Ovarian follicle development in the red deer hind

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ABSTRACT

Ovaries were recovered either 0, 12, 24 or 36 hours or 10 days after CIDR withdrawal, from 25 hinds in which oestrus had been synchronised. All follicles ≥ 2 mm diameter were dissected free of stromal tissue and their health assessed on the basis of oocyte and theca condition, follicular fluid oestradiol content, granulosa cell numbers and oestradiol synthesis.

Hinds had an average of 26.6 ± 3.45 follicles ≥ 2 mm, of which about half were healthy and 1-3 were oestrogenic. Every animal had at least 1 healthy follicle ≥ 7.5 mm diameter. Over the follicular phase, the percentage of healthy follicles ranged from 46-66%, but this did not change significantly with time. Neither did the mean diameter of the largest oestrogenic follicle change over the follicular phase. In summary, the total number of antral follicles varied widely, but every hind had at least one large healthy follicle present, irrespective of stage of the oestrous cycle.

Keywords: deer; antral follicles; oestrous cycle; atresia; oestrogen.

INTRODUCTION

In the red deer hind, the effectiveness of treatments given to synchronise oestrus, to achieve superovulation or to induce ovulation during the non-breeding season, has been variable (Asher *et al.*, 1994; Fennessey *et al.*, 1994). Although there is currently little information available regarding follicle development in this species, the success or failure of these techniques is often arbitrarily attributed to the presence or absence of healthy follicles on the ovaries at the time of treatment, suggesting that there are wide differences in the number or health status of antral follicles between individual hinds. However, two characteristics of reproduction in the red deer hind, namely that twin ovulations are relatively rare (Fisher *et al.*, 1989), and that there is a high degree of synchrony between animals in a herd in the time of onset of breeding activity (Lincoln and Guinness, 1973), would suggest that there is a degree of uniformity in follicle populations between individual hinds. The present experiment was undertaken to assess just how much antral follicle populations do vary in red deer hinds, and to monitor the development of large follicles, particularly that of the presumptive ovulatory follicle, over the follicular phase of the oestrous cycle.

MATERIALS AND METHODS

Animals and management

Oestrus was synchronised in 30 adult red deer hinds (mean live weight 95.8 ± 2.0 kg) during the breeding season (April), by exposure to a 12-day period of progesterone treatment, administered via CIDR devices (AHI Industries, Hamilton, New Zealand). All animals were maintained on pasture as a single group, at Invermay Agricultural Centre, Mosgiel, New Zealand (latitude 45° 50'S). Ovaries were recovered from groups of 5 hinds at the time of (Time zero), or approximately 12, 24 or 36 hours (follicular phase), or 10 days (luteal phase) after CIDR withdrawal, either surgically by ovariectomy (carried out under general anaesthesia; zylazine hydrochloride induced, halothane maintained, N=12), or at slaughter (N=13). The time of onset of oestrus, and the occurrence of a preovulatory LH surge, was monitored in the remaining 5 hinds. These animals were blood-sampled at 2h intervals from 4 h before, until 72 h after withdrawal of the CIDR devices. The time of onset of oestrus was also monitored in these animals by observations made at 2h intervals from the time of CIDR withdrawal. The behaviour patterns associated with oestrus included (i) nuzzling the observer, (ii) tail flicking, (iii) standing immobile to pressure applied to the back, and (iv) adopting a hunched, squatting posture. Hinds were deemed to be in standing oestrus if they remained immobile to the back pressure test.

Follicle dissection

All follicles of ≥ 2.0 mm diameter were dissected free of stromal tissue. To assess follicle health, the vascularity of the thecal tissue and the integrity of the oocyte (healthy, degenerating or not found) was noted. Follicular fluid was aspirated from each follicle and its volume measured. Granulosa cells were scraped free of thecal tissue using a fine-wire loop, and the number of viable cells present in each follicle was estimated. The cells were suspended in phosphate-buffered saline, washed and centrifuged at 900 G for 15 minutes. The supernatant, which contained the contents of dead cells and contaminants of follicular fluid, was discarded and the remaining granulosa cells were counted.

Oestrogen secretion

The oestrogen synthesising capacity (aromatase activity) of granulosa cells was then determined *in vitro*. This involved a known number of granulosa cells being suspended in Dulbeccos medium, substrate (2.0 µg/ml

testosterone) added and the cells being incubated at 37° C for 3 h. Concentrations of oestradiol-17ß in follicular fluid and in granulosa cell incubation media were measured using the method described for sheep by McNatty *et al* (1981).

Follicle classification

Follicles were classified as healthy or atretic according to criteria used to describe sheep follicles (McNatty *et al.*, 1985). Healthy follicles had a vascularized theca interna, follicular fluid that was free of debris, a healthy-looking oocyte and a granulosa cell population that was > 25 percent quartile for a follicle of that diameter. All other follicles were regarded to be undergoing atresia. Non-atretic follicles were classified as 'oestrogenic', if they had a follicular fluid oestradiol concentration of > 50 ng/ml. Within each animal, the dominant non-atretic follicle was deemed to be that healthy follicle that had the highest follicular fluid oestradiol concentration and highest aromatase activity.

Analysis of data

Data on numbers of follicles were first normalised by log-transformation and the values averaged. Differences in follicle numbers and in follicle diameters were assessed by unpaired Student's t-test. All values are means ± s.e.m.

RESULTS

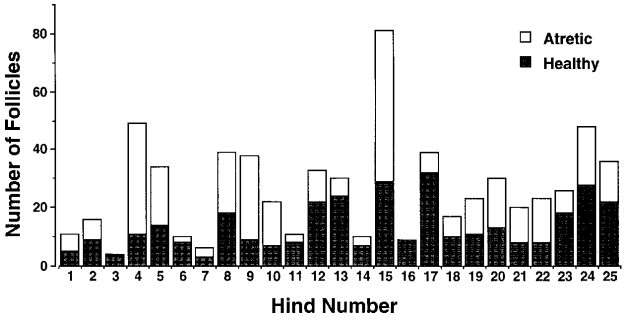
Times of oestrus and LH surge and ovary recovery

In the control hinds, the mean time of onset of oestrus and of the preovulatory LH surge was 45.5 ± 6.8 h and 44.5 ± 6.6 h after CIDR removal, respectively. The nominal times of ovary collection were set as 0, 12, 24 or 36 h after CIDR withdrawal, with the actual mean times of collection being 0 ± 0 h (Group 1, i.e. immediately before CIDR withdrawal), 12.6 ± 0.38 h (Group 2), 27.2 ± 0.65 h (Group 3) and 38.9 ± 0.36 h (Group 4) after CIDR withdrawal.

Antral follicle populations

In total, each hind had a mean number of 26.6 ± 3.45 follicles (range 4 - 81) that were ≥ 2.0 mm diameter, of which 19.6 to 100% were classified as healthy (Figure 1). This antral follicle complement consisted of an average of 16.1 (60.5%), 6.8 (25.6%), 1.7 (6.4%) and 2.0 (7.5%) follicles of the size ranges 2.0 -2.9 mm, 3.0 - 3.9 mm, 4.0 - 4.9 mm and ≥ 5.0 mm, respectively. The mean diameter of the largest follicle was 8.8 ± 0.32 mm. There was a strong correlation between follicle diameter and granulosa cell number (r^2 = 0.784), and for each 1 mm increase in follicle diameter, granulosa cell content increased by an average of 0.41 ± 0.01 x 10^6 cells.

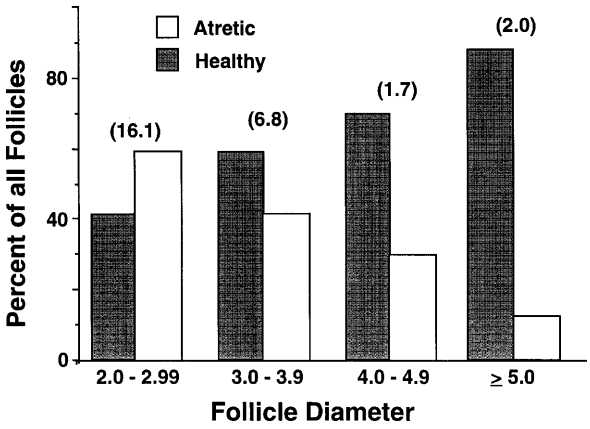
FIGURE 1: Total number of healthy (solid bars) and atretic (open bars) antral follicles ≥ 2.0 mm diameter in ovaries recovered from 25 individual red deer hinds in mid-breeding season.



Classification of health status of deer follicles

Further characterisation of red deer follicles, and classification of their health status, was based on parameters that were assessed in all follicles dissected in this study (a total of 665 follicles recovered from 25 hinds), irrespective of the time of ovary collection. Approximately half of the follicles were classified as healthy (50.8 %). The percentage of follicles that were healthy increased with increasing follicle diameter. Only 41% of follicles in the size range 2-3 mm were healthy, compared with 88% of follicles that were ≥ 5 mm diameter. The distribution of follicle health status in relation to follicle size is shown in Figure 2.

FIGURE 2: Percentage of antral follicles ≥ 2.0 mm diameter classified as healthy (solid bars) or atretic (open bars) according to size-range. Numbers in parentheses indicate mean number of follicles of that size range. The follicles were recovered from red deer hinds in mid-breeding season. N = 25.



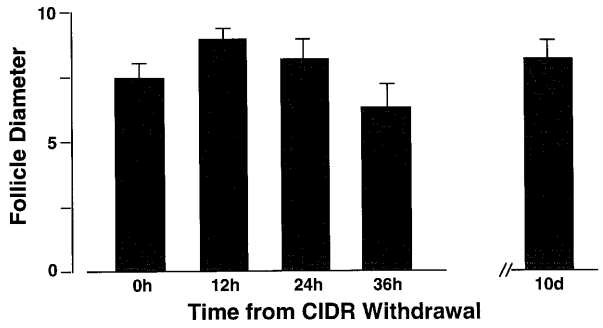
Mean size of largest healthy follicles

Irrespective of the time of ovary collection, there was at least one (range 1 - 3) large (≥ 7.5 mm diameter) healthy, oestrogen-producing follicle present in every hind. The mean diameter of the dominant follicle is shown for each collection time in Figure 3. This did not change significantly over the follicular phase and was not significantly different from that recorded in mid-luteal phase.

DISCUSSION

The primary objective of this study was to determine the extent of between-animal variation in antral follicle populations in red deer hinds. There were two contrasting findings. Firstly, that there was wide variation in both the total number of antral follicles present, and in the percent

FIGURE 3: Mean (± s.e.m) diameter (mm) of the dominant follicle in red deer hinds over the follicular phase or in mid luteal phase of the oestrous cycle. Ovaries were recovered at the time of, or 12, 24 or 36 hours or 10 days after withdrawal of progesterone releasing devices. N = 5 animals per collection time.



of these follicles that were healthy. Secondly, it was found that the number of large, healthy follicles present (and their mean diameter) varied little between animals.

The mean number of antral follicles present in the hind, and the variation between individual animals, was similar to that reported for sheep (McNatty and Henderson, 1987) and for cattle (McNatty *et al.*, 1984), and the proportion of antral follicles that were healthy was also comparable to that reported for these other ruminant species. Reasons for extreme differences in the number of follicles (from 4 to 81) present between individual hinds, and in the percent of follicles that were healthy (from 20 to 100%), remain unclear. These differences were not related to body condition of the hind, to the stage of the oestrous cycle or to the oestrogenic competence of the dominant follicle(s).

The lack of an obvious pattern of preovulatory follicle development over the folliclular phase of the the synchronised cycle is surprising. Previous work in the sheep reported the complete absence of large oestrogenic follicles early in the follicular phase, followed by a progressive increase in the number and diameter of healthy follicles up to the time of ovulation (McNatty *et al.*, 1982). The mean time of onset of the preovulatory LH surge in control animals (44.5 h after CIDR withdrawal) would suggest that in the present study, ovaries were recovered from some animals during the final stages of preovulatory development. However, some ovaries may have been recovered after the time of the LH surge. A further limitation of the experimental protocol used in this study (recovery of ovaries), is that information on follicle status is gathered at fixed time-points from each animal. Methods such as ultrasonography that allow repeated monitoring of follicles in the same animal over the period of preovulatory development, may identify progressive changes in follicle dynamics not observed in this study.

In conclusion, although there was considerable variation in follicle populations between individual hinds, large oestrogenic follicles (presumably capable of ovulating) were present in all animals at all stages of the oestrous cycle. This suggests that failure of treatments given to manipulate reproduction is not due to a block at the ovarian level.

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