CORPUS LUTEUM FUNCTION IN THE BOVINE: IN VIVO AND IN VITRO EVIDENCE FOR BOTH A SEASONAL AND BREEDTYPE EFFECT

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Summary

Breedtype and season appear to modify the reproductive capacity of cattle. This study was designed to elucidate the effect of these factors on the bovine corpus luteum. Six Brahman (B) and six Hereford × Holstein (H) heifers were bled via coccygeal vessel puncture on d 2, 4, 6, 8, 10, 12 and 13 postestrus within 20 d of both the summer and winter solstices. On d 13 postestrus, the corpus luteum (CL) was surgically removed from each heifer following blood sampling. Serum progesterone, total progesterone content of each CL and in vitro progesterone (P₄) production of dispersed luteal cells was determined. Serum progesterone on d 2 through 13 did not vary with breedtype or season. However, the CL weights differed (P<.05) between breeds (H heavier than B), and there was a tendency for CL weight to be greater during the summer than winter among both B and H heifers. CL concentrations of P₄ (µg/g) also differed between B and H. Concentrations of P₄ in B were greater (P<.10) during winter than during summer, but H concentrations of P₄ did not differ between seasons. Total P₄ content was greater (P<.01) in the winter than in summer and in H than in B. Luteal cell viability did not vary with breedtype, season or LH dosage. Breedtype (P<.001) and season (P<.001) influenced the capacity of incubated luteal tissue to release progesterone in response to luteinizing hormone (LH). Luteal tissue from H released a greater amount of progesterone than incubated B tissue. Further, the release of progesterone by winter CL tissue in the LH-challenged cultures was diminished. The minimal LH dosage required to elevate P₄ above concentrations observed at 0 ng LH did not differ between breeds or seasons (5 ng LH). However, the amount of P₄ released at 5 ng LH dosage was highest for tissue from H during the winter, followed by H during the summer, B during the summer and B during winter. In contrast, maximal release of P₄ was associated with the 500 ng LH dosage for H (summer and winter), the 100 ng LH dosage for B during summer and the 50 ng LH dosage for B during winter. At LH dosages above 10 ng, P₄ concentrations were greater in H than B, with summer greater than winter values in both breedtypes. The greatest change in P₄ release (highest P₄ concentrations minus P₄ concentration at 0 ng LH) followed the same trends as above. Significant (P<.001) interaction terms were also detected for breed × season and breed × dose. These data indicate that luteal tissue from H had a greater capacity to release P₄ in vitro than luteal tissue from B. Further, CL removed during the winter had a lessened capability to release P₄ than CL removed during the summer.

(Key Words: Brahman, Corpus Luteum, Bovine, In Vitro, Progesterone, Season.)

Introduction

Beef cattle production in temperate regions such as the southwestern United States is being increasingly influenced by the assimilation of Zebu and Zebu-cross cattle. Although the positive influence of the Zebu genotype on southern beef production operations is well documented (Rhoad, 1955; Cunha et al., 1963; Fowler, 1969; Koger et al., 1973), the greatest single negative factor of Bos indicus is relatively low fertility (Kincaid, 1957; 159
Comparisons of Bos taurus and Bos indicus cattle have shown that the reproductive endocrinology of these two species differs substantially (Clamohoy, 1952; Rollinson, 1955; Plasse et al., 1969; Randel, 1976; Griffin and Randel, 1973; Irvin et al., 1978; Rhodes and Randel, 1978; Rhodes et al., 1978, 1979). Differences in the endocrine events between breedtypes may account for the disparity in fertility between species, particularly when considered in relation to prevailing nutritional and management conditions.

Photoperiodic variation has been long recognized as a major environmental cue synchronizing reproductive function with season in a variety of mammalian species. Among the domesticated bovine species, the Zebu cow exhibits the most distinct seasonality in reproductive capacity. Anderson (1944) reported that reproductive efficiency of Zebu cattle in Kenya paralleled the photoperiod associated with a specific season. Similarly, Tomar (1966) and Plasse et al. (1968) noted that the frequency of estrus and ovulation in Zebu cows was higher during the summer than during the winter. Jochle (1972) observed an increase in conception rates among Zebu cattle during the summer when compared to the winter months. Temperature might modify the seasonal effect of photoperiod on reproductive function. However, the length of daylight appears to be one primary stimulus for shifts in reproductive status due to season (Reiter, 1974; Peters and Tucker, 1978). In agreement with this supposition are demonstrations of enhanced conception rates in cattle herds that received 14 h light/d during a winter breeding season (Sweetman, 1950; Rhodes, 1980). The ability to exploit this physiological phenomenon by application of artificial light during short day lengths would be of economic importance to cattle producers.

Seasonal alteration in luteal function might be one way in which season affects fertility in cattle. An intact corpus luteum (CL) secreting "adequate" progesterone appears to be required for embryo survival (Staples and Hansel, 1961) and pregnancy maintenance (McDonald et al., 1953; Estergreen et al., 1967). Because the data concerning seasonal gonadotropin release in cattle are conflicting and the degree to which season modulates steroidogenesis through a direct neural input unknown, we hypothesized that changes in either of these factors might result in alteration of the functional status of the CL.

The objective of the present study was to investigate the effect of breedtype (Brahman vs Hereford × Holstein) and season (summer vs winter) on (1) serum progesterone concentration on d 2 through 13 postestrus; (2) CL mass; (3) CL progesterone concentration and (4) the in vitro capability of the CL to release progesterone following gonadotropin stimulation.

Materials and Methods

Six Brahman (B) and six Hereford × Holstein (H) 2-yr-old heifers, after exhibiting normal reproductive cyclicity, were bled via coccygeal vessel puncture on d 2, 4, 6, 8, 10, 12 and 13 postestrus within 20 d before both the winter and the summer solstices. The blood was allowed to clot at room temperature for 1.5 h and centrifuged at 4 C. The serum was aspirated, frozen and stored at -20 C until hormone analyses. On d 13 postestrus, all heifers had blood samples collected and CL were removed via mid flank laparotomy. Immediately after surgical removal, the CL was blotted, weighed, halved, and each half was weighed. One-half was snap frozen and stored at -20 C until preparation. The remaining one-half of the CL was placed in cold Nutrient Mixture F-10 (Ham's) and sliced into .5-mm sections with a microtome. The first and last slices of luteal tissue were discarded in order to minimize the amount of capsular material placed in culture. The luteal slices were transferred to a spinning flask containing 10 ml of medium (Ham's) warmed to 32 C. Luteal tissue was dispersed enzymatically by a method similar to that of Simmons et al. (1976).

Two thousand international units of collagenase (Type 1) were added to the swirling medium and incubated for 10 min. Afterward, the medium was exchanged and 500 IU of collagenase were added to the partially digested luteal suspension. The suspension was stirred continuously for 40 min at 32 C. At the termination of centrifugation at 100 x g at room

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5Grand Island Biologicals, Grand Island, NY.
7Sigma Chemical Co., St. Louis, MO.
temperature, the supernatant was decanted and 10 ml of fresh media (32 C) were added. The procedure of medium exchange was repeated four times. After the final wash, dispersed cells and medium were filtered through cheesecloth premoistened with media. The concentration of the cells was then determined with a hemocytometer. No attempt was made to separate the luteal cell populations. One hundred thousand cells were aliquoted per tube and incubated as triplicates.

Luteinizing hormone (NIH-LH-B9) suspended in 1% egg white phosphate buffered saline (1% EW-PBS) was added in doses of 0, 1, 5, 10, 50, 100, 500 or 1,000 ng/tube. Dispersed cells challenged with LH were incubated for 1 h at 37 C in a shaking incubator. During the incubation, 100-µl aliquots of the stock cell suspension incubated with buffer or 1,000 ng LH were aspirated and cell viability was estimated by the trypan blue dye exclusion test (.2% trypan blue in .9% NaCl; Simmons et al., 1976). At the end of the incubation, tubes were placed in an ice bath for 5 min and centrifuged at 200 x g for 10 min at 0 C. A 200-µl aliquot of medium was removed, frozen and stored until steroid analysis.

Progesterone quantification for both serum and medium was accomplished by validated radioimmunoassay by the procedure described by Erb et al. (1976). Antibody RDR-9-P, which was obtained from sheep immunized against 4-pregnen-11α-ol-3,20-dione hemi-succinate:bovine serum albumin, was utilized. The antibody was diluted 1:1,000 and bound 30 to 40% of the 1,2-3H-progesterone with nonspecific binding less than 5%. Five hundred microliters of serum were extracted with glass-redistilled hexane, centrifuged and the aqueous phase discarded. The organic phase was evaporated to dryness under N2 and reconstituted with 100-µl of phosphate buffered saline. Tracer and antibody were then added and allowed to incubate (SC) for 4 h. Samples of medium (200-µl) were assayed directly as organically extracted samples of medium and nonextracted samples yielded similar values in a preliminary study. Separation of bound and free tracer was by charcoal absorption.

Sensitivity of the assay was such that 50 pg of progesterone could be distinguished from zero. Addition of .5, 1.0, 2.0 and 4.0 ng/ml progesterone to serum from an ovariectomized heifer was associated with recovery of .45, .97, 2.10 and 3.96 ng/ml, respectively. Inter- and intraassay coefficients of variation were 4.5 and 8.1%, respectively. Progesterone concentration of luteal tissue was determined by the method of Armstrong et al. (1964).

CL weights and progesterone concentrations were analyzed statistically by an analysis of variance for a completely randomized design with a 2 x 2 factorial arrangement of treatments. Progesterone concentration of serum and culture medium were analyzed by an analysis of variance for a split-plot design (Gill and Hafs, 1971).

Results and Discussion

Serum progesterone increased from d 2 through 13 postestrus, reflecting the functional status of the CL (figure 1). However, there were no detectable differences in serum progesterone concentrations due to breedtype or season (table 1). The absence of a breedtype effect on serum progesterone is similar to the observation of Irvin (1977), who noted no differences in concentrations of serum progesterone between B and H cows on d 4, 8 and 13 of the estrous cycle. Similarly, Randel and Moseley (1977) observed no differences in serum progesterone between B and Hereford heifers 2 d prior to estrus through 1 d after estrus. The lack of a seasonal effect paralleled observations of Harrison et al. (1981), who reported no differences in concentrations of serum progesterone between B and H cows on d 4, 8 and 13 of the estrous cycle. Similarly, Randel and Moseley (1977) observed no differences in serum progesterone between B and Hereford heifers 2 d prior to estrus through 1 d after estrus. The lack of a seasonal effect paralleled observations of Harrison et al. (1981), who reported no differences in concentrations of serum progesterone between B and H cows.

Qualitatively, the B CL were more deeply imbedded in the ovarian stroma and had less distinct papilla than CL of the H heifers. This finding is supportive of past observations that the CL of the B was difficult to detect via rectal palpation (Plasse et al., 1968; Irvin et al., 1978). CL weight was greater (P<.001) in H heifers than in B heifers (table 2). There was also a tendency for CL weight for both breeds to be greater during the winter than summer. Progesterone concentrations (µg/g CL) differed (P<.10) between breedtypes (table 2). The H CL had a greater concentration of P4 than the B CL. A seasonal effect on CL progesterone concentration was ob-
TABLE 1. ANALYSIS OF VARIANCE OF SERUM AND IN VITRO PROGESTERONE CONCENTRATIONS

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Analysis I-serum</th>
<th>Analysis II-in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df(^b)</td>
<td>MS(^c)</td>
</tr>
<tr>
<td>Breedtype</td>
<td>1</td>
<td>.3</td>
</tr>
<tr>
<td>Cow/(breedtype)</td>
<td>10</td>
<td>7.7</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Breedtype X season</td>
<td>1</td>
<td>.1</td>
</tr>
<tr>
<td>Cow X season</td>
<td>10</td>
<td>2.1</td>
</tr>
<tr>
<td>D(^a)</td>
<td>6</td>
<td>45.5</td>
</tr>
<tr>
<td>Breedtype X D</td>
<td>6</td>
<td>.6</td>
</tr>
<tr>
<td>Season X D</td>
<td>6</td>
<td>.6</td>
</tr>
<tr>
<td>Breedtype X season X D</td>
<td>6</td>
<td>.6</td>
</tr>
</tbody>
</table>

\(^{a}\)In analysis I, D represents day of the estrous cycle (i.e., d 2, 4, 6, 8, 10, 12 or 13). In analysis II, D represents LH dosage (i.e., 0, 1, 5, 10, 50, 100, 500 or 1,000 ng).

\(^{b}\)df = degrees of freedom.

\(^{c}\)MS = mean square.

\(^{d}\)NS = not significant (i.e., P>.10).
TABLE 2. SEASON X BREEDTYPE SUBCLASS MEANS FOR CORPUS LUTEUM (CL) WEIGHT (G), PROGESTERONE (P4) CONCENTRATION (µG/G TISSUE) AND PROGESTERONE CONTENT (µG/CL)

<table>
<thead>
<tr>
<th>Luteal measure (means ± SE)</th>
<th>Weight</th>
<th>P4 concentration</th>
<th>P4 content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B^a</td>
<td>H^b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.74 ± .10^c</td>
<td>4.58 ± .44^d</td>
<td>30.8 ± 2.8^e</td>
</tr>
<tr>
<td></td>
<td>3.01 ± .29^c</td>
<td>5.11 ± .49^d</td>
<td>52.6 ± 7.8^f</td>
</tr>
</tbody>
</table>

^aB = Brahman.
^bH = Hereford.
^c,d Means ± SE with different superscripts differ (P<.001).
^e,f,g Means ± SE with different superscripts differ (P<.10).
^h,i,j,k Means ± SE with different superscripts differ (P<.01).

and P4 content. Although luteal progesterone content increased from summer to winter in both breedtypes, this shift was not reflected in systemic serum progesterone concentrations.

In preparing the CL for culture, we noted that 1 g of luteal tissue yielded approximately 2.26 ± .20 × 10^7 cells in both B and H, which is comparable to that reported by Urseley and Leymarie (1979). Previous investigators have reported that the presence of functional luteal cells in culture is characterized by viable cells which have the capacity to release progesterone in response to challenges by either luteinizing hormone (LH) or human chorionic gonadotropin (Simmons et al., 1976). Similarly, in the present study, cultured luteal cells responded to LH in a dose-dependent fashion (figure 2), resulting in LH dosage having a significant effect (P<.001; table 1). Luteal cell viability did not differ between breedtype, season or LH dosage and averaged 72 ± 1.5%.

The minimal dose of 5 ng LH required to elevate concentrations of progesterone above concentrations at 0 ng LH was similar for each breedtype. However, in response to this dose of LH, the amount of progesterone released into the medium varied (P<.001) with both breedtype and season (figure 2; table 3). The winter value exceeded (P<.001) the summer value in the H, while the reverse was true for the B. A breedtype × season interaction was detected (P<.001; table 1). The minimal LH concentration (10^-8 M) required for a significant release of progesterone in the present study is in agreement with Gospodarowicz and Gospodarowicz (1972; 10^-7 M) and Simmons et al. (1976; 10^-8 M). Comparison of magnitude of luteal cell response between studies is difficult due to differences in methods of separation of luteal cell populations and in cell numbers utilized in culture.

Maximal progesterone release declined (P<.001) from summer to winter in B luteal cells. Maximal progesterone release from summer and winter B luteal cells occurred at 100 and 50 ng LH, respectively (table 3). Although no shift in the maximal stimulatory LH dosage was detected (i.e., 500 ng) from summer to winter in the H luteal suspension, a decrease (P<.001) in the P4 concentrations at this LH dosage was observed. The maximal P4 response was consistently higher (P<.001) in the H than in the B incubations and greater (P<.001) during the summer than during the winter. These data indicate that a more dramatic seasonal change in gonadotropin sensitivity occurred in B luteal cells than in that of H luteal cells.

The magnitude of P4 change (highest P4 concentration minus P4 concentration at 0 ng LH) was greatest (P<.05) for the H during the summer, followed by the H during the winter, B during the summer and the B during the winter (table 4). The areas under the LH-induced P4 curves had a tendency to be similar to that noted above (table 5). However, a significant seasonal effect on area under the curve was not observed in the H. The lack of seasonal effect might have been due to a seasonal difference in P4 concentrations at
Figure 2. Effect of season and breedtype on progesterone secretion by luteal tissue challenged with luteinizing hormone (LH) in vitro.

0 ng LH. Winter P₄ values were greater at 0 ng LH than summer P₄ values (figure 2), which could have increased the apparent response of the winter luteal cells.

As the P₄ values at 0 ng LH appeared to differ between season and breedtype (figure 2), it was deemed necessary to adjust the P₄ response curves by subtracting P₄ concentrations observed at 0 ng LH. The analysis of variance of the new data set varied slightly from the analysis of the original data set. Figure 3 represents the adjusted set of curves.

TABLE 3. LUTEINIZING HORMONE (LH) DOSAGE ASSOCIATED WITH MAXIMAL AND MINIMAL PROGESTERONE (P₄) STIMULATION IN VITRO BY SEASON AND BREEDTYPE

<table>
<thead>
<tr>
<th>Season</th>
<th>LH⁵</th>
<th>P₄ d</th>
<th>LH</th>
<th>P₄</th>
<th>LH</th>
<th>P₄</th>
<th>LH</th>
<th>P₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>5</td>
<td>34.5 ± 3.0</td>
<td>5</td>
<td>44.2 ± 5.1</td>
<td>100</td>
<td>66.1 ± 7.0</td>
<td>500</td>
<td>109.2 ± 5.8</td>
</tr>
<tr>
<td>Winter</td>
<td>5</td>
<td>21.5 ± 2.2</td>
<td>5</td>
<td>55.0 ± 5.5</td>
<td>50</td>
<td>40.1 ± 4.0</td>
<td>500</td>
<td>98.7 ± 10.6</td>
</tr>
</tbody>
</table>

aB = Brahman.
bH = Hereford × Holstein.
cLH expressed in ng/10⁶ cells.
dMeans ± SE.
Like the original data set, P₄ values at LH dosages above 10 ng were greater (P<.05) in H than B and summer P₄ values were greater (P<.05) than winter values. When the areas under the P₄ curves were recalculated, a seasonal response was observed in the H (table 5).

Adjusting for P₄ at 0 ng LH did partition some of the variation of P₄ response between seasons. Thus, both breedtype and season have an effect on the in vitro capacity of luteal cells to release progesterone.

Although size and P₄ content of the CL varied with breedtype and season, no differences due to season or breedtype were detected in systemic serum P₄ concentrations. However, when cell numbers were standardized to 10⁵ between breedtype and season (as in the in vitro incubation) and challenged with LH, the H had a greater capacity to release P₄ than the B during both the summer and winter. The manifestation of the seasonal effect (i.e., summer P₄ greater than winter P₄) was particularly evident at LH concentrations above 10 ng. Whether the responses at the higher LH dosages represents an artifact of a pharmacological dosage of LH remains to be determined.

The physiological basis for B having systemic P₄ concentrations similar to (in the face of less P₄ per CL in vivo and less capacity to release P₄ in vitro) those of the Bos taurus counterpart remains unclear. The metabolism of steroids between breedtypes could vary. Rhodes and Randel (1978) and Rhodes et al. (1978) reported striking differences between Bos indicus and Bos taurus with respect to behavioral estrus patterns in response to estradiol-17β. Further, a seasonal shift in steroid metabolic patterns (particularly in the B) may occur. Legan and Karsch (1979) have hypothesized that a shift in estradiol sensitivity may be the "organizer" of the seasonal breeding patterns of another domestic ruminant, the ewe.

Patterns of gonadotropin release differ significantly between Bos indicus and Bos taurus. B cows (both intact and ovariectomized - estrogen-challenged) have lower basal LH concentrations, lower magnitudes of peak LH during the preovulatory surge, and shorter preovulatory LH surge durations than Hereford cows, and also have different timing patterns of LH release (Randel and Moseley, 1977; Rhodes et al., 1978). Recently, Harrison and Randel (1981) reported that basal LH concentrations in B cows were lower during the winter than during the spring. Further, they reported that the peak magnitude of LH surge was lower in B cows during the winter than during the spring. Some of

### TABLE 4. CHANGE IN PROGESTERONE RESPONSE (NG) FROM BASAL TO THE HIGHEST CONCENTRATION REACHED AFTER LUTEINIZING HORMONE STIMULATION

<table>
<thead>
<tr>
<th>Breedtype</th>
<th>Summer P₄</th>
<th>Winter P₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brahman</td>
<td>43.9 ± 5.6a</td>
<td>26.8 ± 2.2b</td>
</tr>
<tr>
<td>Hereford X Holstein</td>
<td>82.7 ± 2.0c</td>
<td>73.3 ± 5.8d</td>
</tr>
</tbody>
</table>

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

### TABLE 5. AREA UNDER THE RAW a AND ADJUSTED b PROGESTERONE CURVES

<table>
<thead>
<tr>
<th>Season</th>
<th>Raw</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>H</td>
</tr>
<tr>
<td>Summer</td>
<td>315.8 ± 29.0c</td>
<td>458.2 ± 26.3g</td>
</tr>
<tr>
<td>Winter</td>
<td>181.7 ± 17.6f</td>
<td>463.7 ± 42.3g</td>
</tr>
</tbody>
</table>

a,b Raw progesterone curve was calculated by integration of the total area within breed and season over luteinizing hormone (LH) dosage. The adjusted progesterone curve was calculated by subtracting the progesterone concentrations at 0 ng LH from P₄ concentrations observed at subsequent LH dosages and integrating the area.

c,B = Brahman.
d,H = Hereford.
e,f,g,h Means ± SE within traits with different superscripts differ (P<.05).
the variation in the in vitro response between breedtypes and season may have been due to the gonadotropic "status" of the animal prior to removal of the luteal tissue. Because in previous studies both basal and peak LH concentrations have been reported to vary in the B cow with season, a gonadotropin receptor-related phenomenon may have also occurred. Harwood et al. (1978) have reported that low concentrations of gonadotropins alter receptor numbers. Since the hypophysiotropic-gonadal axis operates in a classical feedback loop fashion, it is quite possible that differences between breedtype and season, with respect to CL functionality, might encompass all the aforementioned variables: shifts in hormone secretory patterns, receptor numbers and(or) concentrations.

These data indicate that seasonal changes in the bovine CL do occur. These changes, however, were not reflected in serum P₄ concentrations. The transition from summer to winter reduced the capacity of the cultured B CL to respond to exogenous LH. The ovarian changes that transpire during different seasons might affect subsequent fertility. The depression of fertility between seasons observed in the B cow cannot be explained by shifts in the systemic serum P₄ concentrations. Further, these data corroborate differences in the reproductive anatomy and physiology of Bos indicus and Bos taurus and serve as possible indicators of differences in fertility between breedtypes.

**Literature Cited**


