Ultrasonographic monitoring of ovarian function during the oestrous cycle of fallow deer (*Dama dama*)

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ABSTRACT

Follicular and luteal dynamics during the oestrous cycle of fallow deer were investigated using repeated ultrasonographic imaging of ovaries. Nine mature does had their initial oestrus synchronised with intravaginal progesterone CIDR devices. Transrectal scanning (7.5 MHz probe) was performed either daily (perioestrous period) or every second day (mid-cycle) for 49 days from device removal to span two consecutive oestrous cycles. All follicles $\geq$ 3 mm diameter and corpora lutea were mapped for each ovary. Ovaries were visualised on 98% of occasions. All does exhibit two complete oestrous cycles, with mean ($\pm$ sem) durations of 22.0 $\pm$ 0.7 (first cycle) and 21.4 $\pm$ (second cycle) days. A single corpus luteum was observed during each cycle and appeared as a fluid filled sphere that became a solid structure by Days 16-18. An average of only 3.4 follicles $\geq$ 3mm were observed on ovaries throughout the study. The mean number and size varied significantly with individual does and day of cycle ($P < 0.05$). There were no effects of doe genotype, ovary (left vs. right) or proximity of corpus luteum ($P > 0.05$). A single dominant follicle (4+mm) was observed on most days of the cycle except Days 0-4. The 18 observed cycles were classified as having either 2 (n=11), 3 (n=6) or 4 (n=1) dominant follicle waves, with mean ($\pm$ sem) oestrous cycle lengths of 21.2 $\pm$ 0.4, 22.5 $\pm$ 0.9 and 23 days, respectively. It is concluded that the fallow deer oestrous cycle shares many similarities with that of red deer and cattle.

Keywords : Fallow deer, reproduction, ovaries, follicles, ultrasound.

INTRODUCTION

Reproduction in female fallow deer (*Dama dama*) is characterised by precise seasonal entrainment of the timing of conceptions and births, strict monovularity, high fertility (pregnancy rate) and predictable timing of endocrine and ovulatory events during the 21-22 day oestrous cycle (Chapman and Chapman, 1975; Asher, 1985; Asher *et al.*, 1986, 1990). Recent application of artificial breeding technologies within farmed populations has necessitated a better understanding of the precise nature of ovarian follicular activity in order to facilitate improvements in the efficiency of these technologies. For example, multiple ovulation-embryo transfer (MOET) programmes for fallow deer have generally yielded poor results due to inconsistent superovulatory responses and low fertilisation rates (Fennessy *et al.*, 1994) that may partly reflect inappropriate treatment regimens relative to ovarian follicular status.

Ultrasonography has become an important tool for real-time monitoring of ovarian function in a number of domestic ruminant species, including recent studies on red deer (Asher *et al.*, 1997). Our previous experience with fallow deer indicated a high degree of success (98%) in visualising ovaries *in situ* during transrectal scanning of non-pregnant females. The present study aimed to use ultrasonography to describe patterns of ovarian follicular and luteal activity during the fallow deer oestrous cycle.

MATERIALS AND METHODS

Nine mature (4-10 years old) fallow deer does, consisting of 4 European (*Dama dama dama*) and 5 hybrid (*D. d. mesopotamica* x *D. d. dama*) animals located on the Invermay Agricultural Centre (45° 53′ S, 170 ° 21′ E) were used in the study. The does fawned in January 1996 and were subsequently run with a mature vasectomised European buck for the remainder of that year. Surviving fawns (n=8) remained with their dams throughout the study. The deer were contained in a single 1.5 ha enclosure adjacent to covered handling yards, and were grazed in ryegrass-white clover pasture, with additional supplementation with lucerne hay and barley grain in winter.

The does each received an intravaginal CIDR device (Eazi-breed CIDR-G, 0.3 g progesterone per device; InterAg NZ Ltd., Hamilton, NZ) for a 14-day period from 6 May to 20 May to synchronise their initial oestrus. From the day of removal of CIDR devices until 8 July (two complete oestrous cycles), the does were scanned at least every second day. However, during the predicted peri-oestrus period (-3 to +3 days) after removal of CIDR devices and just prior to each return oestrus (ie. starting 18 days after previous oestrus), the does were scanned daily. Oestrous detection involved crayon harnessing of the buck (Asher, 1985) supplemented with direct observation of oestrous behaviour displayed towards operators by some of the tamer does. Blood samples obtained by jugular venepuncture on each day of scanning were analysed for plasma concentrations of progesterone by direct radioimmunassay (Asher *et al.*, 1990).

Rectal ultrasonographic scanning was performed by a single operator using a 7.5 MHz linear array transducer (Aloka SSD 210; Aloka Co. Ltd., Japan). The probe was held rigid in a specially modified aluminum shaft to enable exterior manipulation of the transducer. During

ultrasonography, the does were restrained individually in an upright position in a manually controlled cradle specifically designed for the species. A liberal coating of carboxymethylcellulose was applied to the transducer, which was then inserted carefully into the evacuated rectum until an echo-image of the bladder was observed. The probe was then gently rotated 90° clockwise and 180° counter-clockwise while being moved forward, until the ovaries were located. The diameters of all follicles and luteal structures $\geq$ 3 mm were measured using the inbuilt scanner calipers, and their position and size were recorded on an ovarian map.

Data analysis : Luteal cyclicity was assessed from longitudinal profiles of plasma progesterone concentrations, with a number of cycles verified by observations of oestrous behaviour. Interovulatory interval (hereafter termed as "luteal cycle") was defined as the period between two troughs in progesterone secretion during which plasma concentrations remained below 0.3 ng progesterone ml^{-1} for at least 2 days (periovulatory period), and there was a progressive increase and decrease in progesterone secretion in the intervening period such that peak plasma concentrations exceeded 3.0 ng progesterone ml^{-1} (a luteal phase; after Asher 1985). The day of ovulation (Day 1) was defined as the day of lowest progesterone concentration before a sustained increase, coinciding with the disappearance of a large follicle that had previously been identified and tracked on several occasions. An "oestrous cycle" refers to an observed luteal cycle bounded by valid observations of oestrous behaviour. The day of oestrus (Day 0) was assumed to occur ~ 24 h prior to ovulation (Day 1) (Asher et al., 1990).

A dominant follicle "wave" was characterised by a follicle(s) that was present for 5 or more consecutive days, reached $\geq$ 5 mm diameter, and either became the largest follicle or ovulated. If two or more follicles that met this criteria were recruited from the same cohort, they were deemed to be co-dominant and part of the same follicular wave.

Two approaches were used to describe and examine the follicular dynamics of cyclic does. The first analysis investigated the nature of follicles present on each day of the oestrous cycle. Numbers of small (3 mm), medium (4-5 mm), large ($\geq$6 mm), new and total follicles present, and mean size of all follicles present on any day were recorded. Factors compared included individual, doe genotype, cycle number (ie. first or second cycle) and day of cycle. The

second analysis investigated the characteristics and fate of individually identified follicles that were able to be tracked for at least two consecutive scans. The initial, maximum, last and average diameters of follicles that could be tracked from emergence to disappearance at least two days later, and the number of days the follicle was present, were compared with the following factors: individual, doe genotype, cycle number, ovulatory vs. non-ovulatory follicle, ovary (left or right) and presence or absence of corpora lutea on the same ovary. If a follicle could not be identified from a previous scan, it was deemed to be a new follicle. Abrupt disappearance of tracked follicles $\geq$ 6 mm was deemed to represent ovulatory rupture on the day of disappearance. All comparisons were made by analysis of variance (Steel and Torrie 1980), using the GLM procedure of SAS 6.08 (1992).

RESULTS

All nine does exhibited two complete luteal cycles with the 49-day period following removal of CIDR devices (Figure 1). Oestrus was verified on 13 occasions (48% of potential incidences), with crayon loss being the cause of failed detection. Luteal cycle lengths ranged from 19 to 26 days, with mean ($\pm$ sem) lengths of 22.0 $\pm$ 0.7 days for the first (induced) cycle, and 21.4 $\pm$ 0.4 days for the second cycle ($P > 0.05$). A single corpus luteum was observed during each cycle and generally appeared as a fluid-filled sphere $\geq$ 5 mm diameter 4-6 days after ovulation (Figure 1). Luteinization and contraction of the coele occurred progressively over days 6-16, with most corpora lutea appearing as solid structures by days 16-18. Luteolysis was associated with reduced echo-density and poor visual definition of the luteal remnants, although such remnants were occasionally visible 5-6 days into the next luteal cycle.

Total numbers of follicles $\geq$ 3 mm diameter observed on pairs of ovaries seldom exceeded 7, with an average of 3.4 across all days. Analysis of the numbers and sizes of follicles present on each day of the luteal cycle revealed no significant interactions between the main factors (ie. individual, doe genotype, cycle number and day of cycle) for any parameter investigated ($P > 0.05$). Furthermore, there were no significant associations with cycle number (ie. first or second cycle). The mean numbers of small (overall mean = 1.7), medium (1.2), large (0.5) and total (3.4) follicles were all significantly influenced by individual and day of cycle ($P < 0.05$). Across all cycles (n = 18), the greatest mean numbers were observed on Days 1-3 for small follicles, Days 3-4 and 11-14 for medium follicles, and Days 8-9 and

20-22 for large follicles (Figure 2). The mean numbers of total follicles present was highest (5.3) on Day 3. Mean daily follicular size (4.2 mm) also varied significantly with individual and day of cycle ($P < 0.05$), being highest on Days 13 and 20-23.

Analysis of the characteristics of each follicle that appeared during the observation period again showed no significant interactions between the main factors for any parameter tested ($P > 0.05$). Throughout the analysis there were no significant associations with individual, proximity of the corpus luteum or ovary (left vs. right) ($P > 0.05$). The first oestrous cycle (ie. synchronised cycle) was consistently different from the second cycle (ie. return cycle) for initial size of follicle (3.2 vs. 3.0 mm), day of maximum size (day 11.4 vs. 9.9), maximum size (3.9 vs. 3.7 mm), last day of follicle presence (day 12.8 vs. 11.1) and last size of follicle (3.5 vs. 3.4 mm) ($P < 0.05$), but not for the initial day of follicle appearance or the duration of follicle existence ($P > 0.05$). Comparison of ovulatory follicles (OvF) and non-ovulatory follicles (NOvF) revealed significant differences for all parameters tested, with OvF being consistently larger and occurring later in the oestrous cycle ($P < 0.05$). This was investigated further by analysing data pertaining only to those follicles attaining dimensions of $\geqslant 6$ mm (this included all OvF). In this case, the effects of individual and cycle number on the various follicle parameters were not significant ($P > 0.05$) but the contrasts between OvF and NOvF were highly significant for initial day of appearance (day 13.0 vs. 4.1), day to maximum size (Day 20.8 vs. 10.5), last day of presence (day 21.8 vs. 16.4) and duration of follicle presence (9.8 vs. 13.3 days) ($P < 0.05$). There was no significant effect of ovulatory status on initial size and maximum size of follicles ($P > 0.05$).

Using the aforementioned criteria for describing dominant follicle waves, the 18 observed cycles were classified as having either 2 (n = 11), 3 (n = 6) or 4 (n = 1) such waves, with mean (+ sem) cycle lengths of 21.2 + 0.4, 22.5 + 0.9 and 23 days, respectively.

DISCUSSION

The technique of transrectal ultrasonography was particularly successful for visualising larger ovarian structures (ie. $\geqslant 3$ mm) in fallow deer. Despite an inability to manually manipulate the ovaries by palpation onto the scanner head, we were still 98% successful in locating ovaries throughout the study. This is in contrast to studies on red deer, in which surgical modification of hinds to align the ovaries alongside the vaginal wall was considered

necessary in order to consistently locate them by transvaginal ultrasonography (Asher *et al.*, 1997). The earlier study on red deer, while demonstrating a broad picture of follicular dynamics, was plagued by ovarian abnormalities (eg. cystic structures) that may well have been an artefact of surgical modification. This was certainly not the case in the present study on fallow deer, which subsequently may represent a more suitable model for such research on ovarian dynamic of seasonally-breeding, monovulatory cervids.

The does in the present study exhibited oestrous cycle lengths and plasma progesterone profiles that were in accord with previous studies (Asher, 1985), with no apparent differences observed between European does and hybrid does. The corpus luteum was, in all cases, observed by ultrasonography, appearing as a fluid-filled sphere on and around Days 4-6 and becoming a completely solid structure on and around Days 16-18. This is in marked contrast to studies on red deer in which insufficient density gradient between luteal tissue and surrounding ovarian stroma inhibited observation of luteal development (Asher *et al.*, 1987), and suggests that the red deer corpus luteum does not develop a fluid-filled coele which is easily detected by ultrasonography. A precipitous decline in plasma progesterone secretions at termination of the fallow deer oestrous cycle confirms the secretory demise of the corpus luteum. However, remnant luteal tissues were observable for several days into the next luteal cycle. Loss of ultrasonographic definition of such remnants invariably occurred by Day 5.

The dynamics of follicle appearance, growth and regression in fallow deer during the oestrous cycle has certain similarities with that observed in red deer (Asher *et al.*, 1997) and other domestic ruminant species (Sirois and Fortune, 1988; Lucy *et al.*, 1992; Noel *et al.*, 1993; Bravo *et al.*, 1990, 1991). It is notable, however, that as with red deer, the fallow deer oestrous cycle was characterised by the presence of very few follicles $\geq$ 3 mm diameter (average of 3.4 on any day of the cycle) compared to cattle, sheep and camelids. It is probable that there was a far greater number of smaller recruited follicles (1-2 mm diameter), outside the resolution range of the ultrasound images, that entered atresia before attaining greater size, as perhaps indicated by the ovary dissection data for red deer by McLeod *et al.*, (1996).

With the exception of the first 3-4 days of the oestrous cycle of fallow deer, when small follicles (3 mm) predominated, the ovaries almost invariably contained a single medium (4-5

mm) or large (6+ mm) follicle. Such follicles often persisted for 5-10 days, whereas co-existing small follicles were generally only observed to persist for 2-6 days. This strongly suggests a strong "dominance" effect by a single larger follicle. Analysis of the temporal appearance and disappearance of such follicles revealed a strong non-random pattern during the oestrous cycle, interpreted as dominant follicle "waves". The majority of cycles (ie. 60%) contained two such waves, although three waves were also common (33%). Only one cycle contained four dominant follicle waves. In this respect, the follicular dynamics of fallow deer is similar to that of cattle, which also show a tendency towards increased oestrous cycle length with increased numbers of waves (Sirois and Fortune, 1988). Red deer also exhibit 2-3 waves of dominant follicles, although occasional abnormalities observed by Asher *et al.*, (1997) may have obscured the true nature of these waves.

In the present study, comparison of follicular dynamics between the first (induced) and second (spontaneous) oestrous cycles did not reveal any major differences in events. However, this does not preclude the possibility that the CIDR device treatment regimen occasionally perturbs follicular selection and maturation, leading to some inefficiencies in oestrous/ovulation synchronisation, as demonstrated for cattle (Wishart, 1977; Mihm *et al.*, 1994). Future studies on improving the efficiency of synchronisation techniques may benefit from evaluation of artificial control of the dominant follicle.

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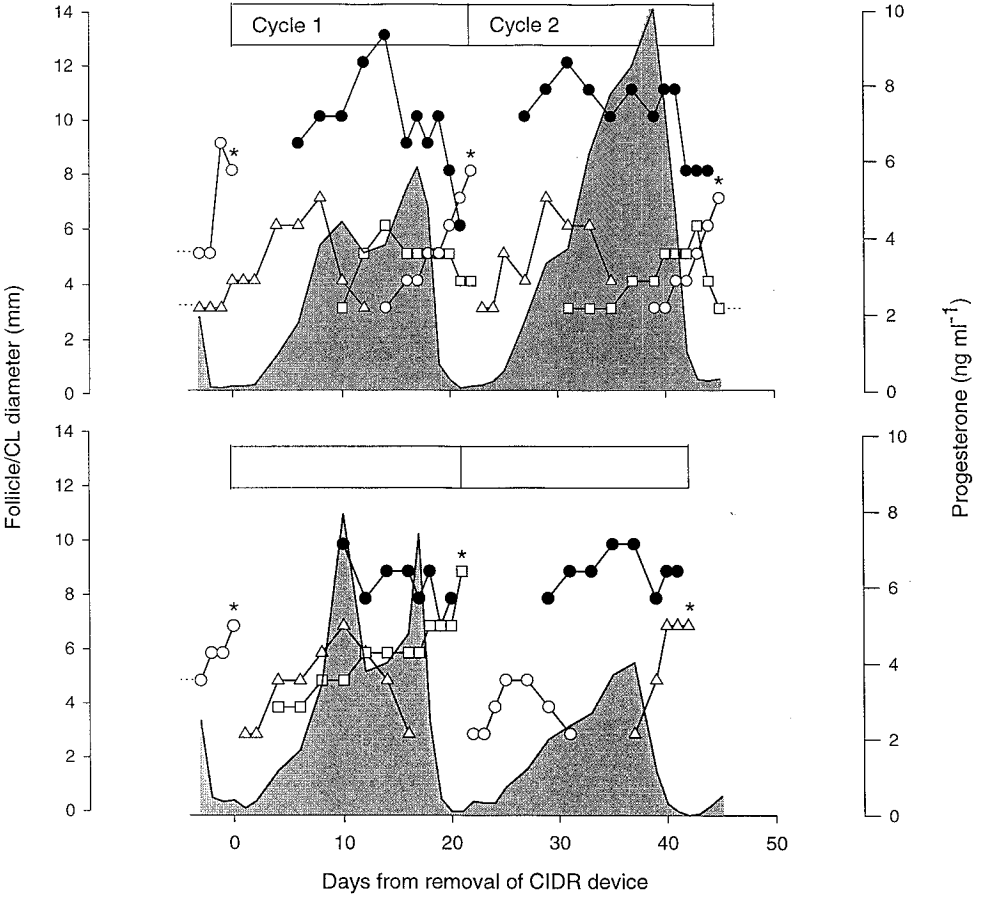
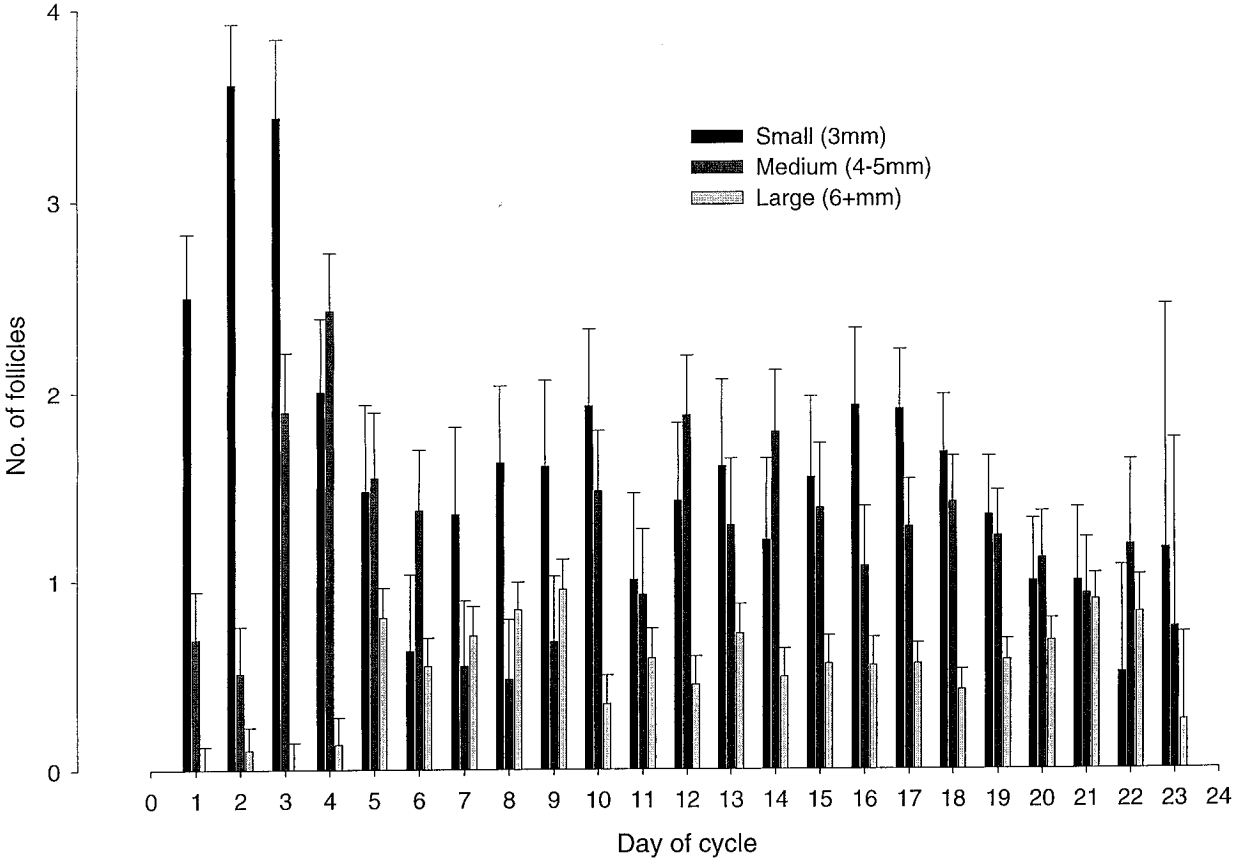
Figure 1 : Representative profiles for two fallow deer does of plasma progesterone concentrations (shaded profiles), diameters of corpora lutea (•) and diameters of dominant follicles (O, △, □) during two consecutive luteal cycles following removal of CIDR devices. Asterisks denote abrupt follicular disappearance indicative of ovulation.

Figure 2 : Mean (+ sem) number of small (3 mm; ■), medium (4-5 mm; ▨) and large (≥ 6 mm; ▩) follicles on each day of the luteal cycle.


Cycle 1
Cycle 2
Follicle/CL diameter (mm)
Progesterone (ng ml^{-1})
Days from removal of CIDR device
0
10
20
30
40
50
2
4
6
8
12
14


Small (3mm)
Medium (4-5mm)
Large (6+mm)
No. of follicles
0
1
2
3
4
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Day of cycle