Selection, dominance and atresia of follicles during the oestrous cycle of heifers

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This study examined the correlation between measurement of follicle growth by ultrasound, and measurement of intrafollicular ratios of oestradiol and progesterone concentrations and the serum concentrations of FSH during selection, dominance and atresia or ovulation of dominant follicles in heifers. Heifers were ovariectomized on days 0 (before LH surge), 1 (after LH surge, preovulation), 1 (postovulation), 3, 6 and 12 of the oestrous cycle. Blood samples were collected at 4–6 h intervals. After ovariectomy all follicles ≥5 mm were measured and follicular fluid was aspirated. Follicles were classified by size according to ultrasound (F1, largest; F2, second largest; F3, all remaining follicles ≥5 mm) and by the ratio of oestradiol:progesterone concentrations. During the follicular phase, a single dominant oestrogen-active follicle increased in diameter while serum concentrations of LH increased and FSH decreased (P < 0.05). On day 1 (after LH surge, preovulation), serum LH and FSH decreased to pre-surge concentrations (P < 0.0001), while follicle size and intrafollicular progesterone concentration increased and oestradiol concentration decreased (P < 0.05). A dominant nonovulatory follicle, classified as oestrogen-active on days 1, 3 and 6 and oestrogen-inactive on day 12, increased in size from day 1 to day 7 and lost dominance during days 10–12, coincident with the growth of multiple oestrogen-active follicles. The serum FSH concentration increased transiently (P < 0.05) before each new wave of dominant follicular growth. The overall correlation of ultrasound measurements of follicle diameter with measures of follicle size after ovariectomy was high. The ratio of oestradiol:progesterone concentrations, but not of size, reliably distinguished potential dominant from atretic follicles. The size of the follicle and the oestradiol concentration were not determinants of subsequent dominance during a selection phase. We conclude that: (1) ovarian follicles go through selection, dominance and atresia phases coincident with transient increases and decreases in FSH; and (2) ultrasound is an accurate measure of follicle growth, but that size alone is not a sufficient measure to ascribe dominance and both ultrasound and the intrafollicular ratio of oestradiol:progesterone concentrations are needed to monitor selection, dominance and atresia of follicles accurately.

Introduction

Antral follicles were originally considered to be in a continuous state of turnover without distinct patterns of growth and atresia during the oestrous cycle of heifers (Choudary et al., 1968; Marion et al., 1968; Dufour et al., 1972). However, the classic histological study of Rajakoski (1960), coupled with the direct follicle-marking studies of Matton et al. (1981), indicated that at least two periods of turnover of antral follicles occur during the oestrous cycle of cattle. One follicle grows to ovulatory size (>10 mm) and undergoes atresia during early dioestrus (days 6–12) and another follicle grows to ovulatory size from luteolysis (day 18) to oestrus (day 0) during the follicular phase and ovulates on day 1 of the cycle (Matton et al., 1981). Ireland and Roche (1982, 1983a, b, 1987) demonstrated that the intrafollicular ratio of oestradiol:progesterone concentrations can be used to distinguish healthy growing from atretic bovine follicles (≥6 mm in diameter), and confirmed and extended the earlier studies on follicular turnover in heifers as follows: (i) a single large (>10 mm) oestrogen-active follicle (ratio of oestradiol:progesterone concentrations >1 in follicular fluid) is present during oestrus and early dioestrus of heifers; (ii) the number of LH receptors increases while the number of FSH receptors decreases during the growth of oestrogen-active follicles during oestrus and early dioestrus (Ireland and Roche, 1983a, b); (iii) the serum concentration of
oestradiol increases in only one, rather than in both, utero-ovarian veins during oestrus, early dioestrus and mid-dioestrus (Ireland et al., 1983); and (iv) oestrogen-active follicles should be classified as dominant because of their similarity to dominant follicles in primates (Goodman and Hodgen, 1983).

These results led to the hypothesis that heifers have three different periods of development of dominant follicles during an oestrous cycle (oestrus, early dioestrus and mid-dioestrus), and that each period of dominant follicle growth has three distinct phases: selection, dominance and atresia or ovulation (Ireland, 1987; Ireland and Roche, 1987). Selection is a hypothetical physiological process whereby 'excess' follicles are reduced to the ovulatory quota, whereas dominance is a process that enables the 'selected' follicle to suppress further growth of other follicles, escape initial atresia and continue to grow until ovulation or atresia (Goodman and Hodgen, 1983). In support of this model of a dominant follicle in heifers, workers in several laboratories have used ultrasound scanning to monitor daily individual follicle growth and confirm that heifers indeed have three (sometimes two and rarely one) different periods of turnover of dominant follicles (Savio et al., 1988; Sirois and Fortune, 1988; Knopf et al., 1989). Nevertheless, the precise correlation between ultrasound analysis of dominant follicle growth, which is now routinely used to monitor growth of antral follicles in both beef and dairy cows (Murphy et al., 1990; Savio et al., 1990; Crowe et al., 1993), and changes in intrafollicular concentrations of oestradiol and progesterone, which is used to distinguish dominant from atretic follicles (Ireland and Roche, 1982, 1983a, b, 1987), has not been examined. The objectives of this experiment were therefore to: (i) examine the interrelationship of ultrasound measurements of follicle growth, intrafollicular ratios of oestradiol and progesterone concentrations and the serum concentrations of FSH during selection, dominance and atresia or ovulation of dominant follicles in heifers; (ii) evaluate the accuracy of ultrasound measurements of follicular size with postovariectomy measurements of follicle diameter and function; and (iii) use the results of this study to re-evaluate our original model for dominant follicle growth in heifers (Ireland, 1987; Ireland and Roche, 1987).

Materials and Methods

Animals, maintenance and synchronization of oestrus

Thirty-three cyclic Simmental crossbred, Hereford crossbred and Charolais crossbred beef heifers, 15–18 months of age and weighing 334–418 kg at the start of the experiment, were housed on slatted flooring, and had free access to grass silage and water and a daily supplement of 2 kg of a 16% crude protein concentrate. To synchronize oestrus, each heifer received a s.c. progestagen ear implant for 10 days (norgestomet: Crestar, Intervet Ireland Ltd, Finglas, Dublin). Two days before implant removal, a single injection (i.m.) of PGF₂α analogue (PGF₂α: Prosolvin: Intervet Ireland Ltd) was administered to initiate luteolysis. After implant removal, heifers were observed for oestrous behaviour for 30 min at 06:00 h, 12:00 h, 16:00 h, 20:00 h and 00:00 h every day until oestrus was detected.

Ultrasound scanning of follicles

From the time of prostaglandin injection until approximately 14 h before ovariectomy, the ovaries of each heifer were scanned daily with a transrectal 7.5 MHz linear transducer (Dynamic Imaging Ltd, Livingston), and the number, size and location of each follicle ≥5 mm was recorded daily, as described by Savio et al. (1988). Follicles were placed into three classes based on ultrasound analysis: F1, the largest or the dominant follicle (as defined by Savio et al., 1988); F2, the second largest follicle; and F3, all other follicles ≥5 mm.

Collection of blood samples

The stage of the oestrous cycle was confirmed by collecting samples of blood (10 ml) daily to measure both oestradiol and...
progesterone concentrations via jugular venepuncture from the time of PGF<sub>2α</sub> injection (2 days before implant removal) until ovariectomy. In addition, samples were collected from each heifer every 4 or 6 h from 36 h after PGF<sub>2α</sub> administration until the time of ovariectomy to establish when the preovulatory LH surge occurred and to determine changes in circulating concentrations of FSH. Each blood sample was maintained at room temperature for 60 min, at 4°C overnight, centrifuged at 700 g for 20 min and the serum was stored at −20°C until assays were performed.

**Hormone assays**

Previously validated radioimmunoassays were used to quantify oestradiol (Moran et al., 1991), progesterone (Ronayne and Hynes, 1990) and LH (Niswender et al., 1969) concentrations. The sensitivities of the progesterone, oestradiol and LH assays were 0.2 ng ml<sup>−1</sup>, 1.5 pg ml<sup>−1</sup> and 0.2 ng ml<sup>−1</sup>, respectively. Serum FSH concentrations were quantified using a heterologous assay as described by Glencross et al. (1992) using the NIDDK anti-oFSH antibody and bovine FSH standard preparation (NIH B1 bFSH). The sensitivity of the assay was 1.6 ng FSH ml<sup>−1</sup>. Interassay coefficients of variation (CV) for the oestradiol assays averaged 11.3 and 15.4% for serum samples containing 0.5 and 20.2 pg oestradiol ml<sup>−1</sup>, respectively. Intra-assay CV for the same serum pools were 10.9 and 8.8%, respectively. Interassay CV for the progesterone assays averaged 9.3 and 6.5% for serum samples containing 0.9 and 3.0 ng progesterone ml<sup>−1</sup>, respectively. Intra-assay CV for the same serum pools were 6.5 and 5.7%, respectively. Interassay CV for the LH assays averaged 15.7 and 12.6% for serum samples containing 3.9 and 26 ng LH ml<sup>−1</sup>, respectively. Intra-assay CV for the same serum pools were 12.9 and 10.8%, respectively. Interassay CV for the FSH assays for three serum pools containing 13.9, 27 and 84 ng FSH ml<sup>−1</sup> averaged 9.9, 11.9 and 11.2%, respectively. Intra-assay CV for the same serum pools were 4.7, 6.9 and 9.5%, respectively.

**Statistical analyses**

Follicular development during the oestrous cycle was evaluated using several different statistical analyses with the computer programs of SYSTAT (1990) and the general linear model of SAS (1986).

A split-plot repeat measure analysis was used to examine whether diameter of follicles determined by the last ultrasound measurement before ovariectomy and measurement of diameter by calliper, volume of follicular fluid, intrafollicular concentrations of oestradiol and progesterone, and the ratio of oestradiol:progesterone concentrations in follicular fluid of the same follicles established after ovariectomy differed (P<0.05) among the six groups (I–VI) of heifers and the three follicle classes. If a significant (P<0.1) statistical interaction was observed, the Bonferroni <i>t</i> test was used to test whether means for the F1 follicle class differed (P<0.05) from the F2 and F3 follicle classes for each group of heifers. There were usually 1–3 follicles ≥5 mm per pair of ovaries the growth of which was accurately monitored by ultrasound. Because the SEM values increased with means, all data were log-transformed (base 10) before statistical analysis; arithmetic means are reported in the text.

Regression analysis was used to determine the correlation of the last ultrasound measurement of follicle size (taken approximately 14 h before ovariectomy) with measurements of diameter, volume of follicular fluid, intrafollicular concentrations of oestradiol and progesterone, and the ratio of oestradiol:progesterone concentrations in follicular fluid from the same follicles established after ovariectomy from each of the six groups of heifers, for each follicle class (F1, F2, F3) and overall (F1 + F2 + F3).

To determine whether follicle size on days 1–6 could be used to predict whether a follicle would become dominant was investigated using regression analysis. This evaluated whether the proportion of the largest follicles on days 1–6 that were classified as dominant by ultrasound on days 6 and 12 varied during days 1–6.

McNemar’s test (Gill, 1978) was used to determine whether there was a difference between the proportion of the largest follicles determined by ultrasound before ovariectomy and the proportion of largest follicles measured after ovariectomy that had the greatest concentration of oestradiol, progesterone, oestradiol:progesterone or progesterone:oestradiol ratios in follicular fluid (P<0.05).

Changes in hormone concentrations throughout the study were evaluated by comparing mean hormone concentrations at different time points using paired <i>t</i> test analysis.

**Results**

The follicular phase of the oestrous cycle of heifers began coincident with luteolysis on day −4 (4 days before oestrus), when the mean serum concentration of progesterone was <2.0 ng ml<sup>−1</sup>, and ended on day 1 (−ov), concomitant with ovulation (Fig. 1a). Day 0 was defined as the time of oestrus and the preovulatory gonadotrophin surges. From day −4 to day −1 each heifer had a single dominant ovulatory follicle (F1) that increased (P<0.05) from 11.6 ± 0.9 mm to 16 ± 0.6 mm in diameter coincident with a sustained basal increase (P<0.05) in the serum concentration of oestradiol, a decrease (P<0.05) in the serum concentration of FSH and progesterone and with no change in LH concentration. After the occurrence of the preovulatory LH and FSH surges on day 0, the serum concentration of LH, FSH and oestradiol decreased (P<0.0001) to pre surge values within 12 h, and the diameter of the dominant ovulatory follicle increased to its maximum size of 18 ± 0.8 mm. As the F2 and F3 follicles were <5 mm in diameter between days −4 and 0, their growth patterns were not measured.

On days 0 and 1 (−ov), dominant ovulatory follicles (F1) were classified as oestrogen-active, whereas F2 and F3 follicles were oestrogen-inactive (Figs 1c, 2). Diameter, oestradiol concentrations, and the ratio of oestradiol:progesterone concentration in follicular fluid were greater (P<0.01) for dominant ovulatory follicles compared with F2 follicles (Figs 1, 2). In contrast, the concentration of progesterone was higher (P<0.01) in F2 than in dominant ovulatory follicles (Fig. 2). Concentrations of oestradiol and the ratio of oestradiol:progesterone concentrations in F1 follicles were two and five
times lower ($P < 0.02$), respectively, whereas the progesterone concentration was higher ($P < 0.01$) in dominant ovulatory follicles on day 1 (−ov) after the preovulatory gonadotrophin surge compared with values on day 0 (Fig. 2). Concentrations of oestradiol, progesterone and the ratio of oestradiol:progesterone concentrations in follicular fluid were highly correlated ($r > 0.75; P < 0.05$) with changes in size of dominant ovulatory follicles on day 0, but not on day 1 (+ov) (Table 1).

The luteal phase of the oestrous cycle of heifers began on day 1 (+ov) after ovulation, when serum concentrations of progesterone were less than 0.5 ng ml$^{-1}$, and ended on day 12. Each heifer had a single dominant nonovulatory (F1) follicle that increased ($P < 0.05$) in diameter from 6.2 ± 0.3 mm on day
1 (+ ov) to 13 ± 0.8 mm by day 7. Thereafter, the size of this
F1 follicle did not change (P > 0.05) up to the time of
ovariectomy on day 12 (Fig. 1c). Coincident with development
of the dominant nonovulatory follicle were two transient
increases (P < 0.02) in the serum concentration of FSH between
days 0.5 and 1.5, and days 8 and 10.5 of the oestrous cycle.
During the early luteal phase, the serum LH concentration
remained unchanged (P > 0.05), whereas transient increases
(P < 0.05) in serum concentration of oestradiol occurred during
days 3–10 when FSH concentrations were low. In contrast to the
time from day −4 to day −1 of the follicular phase, F2 and
F3 follicles were both ≥5 mm during the early diestrus
phase. In addition, the diameter of F2 and F3 follicles increased
(P < 0.05) during days 1–4 and then decreased to <5 mm in
diameter during days 5–10. A new group of F2 and F3 follicles
emerged between days 10 and 12 (Fig. 1c).

Similar to the dominant ovulatory follicle, the dominant
nonovulatory follicle on days 1, 3 and 6 was classified as
oestrogen-active (Figs 1c, 2). It should be noted that the F1, or
the largest follicle was not always the dominant nonovulatory
follicle until after day 4 (Fig. 3). Unlike F2 and F3 follicles on
day 0 and day 1 (− ov), F2 and F3 follicles on days 1 (+ ov)
and 3 of the early luteal phase were oestrogen-active (Figs 1c,
2). The emerging medium follicles of the second wave were
also all oestrogen-active on day 12.

Although concentrations of progesterone in follicular fluid
were greater (P < 0.05) in the F2 and F3 follicles than in the F1
follicle on day 1 (+ ov), size, oestradiol concentration, and the
ratio of oestradiol:progesterone concentrations were similar for
all follicle classes (Figs 1c and 2), and oestradiol, progesterone
and the ratio of oestradiol:progesterone concentrations were
not correlated with the size of follicles (Table 1). On day 3,
only diameter (by ultrasound) and the volume of follicular fluid
were greater for F1 compared with F2 and F3 follicles, and the
size of follicles was correlated (r = 0.59; P < 0.03) with oestra-
diol concentrations in follicular fluid. On day 6, all indices of
follicle size, oestradiol concentration in follicular fluid and the
ratio of oestradiol:progesterone concentrations in follicular
fluid were greater (P < 0.05) for dominant nonovulatory folli-
cles than for F2 follicles, and size of follicles was highly
related (r > 0.70; P < 0.02) with the ratio of oestradiol:
progesterone concentrations in follicular fluid (Table 1). Although all indices for size were greater (P < 0.05) for
dominant nonovulatory follicles compared with F2 or F3
follicles on day 12, the concentration of oestradiol and the ratio
of oestradiol:progesterone concentrations in follicular fluid
were greater (P < 0.05) in F2 and F3 follicles than in the
dominant nonovulatory follicle (F1; Figs 1c, 2). In contrast, the
centration of progesterone was greater in dominant non-
ovulatory follicles (F1) than in F2 follicles on day 12 (Fig. 2).

The size of follicles on day 12 was correlated (r = 0.56;
P < 0.02) only with progesterone in follicular fluid (Table 1).

The overall correlation between ultrasound and calliper
measurements of diameter (r = 0.92; P < 0.0001) and volume
(r = 0.88; P < 0.0001) of F1, F2, and F3 follicles after ovariec-
tomy was very high (Table 1). However, ultrasound measure-
ments of diameter were not correlated (P > 0.05) with diameter
and volume of follicles after ovarioectomy on day 1 (+ ov)
or day 3, or for F3 follicles.

The proportion of the largest follicles with the greatest
concentrations of oestradiol, progesterone, oestradiol:progester-
one or oestradiol:progesterone ratios was similar regardless of
whether follicle size was measured by ultrasound or with
callipers after ovarioectomy (Table 2). The predictability of the
proportion of the largest follicles that became dominant
(P < 0.002) from 0% on day 1 to 100% by day 5 of the
oestrous cycle (Fig. 3).

Discussion

This is the first report to evaluate the relationship between
ultrasound monitoring of follicular growth and measures of
follicular growth and function after ovarioectomy. Our results
indicate that ultrasound and postovariectomy measurements
of follicle size are highly correlated, implying that ultrasound is an
accurate method for monitoring follicular growth. However,
the greatest correlation of ultrasound and postovariectomy
measures of follicular growth occurred when a single dominant
follicle was present during the follicular and luteal phases of the
oestrous cycle. In contrast, when a clear hierarchy of follicles
was not established, as on days 1 and 3 after ovulation,
ultrasound measurements were not significantly correlated with
postovariectomy measures of follicle size. While the reason for
this finding is unclear, results of correlation analysis have
indicated that ultrasound and postovariectomy measurements
of follicular growth are least correlated when follicles are
classified as F3 (usually 5–6 mm in diameter). Thus, the
accuracy of measuring these follicles, regardless of the method
used to estimate size, may be diminished compared with
measurements of larger antral follicles.

In support of the accuracy of ultrasound measurements
for large follicles, both ultrasound and two different

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Fig. 1. Mean (± SEM) changes in (a) serum concentrations of progesterone (○; ng ml\(^{-1}\)) and oestradiol (●; pg ml\(^{-1}\)), (b) serum concentrations of FSH (□) and LH (●) (ng ml\(^{-1}\)), and (c) size of antral follicles during oestrus and early diestrus of the oestrous cycle of heifers. Daily ultrasound measurements were made on each heifer beginning 4 days before oestrus until ovarioectomy. Each point either represents the daily mean (± SEM) for diameter of F1, F2 and F3 follicles, or (b) the mean (± SEM) of four follicles measured for each treatment on each day of the experiment. The size of the dominant follicle was determined by ultrasound on day 0, day 1 after the preovulatory LH surge, and on days 0, 1, 2, 3, 5, and 7 thereafter. The size of the dominant follicle on day 0, 1 and 5 (− ov) is shown by the bar graph. On days 0 and 1 (− ov), F1 is the dominant follicle. On days 1 (− ov) to day 3, ultrasound measurements of follicle size cannot be used accurately to predict which follicle is dominant. However, on days 4–12, F1 is the dominant follicle. Note that all 33 heifers were included between 4 days before oestrus and ovarioectomy (day 0); thereafter, numbers of heifers were reduced by 5–6 after each ovarioectomy (indicated by arrows).
Fig. 2. Changes in the concentrations of (a) oestradiol, (b) progesterone and (c) ratio of oestradiol:progesterone concentrations in follicular fluid for follicles ≥5 mm in diameter during oestrus and early dioestrus of the oestrous cycle of heifers. Ultrasound was used to monitor growth and classify follicles as F1 (largest: ■), F2 (second largest: □) or F3 (remaining follicles ≥5 mm: △) in six different groups of heifers (n = 5–6 heifers per group). Bars represent means (±SE) and asterisks above bars indicate whether means for F1 follicles differed statistically (*P < 0.10, **P < 0.05, ***P < 0.01) from F2 or F3 follicles within a certain day. The number of follicles measured is shown in the top panel. – ov: preovulation; + ov: postovulation.

postovariectomy measurements of follicle size, diameter and volume were used to determine whether the proportion of the largest follicles (excluding the largest follicle on day 12) with the highest concentration of oestradiol, progesterone, oestradiol:progesterone or progesterone:oestradiol ratios differed. Regardless of which method was used to measure follicle size, the results were similar. Finally, the pattern of turnover of follicles of heifers during days 0–12 is similar to previous reports (Savio et al., 1988; Sirois and Fortune, 1988; Knopf et al., 1989; Adams et al., 1992; Badinga et al., 1992). On the basis of results of this study and those of others (Sirois and Fortune, 1988; Knopf et al., 1989; Adams et al., 1992; Badinga et al., 1992), ultrasound analysis is an accurate and consistent procedure for monitoring the size and turnover of follicles ≥5 mm during the oestrous cycle of heifers.

During the oestrous cycle, follicles are recruited into a growing pool and the number selected to continue growing to become dominant follicles is equivalent to the species-specific ovulatory quota (Goodman and Hodgen, 1983). However, the factors involved in selection of dominant follicles are unknown.

The completion of a selection phase is defined in cattle by both ultrasound measurements (Savio et al., 1988; Sirois and Fortune, 1988) and the intrafollicular ratio of oestradiol:progesterone concentrations (Ireland and Roche, 1982, 1983a, b) as the time when an oestrogen-active follicle promotes its own growth and inhibits the growth of other follicles. In our study, we examined whether the size of a follicle influenced whether it would become dominant and whether the largest follicles had the highest concentration of oestradiol. The results indicated that the early dioestrus dominant nonovulatory follicle was usually not the largest follicle (2 of 22 measurements) on days 1 and 2 of the oestrous cycle, but was usually the largest after day 2 (37 of 44). Although the concentration of oestradiol and the ratio of oestradiol:progesterone concentrations were not different for each follicle ≥5 mm on day 1 after ovulation, concentrations of progesterone were greater in the F2 and F3 than in the F1 follicles. These results indicate that factors other than size and oestradiol concentrations, such as progesterone, may be important for establishing which follicle becomes dominant during a selection phase.

Although the largest follicle with the greatest concentration of oestradiol during a selection phase does not always become dominant, functioning dominant follicles during the follicular phase and on day 6 of the oestrous cycle were always the...
Table 1. Correlation of ultrasound measurements of follicle diameter with caliper measurements of diameter, volume of follicular fluid and concentrations of oestradiol, progesterone and the ratio of oestradiol:progesterone concentrations in follicular fluid during the oestrous cycle of heifers

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</table>

*Groups of five or six heifers were ovariectomized on six different days of the oestrous cycle and follicles were classified as follows: F1, largest follicle; F2, second largest; F3, all remaining follicles ≥ 5 mm. The last ultrasound measurement of diameter of follicles ≥ 5 mm was made approximately 14 h before ovariectomy and correlated with measures of diameter, volume of follicular fluid and concentrations of oestradiol, progesterone and the ratio of oestradiol:progesterone concentrations in follicular fluid determined from the same follicles after ovariectomy. Each heifer had 1–3 follicles ≥ 5 mm.

bRegression coefficient.

cP value.

dData for all days of the oestrous cycle were combined and correlations within each follicle class examined: F1, largest follicle; F2, second largest follicle; and F3, all other follicles ≥ 5 mm.

n: number of follicles; ns: not significant (P > 0.05); − ov: preovulation; + ov: postovulation.

Table 2. Proportion of largest follicles of heifers determined by ultrasound or at ovariectomy that had the highest concentrations of oestradiol, progesterone and ratio of oestradiol:progesterone or progesterone: oestradiol concentrations in follicular fluid*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ultrasound diameterb</th>
<th>At ovariectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diameter</td>
</tr>
<tr>
<td>Oestradiol</td>
<td>22/27 (81%)c</td>
<td>25/27 (93%)c</td>
</tr>
<tr>
<td>Progesterone</td>
<td>12/27 (44%)d</td>
<td>14/27 (52%)d</td>
</tr>
<tr>
<td>Oestradiol:progesterone</td>
<td>25/27 (93%)c</td>
<td>25/27 (93%)c</td>
</tr>
<tr>
<td>Progesterone:oestradiol</td>
<td>11/27 (41%)d</td>
<td>11/27 (41%)d</td>
</tr>
</tbody>
</table>

*All heifers were included in this analysis except those ovariectomized on day 12 of the oestrous cycle. Heifers on day 12 were excluded because all the largest follicles were atretic.

bThe last ultrasound measurement was taken approximately 14 h before ovariectomy, and was used to determine the largest follicle.

cValues with different superscripts are significantly different (P < 0.05) between proportions in columns.

largest follicles with the highest concentration of oestradiol (Ireland and Roche, 1983a; Martin et al., 1991). However, the F1 follicle on day 12, despite being the largest follicle, had lost its 'functional dominance' as early as day 10, based on the emergence of a new wave of follicular development. The dominant follicle on day 12 was hormonally classified as
oestrogen-inactive and therefore atretic. Atresia of the
dominant nonovulatory follicle is characterized by a significant
decrease in the number of granulosa cells, a decrease in both
LH and FSH receptors (Ireland and Roche, 1983b) and a
diminished capacity to produce oestradiol between days 7 and
13 of the oestrous cycle in heifers (Badinga et al., 1992). The
factors involved in the regulation of atresia of follicles are not
clear, but it has been demonstrated that decreasing the LH
pulse frequency to luteal concentrations results in the faster
atresia of the dominant follicle (Sirois and Fortune, 1990),
suggesting that the dominant ovulatory follicle fails to undergo
atresia because it is subjected to a higher LH pulse frequency in
the follicular phase. These results, coupled with those of earlier
studies showing that the dominant follicle was not always the
largest during a selection phase, clearly indicate that only
functioning dominant follicles that are present during finite
periods of an oestrous cycle are the largest follicles with the
greatest concentration of oestradiol. Although measuring the
size or the oestradiol concentration alone is not indicative of
whether a follicle will become dominant or whether it is
functionally dominant (e.g. preventing growth of other fol-
cicles), the ratio of oestradiol:progesterone concentrations can
be reliably used to distinguish growing from atretic follicles
\( \geq 5\) mm in diameter in heifers.

Both ultrasound analysis and the ratio of oestradiol:progес-
terone concentrations indicate that there are two different
periods of growth of multiple follicles \( \geq 5\) mm between days 1
and 3 and days 10 and 12. The results reported here indicate
that days 1–3 are the selection phase for development of the
early dioestrous dominant nonovulatory follicle, whereas days
10–12 are not only the selection phase for development of the
next dominant follicle, but are also the period when the first
dominant follicle ceases to function (loses dominance), becomes
oestrogen-inactive and begins to undergo atresia. Functional
dominance of a follicle begins when a selection phase ends
(Goodman and Hodgen, 1983) and is defined as those periods
of the oestrous cycle when only a single antral follicle \( > 5\) mm
grows to ovarioly size. In our study, use of ultrasound
indicated that there were periods of functional dominance
during the follicular phase (4 days before oestrus up to the day
of ovulation) and from day 4 to day 9 of the oestrous cycle, a
finding consistent with previous studies examining follicular
dynamics in heifers (Savio et al., 1988; Sirois and Fortune, 1988;
Knopf et al., 1989). These periods of functional dominance
identified by ultrasound corresponded with the presence of a
single large \( (\geq 10\) mm) oestrogen-active follicle that had
greater concentrations of oestradiol and ratio of oestradiol-
progesterone concentrations than those for other coexisting
follicles (F2, F3). Although our results do not indicate precisely
when the selection phase begins or ends and when dominance
begins, an easily distinguishable hierarchy of follicles was clear
by day 4 of the oestrous cycle. In contrast, despite the presence
of multiple potential dominant follicles on days 1 (after
ovulation), 3 and 12 (based on the ratio of oestradiol:proges-
terone concentrations), a functional dominant follicle was not
present on these days of the oestrous cycle. This finding is
supported by both ultrasound and hormonal analysis indicating
that multiple, rather than a single, oestrogen-active follicles
were growing during these times and that the first dominant
follicle was oestrogen-inactive and undergoing atresia by day
12. Ultrasound analysis also indicates that the F2 and F3
follicles on days 4–10, which were present when the functional
dominant ovulatory follicle was present on the ovary, and the
dominant (F1) nonovulatory follicle on day 12 were either not
growing or had lost functional dominance and were also
identified as oestrogen-inactive.

This is the first study to characterize the relationship
between changing FSH concentrations and intrafollicular changes in the ratio of oestradiol:progesterone concentrations
as follicles develop and regress. Both selection periods of
follicular growth, days 1–3 and days 10–12, are preceded by a
rise in serum concentrations of FSH. Our results, using samples
taken every 6 h and both ultrasound and the ratio of oestradiol:
progesterone concentrations as methods of monitoring follicu-
lar growth, confirm the findings of Adams et al. (1992), who
used daily blood samples and ultrasound to demonstrate that
an increase in FSH occurred 2–4 days before a new wave of
follicle development. FSH has a deterministic role in all stages of
follicular development (Richards, 1980); thus, it is hypoth-
ized that increases in circulating concentrations of FSH initiate
the emergence phase for dominant follicle growth.

This hypothesis is supported by recent data which demon-
strate that delaying the first rise in FSH after ovulation between
days 0 and 3 by administering bovine follicular fluid delays the
Removal of the inhibitory influences of the dominant follicle by
ovariectomy on days 3, 5 and 8 of the oestrous cycle increases
both the FSH concentration and the number of small follicles,
and advances the emergence of a new dominant follicle (Adams
et al., 1992). Thus, our data provide compelling evidence for a
cyclic pattern of FSH secretion during the oestrous cycle of
cattle, and this FSH secretion is responsible for the emergence
and selection of follicle growth. Once selection is complete as
indicated by the presence of one oestrogen-active follicle, the
circulating concentrations of FSH have decreased, suggesting
that the dominant follicle secretes some inhibitory substance(s)
to decrease FSH. It is now well established that both oestradiol
(Price and Webb, 1988) and inhibit (Beard et al., 1990; Rivier
and Vale, 1991; Robertson et al., 1991), produced by the
follicle, control the release of FSH in a negative fashion. The
relative importance of both hormones in the regulation of FSH
concentration is not clear, but the endocrine data generated in
this experiment (Fig. 1) indicate that there is not a clear
negative association between serum concentrations of FSH and
oestradiol at all stages of the oestrous cycle. These findings
suggest that inhibit is important for the regulation of FSH, but
owing to the lack of a specific inhibit assay to measure
biologically active inhibins (Ireland et al., 1994), it is not yet
possible to demonstrate such an effect. Despite this decrease in
the circulating FSH concentration after selection and the atresia
of all but one follicle, the dominant follicle continues growing,
demonstrating that large amounts of FSH are not necessary to
sustain follicular dominance.

We conclude that (1) ovarian follicles go through distinct
phases of selection, growth, dominance and atresia resulting in
the cyclic development of dominant ovulatory and dominant
nonovulatory follicles throughout the bovine oestrous cycle,
and FSH is probably the physiological ‘trigger’ for this cyclic
follicle growth pattern; (2) the methods of ultrasound and the
intrafollicular ratio of oestradiol:progesterone concentrations

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must be combined to monitor dominant follicle growth, function and atresia accurately, and (3) the original model for dominant follicle growth (Ireland and Roche, 1987) was a valid physiological representation of dominant follicle turnover during the oestrous cycle of heifers.

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