**Introduction**

Red deer (Cervus elaphus) are adapted to mixed browsing and grazing (Hoffman, 1985) and have evolved a breeding strategy that copes with seasonal extremes in food supply and quality (Lincoln and Short, 1980). Under farmed conditions in managed pastoral environments, there is considerable misalignment between seasonal breeding in red deer and the seasonal peak of pasture production and quality. In New Zealand, for example, the spring flush of pasture growth occurs in August–October, but red deer hinds calve naturally in November–December (Asher et al., 1996). Thus, peak demands for lactation and the requirements of the young calf for high quality feed occur in late summer when pasture growth and quality is limited and senescence is occurring in pasture plants.

A number of strategies have been used to control breeding in deer with the aim of better aligning calving and the onset of lactation with pasture growth, by inducing ovulation and mating during seasonal anoestrus. These treatments, which typically involve the administration of exogenous gonadotrophic hormones, have also been used to synchronize oestrus during the breeding season or to induce superovulation. However, the responses have been variable and unpredictable in deer, with some hinds failing to ovulate and with widely varying ovulation rates in those that did ovulate (Fennessey et al., 1994; Asher et al., 1995).

In other ruminants that, like red deer, ovulate a single follicle at each oestrous cycle (for example, cows and some breeds of sheep), a number of growing antral follicles enter a growth phase early in the follicular phase of the oestrous cycle. However, only a single follicle completes preovulatory development and ovulates, with the remainder undergoing atretic degeneration (Hirschfield, 1991). The development of this single dominant follicle suppresses the growth and results in atresia of the other large antral follicles present (McNatty et al., 1982; McNatty and Henderson, 1987). The inclusion of antral follicles into the cohort of rapidly growing follicles early in the follicular phase (termed ‘selection’ or ‘cyclic recruitment’) is known to be under the influence of FSH (for review, see McGee and Hseuh, 2000). The administration of exogenous FSH or FSH-like gonadotrophins during the follicular phase will induce superovulation in monovular animals, presumably...
by increasing the number of antral follicles recruited into the growing pool or preventing their degeneration. Equine chorionic gonadotrophin (eCG) is used widely as a source of exogenous gonadotrophin to stimulate superovulation in monovular species. In sheep, eCG increases the numbers of antral follicles ≥ 3 mm in diameter (Dott et al., 1979) and, when administered at the time of luteolysis, eCG prevents the occurrence of atresia in large follicles and enhances their capacity to produce oestriadiol (McNatty et al., 1982).

Equine chorionic gonadotrophin has long been used to induce ovulation in seasonally anoestrous ruminants, including deer, but as occurs in the breeding season, ovulation rates in response to this treatment are sometimes extremely variable (red deer: Adams et al., 1985; fallow deer: Asher and Smith, 1987). An alternative approach has been to treat seasonally anoestrous animals with GnRH administered as either a series of injections or as a continuous infusion to promote endogenous gonadotrophin secretion. In sheep, this approach has proved consistently reliable and has induced ovulation rates comparable to those occurring in the same flock during the breeding season (McLeod et al., 1982, 1983). Similar treatment regimens have been used in Père David’s deer (McLeod et al., 1991) and in red deer (Fisher et al., 1989a). However, in both studies, the percentage of treated animals that ovulated was inconsistent and appeared to be related to the stage of the anoestrous season.

Recently, we reported that the number of antral follicles present in red deer hinds during the breeding season differed widely among individuals (4–81 follicles ≥ 2 mm diameter per hind), and that the proportion of follicles that were healthy increased progressively over the follicular phase of the oestrous cycle (McLeod et al., 2001). Therefore, the reported variability in response to induction–superovulation treatments may simply reflect between-animal differences in the numbers of antral follicles present that are capable of responding to the exogenous hormones. The objective of the present study was to compare the responses to exogenous gonadotrophin (eCG) and to GnRH-induced secretion of endogenous gonadotrophins, in promoting antral follicle development in the breeding and non-breeding seasons, to determine the underlying basis of seasonal differences in ovarian function in red deer. Some of these data (hinds treated with progesterone alone during the breeding season) were included in the earlier report of follicle populations in cyclic red deer hinds (McLeod et al., 2001).

Materials and Methods

Animals and management

A total of 55 parous adult red deer (Cervus elaphus) hinds, aged between 3 and 9 years, were used in this study. Ovaries were recovered from 40 of these animals during either the breeding season (March, n = 20) or seasonal anoestrus (January, n = 20). The remaining 15 hinds (control groups) were monitored on both of these occasions to determine the times of onset of oestrus and the preovulatory LH surge, so that the time of ovary recovery in their herdmates could be related to these endocrine events.

All hinds were maintained on pasture throughout the treatment period, with the exception that those animals from which ovaries were recovered were fasted overnight before ovariection or slaughter. All experimental procedures performed had been given prior approval by the AgResearch Invermay Animals Ethics Committee according to the Animals Protection (Codes of Ethical Conduct) Regulations, 1987.

Treatment and blood sampling

Oestrus was synchronized during the breeding season in the animals studied so that ovaries could be recovered at a known stage of the oestrous cycle. All hinds were subjected to exogenous progesterone treatment for 12 days, administered via controlled intravaginal drug releasing devices (EAZI-BREED CIDR, 0.3 g progesterone; InterAg, Hamilton). A single CIDR, left for 8 days in situ, was replaced with a fresh CIDR for the following 4 days to ensure that increased plasma progesterone concentrations were maintained throughout. All hinds treated during seasonal anoestrus were given an identical progesterone treatment, to allow unbiased comparison of follicle populations between the two reproductive states.

At the time of withdrawal of the second CIDR, hinds were assigned randomly to one of four treatment groups (n = 5 animals group). Animals in groups 1 and 2 (breeding season) and groups 1a and 2a (anoestrus) received no further treatment. Hinds in groups 3 (breeding season) and 3a (anoestrus) were given a single i.m. injection of 300 iu eCG (Folligon; Intervet, Lane Cove, NSW) and those in groups 4 (breeding season) and 4a (anoestrus) were treated with synthetic GnRH (Sigma, St Louis, MO) administered via osmotic minipumps (ALZET 2ML4; Alza Corp, Palo Alto, CA) at the rate of 1.0 μg h⁻¹ for 4 days or until the time of ovariection. These treatment regimens are typical of those used in oestrus synchronization–superovulation programmes in red deer. Both the insertion and removal of minipumps was carried out under local anaesthetic (2% (w/v) xylocaine hydrochloride), injected s.c. at the site of implantation in the neck region. Hinds that were to be blood sampled and monitored for oestrus and ovulation were included in all three treatment regimens (note that groups 1 and 2 received the same treatment). The same blood-sampled animals were assigned to the same treatment protocol in both the breeding and non-breeding seasons.

Ovaries were recovered from hinds (n = 5 per group) either at the time of (groups 1 and 1a), or approximately 36 h after the time of (groups 2, 2a, 3, 3a, 4 and 4a; Table 2) CIDR removal and follicle development within them was assessed. Ovaries were recovered either by ovariection.
carried out under barbiturate-induced and halothane-maintained anaesthesia or within minutes of death at slaughter at a registered deer slaughter facility (Otago Venison Ltd, Mosgiel).

Control animals were monitored for behavioural signs of oestrus and were blood sampled via an indwelling jugular vein catheter to determine the time of onset of the preovulatory LH surge. Blood samples were collected and observations of oestrus were made at 2 h intervals from 4 h before until 72 h after CIDR withdrawal as described by McLeod et al. (2001). The incidence of ovulation was determined in all these animals at laparoscopy, carried out 7 days after CIDR withdrawal under xylazine (Rompun; Bayer NZ Ltd, Petone, NZ) and fentanyl citrate plus azaperone (Fentaz; Smith, Kline and French (NZ) Ltd, Auckland) anaesthesia.

Ovarian tissue collection and preparation

Techniques used in the collection of ovarian tissue and analysis of follicle populations and their health status were as described by McLeod et al. (2001). Briefly, excised ovaries were placed immediately on ice in Dulbecco’s PBS solution (KC Biological Inc., Lenaxa, KS), and transferred to an adjacent laboratory where they were weighed and all follicles > 2.0 mm in diameter were dissected out under a stereomicroscope. After being dissected free of extraneous tissue, the diameter of each follicle was recorded (to the nearest 0.1 mm) and follicle health was assessed. Follicular fluid was aspirated from each follicle, its volume measured and a sample snap-frozen for subsequent analysis of oestradiol content. Granulosa cells were harvested, washed and counted as described by McLeod et al. (2001).

Granulosa cell aromatase activity and cAMP production

Aromatase activity and cAMP production of the harvested granulosa cells was determined in vitro using the methods for deer follicles described by McLeod et al. (2001). Cells collected from each follicle were suspended in ice-cold Dulbecco’s PBS solution containing 0.1% BSA (Sigma Chemical Company), 20 mmol Hepes buffer l–1 and 0.2 mmol 3-isobutyl-1-methylxanthine l–1 (Sigma; Medium A, pH 7.4, 4.0°C), with a final cell concentration of 2–4 × 10^5 cells ml–1. Aliquants (0.5 ml, in duplicate or triplicate depending on the total number of granulosa cells recovered) of these cell suspensions were incubated to assess oestradiol (aromatase activity) or cAMP production.

For the determination of aromatase activity, cells were incubated in either medium alone (time zero incubations), or in medium containing 200 ng LH (NIH-LH-S17) ml–1 (LH incubations), again made up to a final volume of 1.0 ml, and granulosa cell cAMP production was assessed. The LH incubations were placed in a shaking water bath at 37°C for 45 min, after which time the reaction was stopped by immersion in a water bath at 80°C for 15 min. Time zero incubations were immediately incubated at 80°C for 15 min. All tubes were stored at –20°C until the incubation media were assayed for cAMP.

Aromatase activity (expressed as ng oestradiol per 10^6 cells h–1) and production rates of cAMP (mmol per 10^6 cells h–1) in granulosa cells from individual follicles was calculated by subtracting the oestradiol or cAMP content of supernatant from time zero incubations from the values for the same follicle after the testosterone or LH incubations.

Follicle classification

Follicles were classified as healthy or atretic on the basis of the criteria described by McLeod et al. (2001), which had been adapted from the methods of McNatty et al. (1985) used for sheep follicles. The factors considered included the presence or absence of blood capillaries within the thecal tissue and of debris in the follicular fluid, the condition of the oocyte (healthy or degenerating, on the basis of the presence of a cumulus cell matrix, signs of cytolysis, necrosis or loss of spherical shape) and the total granulosa cell complement of the follicle.

Follicle health was classified as one of four categories (classes 1, 2a, 2b and 3) as described by McLeod et al. (2001). Only class 1 follicles are regarded as healthy (non-atretic), with those classed as classes 2a, 2b and 3 representing follicles in progressive stages of degeneration and atresia. Non-atretic follicles were arbitrarily classified as ‘oestrogenic’, or ‘non-oestrogenic’ on the basis of their granulosa cell population, follicular fluid oestradiol content and aromatase activity and cAMP production (McLeod et al., 2001).

Follicles were described as having luteinized if they had a thickened, solid and opaque wall, with or without a fluid-filled cavity, and when there was no evidence of a rupture site.

Hormone assays

Plasma LH concentrations were determined using the heterologous double-antibody radioimmunoassay method described for deer plasma by Meikle and Fisher (1996). All samples were completed within one assay in which the intra-assay coefficient of variation was 11.7%. The limit of detection was 0.08 ng LH ml–1 plasma.

Concentrations of oestradiol in follicular fluid and in granulosa cell incubation media were measured using the tritiated oestradiol radioimmunoassay described for sheep by McNatty et al. (1981) and subsequently validated for deer (McLeod et al., 2001). Results were expressed as ng
oestradiol per 10^6 cells. Follicular fluid oestradiol was measured directly without extraction, in diluted (10–100 fold dilution with 0.1 mol PBS l^{-1}) aliquots of follicular fluid. The limit of detection was 1.0 ng oestradiol ml^{-1}.

Granulosa cell cAMP production was determined using the method described by McNatty et al. (1985), and cAMP production was expressed as pmol per 10^6 cells. Within this study, the inter- and intra-assay coefficients of variation were both < 10% and the limit of detection was 0.02 pmol per 10^6 cells.

Statistical analyses

The definition used to characterize a preovulatory LH surge was that there was a sustained (> 6 h) increase of plasma LH concentrations above 2 ng LH ml^{-1} and that peak concentrations attained during this time exceeded 5 ng ml^{-1}.

Comparison of follicle populations among treatment groups was made by analysis of variance, performed in conjunction with the Neuman and Keuls test, and of follicular fluid oestradiol content and granulosa cell hormone production rates by Student’s t test. The incidence of oestrus and ovulation was compared by chi-squared analysis, and changes in follicle health distribution, by cluster analysis. Unless otherwise stated, all values are presented as the mean ± SEM.

Results

Liveweights, oestrus, the preovulatory LH surge and ovulation rates

There was no significant difference in mean liveweight between hinds treated during the breeding season (n = 35, mean liveweight 100.1 ± 2.0 kg) and those treated in mid-anoestrus (n = 35, mean liveweight 97.1 ± 2.2 kg). Both within and between seasons, there were no significant differences in liveweight among the treatment groups.

The mean incidence and the time of onset of oestrus and the preovulatory LH surge, and ovulation rates are shown for each of the groups of the blood-sampled hinds (Table 1). During the breeding season, four of five hinds treated with CIDRs alone and four of five eCG-treated hinds, but only two of five GnRH-treated hinds, exhibited oestrus during the 76 h period of observation. A preovulatory LH surge was recorded in all except one hind (treated with CIDR alone), and all animals ovulated. When ovaries were observed by laparoscopy 7 days after CIDR withdrawal, two eCG-treated hinds had a double ovulation, and the remaining 13 animals each had ovulated a single follicle. Luteinized follicles were present in three hinds (all eCG-treated). There were no significant differences among treatment groups in the timing of oestrus or the preovulatory LH surge, or in ovulation rates.

When treated during seasonal anoestrus, none of the control animals subjected to CIDR alone or to GnRH exhibited oestrus, had a preovulatory LH surge or ovulated. In contrast, four of five eCG-treated hinds were observed in oestrus and all these animals had a preovulatory surge. However, only two of these animals ovulated (single ovulation) and there was a luteinized follicle present on the ovaries of two hinds (one eCG-treated, one GnRH-treated) that had failed to ovulate.

Antral follicle populations

The mean times of ovary recovery are shown for each of the treatment groups (Table 2). In those animals in which the time of collection was nominally set at 36 h, the mean time of recovery was 38.6 ± 0.34 h after CIDR withdrawal. There were no significant differences in the mean recovery time among groups.

When all treatment groups are combined, the mean number of antral follicles ≥ 2.0 mm in diameter present did not differ significantly between the breeding (mean 19.6 ± 2.81, range 4–49 follicles per hind) and non-breeding seasons (mean 18.6 ± 2.2, range 10–39 follicles per hind). Similarly, the mean number of follicles present was not significantly different among groups in the breeding season (Table 2). However, hinds treated with GnRH during seasonal anoestrus had significantly more (P < 0.05) antral follicles than animals treated with CIDR alone in the same season or animals receiving the same treatment (GnRH) in the breeding season. The numbers of large (≥ 4.0 mm in diameter) follicles did not differ among treatment groups or between seasons.

Follicle health status

The mean number of healthy and oestrogenic follicles, and the mean diameter of the largest follicle, are shown (Table 3). With a single exception, the number of healthy follicles did not differ among treatments or between seasons. The exception was that the number of healthy follicles in animals treated with GnRH during the non-breeding season was significantly higher than it was in the other treatment groups at both reproductive states, and higher than it was in animals subjected to the same treatment during the breeding season. A similar trend was seen in the total number of oestrogenic follicles, but this was significant only between the GnRH-treated group and those treated with CIDRs alone in anoestrus. The number of hinds with at least one oestrogenic follicle present was not significantly different in either the breeding or non-breeding seasons (three of five versus two of five, three of five versus four of five, four of five versus five of five, four of five versus five of five for groups 1–4, respectively). There were no significant differences in the mean diameters of the largest healthy, or largest oestrogenic follicles present.

When animals from all treatment groups were combined and the health status of their follicles expressed as a percentage of all follicles present, there was a significantly higher proportion of healthy follicles present during seasonal anoestrus than during the breeding season (72.2 and 56.6% healthy, respectively, P = 0.019, pooled SD =
The percentage of follicles in each health class is shown for individual treatment groups (Fig. 1). The distribution of atretic follicles across the three classes was significantly different (P < 0.05) between seasons (anoestrus: 19.4, 9.6 and 0.1%; breeding season: 11.2, 25.5 and 6.8% for classes 2a, 2b and 3, respectively), but not among treatment groups.

Oestradiol and cAMP production

Mean concentrations of oestradiol in the follicular fluid of oestrogenic follicles, and in vitro oestradiol (aromatase activity) and cAMP production by their granulosa cells, are shown (Table 4). During both seasons, oestradiol content of follicular fluid was significantly higher (P < 0.05) in GnRH-treated animals than in hinds treated with CIDRs alone. Follicular fluid concentrations in eCG-treated hinds were midway between these two groups. Neither aromatase activity nor cAMP production differed significantly among groups or between seasons.

Discussion

Treatment with exogenous gonadotrophin (eCG) or inducing endogenous gonadotrophin secretion (with GnRH) promoted antral follicle development in red deer hinds, increasing the number of oestrogenic follicles present and their follicular fluid oestradiol content in both the breeding and non-breeding seasons. However, the response was variable and it is likely that few of these follicles would have successfully completed preovulatory development and ovulated. These
hormonal treatments resulted in a double ovulation in only two of the blood-sampled animals in the breeding season and only two of ten animals had an induced ovulation when treated in seasonal anoestrus. There were surprisingly few between-season differences in the parameters of follicle development monitored.

The ovulation rates observed in the hinds in the present study are not dissimilar to those reported previously for a variety of cervid species subjected to these treatments. Comparable doses of eCG administered during or within a few weeks of the start of the breeding season have resulted in double ovulations in 10–50% of treated red and fallow deer (Fisher et al., 1989a; Asher et al., 1992). Multiple ovulations (> 2) can be induced in deer with higher doses of eCG (Kelly et al., 1982; Asher and Smith 1987). At least 50% of treated animals ovulated a single follicle when GnRH was administered 3–6 weeks before the start of the breeding season (red deer: Fisher et al., 1989a; fallow deer: Asher and Macmillan, 1986; Père David’s deer: McLeod et al., 1991). However, few if any hinds ovulated when treated in mid-anoestrus (McLeod et al., 1991). There are also reports of seasonal variation in the ovulatory response to treatment with exogenous gonadotrophins in other species. For example, when sheep were subjected to repeated injections of low doses of LH for an extended period, 100% ovulated when treated in early anoestrus, but only 25% ovulated when treated in mid-anoestrus (Oussaid et al., 1998).

The times of onset of oestrus and of the preovulatory LH surge recorded in the blood-sampled hinds in the present study are similar to those reported earlier in red deer (Asher et al., 1992). They also indicate that follicle collection from the experimental animals would have occurred at a stage equivalent to late follicular phase before occurrence of the LH surge. The time of onset of oestrus and the preovulatory LH surge, both events that are initiated by increasing plasma oestradiol concentrations, was not influenced by treatment with exogenous gonadotrophins. This was in contrast to previous reports in sheep in which there was an inverse relationship between the time of onset of oestrus and ovulation and dose of eCG (Boshoff et al., 1973).

The mean number of large antral follicles recorded in hinds in this study (range 1–10 per hind) was similar to that recorded by ultrasonography in red deer hinds during the breeding season (Asher et al., 1997). In both the present study and that of Asher et al. (1997), there were no significant differences in the numbers of antral follicles present in the breeding season compared with seasonal anoestrus. This finding is in contrast to the findings in sheep reported by McNatty et al. (1984) in which significantly more antral follicles ≥ 1 mm in diameter were present during seasonal anoestrus than during the breeding season. In the present study, there were seasonal changes in the proportion of healthy follicles in untreated hinds (CIDR-alone groups), with follicle populations being skewed towards class 1 (non-atretic) in anoestrus and towards classes 2b and 3 (advanced atresia) in the breeding season. Treatment with gonadotrophins during seasonal anoestrus tended to alter follicle health status towards that seen in the breeding season. Similar seasonal changes in follicle health distribution have previously been reported in sheep (McNatty et al., 1984).

The increase in follicular fluid oestradiol concentrations observed in the gonadotrophin-treated hinds concurs with the pattern reported in both sheep (McNatty et al., 1982) and pigs (Miller et al., 1999) treated with eCG. The increased oestradiol concentrations did not promote the time of onset of oestrus or the onset of the preovulatory LH surge but rather there appeared to be adverse effects on oestrous behaviour, with four of ten of the gonadotrophin-

### Table 3. Mean (± SEM) number of healthy and oestrogenic follicles, and the mean diameter of the largest follicle present, in red deer hinds treated with progesterone (CIDR) alone, or in conjunction with equine chorionic gonadotrophin (eCG) or GnRH during the breeding or non-breeding seasons

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of healthy follicles*</th>
<th>Number of oestrogenic follicles*</th>
<th>Mean diameter (mm) of largest follicle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Healthy</td>
</tr>
<tr>
<td>Breeding season</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>CIDR alone (0 h)</td>
<td>8.0 ± 1.64* (4–13)</td>
<td>0.8 ± 0.37* (0–2)</td>
<td>7.5 ± 0.33*</td>
</tr>
<tr>
<td>2</td>
<td>CIDR alone (36 h)</td>
<td>9.0 ± 2.47* (3–18)</td>
<td>1.0 ± 0.45* (0–2)</td>
<td>8.0 ± 0.63*</td>
</tr>
<tr>
<td>3</td>
<td>CIDR/eCG</td>
<td>8.4 ± 2.46* (3–16)</td>
<td>2.0 ± 0.71* (0–4)</td>
<td>8.0 ± 1.04*</td>
</tr>
<tr>
<td>4</td>
<td>CIDR/GnRH</td>
<td>8.0 ± 2.35* (1–14)</td>
<td>1.8 ± 0.58* (0–3)</td>
<td>7.8 ± 0.88*</td>
</tr>
<tr>
<td>Seasonal anoestrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>CIDR alone (0 h)</td>
<td>8.6 ± 0.75* (7–11)</td>
<td>0.4 ± 0.24* (0–1)</td>
<td>7.4 ± 0.42*</td>
</tr>
<tr>
<td>2a</td>
<td>CIDR alone (36 h)</td>
<td>9.0 ± 1.05* (7–12)</td>
<td>0.8 ± 0.20* (0–1)</td>
<td>7.9 ± 0.25*</td>
</tr>
<tr>
<td>3a</td>
<td>CIDR/eCG</td>
<td>11.0 ± 0.84* (9–14)</td>
<td>1.4 ± 0.24* (1–2)</td>
<td>9.3 ± 0.64*</td>
</tr>
<tr>
<td>4a</td>
<td>CIDR/GnRH</td>
<td>23.6 ± 4.38* (7–33)</td>
<td>2.6 ± 0.68* (1–4)</td>
<td>7.7 ± 0.77*</td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent the range in numbers of follicles per hind. Within columns, values with different superscripts are significantly different (P < 0.05).
treated control hinds failing to exhibit oestrus in the breeding season, although they all ovulated. In addition, despite the significant increase in the number of oestrogenic follicles present, and in their oestriadiol content, evident in their experimental counterparts, none of the GnRH-treated hinds that were blood sampled displayed oestrus, produced a preovulatory LH surge or ovulated when treated during seasonal anoestrus. Meikle and Fisher (1991) showed that a single injection of oestradiol after pretreatment with progesterone induced both an LH surge and oestrus in long-term ovariectomized hinds during anoestrus, except when the treatment was given in mid-anoestrus (January). This is the same time of year in which the seasonal anoestrus experiment was carried out in the present study, in which only four of ten gonadotrophin-treated hinds displayed oestrus and had a preovulatory LH surge, and only two of ten ovulated. It remains to be determined whether the same treatment regimens would promote follicle development and induce oestrus and ovulation at other stages of seasonal anoestrus.

A further abnormality in ovarian function observed in gonadotrophin-treated hinds, was the luteinization of large antral follicles in the blood-sampled animals. Luteinized follicles were observed in five hinds (three breeding season, two anoestrus) in which gonadotrophins had been manipulated. Asher and Smith (1987) reported the occurrence of luteinized follicles in over 50% of fallow deer treated with eCG, and this phenomenon has also been described in sheep treated with GnRH (Gilbert et al., 1990). Luteinization of large antral follicles occurs typically at a very low rate in both red and fallow deer treated with CIDRs alone (< 1%, n > 300, G. W. Asher and I. C. Scott, unpublished) but does appear to be a consequence of the administration of exogenous gonadotrophins. In rats, a single injection of a low dose of exogenous LH given some hours before the expected time of the preovulatory LH surge results in luteinization without ovulation in virtually all of the Graafian follicles present (Mattheij and Swarts, 1995). A cytokine (vascular endothelial growth factor) that induces angiogenesis, which is a feature of follicle luteinization, has been proposed as the mediator of luteinization in women (Anasti et al., 1998), and its production in primate preovulatory follicles is induced by LH (Hazzard et al., 1999).

Despite the incidence of follicle luteinization in the blood-sampled hinds, no luteinized follicles were present in ovaries recovered from the experimental animals. These hinds would have been exposed to exogenous gonadotrophins for a period of < 40 h and the ovaries were recovered before the onset of the preovulatory LH surge, which presumably triggers luteinization.

In summary, manipulating plasma gonadotrophin concentrations in hinds during either the breeding or non-breeding season influences follicle development, but does not change the number of antral follicles that enter the growing phase or the size distribution of the antral follicle population. Nevertheless, the extent to which this promotion of antral follicle growth results in the development of healthy, oestrogenic follicles varies widely among individual animals in both the breeding and non-breeding seasons. This variability is probably a reflection of the numbers of healthy antral follicles present at the time of treatment. As a consequence of this variability, the number of follicles that ovulate will be variable and unpredictable.
**Table 4.** Mean (± SEM) diameter, follicular fluid oestradiol concentration and granulosa cell aromatase activity and cAMP production of oestrogenic follicles recovered from red deer hinds treated with progesterone (CIDR) alone, or in conjunction with equine chori... during the breeding or non-breeding seasons

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>n</th>
<th>Follicle diameter (mm)</th>
<th>Follicle fluid oestradiol (ng ml⁻¹)</th>
<th>Aromatase (ng per 10⁶ cells h⁻¹)</th>
<th>cAMP (pmol per 10⁶ cells h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breeding season</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>CIDR alone (0 h)</td>
<td>4</td>
<td>7.4 ± 0.4</td>
<td>79.7 ± 14.5a</td>
<td>10.8 ± 0.1a</td>
<td>5.8 ± 4.2a</td>
</tr>
<tr>
<td>2</td>
<td>CIDR alone (36 h)</td>
<td>5</td>
<td>5.8 ± 0.6</td>
<td>85.1 ± 18.9a</td>
<td>7.1 ± 1.9a</td>
<td>4.8 ± 3.2a</td>
</tr>
<tr>
<td>3</td>
<td>CIDR/eCG</td>
<td>10</td>
<td>5.6 ± 0.9</td>
<td>120.1 ± 18.3ab</td>
<td>7.5 ± 1.5a</td>
<td>10.2 ± 4.7a</td>
</tr>
<tr>
<td>4</td>
<td>CIDR/GnRH</td>
<td>9</td>
<td>5.6 ± 0.9</td>
<td>158.8 ± 31.4b</td>
<td>5.8 ± 1.2a</td>
<td>6.5 ± 1.5a</td>
</tr>
<tr>
<td><strong>Seasonal anoestrus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>CIDR alone (0 h)</td>
<td>2</td>
<td>7.3 ± 0.2</td>
<td>89.4 ± 16.4a</td>
<td>7.1 ± 2.3a</td>
<td>0.4 ± 0.24a</td>
</tr>
<tr>
<td>2a</td>
<td>CIDR alone (36 h)</td>
<td>4</td>
<td>7.9 ± 0.3</td>
<td>167.9 ± 29.5a</td>
<td>8.9 ± 2.1a</td>
<td>0.8 ± 0.20a</td>
</tr>
<tr>
<td>3a</td>
<td>CIDR/eCG</td>
<td>7</td>
<td>7.6 ± 1.1</td>
<td>264.8 ± 49.3ab</td>
<td>8.6 ± 1.8a</td>
<td>1.4 ± 0.24a</td>
</tr>
<tr>
<td>4a</td>
<td>CIDR/GnRH</td>
<td>13</td>
<td>5.8 ± 0.5</td>
<td>293.5 ± 37.6b</td>
<td>8.7 ± 1.9a</td>
<td>2.6 ± 0.68a</td>
</tr>
</tbody>
</table>

Within columns, values with different superscripts are significantly different (P < 0.05).

Most importantly, these treatments are often associated with abnormalities in follicle development that may result in luteinization without ovulation.

The authors wish to thank Dr Colin Mackintosh for assistance with surgery, the staff of the Invermay Deer Unit for assistance with oestrus treatment, and the staff of the Invermay Deer Unit for assistance with oestrogenic follicles recovered from red deer hinds treated with progesterone (CIDR) alone, or in conjunction with equine chorionic gonadotrophin (eCG) or GnRH during the breeding or non-breeding seasons.

**References**


Downloaded from Bioscientifica.com at 12/12/2018 08:22:51PM via free access
Induced follicle development in red deer hinds


McLeod BJ, Meikle LM, Heath DA, McNatty KP and Fisher MW (2001) Variation in antral follicle development over the follicular phase of the oestrous cycle in the red deer hind Reproduction 120 211–216


