GROWTH AND DIFFERENTIATION OF LARGE ANTRAL FOLLICLES
AFTER SPONTANEOUS LUTEOLYSIS IN HEIFERS: CHANGES IN
CONCENTRATION OF HORMONES IN FOLLICULAR FLUID AND
SPECIFIC BINDING OF GONADOTROPINS TO FOLLICLES

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Summary
The objective of the present study was to characterize changes in growth, concentrations of steroids in follicular fluid (FF) and gonadotropin receptors of individual antral follicles after spontaneous luteolysis in heifers and to reexamine our hormonal method of classifying healthy and atretic follicles. Groups of heifers were ovariectomized (OVX) before regression of corpora lutea on d 17, 18 and 19 of an estrous cycle and after regression of corpora lutea at time periods before, during and after preovulatory gonadotropin surges. Each follicle ≥6 mm in diameter was examined for concentration of progesterone, androstenedione, testosterone and estradiol-17β in FF and capacities of granulosal and thecal cells to specifically bind 125I-bovine follicle stimulating hormone (bFSH) or 125I-human chorionic gonadotropin (hCG). Follicles were separated into two classes, estrogen-active (E-A) or estrogen-inactive (E-I). Concentrations of estradiol in FF were higher than both progesterone and androgens in E-A follicles, whereas either androgens or progesterone was higher than estradiol in E-I follicles. Diameters, volumes of FF and numbers of granulosal cells in E-A follicles increased from d 17 through the end of the preovulatory gonadotropin surges. During this time, concentrations of progesterone, androstenedione and estradiol in FF and specific binding of 125I-hCG to granulosal and thecal cells increased, whereas specific binding of 125I-bFSH to granulosal cells decreased. In E-I follicles, progesterone in FF decreased and androstenedione increased from d 17 through end of gonadotropin surges. The E-I follicles had a higher incidence of granulosal cells with pycnosis than E-A follicles. We concluded that changes in growth, concentration of steroids in FF and gonadotropin receptors in E-A follicles after spontaneous luteolysis were similar to changes we previously reported for E-A follicles after prostaglandin-induced luteolysis, and that ratio of concentration of estradiol in FF to both progesterone and androgens could be used to separate potential ovulatory (E-A) from atretic (E-I) follicles in heifers.

(Key Words: Heifers, Ovulatory Follicles, Follicular Fluid Steroids, Receptors.)

Introduction
After a single injection of prostaglandin F2α (PG) during the luteal phase, estrus occurs within 3 to 4 d in heifers. During this interval, two large follicles (>6 mm in diameter)/pair of ovaries are usually present (Ireland and Roche, 1982). One follicle is estrogen-active (E-A), the other is estrogen-inactive (E-I). The E-A follicles have a higher concentration of estradiol-17β in
follicular fluid (FF) than both progesterone and androgens, whereas E-I follicles have a higher concentration of either progesterone or androgens than estradiol.

During the interval from PG to estrus, sizes, concentrations of progesterone, androgens and estradiol and specific binding of $^{125}$I-human chorionic gonadotropin (hCG) to granulosal or thecal cells in E-A follicles increase, whereas specific binding of $^{125}$I-bovine follicle stimulating hormone (bFSH) to granulosal cells in E-A follicles decreases. The E-I follicles, although similar in size to E-A follicles, contain fewer granulosal cells, a lower concentration of estradiol in FF and a reduced capacity to bind gonadotropins. We concluded from our previous study (Ireland and Roche, 1982) that changes in growth, concentration of steroids in FF and specific binding for E-A and E-I follicles after PG-induced luteolysis were characteristic of changes in ovulatory and nonovulatory follicles, respectively.

Because development of ovulatory follicles in heifers was initiated with an injection of PG at midcycle (Ireland and Roche, 1982), the objective of our present report was to examine the changes in serum hormones, growth of follicles, concentration of steroids in FF and specific binding of gonadotropins after spontaneous regression of corpora lutea in heifers. In addition, we reexamined the efficacy of separating potential ovulatory from atretic follicles based on the relationship of concentration of estradiol in FF to progesterone or androgens.

Methods and Materials

Animals. Hereford heifers (n = 27) weighing 300 to 400 kg were kept on pasture during July and August 1979. All cattle had at least one estrous cycle before this study began. Three days before blood samples were taken, heifers were tied in stalls.

Schedule of Ovariectomy (OVX) and Blood Samples. In an attempt to synchronize occurrence of estrus, heifers between d 7 and 18 of their estrous cycle were each given an injection of 500 μg of a synthetic analog of prostaglandin-F$_2$α* (PG) designed to cause regression of a corpus luteum. Estrus began 2 to 4 d later. During the subsequent estrous cycle, five to seven heifers were OVX at 0600 h 17, 18 or 19 d postestrus, at estrus and 24 h after estrus was first observed. Ovaries were removed from cattle using an ecraseur inserted through an incision in the dorsal wall of the vagina. Heifers that were OVX on d 17, 18 or 19 had blood samples taken via jugular cannula at 8-h intervals beginning 48 h before OVX and ending at time of OVX to establish time of luteal regression. Jugular cannulas were inserted 24 h before blood sampling began. To determine time of preovulatory surges of gonadotropins, heifers OVX at estrus and 24 h after estrus were bled at 2-h intervals from d 19 until OVX.

Beginning of a luteinizing hormone (LH) surge was defined as the first serum LH concentration greater than 5 ng/ml that was followed by two or more LH concentrations of greater magnitude. The end of a surge was defined as the first serum LH level to reach presurge baseline levels.

Samples of blood (10 ml) were stored at 25 C for 8 h and at 4 C for 12 to 24 h, then centrifuged at 800 × g for 30 min. Serum was then decanted and stored at −20 C. Using previously validated radioimmunoassays (RIA), concentrations of LH (Convey et al., 1976) and FSH (Carruthers et al., 1980) were determined for each serum sample and concentrations of progesterone (Louis et al., 1973) were determined for all samples taken at 8-h intervals.

Processing of Ovaries. Ovaries were processed as previously described (Ireland and Roche, 1982). In brief, ovaries were removed and immediately placed in ice-cold phosphate buffered saline (pH 7.0; PBS). Diameter of each follicle ≥6 mm on the surface of each ovary was recorded and follicular fluid (volume recorded), granulosal cells and thecal tissue were removed from each follicle. Each tissue was placed in separate vials (stored in PBS containing 20% glycerol) and frozen. These tissue samples remained frozen at −70 C for 2 to 3 mo before receptor analyses were performed. Although degradation of gonadotropin receptors during storage was not examined, glycerol has previously been shown to substantially minimize loss of receptor activity during storage at low temperatures (Dias et al., 1981). A 2 × 4 mm strip of each follicle wall was taken from most follicles and placed in Bouin’s fixative. Five 10-μm sections of each follicle strip were stained with hematoxylin and eosin and examined under a microscope. If any section of follicle contained granulosal cells

*Cloprostenol, ICI Ltd., U.K.
with pycnotic nuclei, the follicle was considered atretic. A piece of thecal tissue from one heifer/time point was also placed in Bouin's fixative. Most preparations of theca had small patches of granulosal cells. Hereafter, theca is referred to as theca-enriched. The FF was centrifuged at 3,000 × g for 10 min to remove debris. Each follicle was processed at random in the above fashion within 1 to 2 min and all follicles from each group of heifers were processed within 1 to 2 h of OVX.

Samples of follicular fluid (FF) were diluted in PBS 1:100 to 1:10,000. Previously validated RIA were then used to determine concentrations of progesterone (Louis et al., 1973), androstenedione (Kiser et al., 1978), testosterone (Mongkonpunya et al., 1975) and estradiol-17β (Oxender et al., 1977) for each diluted sample of FF. Results of RIA for each steroid are not different if samples are extracted and chromatographed before assay (Ireland and Roche, 1982).

Measurement of Capacity of Granulosal Cells and Theca-Enriched Homogenates to Specifically Bind Radioactive Gonadotropins. A single class of high affinity ($K_d = 10^{-9}$ M), low capacity binding sites specific for βFSH or for hCG, an LH-like molecule, exist for bovine ovarian follicles (Ireland and Roche, 1982). Saturation analysis was used in this study to estimate changes in specific binding of $^{125}$I-hCG (CR-119, 11,600 IU/mg) and $^{125}$I-bFSH (160 × NIH-FSH-B1; see Cheng, 1976) to granulosal cells and $^{125}$I-hCG to theca as previously described (Spicer et al., 1981; Ireland and Roche, 1982). Specific binding was expressed as cpm/μg deoxyribonucleic acid (DNA). Amount of DNA was determined as described by Burton (1956). Specific binding of each labeled hormone to LH or FSH receptor ranged from 1 to 20% of the total amount of radioactive hormone added (200,000 cpm for each hormone). Nonspecific binding (binding in presence of excess NIH-LH-B9, 25 μg/10 μl, or NIH-FSH-B1, 25 μg/10 μl) to follicular tissue or test tubes was less than .5% of the total radioactive hormone added.

Identification of Potential Ovulatory Follicles. In an attempt to separate potential ovulatory from nonovulatory (atretic ?) follicles, we separated follicles retrospectively based on the relationship of concentrations of estradiol in FF to concentrations of progesterone and androgens as discussed in the Introduction. Based on data for sheep (Moor et al., 1978; Hay et al., 1979) and cattle (Ireland and Roche, 1982, 1983), E-A follicles should have granulosal cells with a low incidence of pycnotic nuclei and E-I follicles should have a high incidence of pycnotic granulosal cells.
**Results**

*Serum Hormones.* Estrous cycles vary in length from 17 to 23 d in heifers. Thus, day of cycle (days postestrus) and hormonal milieu are not always well correlated. In this study, two heifers OVX on d 18 and two heifers OVX on d 19 postestrus, each had concentrations of progesterone in serum that were high (2 to 6 ng/ml) 2 d before OVX, but were low (<1 ng/ml) at OVX. Although regression of corpora lutea had occurred in these heifers, gonadotropin surges were not detected. In addition, two heifers OVX during estrus and one heifer OVX 24 h after estrus, each had levels of progesterone <1 ng/ml and each heifer was OVX before onset of preovulatory gonadotropin surges. Data for all seven of these heifers were pooled into a category hereafter referred to as “before surges” (figure 1). Three heifers were OVX 0 to 10 h after onset of preovulatory gonadotropin surges and five heifers were OVX 6 to 18 h after the end of gonadotropin surges, but before ovulation. Data for heifers OVX 0 to 10 h after surges began or 6 to 18 h after surges ended were each pooled. Hereafter, these time periods were referred to as “during surges” or “after surges.”

Only means of serum hormones at time of OVX are presented in figure 1. Bonferroni t-test (Gill, 1978) was used to compare means of progesterone, LH and FSH for d 18 and 19 and before, during and after preovulatory gonadotropin surges with mean of each of these hormones on d 17. Results of this analysis indicated that serum concentrations of progesterone were lower (P<.05) before, during and after surges than on d 17. Serum LH was higher (P<.05) only during a preovulatory gonadotropin surge than on d 17, and serum FSH was higher (P<.05) on d 19 and during surges than on d 17.

*Inventory of Follicles From D 17 Through End of Preovulatory Gonadotropin Surges.* Total number of E-A and E-I follicles during each period of the estrous cycle and average numbers of E-A and E-I follicles/heifer during the estrous cycle are shown in figure 2. Number of follicles >6 mm in diameter ranged from one to seven/heifer. Number of E-A follicles ranged from zero to two/heifer. One heifer OVX before surges had seven E-I follicles. Excluding this heifer, number of E-I follicles ranged from zero to two/heifer. Of 22 heifers OVX from d 17 through end of preovulatory gonadotropin surges, 19 of 22 heifers had at least one E-A follicle, 17 of 22 heifers had at least one E-I follicle and 14 of 22 heifers had E-A and E-I follicles. One heifer OVX on d 17, one heifer OVX on d 19 and one heifer OVX before gonadotropin surges had only E-I follicles. Two heifers OVX before gonadotropin surges and three heifers OVX during gonadotropin surges each had only a single E-A follicle. Based on the above data, E-A and E-I follicles appeared to be equally distributed during most periods of the estrous cycle.

*Development of E-A and E-I Follicles From D 17 Through the End of Preovulatory Gonadotropin Surges.*
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Figure 3. Concentration of progesterone in follicular fluid for estrogen-active (o) or estrogen-inactive (a) follicles during the estrous cycle. Description of when heifers were OVX, numbers of heifers and follicles per time point and how follicles after surges were separated are given in figures 1 and 2. Bars represent means (+- SE).

otropin Surges. Linear regression analysis was used to examine the average changes in diameters, volumes of FF, numbers of granulosal cells, concentrations of steroids and specific binding of gonadotropins in E-A and E-I follicles from d 17 through the end of gonadotropin surges.

Diameters, volumes of FF and numbers of granulosal cells (figure 2) increased (P<.01) from d 17 through the end of gonadotropin surges in E-A, but not E-I follicles. Slopes for these changes were greater (P<.05) in E-A than in E-I follicles. Concentrations of progesterone (figure 3) and estradiol (figure 4) in FF of E-A follicles increased (P<.05) from d 17 through the end of gonadotropin surges. In E-I follicles, concentrations of progesterone in FF decreased (P<.002) from d 17 through the end of gonadotropin surges. Comparisons of slopes for changes in concentrations of steroids for E-A and E-I follicles indicated that they differed (P<.05) for progesterone and estradiol, but not androstenedione. Thus, values for androstenedione from E-A and E-I follicles were pooled (figure 5). Concentration of androstenedione increased from day 17 through end of surges (figure 5). Specific binding of $^{125}$I-hFSH to granulosal cells decreased (P<.0001), whereas specific binding of $^{125}$I-hCG to granulosal cells and theca-enriched homogenates (figure 6) increased (P<.01) in E-A follicles from d 17 through end of surges. During this interval, specific binding of gonadotropins to E-I follicles did not change and slopes of changes in specific binding of gonadotropins differed (P<.05) for E-A and E-I follicles.

Characteristics of Follicles After Preovulatory Gonadotropin Surges Ended. After the preovulatory gonadotropin surges ended, but before ovulation, all follicles were classified as E-I (Ireland and Roche, 1982). Thus, we separated follicles based on size and assumed that the largest follicle after gonadotropin surges, but before ovulation, was ovulatory as shown previously (Dufour et al., 1972). Comparison of the largest follicle with smaller follicles indicated, as expected, that the largest follicle per pair of ovaries had a greater (P<.05) diameter, more (P<.005) FF, more (P<.005) granulosal cells (figure 2) and a greater (P<.05) concentration of estradiol in FF (figure 4). The largest follicle also consistently had at least twice as much estradiol as androgen in FF (figures 4 and 5). The largest follicles after gonadotropin surges were in all respects, except concentration

Figure 4. Concentration of estradiol-17β in follicular fluid of estrogen-active (o) or estrogen-inactive (a) follicles during the estrous cycle. Description of when heifers were OVX, number of heifers and follicles per time point and how follicles were separated after surges are given in figures 1 and 2. Bars represent means (+- SE).
of estradiol in FF, similar (P>.05) to E-A follicles during surges. Estradiol was lower (P<.05) in the largest follicle after gonadotropin surges than E-A follicles during gonadotropin surges (figure 4).

**Correlation of Changes in Diameter of E-A and E-I Follicles with Volume of FF, Number of Granulosal Cells, Concentration of Steroids in FF and Specific Binding of Gonadotropins to Follicles.** An injection of PG was used at midcycle to initiate development of ovulatory follicles in heifers in our previous study (Ireland and Roche, 1982). Because of asynchrony in development of ovulatory follicles after PG, regression analysis was used in that study to examine the relationship of diameter of E-A and E-I follicles to volumes of FF, numbers of granulosal cells, FF steroid concentrations and numbers of gonadotropin receptors. A similar analysis of data generated in our present study should allow a more direct comparison between this and our previous study of the relationships of the aforementioned variables with changes in diameter of E-A and E-I follicles.

In our present study, changes in diameters (6.9 to 19 mm) of E-A follicles (n = 20, the four largest follicles after the surge were excluded) were highly correlated (P<.01, except estradiol) with changes in volume of FF (r = correlation coefficient, b = slope; r = .94, b ± SE = .15 ± .01 ml), number of granulosal cells (r = .80, b = 763,043 ± 129,602), concentration of progesterone (r = .54, b = 7.4 ± 2.6 ng/ml) and estradiol (P<.10, r = .39, b = 56 ± 32 ng/ml), specific binding of 125I-bFSH (r = -.63, b = 195 ± 55 cpm/μg DNA) and 125I-hCG (r = .66, b = 129 ± 34 cpm/μg DNA) to granulosal cells and specific binding of 125I-hCG (r = .62, b = 113 ± 33 cpm/μg DNA) to thecal cells. Changes in diameters (6 to 16.2 mm) of E-I follicles (n = 33) were highly correlated (P<.01) only with changes in volume of FF (r = .78, b = .09 ± .01 ml) and number of granulosal cells (r = .57, b = 585,721 ± 154,928). Slopes of regression lines for E-A and E-I follicles differed (P<.05) for volumes of FF, concentration of estradiol in FF and specific binding of gonadotropins. Slopes of E-A and E-I follicles not presented above were not different (P>.10) from zero.

Comparison of slopes generated from E-A and E-I follicles for the above variables with slopes for these variables generated in our previous study (data not shown, Ireland and Roche, 1982) indicated that slopes were similar for E-A follicles in both studies except that change in concentration of progesterone in FF as diameter of follicles increased was markedly greater (P<.05) after PG-induced than spontaneous luteolysis (b = 25 ± 7 vs 7 ± 2.6 ng/ml).
Slopes for E-I follicles were similar for both studies.

**Relationship of Hormonalty and Histologically Classified Follicles.** Granulosa cells from 42 of 57 follicles were examined histologically for pycnotic nuclei. Follicles without signs of atresia were classified as E-A in 10 of 16 cases and follicles with pycnotic granulosal cells were classified as E-I in 20 of 26 cases. Results of chi-square analysis indicated that there was a high probability (P<.01) that follicles that had granulosal cells without pycnotic nuclei would be classified hormonally as E-A, whereas follicles that had granulosal cells with pycnotic nuclei would be classified hormonally as E-I.

During the estrous cycle, overall means for diameter, volume of FF, number of granulosal cells, concentrations of steroids in FF and specific binding of gonadotropins for E-A follicles or follicles without histological signs of atresia were similar. Means for the above variables were similar when E-I follicles were compared with follicles with histological signs of atresia (table 1). Comparison of E-A with E-I follicles indicated that overall means for diameter, volume of FF, number of granulosal cells, concentration of estradiol in FF were markedly greater (P<.05) in E-A than in E-I follicles. In contrast, concentration of progesterone in FF was greater (P<.05) in E-I follicles (table 1).

**Discussion**

Concentrations of steroids in FF have been shown to reflect capacity of a follicle to produce and secrete steroids (McNatty, 1978; England et al., 1981; Hillier et al., 1981). Thus, changes in the relationship of concentrations of estradiol to other steroids within a follicle during an estrous cycle may reflect alterations in capacity of a follicle to synthesize estradiol. Consequently, when concentrations of progesterone or androgens in FF are higher than estradiol, such a follicle has probably lost the capacity to produce estradiol. Others (Moor et al., 1978; Hay et al., 1979) have reported that follicles with higher concentrations of progesterone or androgens in FF than estradiol have a reduced capacity to produce estradiol in vitro. These follicles were shown to be atretic. First, within 2 to 3 d of estrus, the largest or second largest follicle per pair of ovaries normally ovulates (Dufour et al., 1972). In our study, E-A follicles were larger the E-I follicles and E-A follicles were usually the largest follicle per pair of ovaries after d 17 (figure 2). Second, E-A follicles compared with E-I follicles in heifers had characteristics previously described for healthy (ovulatory) compared with atretic follicles in other species (Bomsel-Helmreich et al., 1979; McNatty et al., 1979; Carson et al., 1981). And, size, concentration of steroids in FF and specific binding of gonadotropins were similar when E-A were compared with nonatretic follicles and when E-I were compared with atretic follicles (table 1). Finally, from d 17 through the end of gonadotropin surges, androstenedione increased in E-A and E-I follicles (figure 5), whereas estradiol remained unchanged in E-I follicles, but increased in E-A follicles (figure 4). This suggested that E-I follicles had lost the capacity to convert androgens to estradiol. Thus, estradiol secretion from E-A and not E-I follicles is likely to be responsible for the rise in concentration of estradiol in serum preceding and during estrus in heifers (Glencross et al., 1973). In support, Staigmiller et al. (1982) have recently reported that the largest follicle removed from heifers during estrus produces 500 to 1,000 times more estradiol in vitro than smaller follicles.

This study and our previous one (Ireland and Roche, 1982) suggest that atretic antral follicles do not lose, at least initially, their capacity to produce progesterone (figure 3) or androgens (figure 5), although capacity of follicle cells to bind LH (figure 6) and number of granulosal cells (figure 2) are reduced. Terranova (1981) has shown that preovulatory follicles lose the capacity to produce estradiol, but retain, at least initially, the capacity to produce progesterone after experimentally induced atresia of preovulatory follicles in hamsters. Progesterone treatments result in atresia in cattle (Maracek et al., 1977), and progesterone blocks FSH-induced increases in estradiol by granulosal cells of rats in culture (Schreiber et al., 1981). In addition, androgen treatments result in atresia and block FSH-induced increases in number of receptors for LH in rats (Farookhi, 1980). Consequently, although several large antral follicles were present during the late luteal phase of heifers in this study, high intrafollicular concentrations of...
<table>
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<tr>
<th>Follicle variables</th>
<th>Estrogen-active follicles (n = 24)</th>
<th>Healthy follicles (n = 18)</th>
<th>Estrogen-inactive follicles (n = 33)</th>
<th>Atretic follicles (n = 26)</th>
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<td>Diameter, mm</td>
<td>13.3 ± .7b</td>
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<td>Volume of follicular fluid, ml</td>
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<td>Number of granulosa cells × 10^6</td>
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<td>8.6 ± .8</td>
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<td>Steroids in follicular fluids, ng/ml</td>
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<td>Estradiol</td>
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<td>Theca cells</td>
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<td>1,106 ± 136b</td>
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*Estrogen-active (E-A) follicles have higher concentration of estradiol in follicular fluid than progesterone, androstenedione and testosterone, estrogen-inactive (E-I) follicles do not. Healthy follicles did not have granulosa cells with pycnotic nuclei, and atretic follicles had granulosa cells with pycnotic nuclei. Data for the largest and data for the remaining follicles on each pair of ovaries after surges were combined with E-A and E-I follicles respectively. Means of E-A compared with healthy follicles and E-I compared with atretic follicles were similar for each variable.*

*bDifference (P < .05) in E-A vs E-I follicles.
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progesterone or androgens in E-I follicles may have blocked ability of these follicles to respond to gonadotropins before preovulatory gonadotropin surges.

After PG, increases in concentrations of progesterone, estradiol and androstenedione in E-A follicles were associated with regression of a corpus luteum, a decrease in concentration of serum progesterone and a presurge rise in serum LH, but not FSH (Ireland and Roche, 1982). Similar results were observed in the present study, although a consistent presurge rise in serum concentration of LH was not observed from d 17 up to beginning of an LH surge. Perhaps this was due to asynchrony of spontaneous estrous cycles compared with PG-induced cycles. Nevertheless, there was a tendency (P< .10) for concentrations of LH to be higher for heifers OVX before surges compared with heifers OVX on d 19 (figure 1). In addition, we observed that concentration of FSH in serum was greater in heifers OVX on d 19 than on d 17. A rise in FSH several days before gonadotropin surges has previously been reported for cattle (Schams and Schallenberger, 1976). We should emphasize that whether or not presurge rises in FSH or LH in serum during the preovulatory time period are detected in heifers may depend upon interval of days before a preovulatory gonadotropin surge that blood sampling begins. Perhaps an increase in FSH from d 17 to 19 before gonadotropin surges that blood sampling begins. Perhaps an increase in FSH from d 17 to 19 before regression of a corpus luteum and a rise in LH in serum from d 19 up to onset of LH surges after regression of a corpus luteum have an important role in promoting growth and differentiation of an ovulatory follicle in heifers.

As antral follicles in cycling rats mature, binding of FSH to granulosal cells does not change until after the preovulatory LH surge (Uilenbroek and Richards, 1979). Clearly, the role of the FSH receptor during development of antral follicles in heifers is unknown. For example, in contrast to observations in rats (Richards et al., 1978) our data and previous results in heifers (Ireland and Roche, 1982, 1983) indicate that maintenance of high levels of receptors for FSH in antral follicles is not necessary for growth of antral follicles, steroid synthesis or an increase in numbers of receptors for LH. Recently, small increases in LH such as those occurring prior to a preovulatory LH surge have been shown to be important for development of ovulatory follicles in intact prepubertal rats (Richards and Bogovich, 1982).

The preovulatory gonadotropin surges may be the endocrine signal for terminal differentiation of a granulosal into a luteal cell. After gonadotropin surges, LH receptor in follicle cells and capacity of these cells to make estradiol are apparently reduced before formation of a new corpus luteum (Ireland and Roche, 1982). There was a tendency (P< .20) for binding of LH to granulosal and thecal cells in the present study to be lower in the largest follicle (presumed ovulatory) after gonadotropin surges than follicles during gonadotropin surges (figure 6). Although numbers of receptors for LH are reduced after gonadotropin surges (Ireland and Roche, 1982), there is no apparent reduction in capacity of a follicle to make progesterone (also shown in figure 3). Perhaps, high concentrations of progesterone in FF after gonadotropin surges reflected selective retention in FF of this steroid by a progesterone binding protein (Fleming and McGaughey, 1982) rather than enhanced synthesis of steroid. Also, note that although progesterone concentrations increased several fold in FF (figure 3) of E-A follicles, no change in serum levels of progesterone was detected (figure 1).

Results of our data and those of others (Moor et al., 1978; Bomsel-Helmreich et al., 1979; McNatty et al., 1979) suggest that ratio of concentration of estradiol to progesterone and androgens in FF is a reliable method of separating healthy (E-A) from atretic (E-I) antral follicles in heifers. However, six of 26 E-I follicles did not have granulosa cells with pycnotic nuclei. Because granulosal cells in FF were discarded during preparation and only the apex of each follicle was examined histologically, signs of pycnosis in these follicles could have been overlooked. It was also possible that changes in concentrations of hormones in FF preceded morphological signs of atresia (Uilenbroek et al., 1980). We also observed that six of 16 E-A follicles had pycnotic granulosal cells. Previously, follicles recovered during estrus in cattle (Priedkalns et al., 1968; Ireland and Roche, 1982) or during the late follicular phase in humans (Bomsel-Helmreich et al., 1979) have been shown to have pycnotic granulosal cells. Thus, ratio of estradiol in FF to progesterone and androgens rather than a histological evaluation of atresia may be a better method of separating healthy from atretic follicles in heifers. Nevertheless, our hormonal procedure of separating healthy from
atretic follicles has been evaluated only for antral follicles >6 mm in diameter during the 3-to-4 d follicular phase of the bovine estrous cycle. This procedure also does not distinguish healthy from atretic follicles during the period from the end of gonadotropin surges to ovulation because progesterone and not estradiol is the dominant steroid in both types of follicles. In heifers, size of follicles after gonadotropin surges, but before ovulation, can be used to separate ovulatory (largest) from nonovulatory follicles (Dufour et al., 1972).

In conclusion, changes in growth, concentrations of steroids in FF and gonadotropin receptors in E-A follicles after spontaneous luteolysis were similar to those changes we previously reported for E-A follicles after PG-induced luteolysis (Ireland and Roche, 1982). And, ratio of concentration of estradiol in FF to progesterone and androgens was reliably used in this and a previous study (Ireland and Roche, 1982) to identify potential ovulatory and atretic antral follicles in heifers.

Literature Cited


estradiol and LH concentrations in mares after an increased photoperiod during winter. Amer. J. Vet. Res. 38:203.


