the more temperate climes of NZ peak forage (pasture) production occurs several months earlier in spring. Often by the onset of lactation, pastoral feed quality has declined due to drought conditions and natural pasture senescence (Asher et al. 1993; Litherland et al. 2002). Considerable research effort has been focussed on better aligning pasture growth with lactation, either by altering forage production systems or by attempting to induce earlier conception/calving patterns in hinds. The latter approach has been largely focussed on exogenous hormone control of seasonal ovulatory patterns of hinds (see Asher et al. 1993), which has found little favour amongst farmers seeking to simplify management systems, reduce operating costs and adhere to more natural production systems. A more recent consideration has been the potential for genetic selection for early seasonal breeding traits in red deer, capitalising on observed differences in conception timing between red deer hinds of differing genotype. Most notable has been the differences in rutting timing, mating dates and calving dates between red deer of Western European origin (*C.e. scoticus*) and those of Eastern European origin (*C.e. hippelaphus*) (Scott et al. 2006). This study demonstrated that the Eastern genotype exhibited earlier breeding characteristics, with

average mating and calving dates being about 2-3 weeks earlier than for the Western genotype. It remains unknown if this represents a slight phase-shift in the breeding season or a difference in the duration of the breeding season favouring a longer period of cyclic activity in Eastern genotypes.

The aims of the present study were (1) to describe luteal patterns in hinds during their first (pubertal) and second (adult) potential breeding seasons, with particular emphasis on differences occurring between ages, and (2) to evaluate the influence of genotype on the onset and duration of the potential breeding season.

## 2. Materials and Methods

### 2.1 *Ethical considerations*

These studies were undertaken with the approval of the AgResearch Invermay Animal Ethics Committee, as required in New Zealand by the Animal Welfare (Codes of Ethical Conduct) Act 1987. All procedures were conducted by fully trained staff from the Invermay Agricultural Centre.

### 2.2 *Pubertal ($R_2$) hinds 2007*

A total of 30 prepubertal hinds were located on the Invermay deer farm (45$^o$ 53'S, 170$^o$ 21'E) in December 2006 (12 months of age). Genotypes represented were: (i) Western European (W) subspecies (predominantly *C.e. scoticus*), progeny of animals imported from the UK within the last 15 years (n = 11); (ii) Eastern European (E) subspecies (*C.e. hippelaphus*), progeny of animals imported from Hungary and Romania within the last 15 years (n = 10); and first-generation *C.e.scoticus x hippelaphus* (F1) crossbred individuals (n = 9) generated through controlled mating or artificial insemination programmes using imported Eastern European sires over Western European dams. The three genotypes were derived from different farm sources just prior to relocation, with the E and W hinds obtained from commercial deer farms and the F1 hinds obtained from the Invermay breeding unit. Subspecies composition of all hinds was verified by DNA microsatellite analysis (**Dodds & Tate in press**) and detailed pedigree records.

Between the time of relocation and 15 January 2007 the hinds were habituated to frequent yarding (at least thrice weekly) to minimise subsequent handling stress. They were grazed outdoors as a

single group and joined with an adult vasectomised stag in early March 2007, remaining within close proximity of the stag throughout the study period. From 15 January (13 months of age) to 10 September 2007 (21 months of age), the period spanning their first potential breeding season, the hinds were yarded at least thrice weekly (Monday, Wednesday and Friday)for blood sampling, with a window of daily yarding between 16 April and 18 May. Blood samples were collected by jugular venepuncture (5ml heparinised vacutainers and 20-gauge needles) while individually restrained in a pneumatic crush. The plasma fraction was decanted into labelled tubes following centrifugation and stored at -20 C until assayed for concentrations of progesterone.

Any overt oestrous behaviour expressed towards handlers (e.g. lordosis) or herdmates during yarding was recorded and used to verify luteal cycle onsets based upon plasma progesterone profiles. Hinds were weighed fortnightly.

During the latter stages of the study period three hinds were removed from the study; two E hinds were euthanized due to the onset of clinical Johne's Disease, detected by weight loss, scouring and serological antibody tests, and one F1 hind was found dead with acute haemorrhagic enteritis (probably Malignant Catarhal Fever virus). All data for these hinds were removed from the subsequent analysis.

### 2.3 *Adult hinds 2008*

The remaining 27 hinds from the 2007 study were retained for further study during the 2008 breeding season as rising-three-year-old, non-parous hinds, representing W (n = 11), E (n = 7) and F1 (n = 9) genotypes. They were again grazed outdoors in the continuous presence of the vasectomised stag. Thrice weekly blood sampling occurred between 14 January and 6 October 2008, with a window of daily sampling occurring between 14 April and 23 May. Hinds were weighed fortnightly. No animal losses or health issues arose during the study period in 2008.

### 2.4 *Plasma progesterone analyses*

Progesterone concentrations in plasma were measured by a direct-addition microtitre plate enzymeimmunoassay (EIA) (Ridgeway Science; St Briavels, Glos., UK) based on the method of Sauer et

al. (1986). Assay sensitivity was assessed as 0.15 ng/ml and the inter- and intra-assay co-efficients of variation were 15% and 12%, respectively.

### 2.5 *Measurement of luteal parameters and statistical analyses*

From the longitudinal profiles of plasma progesterone from each hind, a set of summary statistics relating to luteal events was measured (after Asher et al., 2000). These included: (i) date of "silent" ovulation, (ii) date of first oestrus/viable ovulation, (iii) date of last oestrus/viable ovulation, (iv) duration of "silent" luteal cycle, (v) duration of the breeding season, (vi) number of full oestrous/luteal cycles, and (vii) length of individual oestrous/luteal cycles.

The summary statistics for each hind in each year were analysed by ANOVA, with animal as the blocking factor and genotype, age and their interaction as the treatment structure. The matrix of correlations between summary statistics within each age was calculated and assessed using the normal approximation. To assess whether there was any trend in cycle length throughout the season, luteal cycle lengths for individual hinds were analysed by random coefficient regression using residual maximum likelihood (REML) (Patterson and Thompson 1971). Random effects were given by cycle number nested within individual at each age, and fixed effects were given by genotype, age and their interaction, plus cycle number (having established that interactions between cycle number and genotype and age were not significant ($P>0.05$)).

## 3. Results

There were significant mean live-weight differences between genotypes throughout the study period (Figure 1), with F1 hinds the heaviest and W hinds the lightest at all times. Complete plasma progesterone profiles were obtained across two consecutive breeding seasons for 27 hinds, with a total of 35 short 'silent' cycles and 299 full oestrous cycles identifiable from the profiles. In addition, oestrous behaviours were observed on 65 occasions during yarding for blood sampling. All observations of oestrus coincided with nadirs (ie. <0.5 ng/ml) occurring in respective plasma progesterone profiles (e.g. Figure 2).

Four hinds (3 W and 1 E) failed to enter puberty in 2007, showing no evidence of luteal cyclicity during their first potential breeding season, although they all cycled in 2008 (Figure 3). Summary statistics for

incidences and mean parameters of luteal events are presented in Table 1. The numbers of oestrous cycles exhibited by individual hinds ranged from 0-7 as $R_2$s and 4-9 as adults (Figure 4). It is notable that the F1 hinds exhibited the least variation in number of oestrous cycles at both ages, (6-7 and 5-8 in $R_2$s and adults, respectively), whereas the greatest variation was for the W hinds (0-7 and 4-9 in $R_2$s and adults, respectively).

The onset and duration of the first 'silent' luteal cycle of the breeding season could not be reliably detected in all cyclic animals. Of those detected, the mean lengths ranged from 7.9 to 14.3 days, with a significant genotype effect favouring longer cycles in F1 hinds (Table 1). The mean date of first oestrus, recorded for 23 hinds in 2007 (85.2%) and all 27 hinds in 2008, was significantly earlier for F1 hinds at both ages. The mean dates of 11 April and 19 April in 2007 and 2008, respectively, were about 26 days earlier than for E and W hinds in $R_2$s and 5-10 days earlier than for E and W hinds, respectively, as adults ($P < 0.01$; Table 1). While the mean date of first oestrus was earlier for F1 hinds as $R_2$s than as adults (~ 9 days), for E and W hinds first oestrus was 7-12 days later as $R_2$s than as adults. The last oestrus of the breeding season was on average significantly later for F1 hinds in Year 1, with a 29 day difference between the F1 hinds and the other genotypes ($P < 0.01$). However, there was no effect of genotype on date of last oestrus in Year 2 ($P > 0.05$). Similarly, while there was a marked genotype effect on duration of the breeding season as $R_2$s favouring a 52-day longer season for F1 hinds ($P < 0.001$), there was only a 4-5 day difference as adults ($P > 0.05$). This was also reflected in the mean number of oestrous cycles exhibited (Table 1). It is noted that the dates of termination of oestrous cyclicity may have been under-estimated in four hinds as adults which exhibited evidence of on-going progesterone cycles beyond the termination of blood sampling in early October 2008 (see Figure 6).

Mean oestrous cycle length generally did not vary significantly between genotypes and ages, with overall averages ranging from 20.3–22.5 days (Table 1). There were occasional age x genotype interaction effects observed for some cycles (e.g. 3$^{rd}$ and 5$^{th}$ oestrous cycles, Table 1). There was a significant ($P < 0.05$) random coefficient regression of oestrous cycle length on cycle number, with a slope of 0.16 (SE 0.064) corresponding to an increase of about 1 day in cycle length over 6 cycles. Correlations between the various reproductive summary statistics and March live-weight showed that in $R_2$s live-weight was negatively related to the date of first oestrus ($r = -0.57$; $P<0.05$), while September live-weight in $R_2$s was

positively related to date of last oestrus (0.53; P<0.01), number of oestrous cycles (0.60; P<0.001) and duration of the breeding season (0.64; P < 0.001). However, no such relationships held for adults (P > 0.05).

Individual pubertal $R_2$ hinds generally exhibited fewer normal oestrous cycles than they did as adults (Figure 5). It was also notable that there was generally a higher incidence in observable aberrations within progesterone profiles for $R_2$s. As indicated earlier, 4 hinds remained acyclic (non-pubertal) as yearlings. Erratic patterns of progesterone secretion were observed for most pubertal hinds for 2-4 weeks from the start of the blood sampling regimen (Figures 3 and 5), this being interpreted as adrenal-sourced progesterone as a consequence of the stress of handling. Such erratic patterns of secretion seldom occurred at the start of blood sampling in Year 2. Additionally, one hind (W 75) was observed to exhibit oestrus at each blood sampling between 11 and 30 June in Year 1. This persistent oestrous behaviour was associated with an extended period of low plasma progesterone concentrations (Figure 6), and was interpreted as the possible presence of a persistent cystic, oestrogenic follicle. This event was not repeated in Year 2.

One hind (E 873) was notable for exhibiting unusually long oestrous cycles in both years, exhibiting only two full cycles, one of 30 days and the other of 42 days, as an $R_2$. As an adult the first and last recorded cycles out of a total of 5 oestrous cycles were >40 days in length (Figure 7). The presence of persistent corpora lutea at the end of the breeding season was indicated for one hind as a $R_2$ (Figure 8) and six hinds as adults. In these cases the final luteal event was not associated with any decline in plasma progesterone concentrations, which remained elevated to the end of the blood sampling period. While this occurred most commonly for F1 hinds as adults, it was also observed for single individuals of the other two genotypes.

## 4. **Discussion**

This study has highlighted a number of interesting issues about the processes of puberty in red deer hinds. Previous studies have demonstrated that the potential breeding season of pubertal (R2) hinds is

generally shorter than that of older hinds due to the later onset and earlier cessation of oestrous cycles, with fewer expressed cycles (Duckworth and Barrell 1992, Asher et al. 1997). This is in accord with generally held knowledge that the first calving period of hinds as 2-year-olds is usually 2-3 weeks later than for older hinds due to later conception timing (Beatson et al. 2000, Asher and Pearse 2002). However, the present study has demonstrated a number of anomalies related to puberty in red deer hinds. First, puberty failure, evidenced by failure to express any observable ovulatory and/or luteal activity for the entire duration of the potential breeding season, occurred in four hinds of Western (3) and Eastern (1) genotype, despite the attainment by individual hinds of sufficient body mass to enter the pubertal process (i.e. attainment of >70% of genotype-specific adult body mass; Kelly & Moore 1977, Asher et al. 2005a). Second, in addition to the incidences of puberty failure, these genotypes accounted for the shortest breeding periods (2-5 oestrous cycles). In contrast, the crossbred (F1) hinds all entered puberty in 2007 and expressed 6-7 oestrous cycles. The F1 hinds were notably earlier (by an average of 26 days) to initiate oestrous cycles and later (by an average of 29 days) to terminate oestrous cycles in 2007. These effects on the overall duration of the breeding season were all manifest in 2007, and had largely disappeared in 2008.

The difference in the pubertal breeding season of the F1 hinds relative to the other genotypes was unexpected. Recent studies have shown that the conception date trait in red deer hinds is modestly heritable ($h^2 \sim 0.2$) and there appears to be a breed component favouring earlier conception amongst Eastern genotypes (J.A.Archer unpublished data). Therefore, it was anticipated that the Eastern genotype hinds would initiate cyclic activity earlier than the other genotypes. While it is tempting to attribute the observed results to a direct effect of genotype, favouring the F1 hinds, this is in contrast with earlier observations on a commercial deer farm in which E hinds were considerably earlier to conceive than both W and F1 hinds which had similar conception patterns (Scott et al. 2006). However, in the present study there are confounding effects of live-weight and animal origins to be considered. Clearly, the crossbred hinds were considerably heavier than the other two purebred genotypes. This may partly reflect genetics (eg. a heterotic effect) but also likely reflects their origins, having been born and raised on the research facility. Thus, they were considered by their handlers to be in better physical condition (as reflected in body condition scores) and to be better habituated to the yarding and restraint procedures employed during the study. This latter point is perhaps reflected in a lower incidence of erratic progesterone

secretion for this genotype at the start of the study. The overall association between live-weight and seasonality parameters in $R_2$s also lends some support to a strong influence of the environment on the timing and duration of the pubertal breeding season. We also contend that the stresses of adapting and habituating to a novel farm environment may have influenced the pubertal process, including incidence and seasonal timing, in the purebred genotypes. The high incidences of erratic progesterone secretion for W and E genotype hinds at the start of the trial probably reflected adrenal sources of secretion as a response to acute stress to novel stimuli, as demonstrated previously for red deer (Jopson et al. 1990, Asher et al. 2000) and fallow deer, *Dama dama* (Asher et al. 1989), and may in themselves have strongly influenced reproductive processes through hormonal negative feedback control of hypothalamic/pituitary function (Asher et al. 2000). Whether other aberrant cyclic events are indicative of perturbation of pubertal processes remains to be seen. Certainly, the stand-alone occurrence of persistent oestrus in one pubertal W hind does not indicate a trend. Also, the incidences of persistent corpora lutea, described previously for red deer (Asher et al. 2000), were not confined to $R_2$s, and in fact occurred more commonly in adult hinds.

It does appear that the pubertal process in $R_2$ hinds is perhaps less robust than seasonal cycles in older hinds, and may be particularly susceptible to perturbations from the environment. This argument has been raised previously in relation to high levels of puberty failure, which manifest as low early pregnancy rates in $R_2$ hinds. It has been contended that young hinds reared under low stocking intensities within complex vegetation environments (e.g. New Zealand high-country zones) reputably express higher incidences of puberty and overall reproductive performance than those raised under highly intensive lowland systems at high stocking intensities; the differences being attributable to different levels of acute and chronic stress encountered by the young hinds (Asher and Pearse 2002). While this contention is supported by many producers on the basis that it is intuitive given the behavioural traits of red deer, it is as yet unsupported by a strong objective data set that allows factors such as genotype and other management variables to be evaluated. Studies by Asher et al. (2005a) demonstrated a marked effect of introgression of Wapiti (subspecies *nelsoni, roosevelti* and *manitobensis*) on the incidences of puberty failure across a number of commercial deer farms in New Zealand. This was largely explained by failure of individual hinds with high levels of Wapiti parentage to attain sufficient genotype-specific body mass to enter puberty as rising-two-year-olds. So far, this is the only objective qualification of the influence of

genotype on puberty. In that case it was on the incidence of puberty rather the seasonal timing of its onset. The influence of the introgression of other genotypes on pubertal processes has not yet been evaluated.

It is interesting to note that the F1 hinds in the present study actually initiated cyclic activity 9 days earlier as yearlings than in the subsequent year as adults. In contrast, the Eastern and Western genotype hinds initiated pubertal cycles 7-12 days later than cycle initiation as adults, this being the expected situation (Audige et al. 1999, Asher and Pearse 2002). The reason for this difference between genotypes is unclear. However, irrespective of the differences observed between genotypes and years in the study, it should be noted that the overall timing of the onset of oestrous activity was considerably later than anticipated based on our prior knowledge of conception patterns of red deer genotypes in New Zealand, particularly for adult hinds in 2008. Ultrasonographic scanning of first trimester fetuses/conceptuses has been used frequently to assess fetal age (and hence conception date) based on well-calibrated fetal growth trends and morphological development (White et al. 1989, Revol and Wilson 1991). Recent studies have demonstrated the estimated patterns of conceptions of red deer hinds based on fetal age assessment, and have generally shown that within populations of farmed red deer in New Zealand, adult hinds generally conceive to first oestrus matings from early March to early April, depending upon the actual timing of stag introduction (Beatson et al. 2000, Scott et al. 2006). However, adult hinds in the present study did not initiate cyclic activity until mid to late April in both years, with some hinds not exhibiting their first oestrus until early-mid May. This was also the case for an earlier study conducted within the same facilities with adult red deer hinds of three different genotypes, pure red deer, F1 red deer x wapiti crossbreds and red deer x Pere David's deer crossbreds (Asher et al. 2000). As with the present study, hinds were run with a vasectomised stag for the duration of the potential breeding season and blood sampled at thrice-weekly intervals over this period. The red deer hinds initiated oestrous cycles in mid-April, with the crossbred genotypes being slightly earlier in early April. Interestingly, detailed calving records over the last 10 years on the Invermay breeding unit, the source herd for deer in the study of Asher et al. (2000) and for the F1 hinds in the present study, indicates that, on the basis of a 234-day gestation interval, most hinds generally conceive between late March and early April each year. Although some consideration needs to be given to the unreliability of retrospective assignment of conception date based on calving date due to the environmental plasticity of the gestation interval in red deer (Asher et al.

2005b, Asher 2006, Scott et al. 2008), fetal age scanning of the source herd in 2008 (which included F1 hinds of the same cohort) also showed the earlier conception pattern. The reasons for the apparent discrepancy between the study population and the source population are unclear. One consideration is that maintaining the hinds in a non-pregnant/non-lactating state in 2007 may have influenced the timing of the onset of the 2008 breeding season, but the potential mechanisms behind this are not obvious. Also, the hinds in the earlier study of Asher et al. (2000) exhibited a relatively late entry into their breeding season despite having gestated and reared calves in the previous season. Another important consideration is that the chronic stress associated with repeated yarding and blood sampling of hinds may have impacted on the timing of the onset of oestrous cyclicity. While this appeared to be manifest as erratic fluctuations in plasma progesterone concentrations at the start of the blood sampling regimen in 2007, it is vexing that this was not a feature of progesterone profiles in adult hinds. In fact, handlers considered that all hinds in the study appeared tame and well-habituated to such handling by the start of the regimen in 2008. If indeed the stress of handling was the causal factor behind the late entry into the reproductive season by the adult hinds in 2008, it clearly operated beyond the level detectable from the progesterone profiles and likely involved pathways other than adrenal progesterone secretion. While climatic factors are thought to influence the precise timing of the start of the breeding season in red deer, including putative effects of ambient temperature (Fisher and Johnson 2002), it is difficult to understand how they could have influenced the timing of oestrous cycles in the current trial without affecting other groups of deer nearby. If the apparent late timing of the breeding season in 2008 is actually an artefact of the study methodology, it is reasonable to assume that it has masked the true seasonal nature of the onset of the breeding season. For example, while previous studies have shown that hinds of Eastern genotype can express traits for earlier oestrus onset (Scott et al. 2006) this was not evident in the present study.

The occurrence of ‘silent ovulations’ and short-lived corpora lutea preceding first overt oestrus of the breeding season is well established for red deer (Jopson et al. 1990, Asher et al. 2000) and other cervid species (Curlewis et al. 1988, Asher 1985, Shipka et al. 2007). Most, but not all, hinds in the present study showed clear evidence of ‘silent ovulations’ preceding first oestrus. The ability to reliably detect short-term and low-magnitude plasma progesterone elevations was probably compromised due to sampling imprecision. Some earlier studies on red deer also failed to detect the progesterone output from

silent ovulations (Guinness et al. 1971, Adam et al. 1985), which likely reflects measurement imprecision rather than failure of hinds to exhibit such events.

The overall mean length of the oestrous cycle of around 21.3 days recorded across genotypes and years is slightly longer than has been observed in other studies (18.3-19.6 days; Guinness et al. 1971, Krzywinski and Jaczewinski 1978, Asher et al. 2000), but its estimation in the present study based on thrice-weekly plasma progesterone concentrations generates a degree of measurement imprecision, and the influence of a number of unusually long oestrous cycles (>28 days) was to slightly inflate the average length. Of 299 oestrous cycles observed in the present study, the modal length was 19 days, which accords well with the previous studies. As with previous studies on oestrous cyclicity in red deer (Guinness et al. 1971, Asher et al. 2000), there was a significant increase in oestrous cycle length as the breeding season progressed.

Evidence of persistent corpora lutea at the end of the breeding season of one hind in 2007 and six hinds in 2008 indicates that this is a relatively common phenomenon in red deer. Meikle and Fisher (1996) observed evidence of persistent luteal tissue during anoestrus in two out of eight red deer hinds and Asher et al. (2000) showed similar evidence for four out of nine red deer hinds. Clearly, luteal insufficiency is not a mandatory feature of anoestrus in red deer, as data across two studies have demonstrated that luteal tissue can persist for almost the entire duration of anoestrum. However, as evidenced in these studies by the sudden demise of the persistent corpora lutea immediately before the onset of the next breeding season, the transition from anoestrus to oestrus involves reinstatement of the luteolytic process. Therefore, the presence of persistent luteal tissue does not seemingly interfere with the seasonal breeding process.

In conclusion, this study has demonstrated that puberty in red deer hinds is generally associated with a shorter potential breeding season than for adult hinds, and that perturbation of breeding activity appears to be quite common, leading to incidences of puberty failure and possibly other aberrant cyclic events. Live-weight x genotype interactions may influence puberty processes but do not appear to be as strongly expressed in adults. However, the relatively late onset of oestrous cyclicity in the adult hinds may be an artefact of the study that has masked the true nature of genetic influences on seasonal breeding patterns.

**Acknowledgements**

This project was jointly funded by DEEResearch Ltd and the Foundation of Research, Science and Technology ( FRST) (Contract C10X0709).

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**Figure Captions**

**Figure 1:** Mean live-weight profiles of Western (W), Eastern (E) and crossbred (F1) red deer hinds over the two year period spanning blood sampling in 2007 and 2008 (horizontal bars).Vertical bars represent the s.e.d.

**Figure 2:** Plasma progesterone profiles for the 2007 and 2008 breeding seasons of a crossbred (F1) hind (#5021) which in both years exhibited clear evidence of at least one 'silent' ovulation before the onset of the breeding season, and seven full oestrous cycles. The horizontal bar represents the delineation of each oestrous cycle, with durations (days) indicated for each cycle. Arrows indicate dates for which oestrous behaviour was observed during yarding.

**Figure 3:** Plasma progesterone profiles for the 2007 and 2008 breeding seasons of a Western (W) hind (#76) that failed to exhibit any luteal cycles (ie. did not enter puberty) in 2007 but expressed seven normal oestrous cycles in 2008. The horizontal bar represents the delineation of each oestrous cycle, with durations (days) indicated for each cycle.

**Figure 4:** Frequency histogram of the number of oestrous cycles exhibited by individual hinds in 2007 (yearlings) and 2008 (adults).

**Figure 5:** Plasma progesterone profiles for the 2007 and 2008 breeding seasons of a Western (W) hind (#77) that exhibited three oestrous cycles in 2007 and seven oestrous cycles in 2008. Note the erratic elevations in progesterone concentrations at the start of blood sampling in 2007, interpreted as being of adrenal origin due to handling stress. The horizontal bar represents the delineation of each oestrous cycle, with duration (days) indicated for each cycle. Arrows indicate dates for which oestrous behaviour was observed during yarding.

**Figure 6:** Plasma progesterone profiles for the 2007 and 2008 breeding seasons of a Western (W) hind (#75) that exhibited persistent oestrous behaviour between 11 and 30 June 2007. This was associated with an extended period of minimal progesterone secretion, which may indicate the presence of a cystic, oestrogenic follicle. The hind exhibited nine normal oestrous cycles in 2008. The horizontal bar

represents the delineation of each oestrous cycle, with durations (days) indicated for each cycle. Arrows indicate dates for which oestrous behaviour was observed during yarding.

**Figure 7:** Plasma progesterone profiles for the 2007 and 2008 breeding seasons of an Eastern (E) hind (#873) that exhibited unusually long (> 28 days) oestrous cycles in both years. In 2007 only two long cycles occurred but in 2008 two long cycles occurred either side of three normal oestrous cycles (note: this hind may still have been cycling at the termination of blood sampling in 2008). The horizontal bar represents the delineation of each oestrous cycle, with durations (days) indicated for each cycle.

**Figure 8:** Plasma progesterone profiles for the 2007 and 2008 breeding seasons of an Eastern (E) hind (#930) that exhibited evidence of a persistent corpus luteum at the end of the breeding season in 2007. Note the extended period of elevated progesterone concentrations occurring up to the cessation of blood sampling in early September. The horizontal bar represents the delineation of each oestrous cycle, with durations (days) indicated for each cycle. Arrows indicate dates for which oestrous behaviour was observed during yarding.

**Figure 1**

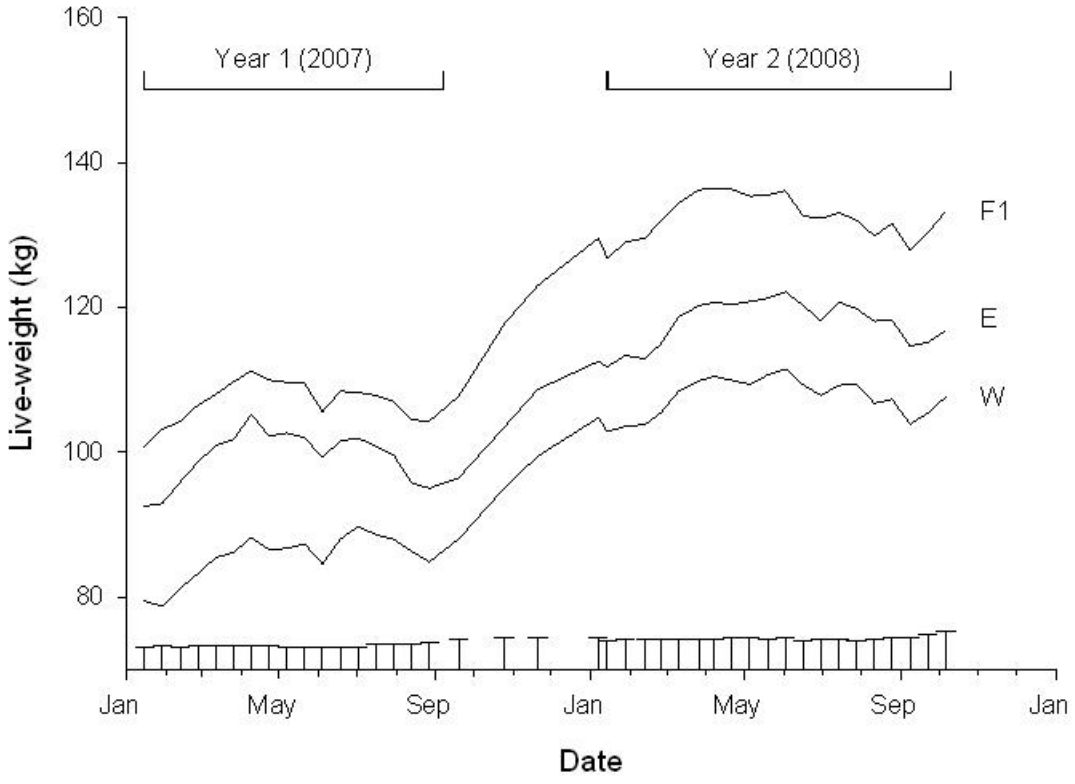
160
140
120
100
80
Year 1 (2007)
Year 2 (2008)
F1
E
W
Live-weight (kg)
Jan
May
Sep
Jan
May
Sep
Jan
Date

**Figure 2**

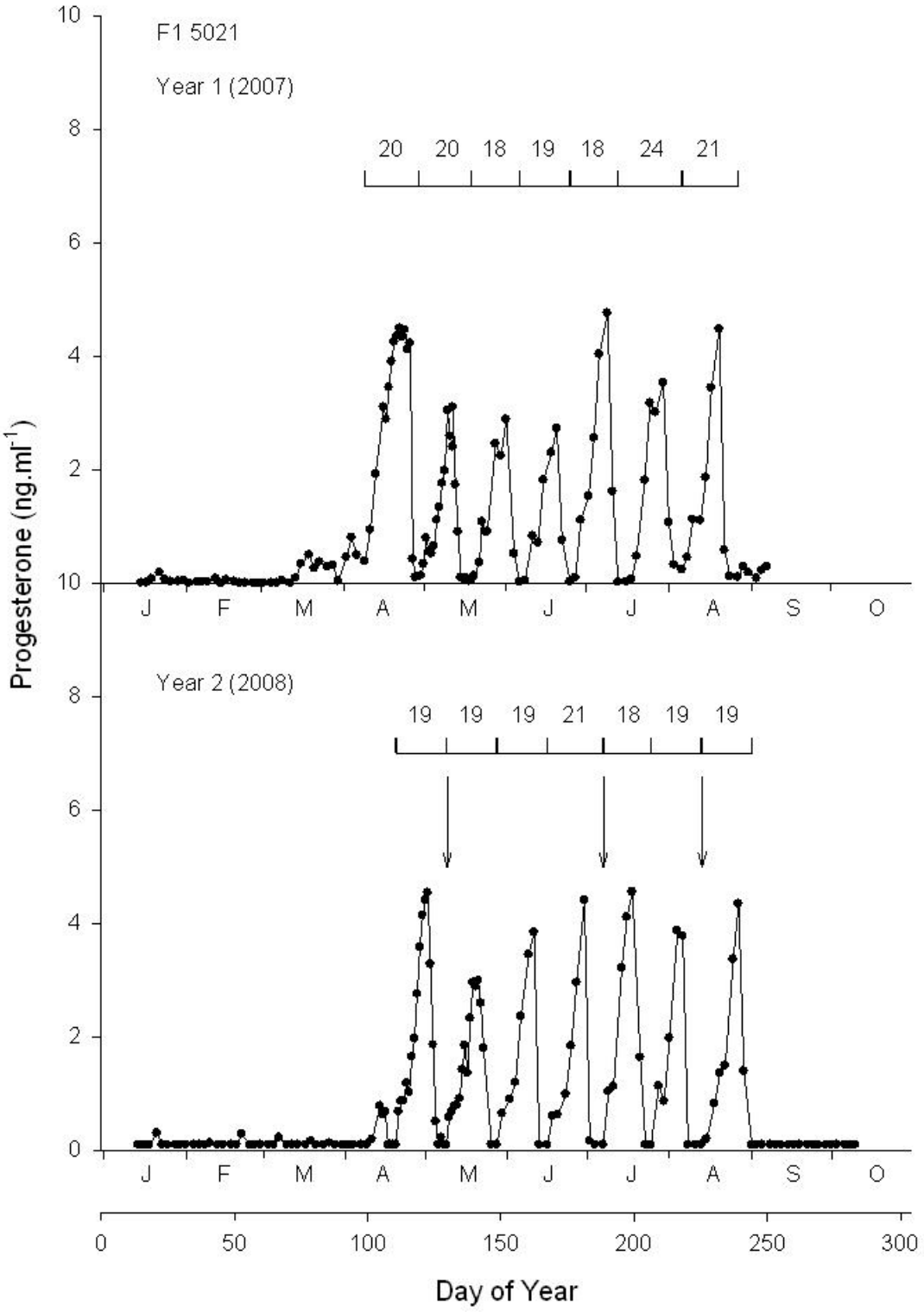
F1 5021
Year 1 (2007)
20 20 18 19 18 24 21
Year 2 (2008)
19 19 19 21 18 19 19
Progesterone (ng.ml-1)
J F M A M J J A S O
Day of Year
0 50 100 150 200 250 300

**Figure 3**

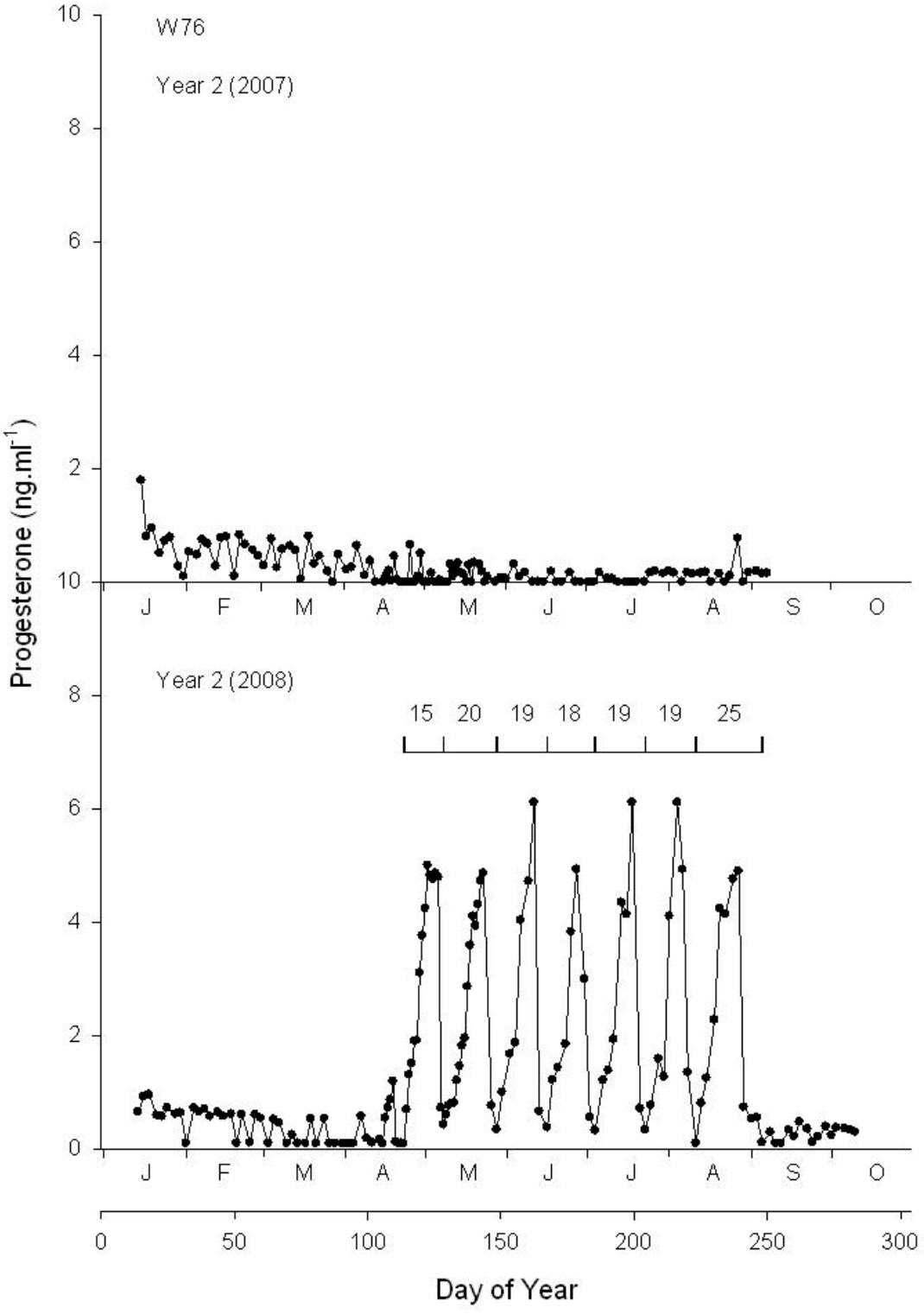
W76
Year 2 (2007)
Year 2 (2008)
15 20 19 18 19 19 25
Progesterone (ng.ml-1)
J F M A M J J A S O
Day of Year
0 50 100 150 200 250 300

**Figure 4**

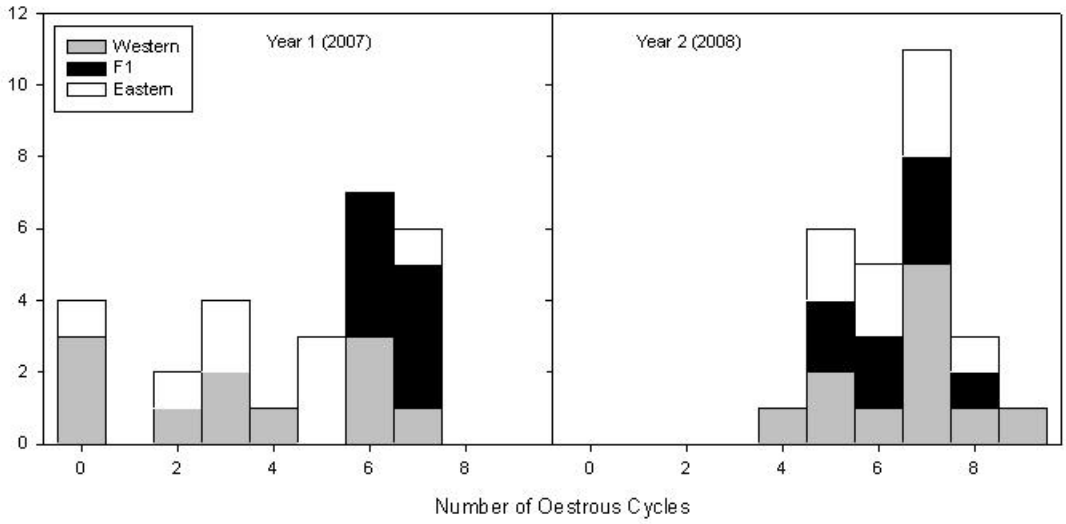
Western
F1
Eastern
Year 1 (2007)
Year 2 (2008)
12
10
8
6
4
2
0
0
2
4
6
8
0
2
4
6
8
Number of Oestrous Cycles

**Figure 5**

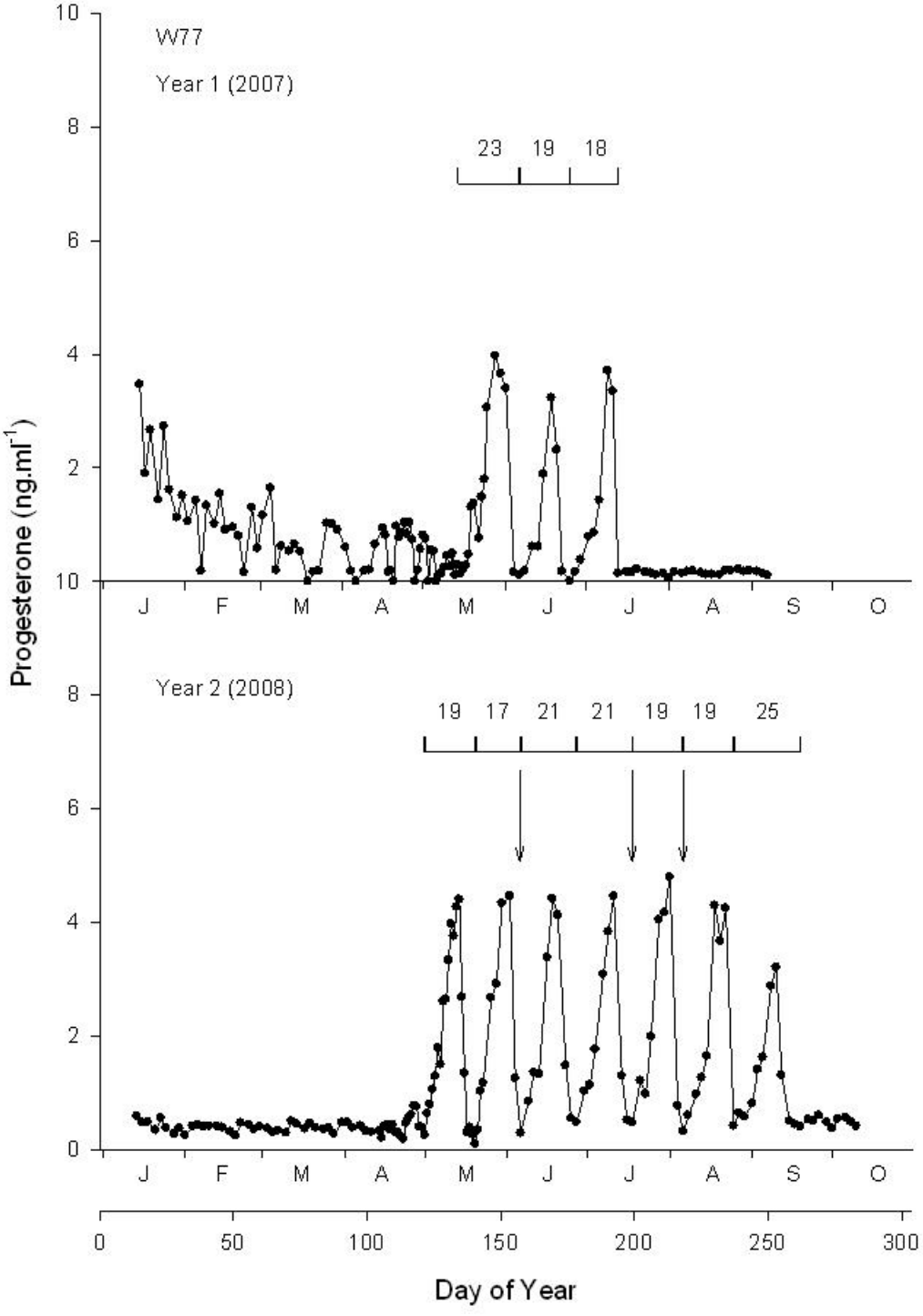
W77
Year 1 (2007)
23 19 18
Year 2 (2008)
19 17 21 21 19 19 25
Progesterone (ng.ml-1)
J F M A M J J A S O
Day of Year
0 50 100 150 200 250 300

**Figure 6**

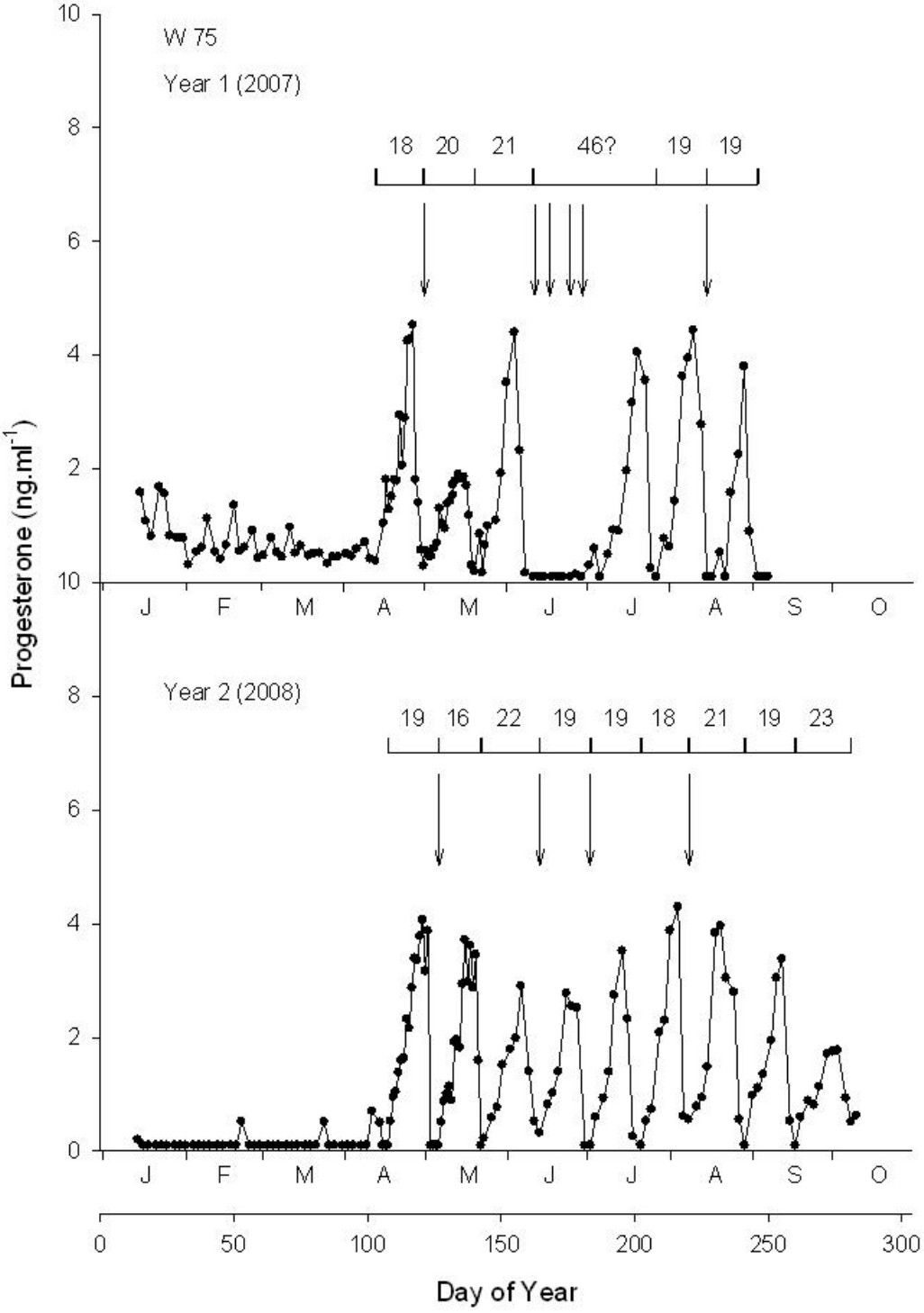
W 75
Year 1 (2007)
18 20 21 46? 19 19
Year 2 (2008)
19 16 22 19 19 18 21 19 23
Progesterone (ng.ml-1)
J F M A M J J A S O
Day of Year
0 50 100 150 200 250 300

**Figure 7**

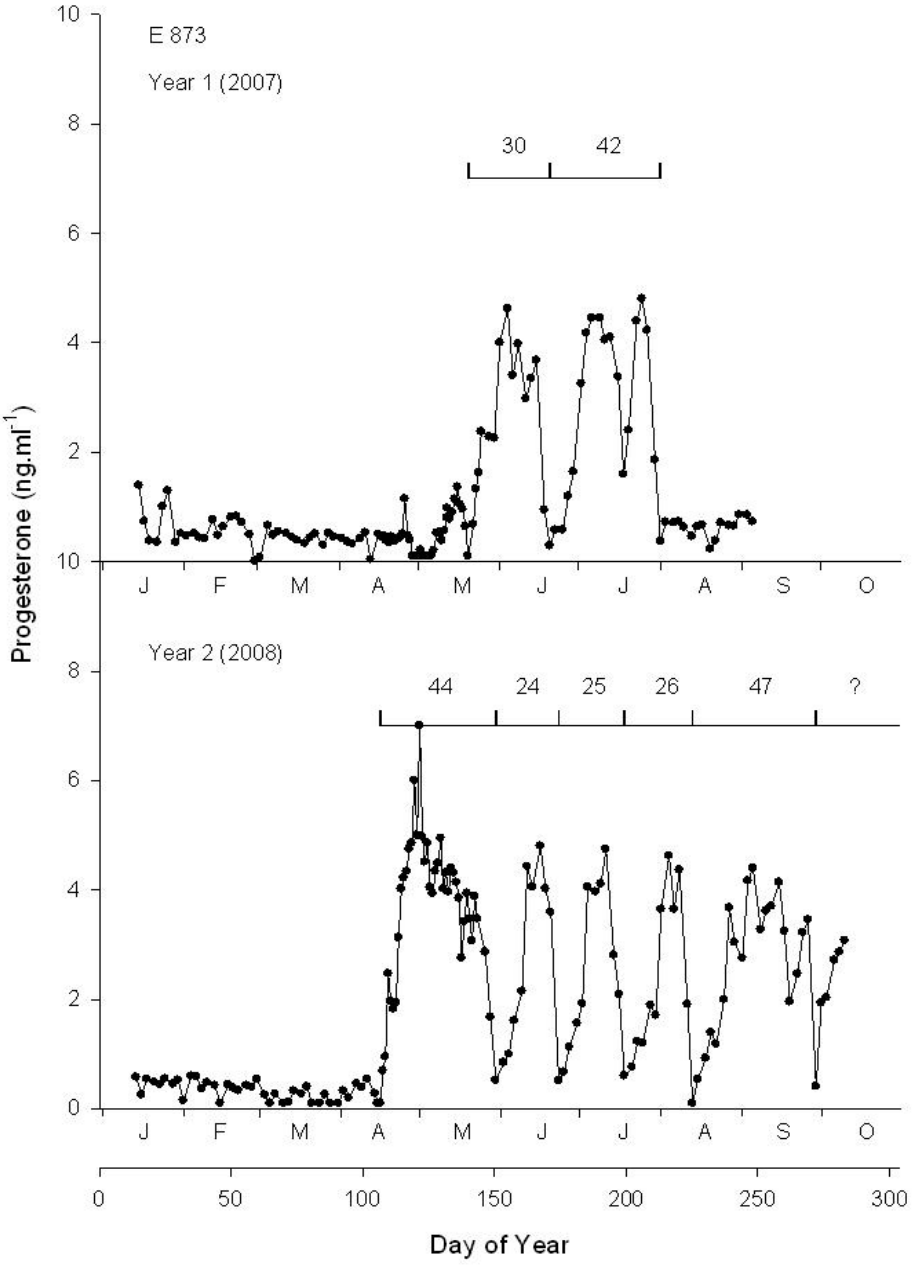
E 873
Year 1 (2007)
30
42
Year 2 (2008)
44
24
25
26
47
?
Progesterone (ng.ml-1)
J
F
M
A
M
J
J
A
S
O
0
50
100
150
200
250
300
Day of Year

**Figure 8**

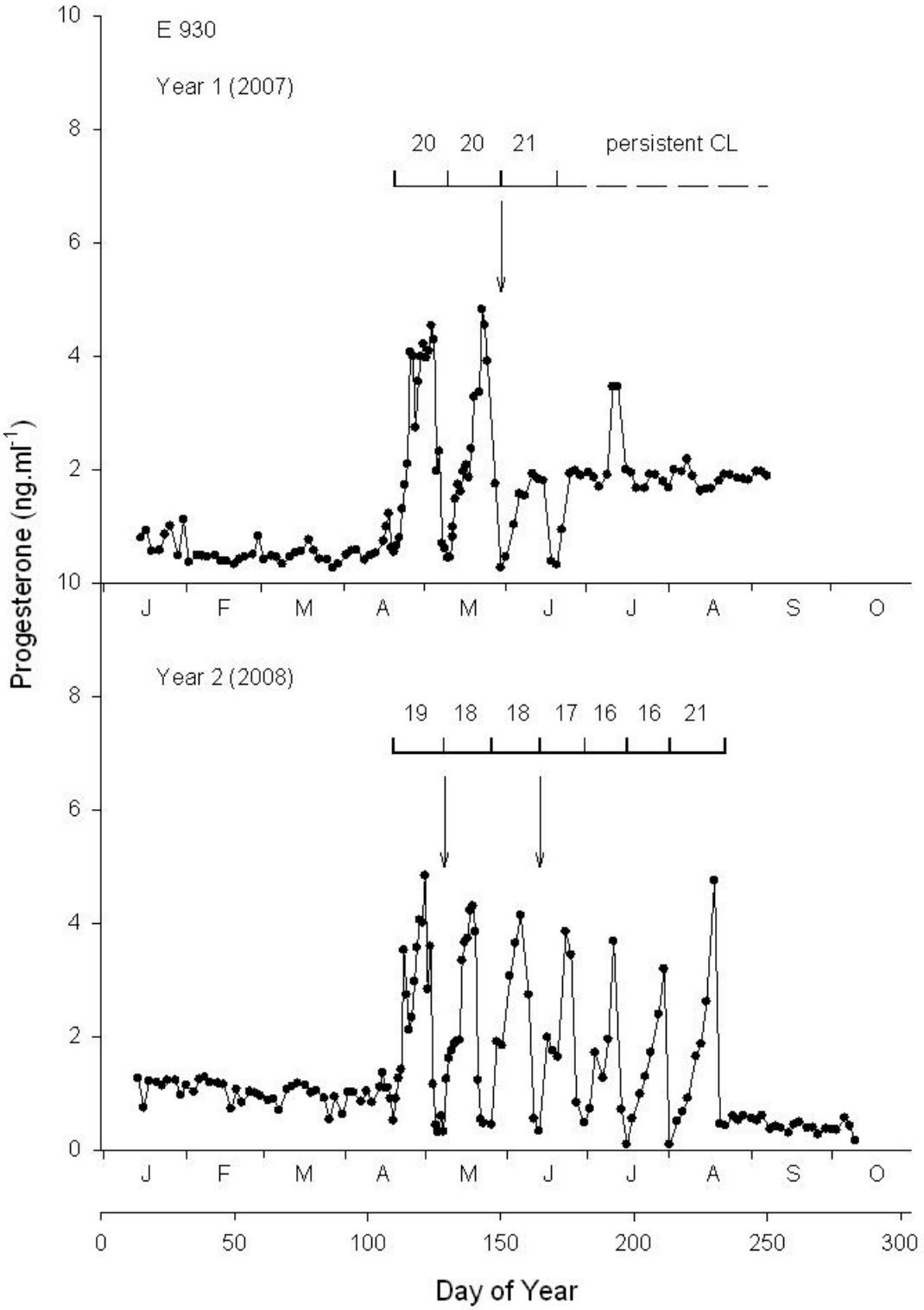
E 930
Year 1 (2007)
20 20 21 persistent CL
Year 2 (2008)
19 18 18 17 16 16 21
Progesterone (ng.ml-1)
J F M A M J J A S O
Day of Year
0 50 100 150 200 250 300

**Table 1**: Summary statistics for luteal cycles and duration of breeding season for red deer hinds of Eastern (E), Western (W) and crossbred ($F_1$) genotypes for 2007 (yearlings) and 2008 ( adults)

| Year | Genotype | n | n acyclic | Means | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Day of silent ovulation | Duration of silent ovulation | Day of first oestrus | Day of last oestrus | Number of oestrous cycles | Duration of breeding season (days) | 1st oestrous cycle length (days) | 2nd oestrous cycle (days) | 3rd oestrous cycle (days) | 4th oestrous cycle (days) | 5th oestrous cycle (days) | Total oestrous cycles (days) |
| 1 | E | 8 | 1 | 112.6 | 10.6 | 126.8 | 213.2 | 3.63 | 86.4 | 20.2 | 22.2 | 25.8 | 17.1 | 27.5 | 22.5 |
| | $F_1$ | 8 | 0 | 89.7 | 14.3 | 100.9 | 241.9 | 6.50 | 138.6 | 22.5 | 21.5 | 20.5 | 21.9 | 21.4 | 21.7 |
| | W | 11 | 3 | 121.7 | 9.9 | 127.4 | 213.4 | 3.27 | 86.0 | 20.0 | 19.7 | 19.7 | 24.9 | 20.2 | 20.7 |
| 2 | E | 8 | 0 | 96.4 | 9.6 | 115.4 | 248.8 | 6.38 | 133.4 | 21.9 | 19.8 | 21.4 | 20.9 | 22.5 | 21.3 |
| | $F_1$ | 8 | 0 | 105.1 | 9.8 | 109.8 | 247.4 | 6.38 | 137.0 | 19.6 | 22.1 | 22.0 | 21.3 | 22.3 | 21.8 |
| | W | 11 | 0 | 112.3 | 7.9 | 119.5 | 251.3 | 6.55 | 131.7 | 18.9 | 19.9 | 20.3 | 20.9 | 19.4 | 20.3 |
| Signif. | s.e.d. | | | 8.5 | 1.9 | 6.9 | 10.3 | 0.86 | 13.5 | 2.5 | 2.2 | 1.4 | 2.6 | 3.1 | 1.5 |
| | genotype effect | | | * | * | ** | ns | * | * | ns | ns | 0.05 | ns | ns | ns |
| | age effect | | | ns | * | ns | ** | *** | *** | ns | ns | ns | ns | ** | ns |
| | age x genotype effect | | | ns | ns | ** | * | *** | ** | ns | ns | *** | ns | *** | ns |

ns not significant
* P<0.05
** P<0.01
*** P<0.001