Plasma concentrations of LH, prolactin, oestradiol and progesterone in female red deer (*Cervus elaphus*) during pregnancy

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Summary. Peripheral blood samples were collected throughout pregnancy from 11 red deer hinds. During the same period, 6 other hinds which mated but failed to produce calves were also sampled. Pretreatment of some of these hinds included synchronization of oestrus alone ($N = 3$) or with injection of 1000 i.u. PMSG ($N = 9$).

During early and mid-pregnancy, LH and prolactin were frequently undetectable. Prolactin concentrations in pregnant and non-pregnant hinds were high (>250 ng/ml) in December–January. The results of the hormone analyses suggested that the amount of progesterone in plasma correlates with the number of corpora lutea (CL) present. The concentrations of oestradiol and progesterone were low from mid-winter onwards in the non-pregnant hinds, suggesting a reduction in ovarian activity at this time. In pregnant animals, progesterone concentrations were high for the first 200 days of gestation. Oestradiol rose to peak values concomitant with declining progesterone concentrations just before parturition.

Introduction

In recent years red deer have been investigated for their potential as a farmed animal (Blaxter, Kay, Sharman, Cunningham & Hamilton, 1974; Drew, 1976) and in New Zealand more than 100 000 are held in captivity for this purpose. The reproductive cycle of the female red deer has been described by Guinness, Lincoln & Short (1971) and Kelly & Moore (1977). Hinds are seasonal breeders, mating in autumn and calving during the following summer after a gestation period of about 233 days. While much is known about the social behaviour and sexual activity of red deer both in the wild and in captivity, the hormonal changes in the hind during pregnancy have not, to our knowledge, been documented. This paper reports on changes in the hormone concentrations of pregnant and non-pregnant hinds.

Materials and Methods

Animals and blood sampling

Blood samples were taken from 19 hinds >2 years of age. These animals were drawn from three groups of 10 hinds each which were run in separate paddocks from 26 March. Animals in Group 1 were kept as a control group, with oestrous activity being monitored about the onset of

the breeding season (~mid-April) with the aid of a vasectomized stag fitted with a Sire-sine harness and crayon (Radford, Watson & Wood, 1960). The hinds in Groups 2 and 3 had intravaginal sponges containing 100 mg medroxyprogesterone acetate (MPA: Upjohn Pty Ltd, N.Z.) inserted on 20 April for 14 days. Subsequent oestrous activity in these hinds was also monitored with vasectomized stags. On Day 14 following the first oestrus after sponge withdrawal (20–21 May) the animals in Group 3 were injected i.m. with 1000 i.u. PMSG (Paynes & Byrne Ltd, England). Two animals in Group 3 did not show oestrus after sponge withdrawal, but were injected with PMSG on 20 May.

From 14 May an entire stag fitted with a Sire-sine harness and crayon was introduced to each group (the vasectomized stag being removed 4 days earlier) and the hinds were inspected daily for mating marks until 29 May. Vaginal smears were taken and examined for spermatozoa on the days that mating marks were recorded. The animals in Group 3 together with the 4 hinds from Group 2 and 5 from Group 1 that were marked by the entire stag, were joined as a single herd on 8 June and run as such until the completion of blood sampling. Entire stags were run with these hinds until August. All hinds were individually identified by numbered ear tags. Mating and calving results of hinds in Groups 2 and 3 have previously been reported (Kelly & Moore, 1977).

Daily peripheral blood samples (15 ml) were obtained from all animals between 08:30 and 10:00 h from 27 April to 29 May. Thereafter a blood sample was collected once weekly from each animal until 22 July and then once every 2 weeks until 2 February (i.e. after the majority of animals were expected to calve). During blood sampling all hinds were restrained by hand. The samples were collected into heparinized tubes, centrifuged within 1 h of collection and the plasma fractions stored at -20°C until analysed for hormones.

The ovaries of all hinds were examined by laparoscopy on 8 June to determine the number of corpora lutea (CL). During the expected time of calving, the animals were inspected daily and all newborn calves were tagged. Observations on suckling activity of the hinds and calves were made during the period of calving to establish the dam of each calf.

To establish whether the heterologous radioimmunoassays could measure changes in LH and prolactin-like activity in deer, one anoestrous animal (not previously used in this study) was injected (i.m.) during January with 100 μg synthetic gonadotrophin-releasing hormone, GnRH (Pierce Chemical Co., Illinois, U.S.A.) and 100 μg thyrotrophin-releasing hormone, TRH (Pierce Chemical Co.). Jugular venous blood samples (15 ml) were collected at 15-min intervals from -15 to $+120$ min from the time of injection of the GnRH/TRH mixture.

Hormone assays

Progesterone was measured by a radioimmunoassay procedure similar to that described by Thorneycroft & Stone (1972) for human plasma. To determine whether the assay was suitable for measuring progesterone in petroleum-ether extracts of plasma without any further purification, samples of pooled plasma from a castrated stag with and without known amounts of authentic progesterone (1–20 ng/ml plasma) were chromatographed through a Sephadex LH-20 column using the method of Murphy (1971). Examination of the results after chromatography with those for samples not subjected to chromatography gave a correlation coefficient of 0.992 ($P < 0.001$; linear regression analysis). The assays for progesterone were therefore performed without a chromatographic step. The progesterone antiserum (Wallaceville-26) was raised in an ovariectomized ewe against progesterone-11-hemisuccinate conjugated to bovine serum albumin and used at an initial dilution of 1:10 000. Major cross-reactions of the steroids in the assay were 11α -hydroxyprogesterone 120%, 11β -hydroxyprogesterone 25%, 5β -pregnane-3-ol-20-one 25%, 20α -dihydroprogesterone 2.5%, androstenedione 0.4%, while those for testosterone, oestradiol-17 β , and oestrone were all $<0.001\%$. The minimum detectable level of progesterone in deer plasma was 0.3 ng/ml, and the within- and between-assay coefficients of variation were $<14\%$.

Oestradiol was assayed by a method similar to that described by Peterson, Fairclough & Smith (1975). The ether extract from plasma was routinely subjected to Sephadex LH-20 column chromatography to remove material which interfered in the assay when performed without chromatography. The ether fraction which also contained the extracted recovery counts of [2,4,6,7-³H]oestradiol-17 β was evaporated to dryness and dissolved in 0.2 ml benzene/methanol mixture (85/15, v/v). This mixture was then added to the top of a Sephadex LH-20 column (0.4 g LH-20 in a 2.5 ml glass syringe barrel and supported by glass fibre paper), and the resulting eluate was discarded. The oestradiol fraction was eventually collected in a 2.0 ml benzene/methanol fraction (85/15, v/v) after an earlier 1.4 ml eluate had also been discarded. The antiserum (Wallaceville-27) used in the assay was raised in an ovariectomized ewe against oestradiol-6-(O-carboxymethyl)-oxime-bovine serum albumin conjugate. It was used at an initial dilution of 1:16 000. The major cross-reactions of other steroids in this assay were: oestrone 7.3%, oestriol 1.4%, oestradiol-17 α 1.4% and androstenedione 0.015%. The minimum detectable level of oestradiol in plasma was 5 pg/ml, and the within- and between-assay coefficients of variation were <16%.

Luteinizing hormone (LH) concentration in deer plasma was assessed using a heterologous radioimmunoassay procedure exactly as described for sheep plasma by Scaramuzzi, Caldwell & Moor (1970). The ovine pituitary LH preparations for both standards and iodinated tracer were NIH-LH-S11. The LH antibody was raised in a rabbit also using NIH-LH-S11 as the antigen, and it was used in the assay at an initial dilution of 1:50 000. The antiserum cross-reacted with other proteins in the following amounts: NIH-P-S12 (0.09%), NIH-TSH-S8 (2.4%), NIH-GH-S11 (0.4%), NIH-FSH-S10 (0.4%), NIH-P-B5 (0.16%), ovine ACTH (Schwarz-Mann) (<0.01%), ovine serum albumin (<0.01%), bovine serum albumin (<0.01%), bovine TSH (Schwarz-Mann) (<0.01%), human prolactin (VLS4) (<0.01%), and human FSH (<0.01%). No deer pituitary preparations or standards were available for testing in the above assay system. Nevertheless, serial dilutions of deer plasma from blood samples taken at oestrus with high concentrations of immunoreactive LH gave inhibition curves parallel to those generated for NIH-LH-S11. Moreover, serial dilutions of NIH-LH-S11 in deer plasma also generated inhibition curves parallel to those generated by NIH-LH-S11 in buffer.

The sensitivity of the assay for LH-immunoreactive material was 0.10 ± 0.03 ng per tube (mean $\pm$ s.d.). The within- and between-assay coefficients of variation were $\leq 11\%$. The accuracy of the assay was tested by adding known amounts of NIH-LH-S11 (10–50 ng/ml) to 7 deer plasmas. The mean recovery was $95 \pm 10\%$ (mean $\pm$ s.d.). Measurement of blood samples recovered from deer around oestrus revealed that immunoreactive 'peaks' of LH could be measured. Further validation of the LH radioimmunoassay was sought by measuring LH in deer plasma following an i.m. injection of 100 μ g GnRH.

Prolactin. These assays were carried out using a heterologous double-antibody assay system identical to that described by McNeilly (1970) except that the serum samples were of deer origin. Ovine prolactin, NIH-P-S11, was used as the reference standard and ovine prolactin LER-860 for iodination. The antiserum, which was raised against ovine prolactin (NIH-P-S6), was used in the assay at a dilution of 1:50 000.

Details of the cross-reactions with the antibody and its characteristics in an homologous sheep plasma system are described by Lamming, Moseley & McNeilly (1974). No deer pituitaries or standards were available for testing. Blood from hinds in April contains a high level of immunoreactive prolactin-like material. Serial dilutions of these samples produced inhibition curves which were parallel with those generated for authentic ovine prolactin (NIH-P-S11).

The sensitivity of the assay for prolactin-immunoreactive material in deer plasma was 0.2 ± 0.03 ng/tube (mean $\pm$ s.d.). The between- and within-assay coefficients of variation were $\leq 15.6\%$ over the range 1–150 ng/ml. The accuracy of the assay was tested by adding known amounts of NIH-P-S11 (75–150 ng/ml) to 7 deer plasmas. The mean recovery was $98 \pm 7\%$ (mean $\pm$ s.d.). Further validation was sought by measuring prolactin in deer plasma following an injection of 100 μ g TRH together with 100 μ g GnRH.

Statistical procedures

The results for all hormones are expressed as means $\pm$ 1 s.e.m. For statistical evaluation the data for each group were analysed by Student's *t* tests (paired or unpaired).

Results

Reproductive performance

The effects of the various treatments on mating to the entire stag, the number of ovulations and subsequent calving performance are shown in Table 1. Five controls (Group 1), 4 MPA-treated (Group 2), and 9 MPA + PMSG (Group 3) treated hinds showed unequivocal evidence (spermatozoa in smear and/or crayon marks) that mating had occurred. Each of the control and MPA-treated animals had one corpus luteum. In Group 3 hinds the number of CL $\sim$ 2 weeks after mating (i.e. 8 June) varied from 1 to 12. All the hinds that calved produced only 1 calf. All but one of the controls calved at the expected time after the recorded mating; for the other (No. 332) the interval from recorded mating to calving indicates conception to a return to oestrus, which occurred outside the period of mating observations. Likewise all Group 2 hinds calved at the expected time following the recorded mating except for No. 445 which showed evidence of conceiving to a subsequent mating. In Group 3, only 3/10 hinds calved to the planned mating. Hind 479 calved in mid-February indicating conception at a later unrecorded mating, and the other hinds failed to calve.

Table 1. Effect of medroxyprogesterone acetate (MPA) and PMSG on mating, ovulation and calving performances of red deer hinds

Group	Hind No.	Mating date (May)	Ovulation† rate	Calving date (January)	Interval from recorded mating to calving (days)
1 (Controls, no treatment)	306	20	1	6	231
	308	15*	1	2	232
	318	19*	1	12	238
	319	20	1	11	236
	332	18*	1	22	249‡
2 (MPA)	401	28	1	22	239
	445	23*	1	>Feb 2	>255‡
	473	23*	1	14	236
	481	23*	1	13	235
3 (MPA + PMSG)	404	25	1	NP	—
	417	—	6	NP	—
	430	23*	3	NP	—
	432	24*	6	20	241
	452	23*	2	NP	—
	474	25*	6	12	232
	479	24*	1	>Feb 2	>254‡
	487	24*	3	15	236
	489	24*	4	NP	—
	499	27	12	NP	—

NP = not pregnant.

* Spermatozoa present in vaginal smear.

† Number of corpora lutea recorded on 8 June.

‡ Returned to service.

LH and prolactin activity in plasma after combined GnRH and TRH test

In response to a single i.m. injection of GnRH and TRH (Text-fig. 1), there was a marked rise in the concentrations of immunoreactive substances with LH and prolactin-‘like’ activity in plasma. It is therefore apparent that the assay techniques were capable of detecting changing concentrations of these two hormones.

Text-fig. 1. Changes of LH and prolactin (logarithmic scale) immunoreactivity in red deer plasma after a single GnRH/TRH (100 µg each) injection (i.m.) at time 0.

Hormone changes

For the 19 hinds for which blood samples were taken from late April to 2 February, the hormone data for two (Nos 445 and 479) were excluded because they calved considerably outside the periods of observation and so precise conception and calving dates were unknown. The results from the remaining 17 animals were subdivided for analysis as follows regardless of treatments: the data for the 11 animals which produced calves were separated from those of the 6 that failed to deliver a calf. For the 11 animals which calved (the ‘pregnant’ animals), the data were further subdivided into two groups based on the number of CL present: ≥ 3 CL (3 animals from Group 3) and 1 corpus luteum (8 animals from Groups 1 and 2). Likewise, for the animals which failed to calve (the ‘non-pregnant’ animals, all in Group 3) the data were also subdivided according to the number of CL present: ≥ 3 CL (4 animals) and 1–2 CL (2 animals).

LH and prolactin. The concentrations of LH and prolactin in the pregnant animals with ≥ 3 CL were not significantly different from those with 1 CL. Likewise no significant differences were noted in the non-pregnant animals. The overall mean concentrations of LH and prolactin in the pregnant animals with respect to day of mating and those for all non-pregnant animals at the

same times of the year are shown in Text-fig. 2. The LH values in the pregnant and non-pregnant animals were not significantly different from one another. For both groups of animals, LH declined to basal or undetectable levels ~14 days after mating and thereafter remained low or undetectable for the rest of the sampling period. Only one exception was found: Hind 332 had an elevated LH value (~10 ng/ml) on about Day 63 of gestation.

Text-fig. 2. Mean concentrations (+1 s.e.m.) for LH-'like', prolactin-'like' and oestradiol concentrations in the plasma of pregnant and non-pregnant red deer hinds. Values without s.e.m. occur when the number of hinds sampled is ≤ 2 , or the concentrations are outside the limits of detection for the particular assay.

The prolactin levels in pregnant and non-pregnant hinds declined to undetectable levels 14 days after mating and thereafter remained low for the next 100 days. After mid-September, the concentrations of prolactin increased significantly in the non-pregnant animals ($P < 0.01$) to values of >250 ng/ml in December and January. A significant rise in prolactin ($P < 0.01$) was also observed in the pregnant animals but this did not begin until mid-November.

Oestradiol. Many samples collected until early July had to be discarded because lysed red blood cells were present and this interfered with the oestradiol determinations despite chromatography.

The concentrations in the pregnant hinds with ≥ 3 CL were not significantly different from those with 1 corpus luteum. Likewise no differences related to number of CL were noted amongst the non-pregnant animals. The overall mean levels of oestradiol in the pregnant animals with respect to day of mating, and the mean levels for all non-pregnant animals over the same times of the year, are shown in Text-fig. 2. In pregnant and non-pregnant hinds, the oestradiol concentrations declined from the start of sampling to reach basal values of 5–10 pg/ml around 160–190 days of gestation or during November. After this, the values for pregnant hinds increased significantly ($P < 0.001$) to peak values (mean 35.0 pg/ml) immediately before parturition. The values for the non-pregnant group showed no such change and remained low until January when the values gradually increased to a mean of 12 pg/ml at the beginning of February.

Progesterone. Throughout pregnancy the three Group 3 hinds with ≥ 3 CL on 8 June had significantly higher ($P < 0.001$) concentrations of progesterone than those in Groups 1 and 2 with only 1 CL (Text-fig. 3). In the latter the mean concentrations of progesterone remained more or less constant at 4 ng/ml until 200 days of gestation, and then declined gradually to basal levels after parturition. In animals with ≥ 3 CL, the progesterone concentrations fluctuated widely from ~8 to ~32 ng/ml over most of pregnancy but the peaks in mean progesterone values appeared to coincide with those in hinds with 1 corpus luteum. After 200 days of gestation the progesterone values in the hinds with ≥ 3 CL declined, to reach basal values after parturition.

Text-fig. 3. Mean progesterone concentration (with associated 1 s.e.m.) in the plasma of pregnant and non-pregnant red deer hinds with 1–2 CL and ≥ 3 CL.

For the non-pregnant hinds, up until about 50 days after mating the concentrations of progesterone in the hinds with ≥ 3 CL were substantially higher than in those with 1–2 CL (Text-fig. 3). Peak values were obtained 20–30 days after mating, and were not significantly different from those recorded in pregnant animals with multiple CL. From 50 days after mating to the end of the study the mean concentrations of progesterone were uniformly low in both groups of non-pregnant animals (< 1 ng/ml). All values from 50 to 220 days after mating were significantly lower ($P < 0.001$) than those in their pregnant counterparts at similar times of sampling.

Discussion

These studies suggest that progesterone is an important hormone of pregnancy in red deer. Furthermore, they suggest that oestradiol may have a role in the initiation of parturition because the pre-partum rise in oestradiol, concomitant with a decline in progesterone, is similar to that recorded for other polyoestrous ungulates and rodents (Heap, Perry & Challis, 1973). The decline in the oestradiol values in plasma from mid-winter (i.e. June) in the pregnant and non-pregnant hinds probably reflects an overall reduction in follicular activity.

From the finding that immunoreactive prolactin is low in plasma during much of early-mid pregnancy, it seems reasonable to propose that this hormone does not have a key role in the maintenance of mid-term pregnancy, although it may have a role in early pregnancy maintenance (R. W. Kelly, unpublished data) and possibly also during the final months before calving. In fact, the marked changes in prolactin values recorded during the sampling period are more closely related to the photoperiod than to the reproductive status of the animals (Text-fig. 2). In Mosgiel, New Zealand, the months of June and July are those with the shortest number of daylight hours (~ 9 h/day) and this corresponds to the period when prolactin is undetectable in blood. Conversely, December and January are the months with the greatest number of daylight hours (~ 15 h/day) and this corresponds to the period when prolactin is at peak levels. The recording of high concentrations immediately after mating is not a stress-related response to the start of sampling, because the hinds had been repeatedly handled and blood sampled in April and May.

It also seems unlikely that LH is required throughout pregnancy, at least at concentrations > 0.3 ng/ml. The recording of only 1 out of 374 values for LH in excess of 2 ng/ml from June until February supports the contention that the frequency of fresh ovulations (i.e. accessory CL formation) or luteinized-follicle formation over this time period may well be extremely low in both the pregnant and non-pregnant hinds (Kelly & Challies, 1978). However, because of the relatively infrequent sampling regimen (7–14-day intervals) the data remain unequivocal on this point.

The progesterone concentrations in animals with only 1 corpus luteum show little change from Days 2 to 220 of gestation, suggesting that the corpus luteum formed in early pregnancy continues to function for the entire gestation period. This conclusion is supported, to some extent, by the consistent 2–8-fold higher peripheral plasma progesterone concentrations in animals with ≥ 3 CL. With these differences in progesterone concentration related to number of CL and the lack of change with stage of pregnancy within CL groupings, it seems reasonable to suggest that luteal tissue provides most, if not all, the progesterone during pregnancy as has been demonstrated for the pig and goat (Heap *et al.*, 1973).

In non-pregnant hinds there was a clear difference in progesterone concentrations during the first 40 days after mating between those animals with 1–2 CL and those with 3–12 CL (Text-fig. 3). It is not known whether any of the non-pregnant animals conceived but subsequently aborted. It is also possible that the failure to observe further cyclic progesterone activity in any of the non-pregnant animals was due to the onset of anoestrus.

While these studies demonstrate that PMSG is capable of inducing multiple ovulations, it did not result in any multiple births. Moreover, only 33% (3/9) of the MPA + PMSG-treated animals that were mated produced calves to that mating compared to a 78% (7/9) success rate in the other mated hinds. The reasons why PMSG had such a detrimental effect on fertility are not known.

In conclusion, these studies suggest that luteal tissue is the major source of progesterone in pregnant red deer and that LH is not always measurable throughout the gestation period. These studies also show that the patterns of prolactin secretion in pregnant and non-pregnant animals correlate with the seasonal changes in photoperiod, and that ovarian follicular activity appears to be low from August until January.

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