ANOVULATION IN POSTPARTUM SUCKLED BEEF COWS.  
I. ASSOCIATIONS AMONG SIZE AND NUMBERS  
OF OVARIAN FOLLICLES, UTERINE INVOLUTION, AND HORMONES  
IN SERUM AND FOLLICULAR FLUID1,2  

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ABSTRACT

Changes in sizes and numbers of ovarian antral follicles, uterine size and weight, serum hormones, and frequency and duration of suckling were examined during the postpartum anovulatory period in primiparous, suckled beef cows. Twenty-one anovulatory, suckled cows (n=4 to 6/d) were slaughtered on d 7, 14, 28 and 42 to 56 after parturition. In addition, a total of 11 postpartum cows that had begun cyclic activity were slaughtered on d 28, 42 or 56. Blood was collected at 10-min intervals for 6 h 1 d before slaughter for measurement of prolactin, cortisol and progesterone in serum. Numbers of medium (4.0 to 7.9 mm) follicles increased fourfold (P<0.05) between d 7 and 42 to 56 in anovulatory cows, whereas numbers of small (1.0 to 3.9 mm) and large (>8.0 mm) follicles did not change (P>0.10). Uterine involution was complete by d 28. In anovulatory cows, a higher (P<0.05) proportion of largest (but not second-largest) follicles was opposite the ovary containing the corpus albicans from pregnancy (CAP). In addition, 90% of these largest follicles opposite the CAP had concentrations of estradiol greater than progesterone. In cyclic cows, however, first ovulations occurred with equal frequency on either ovary. Concentrations of prolactin or cortisol in serum or duration of suckling were not associated with changes in uterine or ovarian measurements.

In conclusion, growth and function of the largest (but not second-largest) follicle were reduced when located on the ovary containing the CAP. We suggest that increased numbers of medium-sized follicles may provide a pool from which ovulatory follicles are selected, as one of the necessary steps leading to first postpartum ovulation.

(Key Words: Reproductive Disorders, Follicles, Uterus, Estradiol, Progesterone, Beef Cattle.)

Introduction

Cows with delayed uterine and cervical involution after parturition prolong the interval to first conception (Kiracofe, 1980; Oltenacu et al., 1983). Cows with suckling calves also have longer intervals to first ovulation after parturition than nonsuckled cows (Edgerton, 1980). However, the hormones involved in delay of ovulatory cycles associated with uterine involution or suckling are unknown. Although prolactin and cortisol are released with suckling, only concentrations of lutelizing hormone consistently increase with time postpartum (Wettemann, 1980). No study has temporally associated timing of uterine involution or changes in suckling behavior with serum hormones or changes in numbers and size of antral follicles during postpartum anestrus in suckled beef cows. In addition, during early postpartum stages there appears to be a block to ovulation on the ovary containing the corpus albicans of pregnancy (CAP; Saiduddin et al.,

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1967; Foote and Peterson, 1968; Marion and Gier, 1968). However, associations between location of CAP and follicular fluid steroids has not been investigated. Thus, the present study was undertaken to characterize the temporal relationship between serum hormones, follicular growth, uterine involution and duration of suckling in postpartum beef cows. We also investigated the relationship among concentrations of steroids in fluid of ovarian antral follicles, size and numbers of follicles and location of CAP.

Materials and Methods

**Animals and Design.** Thirty-two primiparous beef cows (primarily Hereford × Angus) were assigned into five groups (six or eight cows/group) to be slaughtered between July 18 and September 10, on either d 7, 14, 28, 42 or 56 after parturition. Each cow was suckled ad libitum by one calf until time of slaughter. Mean birth weight of calves (26.7 ± 1.4 kg) and mean gestation lengths (278 ± 1 d) were similar among groups (P>0.10). All cows were “halter broken” daily for at least 1 wk before slaughter, and cows were acclimated for approximately 2 h daily to a separate pen at least 4 d before collection of blood. Until day of blood collection, cows were housed at Michigan State University in a barn with one open side and fed 10 kg dry matter of a supplemented 84:16 corn silage:high-moisture corn diet (69% total digestible nutrients and 11% protein).

**Serum Collection and Assays.** Two days before slaughter, each animal was fitted with a cannula in a jugular vein. Between 0800 and 1400 h 1 d later, blood (10 ml) was collected at 10-min intervals for determination of concentrations of prolactin (Koprowski and Tucker, 1973). Progesterone was measured in blood samples collected at 0800 and 1400 h (Convey et al., 1977), and cortisol was quantified in samples collected at 0800, 1000, 1200 and 1400 h (Purchas et al., 1985). During blood collection, cows were restrained loosely with halters, and calves were present with cows. Each suckling event was recorded during this 6-h period. A wall with a one-way mirror separated the cow and calf from the person collecting the blood. Starting 21 d after parturition, additional blood samples were collected via jugular venipuncture every 3 d until time of slaughter. These samples were assayed for progesterone to estimate time of first ovulation which was determined by the first sample with concentrations of progesterone >2 ng/ml. Visual checks for estrus were not conducted on these cows.

After collection, blood was stored at 4 C for 2 to 4 h, incubated at 37 C for 2 h and stored at 4 C for 20 to 24 h. Then, blood was centrifuged at 1500 × g at 4 C for 15 min; serum was decanted and stored frozen at −20 C until hormone measurements were made.

**Uterine and Ovarian Collection Procedures.** Within 30 min of slaughter (0700 to 0900 h), ovaries and uteri were removed, weighed and placed on ice. In addition, external uterine horn (medial) and cervical diameters were recorded. Since all cows were primipara, previously gravid uterine horns were easily discerned and identified.

The numbers and sizes of all follicles 1 mm or greater in diameter on the surface of each ovary were recorded. The two largest follicles from each pair of ovaries were dissected free of ovarian stromal tissue; diameters were determined with a vernier caliper and follicular fluid collected. Follicular fluid was stored at −20 C until concentrations of progesterone and estradiol were estimated by radioimmunoassays described previously (Ireland and Roche, 1982). All follicles measured on the surface of each pair of ovaries were classified into three sizes: small (1.0 to 3.9 mm), medium (4.0 to 7.9 mm) and large (>8.0 mm). Similar classifications have been used previously to inventory follicles during bovine estrous cycles (Kruip et al., 1979; McNatty et al., 1984). Large follicles were separated at >8 mm because follicles that are destined to ovulate average 10 ± 2 mm (Marion et al., 1968).

**Statistical Analyses.** Pulsatile pattern of prolactin (PRL) secretion was analyzed as baseline, amplitude, frequency and overall mean concentrations as described by Leung et al. (1986). Only data of serum PRL obtained during the last 4 h of the 6-h bleeding period were used to study episodic release of PRL because baseline concentrations are exaggerated during early phases of blood collection in cattle (Tucker, 1971).

One-way analysis of variance, with “days after parturition” as the main effect, was used to examine changes in uterine measurements, suckling duration and frequency, serum hormones, CAP weights and follicular size and numbers. Data from cyclic cows were removed before analyses, and data from anovulatory
cows on d 42 and 56 were combined to form a group identified as d 42 to 56 (n=4). Differences among means were determined using Fisher's protected LSD mean test (Ott, 1977). Mean comparisons between anovulatory and cyclic cows of various measurements and between follicular fluid steroids in various size follicles on CAP versus non-CAP sides were conducted by using Bonferroni's t-test (Gill, 1978). Data with heterogeneous variance were analyzed after transformation to natural log (x + 1) (Gill, 1978). Data on proportions of follicles and ovulations on CAP vs non-CAP ovary were analyzed by chi-square.

Results

Ovarian and Uterine Measurements. No cows initiated estrous cycles (as determined by presence of a newly formed corpus luteum or concentrations of progesterone in serum >2 ng/ml) by d 14 after parturition. However, one of six, five of eight and five of six cows had initiated estrous cycles by d 28, 42 and 56, respectively. If weight of CAP was subtracted from total ovarian weight of anovulatory cows, there was no change (P>.10) in non-luteal ovarian weight between d 7 and 42 to 56 after parturition (data not shown).

In anovulatory cows, declines in uterine horn diameter and uterine weight occurred between d 7 and 28 (P<.01; table 1). Coincident with declines in uterine size were declines (P<.01) in weight of regressing CAP and diameter of the cervix (table 1). Diameters of previously pregnant and nonpregnant uterine horns, total uterine weight and CAP weight did not change after d 28 or differ between anovulatory and cyclic cows (P>.10; table 1). Diameters of pregnant and nonpregnant uterine horns were correlated with uterine weight (r=.94 and .90, respectively; P<.01).

Follicular Size, Numbers and Concentrations of Estradiol and Progesterone. Numbers of small and large follicles in anovulatory cows did not change from d 7 to 42 to 56 (P>.05; figure 1). However, numbers of medium follicles increased (P<.05) about twofold between d 7 and 14, and again between d 28 and 42 to 56 (figure 1). Numbers of medium follicles per pair of ovaries were negatively correlated (P<.05) with diameters of previously pregnant (r=-.58) and nonpregnant (r=-.59) uterine horns, and uterine weight (r=-.54).

Diameters of the largest (10.8 ± 3.3 mm) and second-largest (8.0 ± 3.5 mm) follicles were unchanged (P>.10) between d 7 and 42 to 56 in anovulatory cows. However, diameter of largest

<table>
<thead>
<tr>
<th>Days after</th>
<th>Weight</th>
<th>Uterine horn</th>
<th>NP uterine horn</th>
<th>Cervix</th>
<th>Suckling events</th>
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<td>No.</td>
<td>CAP, kg</td>
<td>P</td>
<td>NP</td>
<td>frequency, no./6 h</td>
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<td>7</td>
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<td>14.0b</td>
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<tr>
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<td>6</td>
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<td>3.8c</td>
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<td>5</td>
<td>.34c</td>
<td>3.9d</td>
<td>2.5c</td>
<td>1.7</td>
</tr>
<tr>
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<tr>
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<td>.32c</td>
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aN = number of cows.

b, c, d, Means with different superscripts differ (P<.05).

C = cyclic cows.

SE = pooled standard error of means.
follicles located on the ovary opposite CAP were 1.2-fold greater (P<.05) than diameters of largest follicles on the ipsilateral ovary (table 2). Moreover, a higher proportion of largest follicles (81%) were opposite the ovary containing the previous CAP (P<.05). Location of CAP did not affect (P>.10) size of second-largest follicles, and the second-largest follicle occurred with equal frequency (48 vs 52%) on either ovary (P>.10). Numbers of small and medium follicles on the CAP vs non-CAP ovaries did not differ (P>.10). Size of small follicles did not differ from CAP vs non-CAP ovaries (2.8 ± 1 mm vs 2.9 ± 1 mm), whereas medium follicles were 9% larger (P<.05) on non-CAP vs CAP ovaries (table 2).

Although average concentrations of progesterone and estradiol in fluid of largest and second-largest follicles were not significantly different between contralateral and ipsilateral ovaries in anovulatory cows, the percentage of largest follicles with concentrations of estradiol greater than progesterone were twofold greater on the ovary opposite CAP than on the ipsilateral ovary (table 2). Thus, 90% of all largest follicles with concentrations of estradiol greater than progesterone were present on the ovary opposite CAP (P<.05). Concentrations of progesterone and estradiol in medium follicles were not different between contralateral and ipsilateral ovaries (table 2).

In 5 of 11 cyclic cows, the first postpartum ovulation occurred on the ovary opposite the ovary with CAP, which was not different from the expected proportion of 50% ovulations from right and 50% from left ovaries (P>.10).
Suckling Events. Mean frequency (number/6 h) and duration of suckling events did not change between d 7 and 42 to 56, or differ between anovulatory and cyclic cows (P > .10; table 1). Time spent suckling was 3.3 min/h.

Serum Progesterone. Progesterone averaged .3 to .5 ng/ml of serum from d 7 to 42 to 56 in anovulatory cows with the exception that in two of four anovulatory cows slaughtered on d 42 to 56, transitory rises to 1.7 ± 3 ng/ml occurred 7 ± 1 d prior to slaughter (data not shown). Only luteal tissue from the previous pregnancy was found in ovarian tissue at time of slaughter of anovulatory cows.

Four of 11 cyclic cows showed small transitory rises to 2.3 ± 3 ng/ml 6 ± 1 d before the next increase in progesterone. The remaining seven cyclic cows were in early phases of their first estrous cycle at time of slaughter, and thus we were unable to assess whether small transient peaks of progesterone occurred.

Two of 11 cyclic cows had newly formed CL with progesterone levels in serum <2 ng/ml and 2 of 11 cyclic cows had newly formed CL with progesterone levels in serum <1 ng/ml at time of slaughter. As determined by the first sample with concentrations of progesterone in serum >2 ng/ml, the remaining seven cyclic cows first ovulated 41.3 ± 1.3 d after parturition.

Serum Cortisol. Cortisol in serum averaged 5.8 to 10.5 ng/ml in anovulatory cows and did not change (P > .10) between d 7 and 42 to 56 or differ between anovulatory and cyclic cows (data not shown).

Prolactin. Mean baseline (18.2 ± 2.1 ng/ml), amplitude (22.8 ± 5.7 ng/ml) and frequency (.3 ± 1 pulses/h) of secretory pulses of PRL in serum of anovulatory cows did not change (P > .10) between d 7 and 42 to 56. However, overall mean concentration of PRL tended (P < .10) to be lower on d 42 to 56 (16.7 ± 1.4 ng/ml) than on d 7, 14 and 28 (23.0 ± 3.0 ng/ml). Cows slaughtered on d 7, 14 and 28 were bled on average during late July, as compared with cows in the d 42 to 56 group which were bled during shorter daylengths and cooler temperatures of late August. Changes in concentrations of PRL in fluid of the two largest follicles were similar to those of overall concentrations of PRL in serum (data not shown).

Discussion

Changes in numbers of various sized follicles with time after parturition have not been well characterized for anovulatory cattle. In the present study, a fourfold increase in numbers of medium follicles was observed between d 7 and 42 to 56 after parturition. The increase in numbers of medium follicles must be due to an increase in growth of smaller follicles into this larger size category, since numbers of small follicles did not decline. Thus, growth of follicles less than 1 mm in diameter must also increase to maintain numbers of small follicles. Perhaps the increase in numbers of medium follicles provides an increased pool of antral follicles from which ovulatory follicles can be selected. Whether this larger pool of follicles is necessary for reinitiation of ovulatory cycles after parturition in cattle is unknown. Stockpiling of medium follicles has been suggested to be an important factor in folliculogenesis in rodents (Faddy et al., 1983). Factors regulating growth of medium follicles are also unknown. Perhaps increased luteinizing hormone (LH) in serum plays a role, since overall concentrations of LH, but not follicle stimulating hormone, increased significantly from .9 ng/ml on d 7 to 1.2 ng/ml on d 14 in these same cows (Leung et al., 1986). In addition, declining uterine size was associated with increased numbers of medium follicles, thus some factor related to uterine regression may directly or indirectly be involved (Schirar and Martinet, 1982). Indeed, recent preliminary evidence suggests that removal of the uterus between d 11 and 15 after parturition increases LH secretion in dairy cows (Schallenberger et al., 1984).

In studies utilizing dairy cows, which ovulate 3 to 4 wk earlier than beef cows, it was found that follicles 5 mm and greater appear between d 7 and 14 after parturition (Saiduddin et al., 1968; Wagner and Hansel, 1969; Kesler et al., 1979). In the present study, follicles 8 mm and greater were present on ovaries by d 7 after parturition, with no subsequent change in average size of these follicles. Similarly, Moss et al. (1985) observed that two or more follicles approximately 6 mm in diameter were present by d 5 postpartum in mature beef cows. Thus, results of these studies indicate that appearance of large follicles occurs long before the first postpartum ovulation in cattle.

Diameters of largest follicles in the present study are similar to largest follicles in anovulatory, milked dairy cows on d 7 and 14 after parturition (Wagner and Hansel, 1969). However, these diameters are 2 to 8 mm smaller than diameters of the largest follicles removed.
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from ovaries of cattle during estrus (Donaldson and Hansel, 1968; Marion et al., 1968; Merz et al., 1981; Stagmiller et al., 1982). Factors involved in inhibition of final preovulatory growth of follicles during postpartum anestrus are unknown.

In dairy cattle during the first 20 d postpartum there is an apparent block to ovulation on the ovary containing the CAP (Saiduddin et al., 1967; Foote and Peterson, 1968; Marion and Gier, 1968; Morrow et al., 1968). In the present study, location of CAP (or side of gravid uterine horn) inhibited growth of the largest follicles on the ipsilateral ovary between d 7 and 42 to 56. In addition, 90% of largest follicles that had concentrations of estradiol greater than progesterone were opposite the ovary containing the CAP. This observation is supported by the study of Bellin et al. (1984), who found that on d 5 after parturition in beef cows, 48% of the follicles (>3 mm) on the non-CAP ovary were classified as healthy compared with 18% on the CAP ovary. In contrast, ovaries bearing the CAP and contralateral ovaries contained similar numbers of atretic and non-atretic follicles with diameters of 3.7 mm and greater when collected from dairy cows between d 15 and 35 after parturition (Dufour and Roy, 1985). However, we observed that diameter was 9% smaller for medium follicles located on the CAP vs non-CAP ovary. Similarly, CAP ovaries had follicles that were 10% smaller than non-CAP ovaries on d 5 after parturition (Bellin et al., 1984). Collectively, these data suggest that follicular growth and function on the ovary containing the CAP are reduced during the early postpartum period. The cause of this association between the CAP and follicular function is unknown, but may involve a difference in blood flow across the CAP vs non-CAP ovary, or a difference in secretion(s) from nonpregnant vs previously pregnant uterine horns.

Results of the present study also indicate that the inhibitory effect of the previous pregnancy on follicular growth and function can be present in both early and late postpartum stages, at least in cattle that remain anovulatory. However, in the present study of the 11 cows that ovulated by d 28 or after, there was no effect of the CAP on the location of the new ovulation. Similarly, if ovulation occurs after d 20, less than 60% of the newly formed corpora lutea were opposite the CAP (Saiduddin et al., 1967; Foote and Peterson, 1968; Marion and Gier, 1968; Morrow et al., 1968). These data suggest that inhibitory effects of the CAP are negligible after 4 wk postpartum.

Duration and frequency of suckling events did not change between d 7 and 42 to 56 after parturition. Average time spent suckling in the present study was 3.3 min/h, similar to durations of 2.5 to 5.0 min/h reported by Gimenex et al. (1980). Thus, in contrast to rats (Taya and Greenwald, 1982; Selmanoff and Selmanoff, 1983; Sodersten and Eneroth, 1984) declining frequency and duration of suckling per se does not appear to be associated with spontaneous return to postpartum ovulatory cycles in suckled beef cattle. However, evidence supports the hypothesis that the suckling stimulus becomes a less potent inhibitor of LH secretion as time after parturition progresses (Garcia-Winder et al., 1984). This may explain why basal LH increases with time postpartum (Wettmann, 1980; Leung et al., 1986).

Hormones released in association with suckling, such as glucocorticoids or PRL, are potential inhibitors of postpartum ovulations in cattle (Wettmann, 1980). However, no significant changes in concentrations of cortisol in serum from parturition to first ovulation in beef cows were observed in previous studies (Dunlap et al., 1981; Convey et al., 1983; Humphrey et al., 1983), or in the present study. Average concentrations of PRL in serum do not change with time after parturition (Humphrey et al., 1983) or affect the postpartum anestrous interval in cattle (Williams and Ray, 1980; Montgomery, 1982). Moreover, suckling-induced PRL release increases between d 14 to 42 after parturition in beef cattle (Convey et al., 1983), suggesting that increased PRL due to suckling does not inhibit return to postpartum estrous cyclicity, as hypothesized in rats (Selmanoff and Selmanoff, 1983). In the present study, no change in concentrations of PRL in serum was observed between d 7 and 28, a time in which dramatic shifts in steroid production of large follicles were observed (Spicer et al., 1984). Collectively, the data suggest that concentrations of cortisol and PRL in serum per se are not associated with reestablishment of postpartum estrous cycles in cattle. As described by Leung et al. (1986), using most of the same cows as in the present study, increased secretion of LH is the hormonal change most closely associated with the reestablishment of estrous cycles. Perhaps increased numbers of medium follicles and/or increased
numbers of large follicles with high concentrations of estradiol positively influence secretion of LH to promote further folliculogenesis. Support for this suggestion is found when comparing the number of LH pulses in cows with vs cows without estrogen-active follicles. Cows with estrogen-active follicles (n=12, 1.8 ± 0.3 pulses/6 h) had twice the number of LH pulses as cows without estrogen-active follicles (n=9, 0.9 ± 0.3 pulses/6 h), although the number of medium follicles per cow was similar (8.7 ± 1.4 vs 9.4 ± 2.2, respectively). Overall mean LH in cows with estrogen-active follicles was 0.98 ± 0.11 ng/ml and 1.30 ± 0.11 ng/ml in cows without estrogen-active follicles (data rearranged from Leung et al., 1986).

Results of the present study show that significant increases in numbers of medium follicles occur during the postpartum anovulatory period. In addition, growth and function of the largest follicle is reduced when located on the ovary containing the CAP. Additional research is needed to determine factors involved in such alterations in follicular fluid steroids and(or) changes in follicular numbers, and whether such alterations in ovarian function affect timing of the resumption of postpartum ovulatory cycles.

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