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Out-of-season breeding in thyroidectomized red deer hinds

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ABSTRACT

Thyroid hormones are required for the neuroendocrine processes that cause the onset of the non-reproductive state each spring in red deer. To evaluate the feasibility of achieving out-of-season breeding using thyroidectomized (THX) hinds and a THX stag, oestrous behaviour and ovulation were compared in THX ($n=9$) and thyroid-intact control hinds ($n=6$) following synchronization of oestrous cycles with CIDRs and joining with a THX, vasectomized stag in the breeding and non-breeding seasons. In the control group, oestrus and ovulation were recorded in all (100%) and none (0%) of hinds during May (breeding season) and October (non-breeding season) respectively ($P < 0.05$), however in THX hinds oestrus and ovulation occurred in a proportion of the animals (33-55%) and this incidence was not affected by season ($P > 0.05$). Time of onset after synchronization and duration of oestrus were not significantly affected by thyroidectomy or season ($P > 0.05$). To determine if pregnancy could be established in THX hinds in the non-breeding season, all 15 hinds were synchronized for oestrus using CIDRs plus 200 I.U. PMSG and joined with a fertile, THX stag in early December. Any hinds not mated on this occasion were resynchronized using CIDRs plus 400 I.U. PMSG and again joined with a fertile, THX stag. While the lower dose of PMSG was generally ineffective in inducing oestrus (i.e. only 2 THX hinds mated), 50% of control and 71% of THX hinds which received 400 I.U. PMSG exhibited overt oestrus. In all, seven out-of-season calvings (3 from thyroid intact and 4 from THX hinds) were obtained from 10 matings (all 3 unsuccessful matings occurred in THX hinds). These results imply that although thyroid gland secretions switch off reproductive activity at the end of the breeding season, they do have a supportive role in the expression of overt oestrus and occurrence of ovulation.

Keywords: red deer; reproduction; seasonal breeding; thyroid gland; oestrus.

INTRODUCTION

Considerable research effort has been devoted to advancing the timing of conception in red deer (e.g. Adam *et al.*, 1986; Moore and Cowie, 1986; Fennessy *et al.*, 1986; Duckworth and Barrell, 1988; 1991; Wilson, 1992). This is due primarily to the economic costs of meeting the feed requirements of lactating hinds following calving in December, when pasture in many parts of New Zealand undergoes a seasonal decline in growth rate and quality. Other advantages of advancing the breeding season of deer include being able to grow the young to the desired carcass weight at the time of optimum venison schedules in Northern Hemisphere markets (mid August to late December), reduced weaning stress due to heavier (earlier born) calves and better returns on weaner stags and hinds due to increased live weights (Wilson, 1989).

The hormonal treatments which have been evaluated to date for advancement of breeding in female deer are based on those already employed successfully for other species of livestock such as sheep and cattle (Barrell, 1985; Wilson, 1989). These involve either parenteral administration of PMSG or GnRH in conjunction with a period of intravaginal progesterone treatment, or administration of melatonin (orally or parenterally) in a manner designed to mimic the effects of inductive photoperiods during the breeding season. While all these techniques will induce ovulation prior to the normal breeding season, only the latter has proved successful in advancing the date of calving with any degree of reliability (Asher *et*

al., 1994a). For example, the use of subcutaneous melatonin implants has led to calving advancements of up to 6 weeks (Asher, 1990). This treatment is contra-indicated during pregnancy, however as it may impair lactogenesis (Asher *et al.*, 1994b) and is therefore limited in its potential for changing the breeding season of red deer.

We have recently shown that thyroidectomy of red deer hinds permits oestrous cyclicity to continue during the non-breeding season (Anderson and Barrell, 1998). Manipulation of thyroid gland function may therefore provide a means for artificial control of the breeding season in female deer. The current experiment describes an attempt to induce mating, ovulation and conception in thyroidectomized red deer hinds. If successful out-of-season breeding could be demonstrated in thyroidectomized hinds, treatments might then be developed for on-farm use involving suppression of thyroid gland secretions or blocking thyroid hormones at their site of action during the non-breeding season.

MATERIALS AND METHODS

Experimental design

The experiment used 15 mature red deer hinds (mean live weight at the start of the experiment 102.3 ± 2.3 kg) which were thyroidectomized as previously described (Shi and Barrell, 1992) at the start of the breeding season (March and April) (THX, $n = 9$) or remained thyroid-intact as controls. To test if thyroidectomized hinds exhibit overt oestrus and develop a functional corpus luteum following

oestrus synchronization in the breeding season, ovulation was synchronized in all hinds in May by treating with a controlled internal drug releasing device (CIDR Type G, InterAg, Hamilton, NZ) containing 0.3 g progesterone intravaginally from 15 days previously, renewed after 10 days (Fennessy *et al.*, 1990). A vasectomized stag was run with the hinds from 2 days before until 6 days after CIDR withdrawal. Hinds were continuously observed for oestrus and mating from 15 to 100 hours after CIDR withdrawal (the paddock was illuminated with floodlights at night), and the occurrence of ovulation (i.e. presence of corpora lutea) was determined by laparoscopy 8 days after CIDR withdrawal. To test if the synchronization treatment described above was equally effective during the non-breeding season, hinds were again synchronized for ovulation in late October, and oestrus, ovulation and heart rate were recorded as before. The stag was thyroidectomized in June to prevent the seasonal decline in male reproductive traits such as testosterone concentration, testis diameter and 'rutting' behaviour (Shi and Barrell, 1992).

To test if out-of-season conception can be induced in thyroidectomized hinds following mating by a thyroidectomized stag, hinds were synchronized as above in early December, except that 200 I.U. PMSG (Folligon, Intervet International, Boxmeer, Holland) was administered i.m. at the time of CIDR withdrawal. A non-vasectomized stag which was also thyroidectomized in June was substituted for the vasectomized stag, and hinds were monitored for oestrus and mating from 10 to 80 hours after CIDR withdrawal and for ovulation at 14 days after CIDR withdrawal. Any hinds not mated on this occasion were immediately re-synchronized with an 11 day CIDR treatment (CIDRs renewed after 9 days) in conjunction with 750 µg i.m. cloprostenol (Estrumate, Pitman-Moore, Upper Hutt, NZ) at the time of CIDR renewal and 400 I.U. i.m. PMSG at the time of CIDR withdrawal. Hinds were observed for oestrus from 10 to 100 hours after CIDR withdrawal. Ovulation was not checked for on this occasion. All hinds were pregnancy diagnosed between 44 days and 6 months after mating while restrained in the deer crush using a trans-rectal real-time ultrasonographic transducer (Aloka Echo Camera, model SSD-210DXII, Medtel Teletronics LTD, Auckland, NZ) fitted with a 15 mm diameter probe (model USY-658-5) with an operating frequency of 5 MHz. The presence of placentomes or a foetus was taken to be indicative of pregnancy.

Plasma progesterone concentration was measured throughout the experiment in twice-weekly jugular blood samples, except for periods during and for 1 month following CIDR treatment, when hinds were blood sampled thrice-weekly for plasma progesterone concentration.

Hormone analysis

Plasma progesterone concentrations were determined by ELISA, similar to that which has been described by Elder *et al.* (1987). The sensitivity (95% confidence limit at 0 nmol.l⁻¹) averaged 0.86 nmol.l⁻¹ (6 separate assays involving 113 ELISA microtitre plates). Intra- and inter-assay CV were 11.9% and 12.8% respectively for plasma pools displacing progesterone-3-O-carboxymethyloxime

thyroglobulin conjugate to 91% of the total bound. Plasma total T₃ concentration was assayed using a commercially available radioimmunoassay kit (Coat-A-Count Total T₃, Diagnostic Products Corp., Los Angeles, CA) in duplicate 100 µl aliquots. Sensitivity of the assay averaged 0.03 nmol.l⁻¹ over 2 assays. The intra- and inter-assay CV for a plasma pool which displaced radiolabelled T₃ to 24% of the total bound were 10.1% and 10.7% respectively. Serial dilution of deer plasma produced a binding curve in both the above assays that was parallel to the standard curve.

Data analysis

For determination of oestrous cyclicity, significant episodes of progesterone (taken as indicative of a luteal phase) were defined as 2 consecutive sample concentrations exceeding 2 nmol.l⁻¹, since this concentration divides typical follicular and luteal phase concentrations in red deer (Jopson *et al.*, 1990; Meikle and Fisher, 1996). Plasma concentrations of progesterone measured while CIDRs were in place were not included when determining oestrous cyclicity. For calculation of the number of oestrous cycles during the breeding season, the date used for the transition from the breeding season to the non-breeding season was 5 September (based on results of Meikle and Fisher, 1996). Hinds were deemed to have exhibited overt oestrus if mounted by the stag. Where this occurred, the onset and offset of oestrus for a hind was taken to be the first and last mounting interaction respectively for that hind (whether by mounting other hinds or the stag or by standing to be mounted by other hinds or the stag). The hind was considered to be mated if the stag thrust forward with feet off the ground and nose pointed upward while mounting the hind. Treatment effects on the number of hinds in each group which showed oestrus or ovulated were determined by a chi-squared test for differences between proportions. All other effects were identified by one-way ANOVA. Mean data are presented ± SEM.

RESULTS

Mean plasma T₃ concentrations were 1.5 ± 0.2 nmol.l⁻¹ in euthyroid control hinds and low or undetectable (0.1 ± 0.1 nmol.l⁻¹) in THX hinds ($P < 0.001$). Between early May and early September, THX hinds a similar mean number of progesterone episodes to control hinds (3.6 ± 0.7 and 4.6 ± 0.4 respectively; $P > 0.05$), but in 3 THX hinds progesterone episodes were evident only following CIDR treatment. Overt oestrus was recorded in fewer THX hinds than control hinds in the period following CIDR withdrawal in May ($P < 0.05$). A similar trend was observed for the occurrence of ovulation, but the difference was not significant ($P > 0.05$). In control hinds, the occurrence of oestrus and ovulation was lower in the period following CIDR withdrawal in October compared with July ($P < 0.05$), however in THX hinds there was no significant difference between the two seasons in the proportion of hinds exhibiting oestrus and ovulation ($P > 0.05$) (Table 1). The interval from CIDR withdrawal until the onset of oestrus (64.7 ± 4.5 h) was not affected by thyroidectomy or season and the duration of oestrus in May (10.7 ± 2.4 h) was not affected by thyroidectomy. In

TABLE 1: Percentage of hinds in each group showing oestrus or ovulating following CIDR withdrawal in May and October. Within a parameter, values not assigned common superscript letters are significantly different ($P < 0.05$).

Group	Oestrus		Ovulation	
	May	October	May	October
Control (n = 6)	100.0 ^a	0.0 ^b	100.0 ^a	0.0 ^c
THX (n = 9)	44.4 ^b	33.3 ^b	55.6 ^a	44.4 ^{bc}

TABLE 2: Percentage of hinds in each group exhibiting oestrus or ovulating following CIDR withdrawal in December and injection of 200 I.U. or 400 I.U. PMSG. Within a parameter, values not assigned common superscript letters are significantly different ($P < 0.05$).

Group	% Oestrus		% Ovulation	
	200 I.U.	400 I.U.	200 I.U.	400 I.U.
Control (n = 6)	0.0 ^a	50.0 ^{bc}	33.3 ^a	not measured
THX (n = 9 or 7)	22.2 ^{ab}	71.4 ^c	22.2 ^a	not measured

the 3 THX hinds which exhibited overt oestrus during October, the duration of oestrus (0.5 ± 0.4 h) tended to be much shorter than in May, but this effect did not reach statistical significance due to low numbers.

In early December, the occurrence of oestrus and ovulation was low in both groups following CIDR withdrawal and injection of 200 I.U. PMSG; only 2 matings (both with THX hinds) were recorded. Upon ultrasonic pregnancy diagnosis, one of these 2 hinds was found to be pregnant. However when all other hinds were resynchronized with cloprostenol and progesterone CIDRs in conjunction with 400 I.U. PMSG, the occurrence of oestrus increased in both groups ($P > 0.05$) (Table 2). Time to onset and duration of oestrus (48.6 ± 3.2 h and 3.2 ± 1.1 h respectively) were not affected by thyroidectomy or dose of PMSG ($P < 0.05$). In 3/3 control and 2/5 THX hinds which were mated following 400 I.U. PMSG, successful pregnancies were recorded as indicated by the presence of foetuses and placentomes upon ultrasonic examination. Foetal crown to rump measurement averaged 26.0 ± 0.6 mm on day 45 and 56.0 ± 0.0 mm on day 58 after mating; these values were within the normal range reported by Bingham *et al.* (1990) indicating normal foetal development. Establishment of pregnancy was associated with a sustained increase in plasma progesterone concentration beginning soon after mating and continuing until sampling ceased in early February. This pattern was also evident in all 4 THX hinds which were mated by the fertile stag during December but were not diagnosed as being pregnant by ultrasonic examination, suggesting that conception occurred but the resultant embryos or foetuses were resorbed or aborted prior to ultrasonic examination. All 7 out-of-season pregnancies resulted in birth of live calves on 31 July (n=1) and 9-17 August (n=5), with a mean gestation length of 235 ± 6 days. However 5 of the calvings were characterised by low live weight at birth (mean 6 ± 1.6 kg) and poor udder development in the dams; these calves died within 27 days of birth. The other 2 calves survived (mean birth weight 7.2 kg) and have

grown at better than normal rate (mean live weight at weaning at 4 months = 53 kg, *cf.* 43-48 kg; at 7 months - male calf 82 kg, female calf 74 kg, *cf.* 61 and 56 kg respectively).

DISCUSSION

The results of this experiment indicate that thyroidectomy impairs ovarian function in female red deer, since fewer THX hinds exhibited oestrus following synchronization with progesterone during the breeding season and there was a non-significant reduction in ovulation rate compared with control hinds. However in the non-breeding season the occurrence of oestrus and ovulation was maintained at a similar level in THX hinds, while in control animals oestrus and ovulation ceased to occur following progesterone withdrawal. Collectively these results imply that although thyroid gland secretions are required to activate reproductive inhibition during anoestrus, they also have a supportive effect on various body systems for the occurrence of ovulation and overt oestrous behaviour during the breeding season.

The published literature on the thyroid hormone requirements for successful reproduction suggest considerable variation exists between species. In female cattle, the incidence of overt oestrus following progesterone and oestradiol treatment was higher in thyroidectomized cows than euthyroid cows, although measures of the intensity of oestrus showed no effect of thyroidectomy (Stewart *et al.*, 1993). In contrast, behavioural signs of oestrus were reduced in thyroidectomized mares (Lowe *et al.*, 1987). Similarly, Reddy *et al.* (1996) observed a suppression of behavioural oestrus in female goats induced to hypothyroidism with thiourea and Walkden-Brown *et al.* (1996) reported cessation of oestrous cyclicity in this species following thyroidectomy. Data from several studies in ewes suggest that oestrous cyclicity is not dependent on the thyroid gland (Falconer, 1963; Brooks *et al.*, 1965; Nicholls *et al.*, 1988; Peeters *et al.*, 1989), although Brooks *et al.* noted a reduction in twinning and lower lamb birth weights in thyroidectomized ewes.

Since preovulatory plasma LH concentration was not measured in this experiment, it is not possible to determine if thyroid gland secretions are required at the hypothalamo-hypophyseal level for generation of the increase in gonadotrophin secretion which culminates in the LH surge (Caraty *et al.*, 1995), or at the ovarian level for follicular steroid production. It is possible that the enhanced responses to the higher dose of PMSG reflect a dose-related action of this substance rather than being simply attributable to a second treatment effect. This would imply that the effects of thyroidectomy on oestrous cyclicity were due to inadequate secretion of pituitary hormones during the follicular phase. Also it should be noted that the dose of PMSG required to stimulate ovulation in the current study was higher than that normally employed for successful induction of oestrus and ovulatory responses 2-3 weeks before the onset of natural mating activity (Asher *et al.*, 1994a). Alternatively the present findings may indicate a reduction in ovarian responsiveness to gonadotrophin

stimulation in THX hinds. The results of other studies also strongly suggest an ovarian site of action of thyroid hormones. For example, induction of hypothyroidism was associated with reduced plasma oestradiol and progesterone concentrations and impaired oestrous cyclicity in goats (Reddy *et al.*, 1996) and lowered steroid metabolism in growing follicles and corpora lutea in rats (Mattheij, 1995).

A surprising finding in this experiment is the efficacy of a high dose of PMSG in inducing reasonably successful (50%) out-of season pregnancies in euthyroid control hinds. There are no published reports of attempts to achieve breeding of red deer this far out-of-season using progesterone and PMSG, however when administered in February or March this treatment typically results in 10-20% of treated hinds calving despite induced ovulation rates of 70-85% (e.g. Fisher *et al.*, 1986; Moore and Cowie, 1986; Fennessy *et al.*, 1986; Fennessy and Fisher, 1988). These low calving percentages have been attributed in part to low fertility of stags in February and early March (Fennessy and Fisher, 1988), since in one study fertility was improved (59% of progesterone/PMSG-treated hinds calving) when melatonin-treated stags were used (Moore, 1987), and in another study 79% of progesterone/PMSG-treated hinds conceived following artificial insemination in March (Fennessy *et al.*, 1991). The current results lend some support to this argument, since the stag used for mating in December exhibited rutting behaviour characteristic of the breeding state, and previous studies have shown that thyroidectomized stags do not experience a seasonal decline in reproductive traits such as plasma testosterone concentration, testis diameter and responsiveness of the pituitary gland and testes to exogenous GnRH (Shi and Barrell, 1992).

Another possible reason for successful out-of-season pregnancy in these euthyroid hinds may be social stimulation of ovarian activity. For example, oestrus and ovulation was induced in anovulatory ewes (Zarco *et al.*, 1995) and goats (Restall *et al.*, 1995) by exposure to females in which oestrus had been induced. Such a social cue may have been provided by cycling THX females in the current experiment. Furthermore, the presence of a stag in which rutting behaviour was advanced by melatonin treatment has been shown to advance the breeding season in adult red deer hinds (Moore and Cowie, 1986; Wilson, 1992), and in yearling hinds the timing of puberty was slightly advanced if they were reared with a stag in the same paddock (Fisher *et al.*, 1995). In the current experiment, the hinds which were mated in late December had been kept in one paddock with either a vasectomized or fertile rutting stag for over 2 months. Both stags showed typical herding behaviour during this time and interacted closely with the hinds. This contact would presumably have provided powerful olfactory, visual and auditory stimuli for reproductive activity in the hinds. Such stimulatory effects of male animals on reproduction in anovulatory females have been described for sheep (Martin *et al.*, 1986). The argument that successful out-of-season breeding in euthyroid hinds in the current experiment may have been achieved at least in part by social stimulation

and mating with a fertile stag implies that the current inability to alter markedly the breeding season of deer on farms is not primarily due to a lack of responsiveness of the hind to hormonal treatments. The use of thyroidectomized stags to provide stimulatory social cues to the hinds and for out-of-season mating could prove to be a useful tool for further investigating this possibility and for future out-of-season breeding studies.

As the incidence of low birth weight, poor calf survival and compromised lactation in July/August calvings was not confined to thyroidectomized hinds, it is possible that calving at such early dates is detrimental to calf welfare. This requires further investigation.

In summary, while thyroidectomized hinds did not display a seasonal decline in ability to ovulate or display oestrus behaviour following synchronization with progesterone, the incidence of ovulation and overt oestrus was low in both seasons, suggesting that a side-effect of thyroidectomy may be impaired fertility. Following gonadal stimulation with a high dose of PMSG and joining with a fertile, rutting stag, seven out-of-season pregnancies were obtained from ten matings. However because three of these pregnancies occurred in euthyroid control hinds no improvement in out-of-season reproductive performance could be attributed to thyroidectomy. An important implication of these results is that before the effects of the thyroid glands on seasonal reproduction can be exploited to achieve practical out-of-season breeding, techniques must first be developed for blocking their specific effects on the reproductive neuroendocrine centres without causing hypothyroidism.

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