
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

*ISSN 0370-2731/2001* *Proceedings of the New Zealand Society of Animal Production* **61:** 141-143

# The influence of reproductive status on pituitary gland function in seasonally anoestrous red deer hinds

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## ABSTRACT

Changes in pituitary responsiveness to exogenous hypothalamic hormones were assessed during seasonal anoestrous in dry, pregnant and lactating hinds. Of the fifteen adult red deer hinds monitored, five were non-pregnant, five had their calves removed immediately after birth, and five suckled their calves. All hinds were given a single i.v. injection of hypothalamic releasing hormones (5µg each of GnRH, GRF and TRH) on four occasions between Nov/Dec (10 days prior to calving) and March. The pituitary response was determined from changes in plasma concentrations of LH, growth hormone, and prolactin in samples obtained by jugular venepuncture at 0, 15 and 30 min after injection. Reproductive state significantly (P<0.001) affected LH release with a mean response (over all sample dates) of 1.12 ng/ml in dry hinds compared with 0.85 ng/ml in hinds that had their calves removed and 0.60 ng/ml in lactating hinds. Neither growth hormone (average 5.65 ng/ml) nor prolactin (average 33.9 ng/ml) responses were affected by reproductive status.

**Keywords:** red deer hind; pituitary; hypothalamus; LH; prolactin; growth hormone

## INTRODUCTION

Red deer hinds are highly seasonal, mating in autumn, calving in late spring or early summer, and at least in the majority of farmed animals, continuing to lactate until immediately prior to the next breeding season. Not surprisingly, red deer exhibit a range of seasonal physiological transformations including changes in voluntary food intake, body growth rate and coat growth, and in reproductive function and the expression of sexual behaviour (Guinness *et al.*, 1971; Loudon & Jabbour, 1997). These seasonal changes in physiology are principally regulated by central neuroendocrine or hypothalamic mechanisms. However, the contribution of other mechanisms such as the response of the pituitary gland to hormonal signals from the hypothalamus is also important (see Meikle & Fisher, 1996, for example). A number of releasing hormones secreted by the hypothalamus induce the release of pituitary hormones that are involved in the control of reproduction, lactation and growth. These include gonadotrophin releasing hormone (GnRH) that promotes the release of two major reproductive hormones luteinising hormone (LH) and follicle stimulating hormone (FSH), growth hormone releasing factor (GRF) that induces secretion of growth hormone (GH) and thyroid hormone releasing hormone (TRH) that causes release of prolactin. The aim of this study was to assess the effects that pregnancy, lactation and/or suckling may have on pituitary responsiveness to these hypothalamic exogenous hormones during seasonal anoestrus. The three hypothalamic hormones (GnRH, GRF, and TRH) were administered simultaneously, and the response of the pituitary in terms of release of LH, growth hormone and prolactin monitored.

## MATERIALS AND METHODS

Fifteen farmed adult red deer (*Cervus elaphus*) hinds (89 – 110 kg live weight at mating) were used in this study. Five were non-pregnant (having failed to conceive following a single cloprostenol-induced synchronised mating – see Fisher *et al*., 1994) and ten were pregnant at the beginning of the study (29 November). All pregnant hinds calved over a six-day period (mean calving date 17 December), five had their male calves removed 12-24h after birth for artificial rearing for a separate experiment (McMahon *et al*., 1997) and five suckled their calves. Lactational status was confirmed by udder inspection on two occasions during the experiment (18 January and 5 March).

All hinds were given a single intravenous injection containing a mixture of 5 µg each of GnRH, GRF, and TRH (all Sigma Chemical Co., St Louis, US) in 1ml saline containing 1% BSA, on four occasions during seasonal anoestrus. The treatment was carried out on either 29 November or 7 December, and again on 18 January, 8 February and 1 March. These times corresponded to approximately 10 days prior to, and 4, 7 and 10 weeks after calving in those hinds that were pregnant at the start of the experiment, and extended over the period of seasonal anoestrus. At Invermay the breeding season in red deer hinds extends from late March until September (Meikle & Fisher, 1996). Blood samples (10 ml) were taken by jugular venepuncture at 0, 15 and 30 minutes after each injection, with hinds constrained in a compressed-air-operated deer crush for this procedure. Plasma samples were analysed by radioimmunoassay for LH and growth hormone according to the methods of Meikle & Fisher (1996) and Webster *et al.* (1996), respectively. Plasma prolactin samples were measured using an ovine assay that used ovine prolactin antiserum (NIDDK–anti–oPrl-2) and ovine prolactin (NIDDK-oPrl-I-2) for iodination and reference standards, with serial dilutions of cervine plasma attaining near parallelism to the ovine standards. Intra-assay coefficients of variation calculated on 20 duplicate pairs were 6.06, 9.49, and 9.33% for LH, growth hormone and prolactin, respectively. For each hormone, the pituitary response was calculated as the mean hormone concentration at times 15 and 30 minutes, minus the concentration at time zero. All data are expressed as means (± sem) and differences between reproductive status and time of treatment were compared by analysis of variance and Student's t-test.

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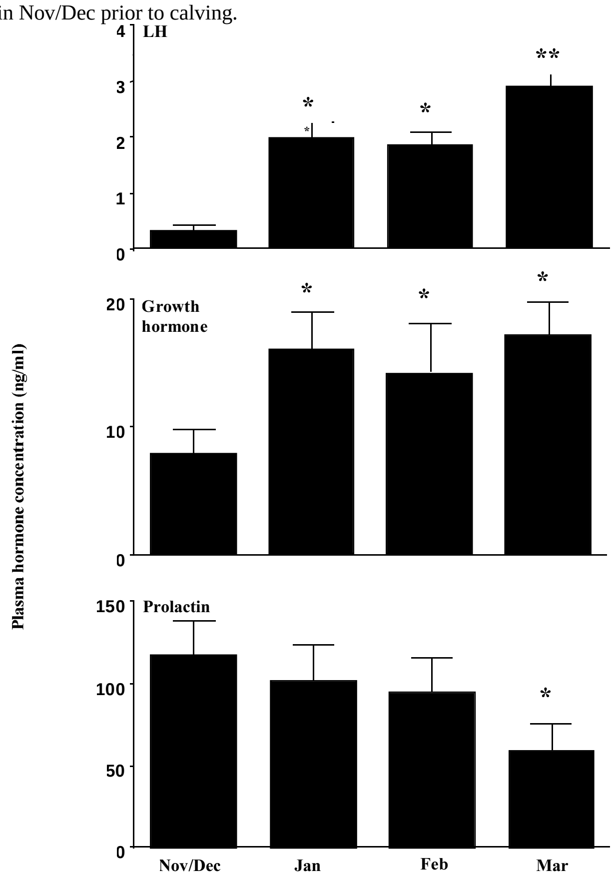
## RESULTS

At all four treatment times, there was a significant increase in plasma concentrations of LH, growth hormone and prolactin following administration of the hypothalamic hormones (Figure 1). On 158/173 occasions, the highest hormone concentrations were recorded in the samples taken 15 minutes after administration. When data from all animals were combined to assess the pituitary response in relation to treatment date, LH release increased significantly (P<0.001) at the January and again at the March treatment time (mean response 0.33 ± 0.08, 2.01 ± 0.27, 1.87 ± 0.21 and 2.93 ± 0.22 ng/ml in Nov/Dec, Jan, Feb and March, respectively). Similarly, there was a significant increase (P<0.001) in the growth hormone response at the January, February and March challenges (16.09 ± 2.87, 14.28 ± 3.75 and 17.22 ± 2.53 ng/ml) compared with that in November/December (8.00 ± 1.83 ng/ml). In contrast, the prolactin response did not differ significantly over the November/December, January and February treatments (118.19 ± 19.83, 102.56 ± 20.56, 103.24 ± 19.34 ng/ml) but was significantly lower (P<0.05) at the March treatment (60.27 ± 15.76 ng/ml, see Figure 1).

**FIGURE 1:** Mean (± sem) LH, growth hormone and prolactin responses following intravenous administration of a mixture of 5 μg each of GnRH, GRF and TRH on four different occasions during seasonal anoestrus. Asterisks indicate the significance of the comparison with the first response in Nov/Dec prior to calving.



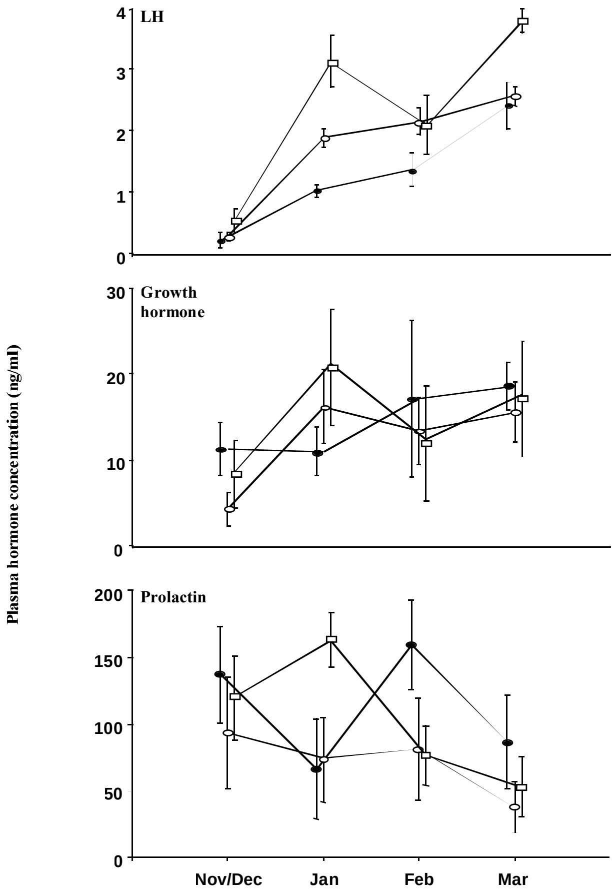

There was a trend for the LH response to be lower in pregnant (0.12 ± 0.04 ng/ml) than in non-pregnant hinds (0.27 ± 0.10 ng/ml plasma), but this difference was not significant. Neither growth hormone (pregnant: 3.56 ± 1.14 and non-pregnant: 4.32 ± 2.27 ng/ml) nor prolactin (pregnant: 40.24 ± 11.62 and non-pregnant: 37.68 ± 13.95 ng/ml) responses were affected by pregnancy status.

**TABLE 1:** Mean (± sem) plasma LH, growth hormone and prolactin responses following intravenous administration of a mixture of 5 μg each of GnRH, GRF and TRH. Values shown are the mean of three post-calving challenges over a 3-month interval.

| **Mean hormone response** | | **Group** | |
|---|---|---|---|
| **(ng/ml plasma)** | **Lactating** | **Calf removal** | **Dry** |
| LH | 0.78 ± 0.11 | 1.09 ± 0.06 | 1.49 ± 0.14 |
| Growth hormone | 7.13 ± 1.56 | 7.00 ± 1.02 | 8.04 ± 1.83 |
| Prolactin | 36.45 ± 10.23 | 24.85 ± 8.03 | 39.90 ± 8.69 |

When all three post-calving releasing hormone challenges were combined, the LH response was significantly (P<0.001) influenced by reproductive status after calving, with the mean response being lowest in lactating hinds and highest in dry hinds, with that recorded in hinds whose calves had been removed being intermediate (Table 1). Neither the growth hormone nor prolactin responses were significantly affected by physiological status. However there were interactions between reproductive status and time. The mean LH response was significantly different between all 3 groups at the first post-calving challenge, and at the last challenge between dry hinds and hinds that had calved (Figure 2). In addition, the prolactin response was significantly higher in dry hinds than in hinds that had calved at the first post-calving challenge.

**FIGURE 2:** Mean (± sem) LH, growth hormone and prolactin responses following intravenous administration of a mixture of 5 μg each of GnRH, GRF and TRH in pregnant/lactating (•), pregnant/calf removal (○) and dry (❑) red deer hinds on four different occasions during seasonal anoestrus.



## DISCUSSION

The physiological status of the hind has a major influence on the response of the pituitary gland to exogenous GnRH, with LH concentrations being affected by the time of the season, lactation and/or suckling, and calf removal in the present study. This result complements previous studies that have implicated factors such as season, pregnancy, and ovariectomy affecting pituitary function in a number of species of female deer (Curlewis *et al.* 1991; Baker *et al.* 1995; Meikle & Fisher, 1996). Although seasonality is likely to be driven by a central neuroendocrine mechanism, these findings highlight the contribution that other mechanisms, such as changes in pituitary responsiveness, have in the regulation of seasonal physiology.

In the hind, seasonal anoestrus coincides with the post-partum period, which itself influences reproductive function in many species (see Lamming, 1978). Earlier studies have reported changes in LH response to the administration of GnRH during seasonal anoestrus, notably that there is a marked depression early in anoestrus followed by an increase prior to the breeding season, in Pere David's deer (Curlewis *et al.* 1991), wapiti (Baker *et al.* 1995) and red deer (Meikle & Fisher, 1996). To our knowledge, the additional effect of lactation and/or suckling on pituitary function noted in the present study has not previously been reported in the hind. Nevertheless, lactation has been implicated in depressed reproductive performance. For example, it is well documented that in wild populations of red deer in Scotland, lactating hinds have lower calving rates and later calving dates than dry hinds (Lowe, 1969; Guinness *et al.*, 1978). Similarly, when hinds were mated through autumn and winter, the shortest interval between calving and a return to oestrus was 10 weeks, unless the calf was lost at birth and the hind ceased lactating, when the interval could be as short as 10 days (Guinness *et al.*, 1971). These effects of lactation and/or suckling may not be so obvious in farmed deer that are usually in better body condition and under better nutrition than their wild counterparts in more harsh environments. However, Adam *et al.* (1985) reported a 10-day delay in the onset of the breeding season in lactating hinds compared to weaned hinds in Scotland, and Pollard *et al.* (2000) a similar 12-day difference in the mean date of conception in New Zealand.

Both growth hormone and prolactin are known to display seasonal patterns of secretion (Kelly *et al.*, 1982; Curlewis *et al.*, 1992) and both have been implicated in lactation in several species (Tucker, 1988). A role for prolactin in lactation in red deer is supported by the significant difference in the prolactin response between dry hinds and recently calved hinds at the first post-calving challenge (Figure 2). Further support is given by the increased prolactin response at the subsequent challenge in hinds that had had their calves removed. There is no evidence from the current study that growth hormone is involved in lactation in this species.

In conclusion, the LH response of the pituitary gland to GnRH is significantly affected by lactation and/or suckling and by the stage of seasonal anoestrus. The prolactin response to TRH is similarly affected by lactation, but growth hormone release is not.

## ACKNOWLEDGEMENTS

The Deer Crew and especially Rob Labes are thanked for managing the hinds through calving and assisting with sampling.

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