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Expression of Follicle-Stimulating Hormone and Luteinizing Hormone Receptor Messenger Ribonucleic Acids in Bovine Follicles during the First Follicular Wave[1]

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ABSTRACT

The objective of the present study was to characterize expression of mRNAs encoding FSH and LH receptors during follicular development and at different stages of the first follicular wave in cattle. Following estrus, groups of heifers (3–5 per group) were ovariectomized on the day of initiation of the first follicular wave (as determined by ultrasonography; Day 0), or on Days 2, 4, 6, 8, or 10 after initiation of the first wave. FSH and LH receptor mRNAs were detected within follicles ≥ 4 mm and in some smaller follicles by in situ hybridization and were quantified by image analysis. FSH receptor mRNA was expressed in granulosa cells of all growing follicles, starting in some follicles with only one layer of granulosa cells. Irrespective of day of the follicular wave, the level of expression of FSH receptor mRNA in granulosa cells of healthy antral follicles ranging from 0.5 to 14 mm in diameter did not vary significantly with follicular size ($r = 0.02$, $p > 0.10$). Expression of LH receptor mRNA was first observed in theca interna cells of follicles shortly after antral formation. Irrespective of day of the follicular wave, the levels of LH receptor mRNA in theca interna cells of healthy antral follicles ranging from 0.5 to 14 mm increased with follicular size ($r = 0.39$, $p < 0.01$). In granulosa cells, LH receptor mRNA was expressed only in healthy follicles > 9 mm in diameter and was first observed in the dominant follicles collected on Day 4. Expression of mRNA for LH receptor, but not for FSH receptor, changed ($p < 0.01$) with the stage of the first follicular wave. LH receptor mRNA levels were highest in theca interna cells of dominant follicles collected on Day 4 and did not differ on other days. Only the healthy dominant follicles collected on Days 4, 6, and 8 expressed LH receptor mRNA in the granulosa cells. Levels of LH receptor mRNA in granulosa cells were similar on Day 4 and Day 6 but were reduced ($p < 0.05$) on Day 8. Atresia was associated with a loss of LH receptor mRNA in granulosa cells and with reduced expression of LH receptor mRNA in theca cells and FSH receptor mRNA in granulosa cells. In summary, acquisition of LH receptors in granulosa cells between Day 2 and Day 4 may be important in the establishment of follicular dominance during the first follicular wave.

INTRODUCTION

Follicular growth during the estrous cycle in cattle is usually characterized by two or three follicular waves [1–4]. During each follicular wave, a cohort of follicles is initiated to grow beyond 4 mm in diameter. From this cohort, a single follicle is selected and grows larger than the rest of the follicles in the cohort to become the dominant follicle. The other follicles in the cohort regress while the dominant follicle continues to grow until it reaches maximum size. If the dominant follicle develops during the follicular phase, it ovulates. However, dominant follicles developing during the luteal phase of the estrous cycle regress, rather than ovulate, because of the lack of the preovulatory LH surge [5–7].

Although the pattern of follicular growth during a follicular wave has been well characterized, the mechanisms responsible for recruitment of the cohort of follicles at the start of a follicular wave and the subsequent selection, maintenance, and later regression of the dominant follicle are not clear [7]. Recent studies have demonstrated that a transient increase in the concentration of FSH precedes the initiation of each follicular wave [4, 8]. This transient increase in FSH could be the stimulus for recruitment of the cohort of follicles [9]. Once a follicle has been selected to become dominant, it is able to continue to grow in a hormonal milieu that is suppressive to the development of other less-developed follicles. Dominant follicles have acquired LH receptors on the granulosa cells, and this renders them capable of responding to both FSH and LH stimulation [10, 11]. Furthermore, dominant follicles have the capacity to produce large amounts of steroids, especially estradiol-17β [10, 12, 13]. Increased estradiol-17β production may locally increase the responsiveness of the dominant follicle to gonadotropin stimulation [12, 14].

Although gonadotropin receptor binding in dominant follicles of cattle has been characterized, the changes in gonadotropin receptors or their mRNAs during follicular recruitment and selection and during the subsequent atresia of the dominant follicle have not been clearly described. In a previous study [15], the expression of mRNAs for cytochrome P450 side-chain cleavage ($P450_{scc}$), cytochrome P450 17α-hydroxylase (P450c17), and cytochrome P450 aromatase ($P450_{arom}$) in bovine follicles collected at different stages during the first follicular wave were characterized. While there were specific changes in the expression of all three of these steroidogenic enzymes during the first

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follicular wave, such changes are unlikely to be responsible for selection of the dominant follicle. Our hypothesis is that changes in expression of mRNAs for the FSH and LH receptor, on the other hand, may be important for recruitment of a cohort of follicles and selection and atresia of the dominant follicle in cattle. Therefore, the objective of the present study was to characterize, by in situ hybridization, the changes in expression of FSH and LH receptor mRNAs in bovine follicles collected at different stages during the first follicular wave. In addition, attempts were made to further characterize the expression and localization of mRNAs for these receptors during follicular development.

MATERIALS AND METHODS

Animals and Treatments

The study was conducted according to approved University of Missouri animal use protocol number 2174. Crossbred beef heifers were injected with 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Lutalyse; Upjohn, Kalamozoo, MI) to induce luteal regression. Starting from the second day after $PGF_{2\alpha}$ injection, heifers were observed three times daily for estrous behavior. Following onset of estrus, animals were examined daily by real time ultrasonography to monitor the growth of all follicles $\geq$ 4 mm in diameter. Initiation of the first wave of follicles was identified by the growth of a cohort of follicles at least one of which was $\geq$ 4 mm in diameter. Groups of heifers (3–5 per group) were ovariectomized through a flank incision on the day of initiation of the first follicular wave (designated Day 0) or on Days 2, 4, 6, 8, or 10 after initiation of the first wave. The time of ovariectomy was chosen in order to obtain follicles at different stages of development during a follicular wave.

Tissue Preparation

Following their removal, ovaries were placed on ice and transported to the laboratory. Blocks of ovarian tissue containing all follicles $\geq$ 4 mm in diameter and some smaller follicles were excised, frozen in liquid nitrogen, and stored at −80°C until sectioned. All follicles were frozen within 30 min of ovariectomy. The size of each follicle was measured from the ovarian surface with a divided ruler before freezing and was confirmed, whenever possible, after sectioning.

Template Generation and cRNA Probe Synthesis

A 637-bp cDNA encoding a portion of the extracellular domain of the bovine FSH receptor was generated by the reverse transcriptase-polymerase chain reaction using bovine preovulatory follicle RNA and primers corresponding to 100% conserved sequences present in the nucleotide sequences of rat and human FSH receptors [16, 17]. Similarly, a 730-bp cDNA encoding a portion of the extracellular domain of the ovine LH receptor was generated from an ovine small luteal cell library by polymerase chain reaction using primers corresponding to 100% conserved sequences present in the cDNA sequences of rat, porcine, and human LH receptor [18–20]. The resulting bovine FSH receptor cDNA (corresponding to nucleotides 104–740 of the human FSH receptor cDNA) and ovine LH receptor cDNA (corresponding to nucleotides 193–922 of porcine and human LH receptor cDNAs) were subsequently ligated into the p-Bluescript plasmid (Stratagene, La Jolla, CA) and subjected to dideoxy sequencing to verify identity. The bovine FSH receptor cDNA was 98%, 91%, and 86% identical to the reported nucleotide sequences of ovine, human, and rat FSH receptors, respectively [16, 17, 21]. The ovine LH receptor cDNA was 95%, 90%, and 86% identical to the reported nucleotide sequences of porcine, human, and rat LH receptors, respectively [18–20].

Both antisense and sense [^{35}S]UTP-labeled cRNA probes were transcribed from linearized cDNA templates using a transcription kit (Stratagene) according to the manufacturer's recommendations. The cRNA probes were purified by centrifugation on a Sephadex G-50 column (Sigma Chemical Co., St. Louis, MO) and used for hybridization within 2–3 days. For hybridization, the labeled probes were diluted in hybridization buffer (50% formamide, 0.3 M NaCl, 10 mM Tris [pH 8.0], 1 mM EDTA [pH 8.0], single-strength Denhardt's solution [single-strength = 0.02% (w/v) ficoll, 0.02% (w/v) polyvinylpyrrolidone, and 0.02% (w/v) BSA], 10 mM dithiothreitol, 500 μg/ml yeast tRNA, and 10% dextran sulfate) to about 2×10^7 cpm/ml.

In Situ Hybridization

Sections (14 μm) of follicular tissue were cut at −20°C using an IEC (International Equipment Co., Needham Heights, MA) cryostat and mounted onto prechilled microscope slides (Superfrost/Plus; Fisher Scientific, Pittsburgh, PA). Sections were fixed in 4% formaldehyde in 0.01 M PBS for 5 min, washed in PBS, dehydrated in ethanol, and stored at −80°C in desiccated, air-tight boxes until hybridization.

Before hybridization, slides were allowed to warm to room temperature. Sections were then hydrated in double-strength SSC (single-strength SCC = 0.3 M NaCl and 0.03 M Na_3 citrate, pH 7.0), acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min, rinsed in double-strength SSC, dehydrated in ethanol, and air dried. Hybridization was performed by application of just enough of the diluted probes (50–100 μl) to cover each section. The slides were loaded into plastic boxes moisturized with a buffer identical in osmolarity and formamide concentration to the hybridization buffer and were incubated in a humidified oven at 55°C for 20 h. After hybridization, excess hybridization buffer was removed by dipping slides into double-strength SSC. Slides were washed twice in double-strength SSC for 15 min at room temperature and treated with RNase A (50 μg/ml in 0.5 M NaCl, 10 mM Tris, and 1 mM EDTA)

for 30 min at 37°C. Slides were then washed at 55°C in double-strength SSC for 15 min, single-strength SSC for 15 min, single-strength SCC/50% formamide for 30 min, and 0.1-strength SCC for 30 min. Slides were dehydrated, air dried, dipped in Kodak NTB-2 (Eastman Kodak, Rochester, NY) emulsion, and exposed for 2 wk at 4°C. The slides were developed, lightly counterstained with hematoxylin and eosin, and mounted for examination under the microscope. For each follicle, two sections were hybridized to the antisense probe and one section was hybridized to the sense probe, for each of the receptors. Sections of follicles from animals ovariectomized on different days were balanced in each hybridization run to minimize biases due to variation among runs.

Hybridization intensity was quantified through use of the Bioquant image analysis system (R & M Biometrics Inc., Nashville, TN). Within a marked area of interest, the system determined the number of graphic pixels occupied by the silver grains (identified by a set grey threshold). The system also counted the total number of pixels in the marked area. Hybridization intensity was then defined as the number of occupied pixels per total pixels. For each follicle, four fields at roughly 90° angles were measured for both of the sections hybridized to the antisense probe and the one section hybridized to the sense probe. Specific hybridization intensity for each follicle was defined as the average hybridization intensity for the two sections hybridized to the antisense probe minus the average hybridization intensity for the section hybridized to the sense probe. All follicles $\geq$ 4 mm in diameter and those 0.5–4 mm in diameter that resided on the same section as the larger follicles were measured.

Classification of Follicles

All measured follicles were morphologically classified as healthy follicles or were placed into one of three classes (early, advanced, and late) of increasing degree of atresia [22]. Briefly, early atretic follicles had minor degenerative changes such as the presence of a few pyknotic nuclei and/or local destruction of the basal membrane. Advanced atretic follicles were identified by the presence of many degenerated granulosa cells, a more severe destruction in the follicular structure such as lack of the basal membrane and invagination of the theca layer into the granulosa layer, and/or a noticeable decrease in the number of granulosa cells. Late atretic follicles showed more severe degenerative changes than advanced atretic follicles, such as the presence of numerous pyknotic nuclei, disintegration of the granulosa layer, and a great decrease in the number of granulosa cells.

Statistical Analysis

Analysis of variance was used to test the effects of day of ovariectomy or health status of the follicles on the levels of gonadotropin receptor mRNA expression. When a main effect was significant, differences between means were compared with Duncan's Multiple Range test. Pearson correlation was used to establish the linear relationships between follicle size of healthy follicles and levels of mRNA expression. For analysis of the effects of follicle size (as measured on section) or health status on mRNA expression, data from all follicles $\geq$ 4 mm in diameter and the smaller follicles, regardless of the stage of follicular wave, were used. To account for differences in follicle size among health status classes, follicle size was fitted as a covariate in the analysis of the effect of health status of follicles on mRNA expression. To test the effect of day of ovariectomy on expression of LH and FSH receptor mRNAs, data from all healthy follicles $\geq$ 4 mm in diameter that were collected on Day 0 and Day 2, as well as the dominant follicles collected on Days 4, 6, 8, and 10, were used. All analyses were performed through use of the Statistical Analysis System [23] and, unless specified otherwise, results are reported as mean $\pm$ SEM.

RESULTS

Follicular Characteristics

Initiation of the first follicular wave was detected between 1 and 3 (1.9 $\pm$ 0.13) days after onset of estrus. Characteristics of follicles present on ovaries collected on various days of the first follicular wave have been previously reported [15]. On the day of detection of the first follicular wave, a variable number [1–5] of healthy follicles $\geq$ 4 mm in diameter were present on the ovaries of each animal. The number of follicles was similar two days later. On Day 2, it was still not possible to identify the dominant follicle on the basis of size. On Day 4, there was only one healthy follicle $\geq$ 4 mm in diameter in each animal, and this follicle was larger than the second largest follicular structure by at least 2.5 mm. On Day 6, there was one healthy dominant follicle in each heifer. On Day 8, there was one large follicle in each of the four animals. One of these large follicles was in the advanced stage of atresia and the remaining three were morphologically healthy. The second follicular wave had not been initiated by Day 8. On Day 10, all dominant follicles from the first wave were in the advanced stage of atresia and the second follicular wave had been initiated.

Expression of FSH Receptor mRNA

FSH receptor mRNA was localized to the granulosa cells of growing follicles, starting in some follicles with only one or two layers of granulosa cells (Fig. 1, A and B). Irrespective of the stage of the first follicular wave, the level of FSH receptor mRNA expression in granulosa cells of healthy antral follicles ranging from 0.5 to 14 mm in diameter did not correlate linearly with follicle size ($r = 0.02$, $p > 0.10$). The

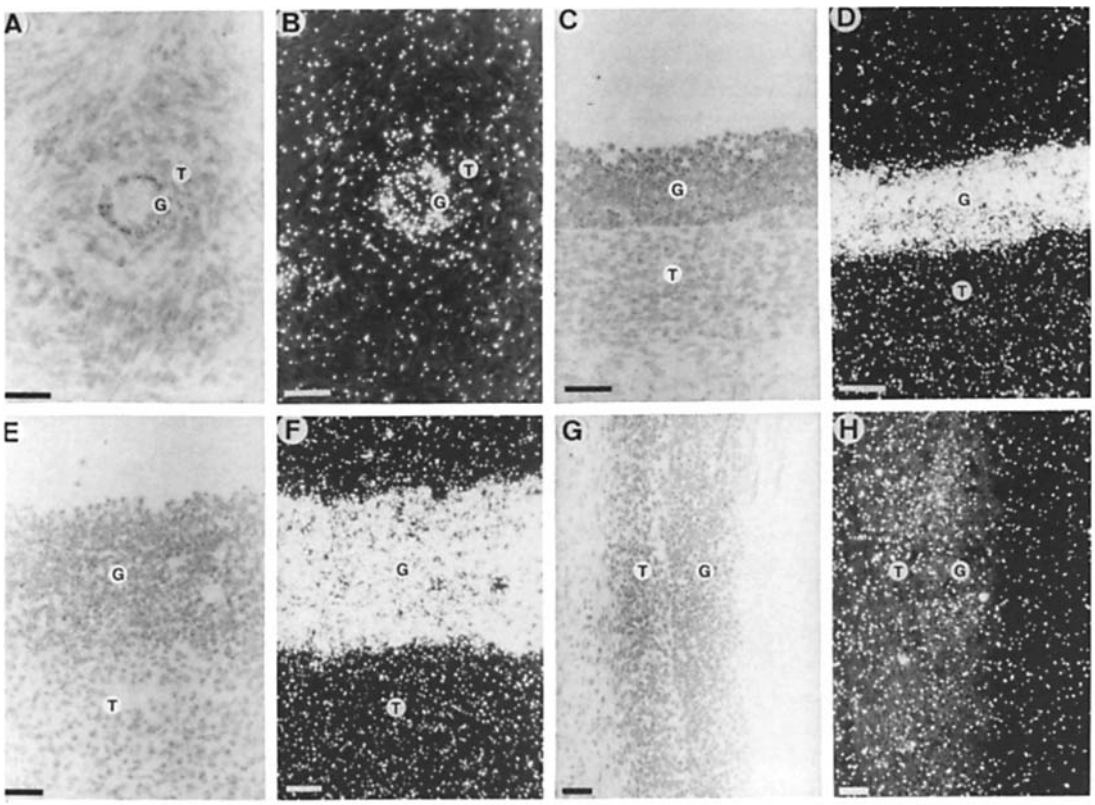

FIG. 1. In situ hybridization of FSH receptor mRNA in cryosections of bovine follicles collected at different stages during the first follicular wave. **A** and **B**) Brightfield and darkfield views of a small preantral follicle with only one layer of granulosa cells hybridized to the antisense probe, showing specific hybridization in the one layer of granulosa cells. **C** and **D**) Brightfield and darkfield views of a 3-mm antral follicle hybridized to the antisense probe. **E** and **F**) Brightfield and darkfield views of a Day 4 dominant follicle hybridized to the antisense probe. **G** and **H**) Brightfield and darkfield views of a dominant follicle hybridized to the sense probe. G, granulosa layer; T, theca layer. Bar = 50 μm.

effect of stage of the first follicular wave on the level of expression of FSH receptor mRNA failed to reach statistical significance ($p = 0.13$), despite a trend toward an elevated expression of FSH receptor mRNA in follicles collected on Day 4 (Table 1). Regardless of the stage of the follicular wave, FSH receptor mRNA expression in healthy follicles was similar to that observed in early atretic follicles but was greater ($p < 0.05$) than in follicles at the advanced and late stages of atresia (Table 2).

TABLE 1. Expression of FSH and LH receptor mRNAs (mean ± SEM) in bovine follicles collected on different days after initiation of the first follicular wave.

Day	No. animals (no. follicles)*	LH receptor		FSH receptor
		Theca	Granulosa	Granulosa
0	3 (8)	15.0 ± 6.5^{a}	0^{a}	15.1 ± 4.9
2	4 (12)	13.1 ± 5.8^{a}	0^{a}	24.7 ± 4.4
4	4 (4)	58.7 ± 10.3^{b}	26.1 ± 10.4^{b}	30.5 ± 11.3
6	5 (5)	21.0 ± 9.8^{a}	24.2 ± 7.6^{b}	14.2 ± 4.1
8	4 (4)	27.7 ± 9.7^{a}	9.0 ± 6.8^{a}	12.6 ± 5.5
10	3 (3)	10.4 ± 6.5^{a}	0^{a}	6.0 ± 2.8
Effect		$p < 0.01$	$p < 0.01$	$p = 0.13$

*For Days 0 and 2, the number of healthy follicles ≥ 4 mm in diameter; for Days 4, 6, 8, and 10, the number of dominant follicles only.
[a,b]Values within each column with no common superscripts differ ($p < 0.05$).

Expression of LH Receptor mRNA

LH receptor mRNA was first detected in theca interna cells of small antral follicles shortly after antrum formation (Fig. 2, A and B). Level of LH receptor mRNA expression in the theca interna of healthy follicles increased linearly with follicle size ($r = 0.39$, $p < 0.01$). LH receptor mRNA levels in theca were highest in dominant follicles collected on Day 4 and were not different among other days (Table 1). LH receptor mRNA expression in theca interna cells of healthy

TABLE 2. Expression of FSH and LH receptor mRNAs (mean ± SEM) in healthy and atretic bovine follicles.*

Health status	No. of follicles	LH receptor: Theca	FSH receptor: Granulosa
Healthy	60	16.2 ± 2.2^{a}	19.7 ± 1.7^{a}
Early atretic	20	11.8 ± 3.8^{ab}	16.5 ± 2.9^{a}
Advanced atretic	20	11.6 ± 3.8^{ab}	5.9 ± 2.9^{b}
Late atretic	24	6.9 ± 3.5^{b}	0.1 ± 2.7^{b}

*The effect of health status of follicles on expression of LH receptor mRNA in granulosa cells was not examined, as no follicles classified as either early or late atretic were >9 mm in diameter, the size at which expression of LH receptor mRNA in granulosa cells was first observed.
[a,b]Values within each column with no common superscripts differ ($p < 0.05$).

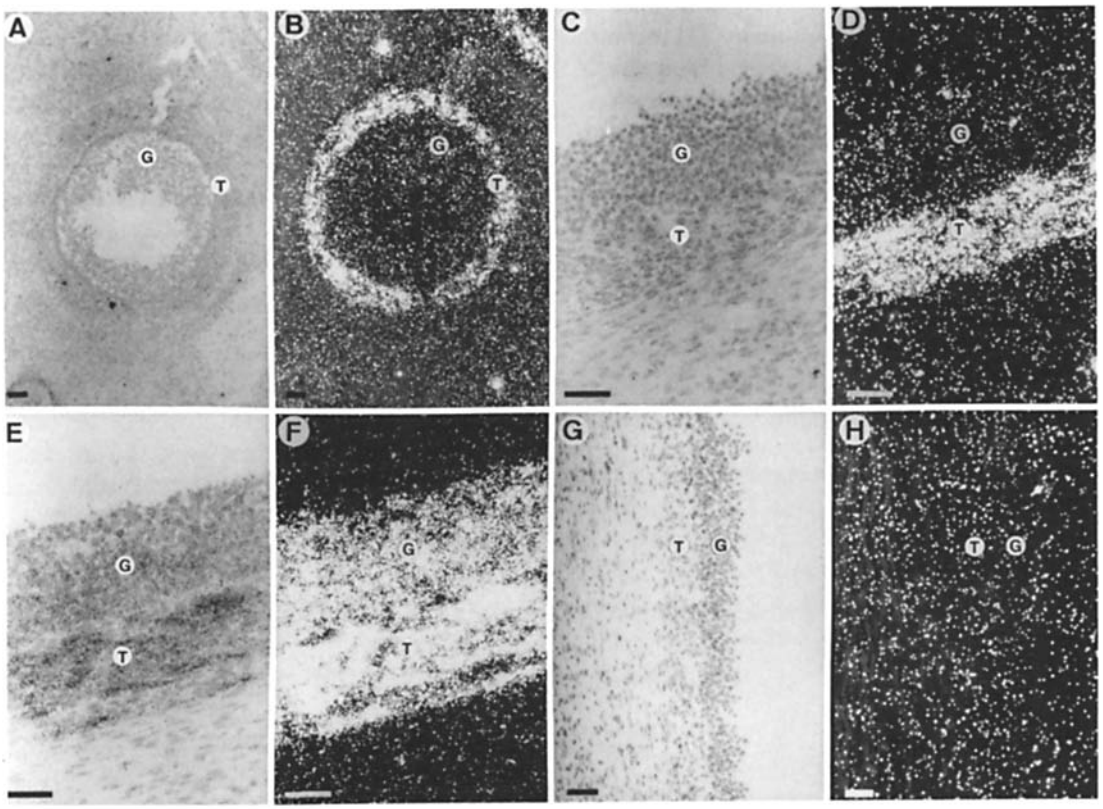

FIG. 2. In situ hybridization of LH receptor mRNA in cryosections of bovine follicles collected at different stages during the first follicular wave. **A** and **B**) Brightfield and darkfield views of a 0.45-mm antral follicle hybridized to the antisense probe, showing specific hybridization in the theca layer. **C** and **D**) Brightfield and darkfield views of an 8-mm healthy follicle hybridized to the antisense probe, showing only hybridization to the theca interna layer. **E** and **F**) Brightfield and darkfield views of a Day 4 dominant follicle hybridized to the antisense probe, showing intense hybridization in both the theca and granulosa cells. **G** and **H**) Brightfield and darkfield views of a dominant follicle hybridized to the sense probe. G, granulosa layer; T, theca layer. Bar = 50 μm.

follicles was similar to that of follicles at the early and advanced stages of atresia but was greater ($p < 0.05$) than that of follicles at the late stage of atresia (Table 2).

In granulosa cells, LH receptor mRNA was detected only in healthy follicles > 9 mm in diameter and was first observed in the dominant follicles collected on Day 4 (Fig. 1, C-F). The level of LH receptor mRNA expression in dominant follicles was similar on Day 6 as compared to Day 4 but was reduced on Day 8 (Table 1). Expression of LH receptor mRNA in granulosa cells was not detected in the regressing dominant follicles collected on Day 10 (Table 1).

DISCUSSION

In this study, expression of mRNAs for FSH and LH receptors during follicular development and at different stages of the first follicular wave was characterized. FSH receptor mRNA was expressed in granulosa cells shortly after the time follicles leave the primordial pool. This observation is similar to findings from studies in rats showing that FSH receptor or its mRNA was present on granulosa cells of small preantral follicles [12, 24]. These results raise questions about the possible role of FSH during early follicular growth, because follicles can grow to the early antral stage in the absence of gonadotropin support [25–27]. Nevertheless, it has been found that FSH was able to increase the number of small preantral follicles in hypophysectomized mice [28]. In addition, despite the fact that FSH is required for supporting follicular growth beyond the early antral stage, the steady state FSH receptor mRNA level in healthy follicles did not correlate with follicle size, nor did the level of FSH receptor mRNA change with stage of the first follicular wave. Previous studies have shown that the ability of granulosa cells to bind FSH either did not vary significantly with follicular size [12, 29–31] or decreased with increasing follicle size [10, 11, 32]. These results show that while FSH is necessary for follicular growth, potential differences in responsiveness to FSH, if such differences exist, are not mediated via differences in FSH receptor concentrations. Therefore, it is unlikely that differences among follicles in FSH receptor numbers underlie the mechanisms for recruitment and selection of dominant follicles in cattle.

LH receptor mRNA was expressed in theca interna cells of antral follicles. During follicular growth, the level of LH

receptor mRNA expression increased with follicle size, in agreement with results in rats [24, 33]. Similarly, LH receptor concentrations in theca interna cells increased with size in estrogen-active follicles collected during the follicular phase [10, 11, 30, 34]. However, no relationship between LH binding to theca interna tissue and follicular diameter was found in bovine or ovine follicles collected at unknown stages of the estrous cycle [29, 32, 35]. The reasons for this discrepancy are not known and could relate to differences in tissue preparation and identification of healthy follicles. LH receptor mRNA expression in theca interna cells also varied with stage of the first follicular wave, being higher in dominant follicles collected on Day 4 than on any other day. This is in agreement with a previous study showing that the capacity of theca-enriched homogenates to bind hCG was greater in estrogen-active follicles on Days 5 and 7 than on Day 3 of the bovine estrous cycle [30]. We have also found previously that theca interna cells of dominant follicles collected on Day 4 after initiation of the first follicular wave had the highest levels of mRNAs for $P450_{scc}$ and P450c17 [15]. These features of dominant follicles on Day 4 ensure that the theca interna cells of these follicles are capable of producing large amounts of androgen substrates for estradiol-17β biosynthesis in the granulosa cells.

The present study showed that in bovine granulosa cells, LH receptor mRNA was detected only in healthy follicles > 9 mm in diameter. Previous studies demonstrated that LH receptor or its mRNA was expressed only in granulosa cells of large antral follicles [10, 12, 29, 33, 34, 36]. While the small number of follicles expressing LH receptor mRNA in the present study did not allow a relationship to be established between follicular size and LH receptor mRNA expression in granulosa cells, previous studies have shown that LH receptor concentrations in granulosa cells increased with follicle size [10, 11, 29, 32, 34]. Recently, Jolly et al. [37] reported that granulosa cells from a minority of healthy bovine follicles as small as 4.5 mm in diameter could produce a cAMP response following LH stimulation. The reasons for this discrepancy in the size at which granulosa cells first acquire LH receptors are not known and could relate to differences in the sensitivity and/or specificity of the techniques used in these two studies. In support of our present study, Jolly et al. [37] concluded that the cAMP response to LH was generally low or not detected in granulosa cells from 4–8-mm follicles; it then increased linearly with increasing follicle diameter ≥ 8 mm. In the present study, significant expression of LH receptor mRNA in granulosa cells was first observed in dominant follicles collected on Day 4 after initiation of the first follicular wave. Prior to physical identification of the dominant follicles (i.e., on Days 0 and 2), no follicles expressed LH receptor mRNA in the granulosa cells despite the fact that some of these follicles were as large as 8 mm in size. Similarly, Ireland and Roche [30] showed that granulosa cells of estrogen-active follicles collected on Day 7 of the estrous cycle had a much greater capacity to bind LH than granulosa cells of follicles collected on Days 3 and 5. Thus, critical changes must have taken place between Day 2 and Day 4 in the present study that enabled one follicle in each animal to assume dominance. While the present study showed a definite association between the expression of LH receptor in granulosa cells and dominance, a cause-effect relationship cannot be established. A more frequent sampling regime between Day 2 and Day 4 may reveal whether or not only one follicle from among the cohort of recruited follicles acquires LH receptors in the granulosa cells.

Multiple mRNA transcripts for the LH and FSH receptor have been shown to be expressed in the ovary [38]. In general, changes in the expression of the various transcripts during up- and down-regulation of FSH and LH receptor expression follow a similar pattern under several experimental models [38]. However, it remains to be determined whether or not the various transcripts are regulated differently during follicular development and atresia in cattle.

Both FSH and estradiol-17β are required for stimulating the expression of LH receptor or its mRNA in granulosa cells [12, 28, 33, 39]. However, no divergence in the pattern of expression of FSH receptor and aromatase mRNAs was apparent prior to physical identification of the dominant follicle [15]. These results suggest that selection of the dominant follicle may be a passive process in which the first follicle that acquires LH receptors in its granulosa cells is selected to become the dominant follicle. The acquisition of LH receptors in granulosa cells will enable these follicles to respond to LH in addition to FSH, whose concentrations in blood have declined to basal levels at the time of selection [4, 8].

The present study showed that atresia was associated with reduced expression of gonadotropin receptor mRNAs, which is in agreement with previous studies on receptor binding [10, 11, 29, 30, 35] and mRNA expression [40]. The expression of LH receptor mRNA in thecal cells was less affected by atresia, with follicles in the late stage of atresia still expressing detectable levels of LH receptor mRNA. Previously, Henderson et al. [35] also could not demonstrate significant differences between theca interna prepared from healthy and atretic follicles in their capacity to bind hCG. Dominant, morphologically healthy follicles collected on Day 6 after initiation of the first follicular wave expressed abundant $P450_{arom}$ and LH receptor mRNAs in their granulosa cells but low levels of $P450_{scc}$ and P450c17 mRNAs in the theca interna [15]. These results suggest that regression of the dominant follicles may be due to a reduction in thecal androgen production rather than to loss of aromatase activity or LH receptors in granulosa cells [15].

In conclusion, the present study showed that the acquisition of LH receptors in granulosa cells may be critical to the establishment and maintenance of follicular dominance,

whereas FSH receptors may only play a permissive role. Reduction in LH and FSH receptors in the theca interna and/or granulosa cells may be secondary to the loss of steroidogenic capacity of the theca interna in causing regression of the dominant follicles of the first follicular wave.

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