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Current Concepts for Understanding Ovarian Follicular Growth and Function in Cattle
Leon J. Spicer and Sherrill E. Echternkamp

Introduction
Ovarian follicular development in cattle during either the estrous cycle or postpartum anestrus is not presently well understood. Although several investigators have suggested that follicular development is continuous, the notion that follicular growth occurs in waves still persists. A better understanding of bovine folliculogenesis is required to solve beef production problems such as prolonged postpartum infertility and variable responses to superovulation and estrous synchronization treatments. Therefore, research was conducted at MARC and results compared with earlier studies to explain follicular growth and function in cattle during postpartum anestrus.

Follicular Growth
Folliculogenesis may be defined as formation of Graafian (mature, preovulatory) follicles from a pool of primordial (non-growing) follicles (Fig. 1). The pool of primordial follicles remains stable from birth until about the fourth yr of life, and then subsequently declines. In comparison, numbers of antral follicles (follicle with a fluid filled cavity) remain constant (30 to 60 per pair of ovaries) in cows up to 10 yr of age and then decline to less than 50% of maximal numbers at 15 to 20 yr of age. Measurements such as numbers of follicles within various size categories and mean sizes (i.e., diameter) of various types of follicles have been the predominant criteria for assessment of follicular growth in cattle. Several researchers have reported significant variation in numbers of various sized antral follicles during both the bovine estrous cycle and postpartum anestrus.

The limited information available on growth of antral ovarian follicles in cattle during postpartum anestrus suggests that follicular growth increases markedly after the first wk postpartum and that large antral follicles (> 10 mm diameter) may be present up to 5 wk prior to the first postpartum estrus. We have shown that large follicles (≥ 8 mm) are present on the ovarian surface as early as 7 days after parturition and that the number of medium-sized follicles (4-7.9 mm in diameter) increases significantly during the first 7 wk postpartum. Thus, large antral follicles are present during postpartum anestrus, but they do not ovulate soon after they appear. Although large doses of gonadotropin-releasing hormone (GnRH) or estradiol can induce normal gonadotropin surges by this time, these large follicles may be incapable of producing sufficient estradiol to increase estradiol in blood (10 to 15 pg/ml of blood) to concentrations required to induce estrual behavior and to stimulate preovulatory gonadotropin surges (and ovulation). We and other researchers have been able to stimulate (in 70-80% of the cows) follicular estradiol production and (or) ovulation by administering multiple low-dose injections of GnRH in suckled beef cattle (Table 1). However, multiple low-dose injections of GnRH have not induced ovulation consistently, which may be due to numerous factors such as dose and injection schedules or nutritional status of dams. Collectively, data from macro- and (or) microscopic evaluation of ovaries of dairy and beef cows suggest that there is growth of small antral follicles into larger follicles during postpartum anestrus. Rate of replacement of large antral follicles within the ovaries during postpartum anestrus is unknown.

Ovarian Follicular Steroidogenesis
The ovaries of cattle are the major source of estrogens, androgens, and progesterone found in peripheral blood. Consequently, concentrations of these steroids in peripheral blood may measure follicular development and ovarian function. In addition to concentrations of steroids in blood, follicular steroidogenic capability may be ascertained by quantifying concentrations of steroids in follicular fluid. There is strong evidence to indicate a high positive correlation between in vitro follicular cell steroid production and concentration of steroids in follicular fluid. Thus, the next section will describe changes in concentrations of estradiol, androgens and progesterone in blood and follicular fluid during postpartum anestrus in cattle.

Steroids in Peripheral Blood
Estriadiol. In cattle, concentrations of estradiol in peripheral blood decrease sharply at parturition to basal levels of 1-5 pg/ml within 2 to 6 days and then increase just before the first postpartum estrus. This increase in preovulatory estradiol is similar in duration and magnitude to that observed during proestrus and estrus in repetitive estrous cycles. However, first postpartum ovulations that occur without estrual behavior are often followed by a short luteal phase. It is unknown if increases in blood estradiol are “normal” before these short luteal phases. Our studies would suggest that ovarian follicles obtain an increased ability to synthesize estrogens at least 2 wk before the first postpartum ovulation.

Figure 1—Schematic of various types of follicles present in ovaries of cattle. 1 = primordial follicle; 2 = growing preantral follicle; 3 = small antral follicle; 4 = large antral follicle (i.e., Graafian follicle). a = antrum (i.e., cavity filled with follicular fluid); g = granulosa cells; o = oocyte (egg); t = thecal cells.

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Table 1—Comparisons among follicular fluid (FF) steroids and gonadotropin binding sites in granulosa cells (GC) and thecal cells (TC) or large follicles (≥ 8 mm) categorized as either estrogen-active (EA) or estrogen-inactive (EI) after 48 or 96 hr of either LHRH (L) or saline (S) injections in suckled beef cows.

<table>
<thead>
<tr>
<th>Follicle group</th>
<th>n&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Average diameter (mm)</th>
<th>FF estradiol (nmol FF/ml)</th>
<th>GC binding of 125I-hCG (cpm/μg DNA)</th>
<th>TC binding of 125I-oFSH (cpm/μg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48S-EI</td>
<td>6</td>
<td>8.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>829&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,183&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
<tr>
<td>48S-EA</td>
<td>6</td>
<td>10.9&lt;sup&gt;de&lt;/sup&gt;</td>
<td>146&lt;sup&gt;d&lt;/sup&gt;</td>
<td>582&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,928&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
<tr>
<td>48L-EI</td>
<td>11</td>
<td>9.8&lt;sup&gt;de&lt;/sup&gt;</td>
<td>12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,550&lt;sup&gt;d&lt;/sup&gt;</td>
<td>696&lt;sup&gt;ae&lt;/sup&gt;</td>
</tr>
<tr>
<td>48L-EA</td>
<td>6</td>
<td>11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>157&lt;sup&gt;d&lt;/sup&gt;</td>
<td>802&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,120&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
<tr>
<td>96S-EI</td>
<td>10</td>
<td>9.8&lt;sup&gt;de&lt;/sup&gt;</td>
<td>9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>594&lt;sup&gt;c&lt;/sup&gt;</td>
<td>570&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>96L-EI</td>
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<td>1,965&lt;sup&gt;d&lt;/sup&gt;</td>
<td>583&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>96L-EA</td>
<td>7</td>
<td>12.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>208&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,881&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,008&lt;sup&gt;ce&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>EA = Estradiol concentration > progesterone concentration in FF; EI = progesterone concentrations > estradiol concentration in FF.

<sup>b</sup>Number of follicles.

<sup>c</sup>Means that do not have a common superscript within a column differ (P < .05). Only one follicle was EA in 96 hr saline-injected group and was deleted from the table.

Androgens. Studies reporting androgens in peripheral blood during the postpartum anestrous period were not identified.

Progestrone. Concentrations of progesterone in serum are low (<1.0 ng/ml) at parturition due to the preparturient regression of the corpus luteum of pregnancy and cessation of steroidogenesis by the placenta. Concentrations of progesterone remain low in cows until initiation of estrous cycles. In 40 to 70% of cows examined, a small progesterone peak (<2 ng/ml) occurs 1 to 6 days before the first postpartum estrus. This increase in concentration of progesterone in peripheral blood, which precedes the first postpartum estrus, may result from formation of a transitory corpus luteum or luteinization of some follicles. However, these structures are unable to maintain normal luteal phase progestosterone secretion. The cause of the shortened life span of these corpora lutea (or luteinized follicles) is unknown, but may involve excess prostaglandin production by the uterus or insufficient numbers of follicular receptors for LH.

Follicular Fluid Steroids

Estrogens. Recent studies have reported that concentrations of estradiol in fluid of large follicles (≥ 8 mm diameter) increase significantly between the second and fourth wk postpartum in suckled beef cows. Ovulation did not occur until the sixth wk postpartum in this same study. Thus, it appears that the ability of large follicles to produce estrogens increases about 2 wk before the first postpartum ovulation. These studies also suggest that functional capabilities of ovaries may be limiting reestablishment of estrous cycles during the first 2 to 4 wk postpartum in beef cattle. However, there is no change in the steroidogenic capacity (as measured by concentrations of steroids in follicular fluid) of smaller follicles (1-7.9 mm diameter) during postpartum anestrus. Collectively, studies suggest that circumstances shortly after parturition may allow follicles to develop to a large size but not the development of enzyme systems required for adequate production of estrogen. Recent evidence also indicates that the level of nutrition during the postpartum period may influence follicular estradiol production.

Androgens. In suckled beef cows, concentrations of androstenedione are 2- to 10-fold greater in fluid of medium-sized follicles (4-7.9 mm diameter) than in large-sized follicles (≥ 8 mm diameter) and do not change significantly during the postpartum interval. This suggests that low follicular estradiol secretion may not be due to a lack of aromatizable precursor (androstenedione).

Progestins. Recent studies have reported that concentrations of progesterone in fluid of large follicles (≥ 8 mm in diameter) increase between the first and second wk postpartum in suckled beef cows. This indicates that increased progesterone production preceded estradiol production by large follicles (Fig. 2).
Binding of Gonadotropins to Ovarian Follicles

The concept that the responsiveness of follicles depends not only on changes in concentrations of gonadotropins in serum but also on changes in the concentration of hormone binding sites (or receptors) in cellular membranes of follicles has gained considerable attention during the past few years. Changes in follicular function may be associated with changes in numbers of follicular gonadotropin binding sites.

Numbers of LH receptors in pooled follicular homogenates are significantly higher on day 25 after parturition in nonsuckled vs suckled cows. Since nonsuckled cows were approaching first estrus, this may indicate that receptors for LH increase in follicles prior to the first postpartum ovulation. This increase in numbers of LH receptors also is observed near ovulation in cyclic heifers. Number of follicular FSH receptors did not differ between nonsuckled and suckled cows. Similarly, there is no change in number of FSH receptors during the estrous cycle of cattle. Recently, we have found that in anestrous beef cows injected every 2 hr with LHRH (500 ng), the largest follicle per pair of ovaries responds within 96 hr with an increase in number of LH binding sites for both thecal and granulosa cells with no change in number of FSH binding sites (Table 1). This increase in LH binding sites coincided with an increase in concentrations of estradiol in follicular fluid at 96 hr.

Changes in numbers of ovarian LH or FSH receptors were not associated with increased estradiol production in large follicles during the postpartum anovulatory period in suckled beef cattle. This suggests that reduced ovarian function in postpartum cattle is not due to a lack of receptors for gonadotropin hormones.

Conclusions

The mechanism for selection of the follicle(s) destined to ovulate at estrus in cyclic cows or of the follicle(s) destined to ovulate and reinitiate estrous cycles in postpartum anestrous cows is unclear. It is clear, however, that just the presence of a large (≥10mm) follicle on the ovarian surface of postpartum cattle is not a sign of imminent ovulation. Rising titers of estradiol in peripheral blood during the preovulatory periods may be due to increased production of estradiol by large follicles which, in turn, may be due to an increased frequency of pulses of LH in blood and an increased responsiveness to LH within the follicle (i.e., increase in LH receptors). Specific proteins (e.g., inhibin, FSH-binding inhibitor, aromatase inhibitor; insulin-like growth factors) produced by the "selected" preovulatory follicle may aid in its assurance to ovulate.

One cannot rule out potential interactions these intrafollicular peptides have with steroids on ovarian follicles and the anterior pituitary gland. Substances that regulate ovarian blood flow also may be involved in follicular selection, also.

Our current and future research goals are to characterize and define morphological and biochemical markers that could be used to predict ovulatable follicles in cyclic and postpartum cattle. We hope that such markers could be used in such a way as to enhance the success rates of superovulation and ovulation after estrous synchronization treatments.