INSULIN-LIKE GROWTH FACTORS (IGF), LH AND IGF-I RECEPTORS, AND STEROIDS IN DOMINANT FOLLICLES DURING THE FIRST FOLLICULAR WAVE IN DAIRY CATTLE

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Story in Brief

Objectives of this study were to determine if concentrations of steroids, insulin-like growth factor-I (IGF-I) and IGF-II in follicular fluid, and numbers of LH and IGF-I receptors change during growth of the dominant follicle. Ovarian follicular development was monitored daily via ultrasound in lactating Holstein cows. Animals were bilaterally ovariectomized when the dominant follicle (DF) was first identified or when it stopped growing. Numbers of specific ^{125}I-hCG/LH binding sites in thecal cells were greater in DF compared with large (LG) and small (SM) follicles of both Early (day 5 post-estrus) and Late (day 10 post-estrus) cows. Numbers of specific ^{125}I-hCG/LH binding sites in granulosa cells were similar for follicle sizes in Early cows, but in Late cows were greater in DF compared with SM follicles, and were greater in Late DF compared with Early DF. Numbers of receptors for IGF-I in thecal cells were twofold greater in DF and LG compared with SM in Late cows. Numbers of IGF-I receptors in granulosa cells were unaffected by size or growth of follicles, but were greater than in theca cells. Concentrations of estradiol were greater in DF compared with LG and SM in both Early and Late cows. Concentrations of androstenedione in Early cows were greater in DF and SM compared with LG follicles. Concentrations of progesterone and IGF-I did not differ among follicle classes. Concentrations of IGF-II did not differ between Early and Late cows but were greater in SM than DF or LG follicles. We conclude that increased thecal binding sites for hCG/LH and increased estradiol in follicular fluid appear to be related to establishment of the dominant follicle during the first follicular wave in dairy cattle exhibiting regular estrous cycles during late lactation.

(Key Words: Ovarian Follicles, Estradiol, IGF Receptors, Luteinizing Hormone Receptors, Cattle.)

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Introduction

In cattle, follicular growth during the estrous cycle as assessed by ultrasonography has been associated with usually two to three follicular waves, with each wave consisting of a dominant follicle and associated subordinate follicles (Fortune, 1994). However, the endocrine and(or) intraovarian factors responsible for these changes in follicular growth are uncertain. One potential intraovarian regulator is insulin-like growth factor-I (IGF-I), which has been shown to be produced locally within the ovary, and concentrations of which increase with follicle diameter (Spicer and Echternkamp, 1995). Previous research has shown that increased luteinizing hormone (LH) binding sites in theca cells were associated with increased diameters of estrogen-active follicles during d 3 to 7 of the bovine estrous cycle, whereas follicle-stimulating hormone (FSH) binding sites in granulosa cells of estrogen-active follicles did not change during the same interval (Ireland and Roche, 1983). Also, estrogen-inactive follicles from d 9 to 13 were smaller with less granulosa cells and fewer thecal and granulosa LH binding sites. Research examining differences in IGF-I binding sites and IGFs between growing and regressing nonovulatory dominant follicles has not been done. Therefore, our objective of this study was to determine if concentrations of steroids, IGF-I, IGF-II, and numbers of receptors for IGF-I and LH change during the transition from a growing phase to a nongrowing (static) phase of first-wave dominant follicles in cattle exhibiting regular estrous cycles during late lactation.

Materials and Methods

Thirteen lactating Holstein cows were milked twice daily (0300 and 1500 h), housed on pasture, and group-fed a total mixed ration consisting of sorghum silage, alfalfa hay, whole cottonseed, and concentrate ad libitum. Estrus was monitored twice daily. Estrous cycle interval, body weight at the termination of the experiment, days postpartum, daily milk production, and average number of lactations were recorded.

Estrous cycles were synchronized using two injections (i.m.) of prostaglandin $F_{2\alpha}$ (Lutalyse®, 25 mg) 11 days apart. Starting at estrus, follicular development was monitored daily for an entire estrous cycle via ultrasonography, using an Aloka 500V with a 7.5 MHz probe. Blood samples were obtained daily via jugular venipuncture from d 0 until ovariectomy. Ultrasound examinations were performed daily, and as soon as the first dominant follicle could be identified from its subordinates (i.e., the first day the largest growing follicle diameter was $\geq$ 2 mm from any other $\geq$ 5 mm follicle), cows were either bilaterally ovariectomized or follicular development was monitored until the first dominant follicle slowed in its growth or stopped

growing (i.e., the first day the dominant follicle grew ≤ 1 mm from the previous day). Both ovaries from each cow were removed via lateral incision through the left paralumbar fossa area after local anesthesia (2% lidocaine; 60 to 80 ml s.c. and i.m.). After each ovariectomy, ovaries were put on ice, and transported to the laboratory where diameters of all follicles ≥ 6 mm in diameter were recorded, the numbers of all follicles ≥ 1 mm in diameter on the ovarian surface were determined, and ovarian tissue and fluid were collected.

Follicular fluid from individual follicles ≥ 6 mm in diameter (classified as Dominant or Large) was aspirated and centrifuged to obtain granulosa cells (Spicer et al., 1993), and follicular fluid from follicles < 6 mm in diameter (Small) was pooled within ovaries and then centrifuged to obtain granulosa cells. The theca interna layer was removed by blunt dissection. For Small follicles, the follicular wall was removed by blunt dissection after bisection of each follicle; granulosa cells were not separated from the theca interna. Granulosa cells separated from follicular fluid of small follicles were combined from both ovaries. Each corpus luteum (CL) was dissected free of ovarian stroma, and weighed. All tissue was stored at -70°C in phosphate-buffered saline containing 20% glycerol.

Concentrations of androstenedione, estradiol, progesterone, IGF-I and IGF-II in follicular fluid were determined by radioimmunoassays. Numbers of binding sites for IGF-I and LH in bovine thecal and granulosa cells were determined using radioreceptor assays.

Follicular fluid hormonal and follicular cell receptor data were analyzed by ANOVA. Main effects, consisting of time of ovariectomy (Early and Late) and follicle category (Small, Large and Dominant follicle) and their various interactions were analyzed. Daily plasma hormonal data (progesterone and IGF-I) were analyzed using a split plot ANOVA for repeated measures over time; effects of time of ovariectomy were tested using cow within time group as the error term and effects of day and time group by day were tested using the residual error term. Specific differences between means were determined using Fisher's protected least-significant difference procedure if significant main effects were detected.

Results

Physiological characteristics for cows ovariectomized early versus late did not differ (P>.10): estrous interval, final body weight, days postpartum, daily milk production and lactation number averaged (± SE) 23.2 ± 1.4 d, 658 ± 43 kg, 319 ± 59 d, 17.5 ± 4.0 kg/day and 2.8 ± .7, respectively, for cows ovariectomized early and 21.1 ± 1.1 d, 707 ± 34 kg, 336 ± 47 d, 16.0 ± 3.1 kg/d and 3.3 ± .5, respectively, for cows ovariectomized late. Total ovarian weight, numbers of small and large follicles and average diameter of large follicles

(excluding dominant follicles) did not differ (P>.10) between Early and Late cows. However, day of cycle, CL weight and average diameter of Dominant follicles were greater in Late (d 10, 4.6 g, and 17 mm, respectively) than Early (d 5, 2.4 g and 12 mm, respectively) cows.

Follicle category affected (P<.01) follicular fluid concentrations of estradiol whereas day of ovariectomy and follicle category by day of ovariectomy interaction were not significant. Concentrations of estradiol in follicular fluid were greatest (P<.01) in Dominant (155 ± 18 ng/ml) compared with Large (19 ± 11 ng/ml) and Small (2 ± 14 ng/ml) follicles in cows ovariectomized both early and late during the first follicular wave. For both early and late groups, Large and Small follicles had similar (P>.10) concentrations of estradiol.

Follicle category and its interaction with day of ovariectomy had no effect (P>.10), whereas day of ovariectomy tended (P<.10) to influence concentrations of progesterone in follicular fluid (data not shown). Averaged across follicle category, concentrations of progesterone tended to be greater (P<.10) in follicles from cows ovariectomized late (116 ± 18 ng/ml) compared with cows ovariectomized early (65 ± 21 ng/ml) during the follicular wave.

Follicle category affected (P<.05) follicular fluid androstenedione concentrations, whereas day of ovariectomy and its interaction with follicle category was without effect (P>.10). Averaged across Early and Late groups, concentrations of androstenedione in follicular fluid were 5.6-fold greater (P<.05) in Dominant follicles (141 ± 20 ng/ml) compared to Large follicles (25 ± 13 ng/ml) and greater (P<.05) in Small follicles (58 ± 16 ng/ml) compared to Large follicles. Dominant and Small follicles from cows ovariectomized early or late during the first follicular wave had similar concentrations of androstenedione (data not shown).

Concentrations of IGF-I in follicular fluid did not differ (P>.10) among follicle categories. However, cows ovariectomized early during the first follicular wave tended to have lower (P<.10) IGF-I concentrations in large follicles than cows ovariectomized late (data not shown). No significant time of ovariectomy by follicle category interaction was detected. Although diameter of large follicles tended to correlate with concentrations of follicular fluid IGF-I (r=.27, P<.10), the percentage of follicular fluid IGF-I to plasma IGF-I did not differ (P>.10) among follicle categories and averaged 79 ± 7%.

There were no differences (P>.10) in progesterone and IGF-I concentrations during the first 5 d of the estrous cycle between cows ovariectomized early or late during the first follicular wave (data not shown). Also, no significant interaction between day of cycle and ovariectomy group existed for plasma progesterone and IGF-I concentrations. Concentrations of IGF-I in plasma averaged 89 ± 9 ng/ml and did not (P>.10) change between d 0

and 5, whereas plasma progesterone increased (P<.05) from .14 ng/ml on d 0 to .78 ± .08 ng/ml on d 5 of the estrous cycle.

Concentrations of IGF-II in follicular fluid were not affected (P>.10) by day of ovariectomy or its interaction with follicle category. However, follicle category tended (P<.10) to influence follicular fluid IGF-II concentrations such that Small follicles had greater IGF-II concentrations (227 ± 15 ng/ml) than did Dominant (183 ± 18 ng/ml) or Large (188 ± 16 ng/ml) follicles. Plasma concentrations of IGF-II on the day of ovariectomy averaged 110 ± 10 ng/ml and 110 ± 8 ng/ml for Early and Late cows, respectively, and did not differ (P>.10). The percentage of follicular fluid IGF-II to plasma IGF-II was affected (P<.05) by follicle category but not day of ovariectomy or their interaction (data not shown). Averaged across Early and Late cows, Small follicles had a greater (P<.05) percentage of follicular fluid IGF-II to plasma IGF-II (196 ± 11%) than Dominant (162 ± 13%) or Large (155 ± 12%) follicles.

Day of ovariectomy and its interaction with follicle category had no effect (P>.10), whereas follicle category influenced (P<.01) specific binding of ^{125}I-IGF-I to thecal cells (Figure 1A). Specific binding of ^{125}I-IGF-I to thecal cells was similar (P>.10) between Dominant and Large follicles from cows ovariectomized both early and late (Figure 1A). Also, specific binding of ^{125}I-IGF-I to thecal cells did not differ (P>.10) between Small and Large or Dominant follicles from cows ovariectomized early, but was lower (P<.05) in Small than in Dominant and Large follicles of cows ovariectomized late (Figure 1A). Specific binding of ^{125}I-IGF-I to granulosa cells was unaffected (P>.10) by day of ovariectomy, follicle category or their interaction (Figure 1B).

Day of ovariectomy and its interaction with follicle category had no effect (P>.10), whereas follicle category influenced (P<.01) specific binding of ^{125}I-hCG to thecal cells. Specific binding of ^{125}I-hCG to thecal cells was greater (P<.01) in Dominant follicles compared to Large and Small follicles, respectively, in cows ovariectomized early in the growing phase, and greater (P<.05) in Dominant follicles compared to Large and Small follicles, in cows ovariectomized late in the growing phase (Figure 2A). Thecal cells from Large and Small follicles in both groups had similar (P>.10) specific binding of ^{125}I-hCG.

Day of ovariectomy influenced (P<.05) specific binding of ^{125}I-hCG to granulosa cells, whereas follicle category and its interaction with day had no effect (P>.10). Specific binding of ^{125}I-hCG to granulosa cells did not differ between Dominant, Large or Small follicles from cows that were ovariectomized early (Figure 2B). However, in cows that were ovariectomized late, specific binding of ^{125}I-hCG to granulosa cells was greater (P<.05) in Dominant follicles than in Small follicles. Also, specific binding of ^{125}I-hCG

was greater ($P<.05$) in granulosa cells of Dominant follicles from cows ovariectomized late compared to Dominant follicles in the early growing phase.

Discussion

Estradiol concentrations were greatest in dominant follicles compared to subordinates regardless if they were in the early or late growing phase, which agrees with earlier reports in cattle (Fortune, 1994; Ireland and Roche, 1983). As the first dominant follicle regresses, concentrations of estradiol in its follicular fluid decrease and are associated with a decrease in follicular P450 aromatase mRNA (Price et al., 1995; Xu et al, 1995a). The loss in the ability of estrogen-active follicles to produce estradiol during the first two weeks of an estrous cycle has been associated with decreased numbers of granulosa cells and reduced numbers of LH receptors in granulosa and thecal cells (Ireland and Roche, 1983). Levels of androstenedione also were greater in dominant follicles early in their growth phase compared to their subordinate follicles. These greater androstenedione concentrations in dominant follicles of the present study were related to a greater number of LH/hCG receptors in thecal cells since these two variables were correlated ($r = .41$, $P<.01$). In addition, concentrations of estradiol were correlated with androstenedione concentrations ($r = .64$, $P<.01$) and numbers of LH/hCG receptors in thecal cells ($r = .36$, $P<.05$) in dominant and large follicles. Thus, it appears that both thecal and granulosa cell steroidogenic enzyme activity are coincidentally increased in the early developing dominant follicle.

Concentrations of IGF-I did not differ between dominant and subordinate large or small follicles during the first follicular wave of an estrous cycle in cattle, an observation not previously reported. Similarly, IGF-I concentrations in follicular fluid of large preovulatory follicles with high estradiol concentrations do not differ from those in small (< 4 mm) or medium follicles (4 to 7.9 mm) with low estradiol concentrations (Spicer et al., 1991; Simpson et al., 1994).

Concentrations of IGF-II did not differ between dominant and subordinant large follicles during the first follicular wave of an estrous cycle in cattle. However, IGF-II concentrations tended to be greater in small than in dominant or large follicles which is in general agreement with a previous report in ewes and cattle (Spicer and Echternkamp, 1995).

Numbers of IGF-I binding sites in thecal cells did not differ between early dominant and large or small subordinate follicles but were greater in late (nongrowing) dominant and large follicles than small follicles. In contrast, numbers of IGF-I binding sites in granulosa cells did not significantly differ among follicle categories although dominant and large follicles had numerically a twofold greater ($P = .09$) number of IGF-I receptors than small

follicles. Previously, Spicer et al. (1994) demonstrated that granulosa cells from large (≥ 8 mm) bovine follicles have a severalfold greater number of IGF-I receptors than do small (≤ 5 mm) follicles after culture in vitro. In addition, specific IGF-I binding to granulosa cells was greater than that found in thecal cells. Thus, granulosa cells which contain a greater number of IGF-I receptors than thecal cells may be more sensitive to changes in local concentrations of bioavailable IGF-I than thecal cells.

Binding sites for LH/hCG in thecal cells were greater in growing (early) and late dominant follicles, as compared with subordinate large and small follicles. In granulosa cells, LH/hCG binding sites were greater in the dominant follicle than subordinate follicles only during the late growing phase. Recently, Xu et al. (1995b), using in situ hybridization, reported that FSH receptor mRNA in granulosa cells of the dominant follicle does not change between d 2 and 10 of the first follicular wave, whereas thecal and granulosa cell LH receptor mRNA increases between d 2 and 4 of the first follicular wave in beef heifers. In nonovulatory follicles, binding of LH/hCG to thecal and granulosa cells was highest on d 7 of the estrous cycle (Ireland and Roche, 1983), which would be during the middle of the first wave of follicular growth, at a time of maximum growth of the dominant follicle (Savio et al., 1988; Adams et al., 1992). This increase in thecal LH receptors could be one key factor that allows dominance to be manifested. However, the factor(s) responsible for the increase in thecal LH/hCG receptors in early dominant follicles is(are) unknown. Regardless of the stimulus, an increase in thecal LH receptors may allow for greater thecal androgen production and subsequently greater estradiol production by granulosa cells.

In conclusion, results of the present study support the hypothesis that the procurement of LH receptors in thecal cells and increased estradiol in follicular fluid may be critical to the establishment of follicular dominance whereas IGF-I receptors may only play a permissive role. With a better understanding of the physiological changes occurring within ovarian follicles during their growth and dominance, it is hoped that procedures can be developed to improve reproductive efficiency in dairy cattle.

Literature Cited

Adams, G.P. et al. 1992. J. Reprod. Fertil. 94:177.
Fortune, J.E. 1994. Biol.Reprod. 50: 225-232.
Ireland, J.J. and Roche, J.F. 1983. Endocrinology 112:150.
Price, C.A. et al. 1995. J.Reprod. Fertil. 103:63.
Savio, J.D. et al. 1988. J.Reprod. Fertil. 83:663.
Simpson, R.B. et al. 1994. J. Reprod. Fertil. 102:483.
Spicer, L.J. et al. 1994. Mol. Cell Endocrinol. 102:69.

Spicer, L.J. et al. 1991. Dom. Anim. Endocrinol. 8:431.
Spicer, L.J. and S.E. Echternkamp. 1995. Dom. Anim. Endocrinol. 12:223.
Spicer, L.J. et al. 1993. J. Anim. Sci. 71:1232.
Xu, Z. et al. 1995a. Biol.Reprod. 53:951.
Xu, Z. et al. 1995b. Endocrinology 136:981.

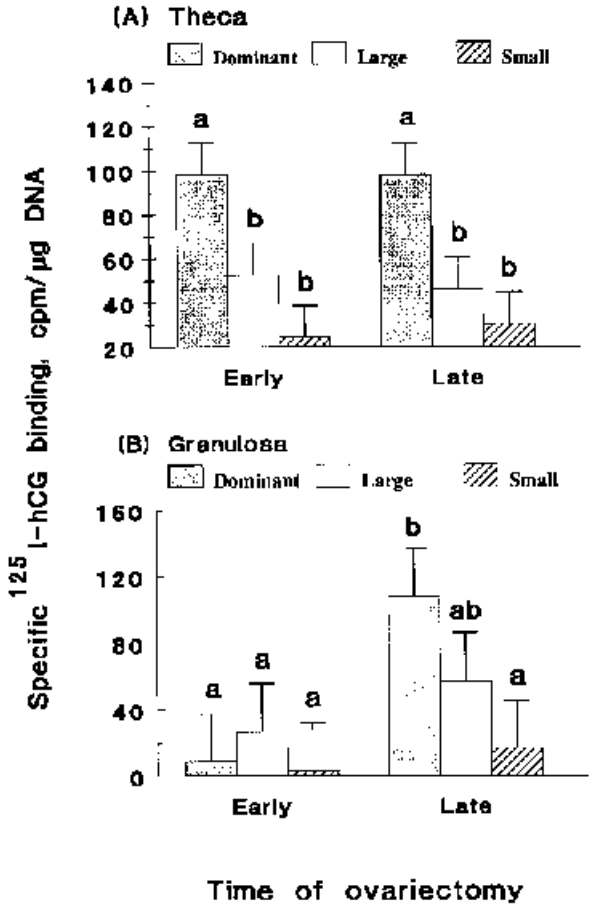

Figure 1. Specific binding of ^{125}I-IGF-I to thecal (Panel A) and granulosa (Panel B) cells collected from Dominant, Large (≥ 6 mm in diameter excluding Dominant), and Small (< 6 mm in diameter) follicles from cows ovariectomized early or late during the first follicular wave of an estrous cycle.

[a]Late Small differs from Late Dominant and Large (P<.05).

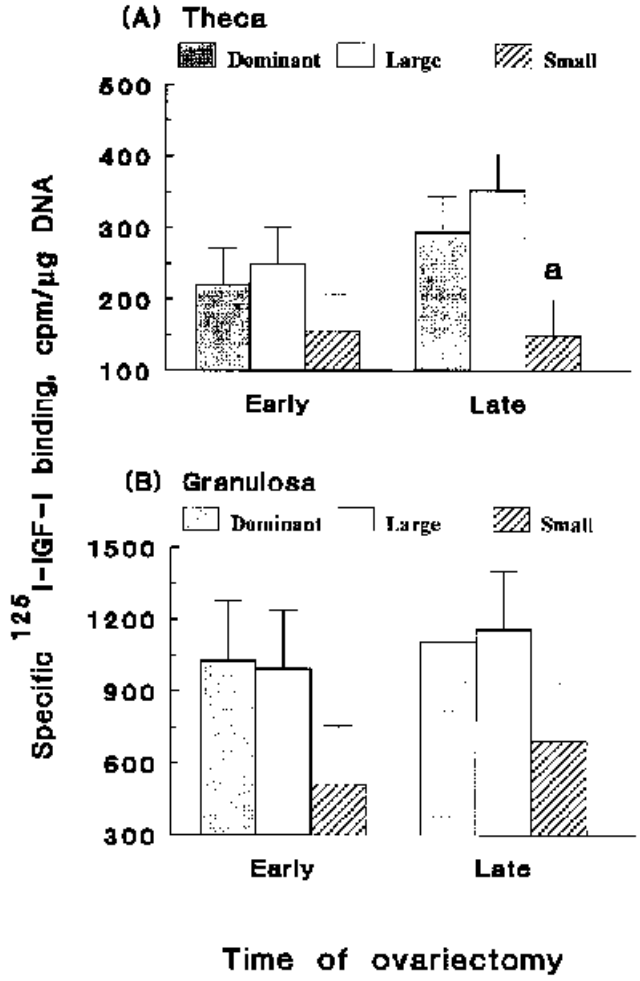

Figure 2. Specific binding of ^{125}I-hCG to thecal (Panel A) and granulosa (Panel B) cells collected from Dominant, Large (≥ 6 mm in diameter excluding Dominant), and Small (< 6 mm in diameter) follicles from cows ovariectomized early or late during the first follicular wave of an estrous cycle.

[a, b] Within a panel, means without a common letter differ (P<.05).