
VOLUME 62 2002

Puberty in red deer hinds: (1) onset of luteal and mating activity

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ABSTRACT

Pregnancy rate of yearling (15-18 months) red deer hinds may be influenced by time that puberty is attained, especially if stags are removed before all hinds have had a chance to conceive. This paper reports on two studies that investigated onset of puberty in red deer hinds. In Study 1, ovarian follicle populations and luteal activity of pubertal and adult red deer hinds were monitored for three months during their transition into the breeding season. Yearling (n = 5) and adult (n = 5) hinds were grazed in one group and weighed monthly. A fertile stag was run with them from 20 March until 11 May. Yearling hinds were significantly lighter than adults in March (85.8 vs 91.3 kg, SED = 2.1). There were no significant differences between yearling and adult hinds in mean day of first (14 April vs 8 April, SED = 3.5) or second (23 April vs 16 April, SED = 3.7) ovulations, or interval between ovulations (9 vs 8 days, SED = 0.7). First ovulation was invariably followed by a transient rise in plasma progesterone concentration and then a second ovulation preceded by oestrus. All hinds conceived to their first mating, demonstrating the ability of red deer hinds to exhibit high conception rates. In Study 2, daily observations for mating activity of yearling (n = 80) and adult (n = 80) hinds were carried out continuously from dawn until dusk for the period 25 March-27 April. Hinds were grazed on pasture in one of five single sire mating groups: Gp1, adults only (n = 40); Gp2, yearlings only (n = 40); Gp3, adults (n = 20) + yearlings (n = 20); Gp4, adults (n = 10) + yearlings (n = 10); Gp5, adults (n = 10) + yearlings (n = 10). Hinds were scanned by rectal ultrasonography to determine pregnancy status and estimate fetal age. Yearling hinds were significantly lighter than adults in March (101 vs 119 kg, SED = 1.3). The stag in Gp3 was replaced due to exhaustion and consequently, mean conception date of yearlings in that group was 16 days later than that of the yearlings in Gps 2,4 & 5, which had a mean conception date 12 days later than that of the adults (1 May vs 15 April vs 3 April, SED = 1.1; $P < 0.001$). There was no significant difference between age groups in the number of non-pregnant hinds. These data indicate that yearling red deer hinds reach puberty and are mated in mid-April, about two weeks later than adult hinds. Conception failure was not a limiting factor to yearling reproductive performance; therefore, under standard farming conditions, it is unlikely that stags will be removed from the mating mob before yearling hinds have had a chance to conceive.

Keywords: red deer hinds; puberty; reproduction.

INTRODUCTION

Reproductive performance of yearling (15-18 months) red deer hinds has been identified as a major determinant of deer farming productivity in New Zealand, and seems to vary greatly from farm to farm. Pregnancy rate of yearling hinds (as assessed from ultrasound scanning results) has been reported to range from less than 50% to 100% (Audigé *et al.*, 1999a). Industry statistics (collected from eighteen farms that participated in producing the Deer Industry Manual) indicate an average yearling pregnancy rate between 77 and 86% (Beatson *et al.*, 2000). Furthermore, yearling hinds conceived on average 17 days later than mixed age hinds.

The minimum threshold live weight for red deer hinds to attain puberty was established by Kelly and Moore (1977) as being 65 kg, or at least 65-70% of their mature live weight. This is unlikely to be a limiting factor, as average pre-mating live weights of greater than 80 kg are usually attained (Beatson *et al.*, 2000). It is not known if poor reproductive performance in yearling hinds is associated with hinds failing to begin reproductive cycles in time to be mated, or if they are in fact cycling but fail to conceive.

The aim of the present study was to monitor the onset of cyclic ovarian and mating activity and the subsequent estimated conception date in red deer yearling and adult hinds.

MATERIALS AND METHODS

Animals and treatments

This study was conducted at Invermay Agricultural Centre (latitude 45^{o} 51′ S) and approved by the AgResearch Invermay Animal Ethics Committee.

Study one: Five yearling and five weaned adult red deer hinds were grazed outdoors on mixed ryegrass/white clover pasture as a single group from 5 March until 4 May. Live weight was recorded monthly during this period and their ovaries were examined by rectal ultrasonography to monitor ovarian follicle populations every Monday, Wednesday and Friday. Blood samples were obtained by jugular venipuncture at the same time. After centrifugation, the plasma fraction was decanted into labelled tubes and stored at -10°C until assayed to measure progesterone concentration. A fertile stag was run with the hinds from 20 March until 11 May. Hinds were scanned by rectal ultrasonography to determine pregnancy status and estimate fetal age in mid-June.

Study two: Adult and yearling red deer hinds were grazed on mixed ryegrass/clover pasture in paddocks that were visible from an observation platform. Hinds were allocated to one of five single sire mating groups: Gp1, adults only (n = 40); Gp2, yearlings only (n = 40); Gp3, adults (n = 20) + yearlings (n = 20) with stag replacement 23 April; Gp4, adults (n = 10) + yearlings (n = 10); Gp5, adults (n = 10) + yearlings (n = 10). Daily observations for mating activity were carried out continuously from dawn until dusk for the period 25 March to 27 April. Hinds were scanned by rectal ultrasonography to determine pregnancy status and estimate fetal age in mid- (adults) or late- (yearlings) May. When results from the first scan were inconclusive, hinds underwent a second scan three weeks later.

Ultrasonography

A single operator using a 7.5 MHz linear array transducer (Aloka SSD 500; Aloka Co. Ltd., Japan) performed all the rectal ultrasonographic diagnoses. During ultrasonography, hinds were restrained individually in an upright position in a pneumatic crush. A liberal coating of carboxymethylcellulose was applied to the transducer before it was inserted carefully into the rectum until an echo-image of the bladder was observed. The transducer was then gently rotated 90° clockwise and 180° counter-clockwise while being moved forward until the ovaries were located. The diameters of all luteal structures and follicles ≥ 3 mm were measured using the inbuilt scanner callipers, and their position and size were recorded on an ovarian map. Fetal age was estimated by measuring fetal size according to the method of Revol and Wilson, (1991).

Hormone analyses

Plasma progesterone concentrations were measured in duplicate by direct radioimmunoassay. All samples were included in a single assay that has been previously validated for red deer plasma (Asher, 1990). Control samples with low, medium and high concentrations of progesterone were included at frequent intervals within the assay. The intra-assay coefficients of variation were 6.1% for the low control (mean 2.00 ng/ml), 7.1% for the medium control (mean 3.48 ng/ml) and 8.2% for the high control (mean 9.45 ng/ml) samples. Sensitivity of the standard curve, defined as the first point that was significantly different from 0, was 0.15 ng/ml.

Statistical analyses

Study 1: Live weight data and summary statistics for ovulation and progesterone data were analysed by analysis of variance (ANOVA), fitting age group. The pattern of change in progesterone concentration following the second ovulation was clearly linear and was analysed by ANOVA of the linear contrast over time.

For each observation, the ovarian follicle populations from both ovaries of each hind were categorised according to the following five categories: (1) new recruits, defined as appearance of a follicle that was not present at the previous observation; (2) 3 mm; (3) 4-6 mm; (4) > 6 mm; (5) follicle regressed, defined as diminished size or disappearance of a large follicle that was present the previous observation. Each category was then entered into a first-order five-state Markov chain. Follicles could not be individually identified between times, but the number of follicles in each size class (states 2, 3 and 4) at each time was known. Therefore, a rule was devised to allocate follicles to transitions between times, such that (a) follicles could not move to a numerically lower state and (b) a smaller follicle could not overtake a larger follicle. This enabled the calculation of a transition matrix for the ovaries of each hind. These matrices were aggregated for each age group, and age groups were compared by maximum likelihood using the multinomial distribution for transitions from each state.

Study 2: Having established that the random effect of group was negligible, mating and conception dates were analysed by least squares, fitting age group (adult vs yearling). A second sire had to be used in Group 3 after the adults had finished mating, therefore Group 3 yearlings were in a separate category. Conception date was then regressed on hind live weight, allowing separate slopes and intercepts for each group.

RESULTS

Study 1

Live weight

One yearling hind repeatedly avoided capture in the crush so was removed from the study after three weeks.

Yearling hinds were significantly ($P < 0.05$) lighter on average than adults at the start of the study (85.8 vs 91.3 kg, SED = 2.1) and gained a similar amount of live weight over the three months of the study (3.8 vs 3.9 kg, SED = 0.9).

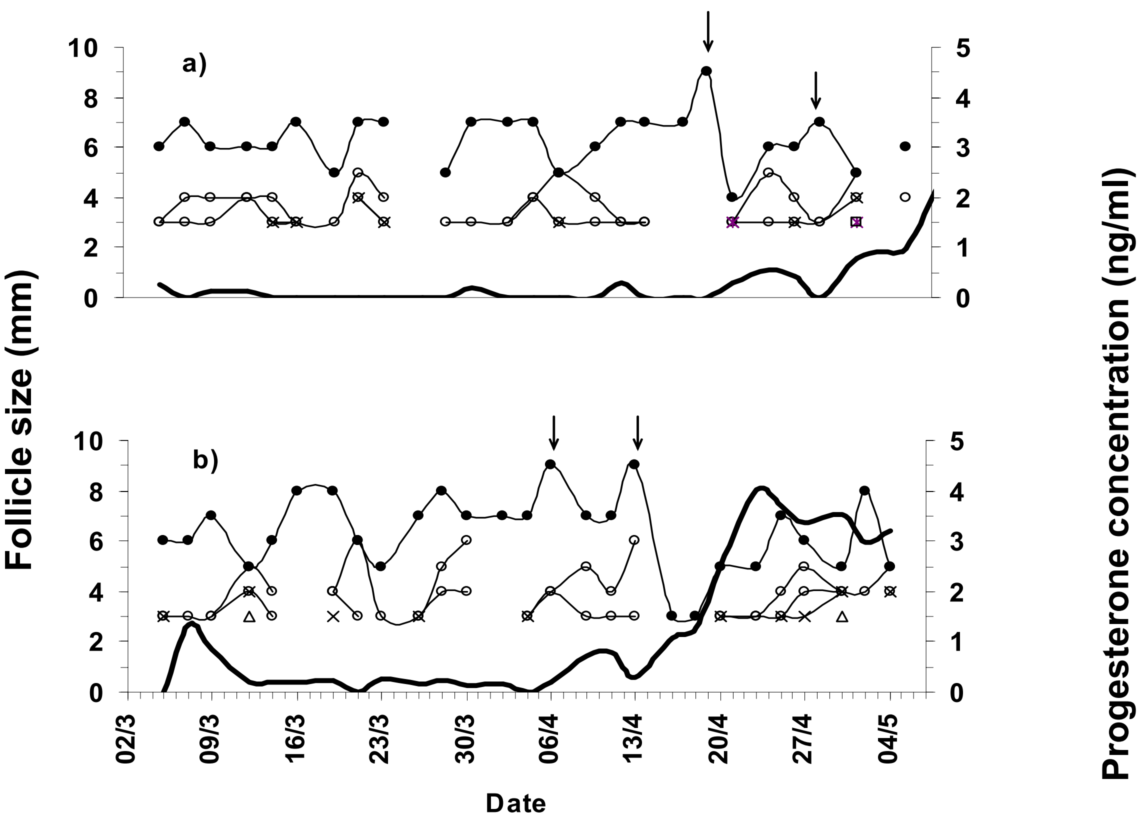
Follicular dynamics

All nine hinds had regular patterns of a dominant follicle growing to 6-9 mm diameter before regressing or suddenly disappearing. There were no significant differences between age groups in mean number and size of follicles present at each observation or their transition between size categories (Table 1).

TABLE 1: Estimated probability matrices between follicle size-classes and states for yearling and adult hinds

Yearling						
		To:				
		new recruit	3 mm	4-6 mm	> 6 mm	regressed
From:	new recruit	0.00	0.82	0.15	0.04	0.00
	3 mm	0.00	0.27	0.28	0.18	0.26
	4-6 mm	0.00	0.00	0.18	0.23	0.59
	> 6 mm	0.00	0.00	0.00	0.39	0.61
	regressed	0.00	0.00	0.00	0.00	1.00
Adult						
		To:				
		new recruit	3 mm	4-6 mm	> 6 mm	regressed
From:	new recruit	0.00	0.76	0.21	0.04	0.00
	3 mm	0.00	0.31	0.23	0.16	0.31
	4-6 mm	0.00	0.00	0.18	0.24	0.59
	> 6 mm	0.00	0.00	0.00	0.35	0.65
	regressed	0.00	0.00	0.00	0.00	1.00

FIGURE 1: Profiles for (a) a yearling and (b) an adult hind of the size of the largest (●) and subordinate (○) follicles relative to plasma progesterone concentration (solid line) during ultrasonographic monitoring over the transition phase and into the breeding season. ↓ indicates last day a follicle was detected before ovulation.



Ovulation, confirmed from progesterone profiles, was characterised by the disappearance of a large (≥ 6 mm) follicle (Figure 1). Day of first ovulation was not significantly different between yearling and adult hinds (Table 2), and within three days, all hinds grew a large follicle which ovulated 7-10 days after the first ovulation. The interval between first and second ovulation and mean size of the first and second ovulatory follicles was not significantly different between age groups (Table 2).

TABLE 2: Mean day of first and second ovulation, interval between first and second ovulation and size of first and second ovulatory follicles in yearling and adult hinds.

	Yearling	Adult	SED	Significance
Number of hinds	4	5		
Day of first ovulation	14 April	8 April	3.5	NS
Day of second ovulation	23 April	16 April	3.7	NS
Interovulatory interval (days)	9	8	0.7	NS
Follicle size first ovulation (mm)	7.5	7.4	0.8	NS
Follicle size second ovulation (mm)	6.5	7.8	0.6	NS

Luteal structures and progesterone concentrations

Identification of luteal structures by ultrasonography was possible on only some occasions, despite indicative elevated plasma progesterone concentrations.

Onset of the breeding season was characterised by a single transient (5 - 9 days) elevation of plasma progesterone concentrations in all hinds. This event was preceded by the disappearance of a large follicle and was considered to be as a consequence of 'silent' ovulation (i.e., ovulation not preceded by overt oestrus). Mean maximum progesterone concentrations of this luteal phase did not differ significantly between yearlings and adults (Table 3), with peak values exceeding 1 ng/ml in only two hinds.

Conception date (as assessed from fetal aging) of all but one hind coincided with the presumed first oestrous preceding second ovulation. One adult hind, whose second ovulation coincided with a 24-h period of separation from the stag, had a normal (18 days) luteal cycle and was mated and became pregnant to the oestrus preceding her third ovulation. Both yearling and adult hinds had a linear increase in progesterone secretion of 0.29 ng/ml/day (SED = 0.039) for the first ten days following second ovulation. There were no significant differences between age groups in mean progesterone concentrations on days three, five, seven and ten following ovulation (Table 3).

Study 2

Live weight

Mean live weight of yearling hinds was significantly ($P < 0.05$) lighter than that of adult hinds (101 vs 119 kg, SED = 1.3) when weighed in the second week of March before the study commenced. For yearlings the range was 86.5-114.0 kg (excluding two outliers), while for adults the range was 98.5-138.5 kg.

TABLE 3: Mean maximum plasma progesterone concentration during the short luteal phase and plasma progesterone concentrations 3, 5, 7 and 10 days after second ovulation.

Mean plasma progesterone (ng/ml)	Yearling	Adult	SED	Significance
Max. short luteal phase	0.66	0.89	0.19	NS
Day 3	0.65	0.70	0.17	NS
Day 5	1.27	1.61	0.17	NS
Day 7	1.61	2.27	0.35	NS
Day 10	3.47	3.90	0.51	NS

Oestrus and conception

A total 56 observations of mating were made. Of these, 13 yearling and 34 adult hinds were individually identified as having been mated. The stag in Group 3 became exhausted after mating the adults and had mated only two of the yearlings before being replaced on 23 April. Four yearling hinds that were scanned non-pregnant, but were later identified as having calved, were removed from the analysis of conception data. The mean mating and conception dates were 12 days later for yearlings in Groups 2, 4 and 5 than for adults ($P < 0.001$; Table 4). As a consequence of the stag becoming exhausted and not being replaced until 23 April, the mean conception date for Group 3 yearlings was 16 days later than for the other yearling groups ($P < 0.001$; Table 4). All adult hinds that were observed to mate (n = 34) conceived to that mating, but one of the yearling hinds observed to mate (n = 13) was scanned non-pregnant and one died before scanning. The remaining eleven hinds conceived to the observed mating.

TABLE 4: Mean mating and conception dates classified by age group, with standard error of difference (SED) between adult and yearling hinds. (*** P < 0.001).

	Yearlings (Gps 2, 4 & 5)	Yearlings (Group 3)	Adult	SED	Significance
Observed mate date	15 April	17 April	3 April	1.5	***
n	11/60	2/20	34/80		
Conception date	15 April	1 May	3 April	1.1	***
n	54/57	18/18	78/80		

There was no significant relationship between conception date and live weight found except for the yearling hinds in Group 3, where conception day was on average 4.7 (SEM 1.9) days earlier for a 10 kg increase in live weight.

DISCUSSION

The technique of transrectal ultrasonography to monitor the *in situ* developmental dynamics of follicles ≥ 3 mm in red deer was successful in this study. In an earlier study on the follicular dynamics of red deer (Asher *et al.*, 1997), hinds were surgically modified to align the ovaries alongside the vaginal wall. This was considered necessary to consistently locate the ovaries without rectal manipulation of the reproductive tract. That study had a high incidence of abnormally large debris-filled structures that were considered to be artefacts due to scar tissue interfering with the normal rupture of ovulating follicles. No such structures were observed in the present study.

An inability to reliably locate the corpus luteum was consistent with a previous study (Asher *et al.*, 1997) and is considered a limiting factor in ultrasonographic monitoring of reproductive cycles in red deer. It is possible that the luteal tissue of red deer has a similar echo density to the surrounding ovarian stroma, as we have been successful in identifying luteal tissue in fallow deer (Asher *et al.*, 1998). However, the abrupt disappearance of a large (≥ 6 mm) follicle followed by a sustained increase in progesterone concentration was indicative of ovulation and formation of a corpus luteum.

A single 'silent' ovulation followed by a short luteal phase at the transition into the breeding season, as occurred in the present study, has previously been reported to occur in pubertal (Webster & Barrell, 1985) and adult (Jopson *et al.*, 1990) red deer hinds. This pattern of luteal activity has also been reported in fallow (Asher, 1985) and Père David's (Curlewis *et al.*, 1988) deer, and is a characteristic of the transition into the breeding season of many other species (e.g. sheep; McLeod *et al.*, 1982). Progesterone priming is considered essential to ensure normal luteal function at the subsequent ovulation in sheep (McLeod *et al.*, 1982), and Asher (1985) considered that 'silent' ovulations helped synchronise first oestrus in fallow deer, with the spread of first oestrus corresponding to the approximate longevity of the short-lived corpora lutea.

The yearling hinds had a later mean conception date than adult hinds, which is in agreement with the data of several other authors (e.g. Bray & Kelly, 1979). Moore *et al.* (1985) have suggested that the difference in fertility between yearling and older hinds may be at least partly due to a difference in live weight, and Audigé *et al.* (1999b) recommended that to increase the probability of yearling hinds conceiving early, they should be selected for high live weight prior to mating and joined with young stags late in summer. Hamilton and Blaxter (1980) calculated that for yearling red deer hinds in Scotland, for each 1 kg increase in live weight at mating, calving occurred 0.3 days earlier. Our second study found a similar relationship for yearling hinds in Group 3, but not for those in Groups 2, 4 & 5.

Of particular interest was the observation that the stag in Group 3 appeared to become exhausted after mating the adult hinds. The stag:hind ratio in this group was 1:40, which is within the recommended 1:50 ratio for an experienced stag (Moore *et al.*, 1985). The yearling hinds in that group had a mean conception date 16 days, or about one cycle, later than those in the other groups. This indicates that Group 3 yearlings may have first come into oestrus about the same time as the other yearlings, but the stag was just too exhausted to mate them.

The ability of red deer hinds to exhibit high conception rates has previously been demonstrated (Kelly & Moore, 1977). All of the yearling hinds in Study 1 became pregnant to their first mating, and from the yearlings in Study 2 with known mating dates (13), only one was non-pregnant while the rest became pregnant to their first mating. This indicates that conception failure was not a limiting factor to yearling reproductive performance in the Invermay herd.

CONCLUSION

At the transition into the breeding season both yearling and adult hinds have a single silent ovulation followed by a short, low progesterone amplitude luteal phase. However, yearling hinds begin cyclic ovarian activity later, and therefore have a later conception date, than adult hinds.

ACKNOWLEDGEMENTS

We thank Tim Manley for performing the progesterone radioimmunoassays and the Invermay Deer Crew for all aspects of animal husbandry. This research was funded by the Foundation for Research, Science and Technology.

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