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Ovarian and endocrine characteristics during an estrous cycle in Angus, Brahman, and Senepol cows in a subtropical environment[1,2,3]

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ABSTRACT: To determine breed differences in ovarian function and endocrine secretion, daily rectal ultrasonography was conducted on multiparous lactating Angus (temperate *Bos taurus*; n = 12), Brahman (tropical *Bos indicus*; n = 12), and Senepol (tropical *Bos taurus*; n = 12) cows during an estrous cycle in summer. Blood was collected daily to quantify plasma concentrations of FSH, LH, progesterone, estradiol, GH, insulin-like growth factor (IGF)-I, IGF-II, IGF binding proteins (IGFBP), insulin, glucose, and plasma urea nitrogen (PUN). Numbers of small (2 to 5 mm), medium (6 to 8 mm), and large follicles (≥ 9 mm) were greater ($P < .05$) in Brahman than in Angus and(or) Senepol cows. Length of the estrous cycle (SEM = .6 d) was similar ($P > .10$) among Senepol (20.4 d), Angus (19.5 d), and Brahman (19.7 d) cows. Senepol cows had greater ($P < .05$) diameters of the corpus luteum (CL) and a delayed regression of the CL as compared with Angus cows. The secondary surge of FSH (between d 1 and 2; d 0 = estrus) was greater in Angus than Brahman or Senepol cows (breed × day, $P < .05$). Between d 2 and 14 of the estrous cycle, concentrations of progesterone, LH, IGF-II, and binding activities of IGFBP-3, IGFBP-2, and the 27- to 29-kDa IGFBP in plasma did not differ ($P > .10$) among breeds. Concentrations of GH, IGF-I, insulin, and PUN were greater ($P < .001$) and binding activities of the 22-kDa and 20-kDa IGFBP tended ($P < .10$) to be greater in plasma of Brahman than in Angus or Senepol cows. Plasma glucose concentrations were greater ($P < .05$) in Senepol than in Brahman or Angus cows. In conclusion, Brahman (*Bos indicus*) and Senepol cows (tropical *Bos taurus*) had greater numbers of follicles in all size categories and greater diameter of CL than Angus (temperate *Bos taurus*) cows. These ovarian differences may be due to changes in the pattern of secretion of FSH, insulin, IGF-I, and GH but not LH, IGF-II, or IGFBP-2 or -3.

Key Words: Beef Cows, Binding Proteins, Cattle Breeds, Follicles, FSH, Insulin-Like Growth Factor

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Introduction

Differences in the reproductive characteristics between *Bos taurus* and *Bos indicus* cattle include longer gestation length for Brahman than Angus cows (Foote et al., 1960; Reynolds et al., 1980), and shorter and less-intense estrus in *Bos indicus* than *Bos taurus* females (Rhodes and Randel, 1978; Galina et al., 1982; Randel, 1984). In addition, puberty occurs at an older age in *Bos indicus* than in *Bos taurus* cattle (Plasse et al., 1968; Baker et al., 1989), and twinning rate is less in *Bos indicus* than in *Bos taurus* cattle (Rutledge, 1975). Disparities in several reproductive characteristics, including gonadotropin (Rhodes et al., 1978) and steroid (Adeyemo and Heath, 1980; Segerson et al., 1984) secretion, follicular growth and luteal development (Segerson et al., 1984; Simpson et al., 1994), and(or) the secre-

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[3]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product or the exclusion of others that may also be suitable.

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tion of metabolites and metabolic hormones (Simpson et al., 1994; 1997) may account for these differences in reproductive performance.

Senepol cattle (tropically adapted *Bos taurus*) were developed in the early 1900s on St. Croix, U.S. Virgin Islands, from a cross between West African (N'Dama) and European (Red Poll) breeds of cattle (Williams et al., 1988). Several traits, such as heat tolerance and disease and parasite resistance, make Senepol cattle a suitable breed for beef production in subtropical environments (Hupp, 1981; Hammond and Olson, 1994; Hammond et al., 1996). However, reproductive characteristics of Senepol females have not been well characterized. Ovarian function and endocrine secretion have not been compared among Angus, Brahman, and Senepol cows. Therefore, we set out to test the hypothesis that ovarian function (follicular growth and luteal development) and endocrine secretion of Senepol cows was more like Brahman than Angus cows maintained in a subtropical environment.

Materials and Methods

Animals, Management, and Estrous Synchronization. This study was conducted during summer (July and August) of 1995 at the Subtropical Agricultural Research Station (STARS) located near Brooksville, Florida. The average maximum and minimum daily temperatures during July were 32.6 and 22.8°C and during August were 32.0 and 23.6°C, respectively; the highest and lowest temperatures during July were 35.0 and 21.1°C and during August were 35.5 and 21.1°C, respectively; and total rainfall in July was 177 mm and in August was 360 mm. Total rainfall for August of 1995 was 132 mm above normal. Twelve multiparous lactating cows were randomly selected from each of the Angus (temperate *Bos taurus*; age = 6.5 ± .40 yr; BW = 417 ± 9.4 kg; body condition score = 5.2 ± .21, 1 = thin to 9 = obese; days postpartum = 148 ± 5.0 d), American Gray Brahman (tropical *Bos indicus*; age = 6.9 ± .43 yr; BW = 490 ± 15.7 kg; body condition score = 5.2 ± .27; days postpartum = 134 ± 3.0 d) and Senepol (tropical *Bos taurus*; age = 6.3 ± .22 yr; BW = 464 ± 11.0 kg; body condition score = 4.4 ± .23; days postpartum = 120 ± 5.1 d) cow herds at STARS. Within breed, no more than two cows with the same sire were selected and 100% of the Angus, 67% of the Brahman, and 100% of the Senepol cows also had calved the previous year. Cows and calves were maintained in a drylot (with access to shade) as one group and given ad libitum access to mixed rhizoma perennial peanut (*Arachis glabrata* Benth)-bahiagrass (*Paspalum notatum*) hay (large round bales; 10.3% CP and 47.8% TDN, DM basis) and custom mineral mixture (25 to 32% salt, 15 to 18% Ca, 5 to 8% P, ≥ .94% Fe, ≤ .15% Fl, ≥ .10% Cu, ≥ .01% Co, and .0010 to .0015% Se). During the study, ADG was −.3 ± .08 kg/d for Angus, −.2 ± .08 kg/d for Brahman, and .1 ± .12 kg/d for Senepol. Calves were allowed to suckle ad libitum. Estrus was synchronized using prostaglandin-$F_{2\alpha}$ (**PGF**; Lutalyse, Upjohn, Kalamazoo, MI). The estrous synchronization procedure consisted of an initial injection of 25 mg of PGF (i.m.), followed by injections of 12.5 mg of PGF 11 and 12 d later (Santos et al., 1988). Cows were visually observed for signs of estrus for 1 h at 12-h intervals from 1 to 4 d after the last injection of PGF. Sterile bulls fitted with chin ball marker devices were used to assist in the detection of estrus. Onset of estrus was considered to have occurred when a cow stood to be mounted by another cow or marker bull. Observations for estrus resumed 17 d after estrus to determine the length of the estrous cycle. Five cows were removed from the original sample group of 36; one cow was removed due to the presence of a cystic follicle, two of the cows failed to exhibit behavioral estrus, and two other cows had irregular estrous cycle lengths and irregular progesterone profiles. All experimental procedures were approved by the STARS Animal Care and Use Committee.

Ovarian Ultrasonography. After the synchronized estrous cycle, beginning on d 0 (estrus), ovaries were examined by rectal ultrasonography (Aloka 210 ultrasound scanner equipped with a 7.5-MHz probe, Corometrics Medical Systems, Inc., Wallingford, CT) once daily (beginning at 0800) during the second estrous cycle following the synchronized estrus. Images of each ovary were recorded using a VCR (General Electric 1CVD5025X, General Electric Co., Portsmouth, VA). Tapes were played back, images were projected on a monitor, and a diagram depicting the relative location of follicles ≥ 2 mm and the corpus luteum (**CL**) was drawn for each ovary. Numbers and sizes of ovarian follicles and size of the CL (height, width, and cavity) were determined for each day. Ovulation was determined by the disappearance of the dominant follicle and subsequent formation of a CL at the same location in the ovary. Maximum diameter and growth rate of the dominant follicle of the first follicular wave and of the ovulatory follicle were also estimated. Growth rate of the dominant follicle was determined from the day the dominant follicle was first identified to the day that the diameter of the follicle no longer increased more than 1 mm. Growth rate of the ovulatory follicle was determined from first detection to ovulation. Number of follicular waves during the estrous cycle was determined using the identity method (Knopf et al., 1989), in which individual follicles on each ovary (> 5 mm) were identified and assigned a position relative to the other ovarian structures present (i.e., CL and follicles). A wave of follicular growth was identified by the formation of a dominant follicle and a group of growing follicles (subordinates) associated with the dominant follicle. The day of emergence was defined as the last day a group of follicles were 4 mm as indicated by increasing diameters on subsequent days. The dominant follicle was defined as the largest follicle present on either ovary before d 12, and a subordinate follicle was defined as one that originated from the same follicular pool as the dominant follicle and was indicated by: 1) its first

detection within 2 d of the first detection of the dominant follicle; and 2) had a subsequent increase in diameter. Numbers of follicles were divided into three distinct size classes for analyses: small (2 to 5 mm), medium (6 to 8 mm), and large (≥ 9 mm) follicles.

Blood Collection and Metabolite and Hormone Analyses. During the synchronized estrous cycle and the subsequent natural estrous cycle (when ovarian ultrasound was conducted), a single blood sample was collected daily from each cow (beginning at 0800) by jugular venipuncture into 9-mL blood collection tubes containing EDTA (Monovette Sarstedt, Newton, NC). Blood samples were placed on ice immediately after collection, and plasma was separated by centrifugation (4°C, 1,750 × *g* for 30 min). Plasma samples were frozen and stored at –20°C until metabolite and hormone analyses were performed. For each metabolite and hormone analysis, all breeds were represented in each assay.

Plasma concentration of glucose was determined in daily samples by an automated colorimetric method (Technicon AutoAnalyzer II; Industrial Method 339-19, Technicon Industrial Systems, Tarrytown, NY) based on the glucose oxidase procedure as described by Gochman and Schmitz (1972). Concentration of PUN was determined in daily samples by an automated colorimetric procedure (Technicon AutoAnalyzer II; Industrial Method 339-01, Technicon Industrial Systems, Tarrytown, NY) based on the diacetyl monoxime method described by Marsh et al. (1965).

Concentration of IGF-I in plasma collected every other day was determined by double-antibody RIA after acid-ethanol extraction (16 h at 4°C) as described previously (Echternkamp et al., 1990). Intra- and interassay CV for five plasma IGF-I assays were 12.1% and 23.4%, respectively. Sensitivity of the assay, defined as 90% of total binding, was 3.7 ± .6 ng/mL.

To compare follicular phase and luteal phase IGF-II levels, plasma concentration of IGF-II for samples collected on d 0 and d 10 was determined by a double-antibody RIA after formic acid-acetone extraction as described previously (Stewart et al., 1996). All samples were evaluated in one assay; the intrassay CV was 2.7% and sensitivity was 50 ng/mL.

Plasma concentration of progesterone was determined in daily samples using a solid-phase RIA kit (Diagnostics Products Corporation, Los Angeles, CA) as previously described (Stewart et al., 1996). Intra- and interassay CV for 11 assays were 4.4% and 10.6%, respectively. Sensitivity of the assay was .05 ± .002 ng/mL.

Concentration of FSH in plasma was quantified in daily samples by a double-antibody RIA as previously described (Vizcarra et al., 1997). Intra- and interassay CV for 10 assays were 11% and 27.6%, respectively. Sensitivity of the assay was .05 ± .01 ng/mL.

Plasma concentration of insulin was measured in daily samples using a solid-phase RIA kit (ICN Pharmaceuticals Inc., Costa Mesa, CA) as described previously (Simpson et al., 1994). Intra- and interassay CV for 11 assays were 9.9% and 13.5%, respectively. Sensitivity of the assay was .12 ± .01 ng/mL.

Concentration of estradiol in plasma was determined in daily samples using a solid-phase Serono Estradiol MAIA assay kit (Polymedco Inc., Cortlandt Manor, NY) after extraction with ethyl acetate as previously described (Vizcarra et al., 1997). Intra- and interassay CV for 9 assays were 15.0% and 24.3%, respectively. Sensitivity of the assay was .63 ± .06 pg/mL.

Growth hormone concentration in plasma was quantified in daily samples by a double-antibody RIA as previously described (Yelich et al., 1995). Intra- and interassay CV for five assays were 8.0% and 15.8%. Sensitivity of the assay was 8.4 ± 1.4 ng/mL.

Plasma concentration of LH was determined in daily samples by a double-antibody RIA as previously described (Bishop and Wettemann, 1993). Intra- and interassay CV for five assays were 12.1% and 19.2%, respectively. Sensitivity of the assay was .31 ± .09 ng/mL.

Ligand Blots. Plasma IGF binding proteins (IGFBP) were analyzed in samples collected on d 10 by one dimensional SDS-PAGE, as previously described (Echternkamp et al., 1994; Simpson et al., 1997). Briefly, samples (4.0 μL added to 21.0 μL of buffer) were heat-denatured and then separated on a 12% polyacrylamide gel via electrophoresis. After separation, proteins in gels were electrophoretically transferred to nitrocellulose, and ligand-blotted overnight with [^{125}I]IGF-II. After washing and then exposure to x-ray film at –70°C for 48 h, band intensity on autoradiographs was determined using a PDI Model DNA 35 scanner and Quantity One (Version 2.4) software for quantification by scanning densitometry.

Statistical Analyses. The experiment was a completely randomized design with repeated measures. The data were analyzed using PROC MIXED (Littell et al., 1996), with sources of variation including breed, cow within breed (error term for breed), day, breed × day interaction, and residual. An autoregressive with lag equal to one model was used to model the covariance structure of the repeated measurements. If the breed × day interaction was significant, simple effects of breed were analyzed using the SLICE option for the LSMEANS statement. Satterthwaite's approximation was used for calculation of the degrees of freedom of the pooled error term. If the breed × day interaction was not significant, the main effects, if significant, were analyzed using LSMEANS with the PDIFF option (SAS, 1989). For certain variables (estradiol, CL diameter, progesterone, LH), the maximum value over the estrous cycle was also analyzed. The resulting design was a completely randomized design and PROC GLM was used to analyze these data with LSMEANS reported (SAS, 1989). Number of waves of ovarian follicular development was analyzed by PROC FREQ using chi-square analysis (SAS, 1989). Data are presented as least squares means ± SEM.

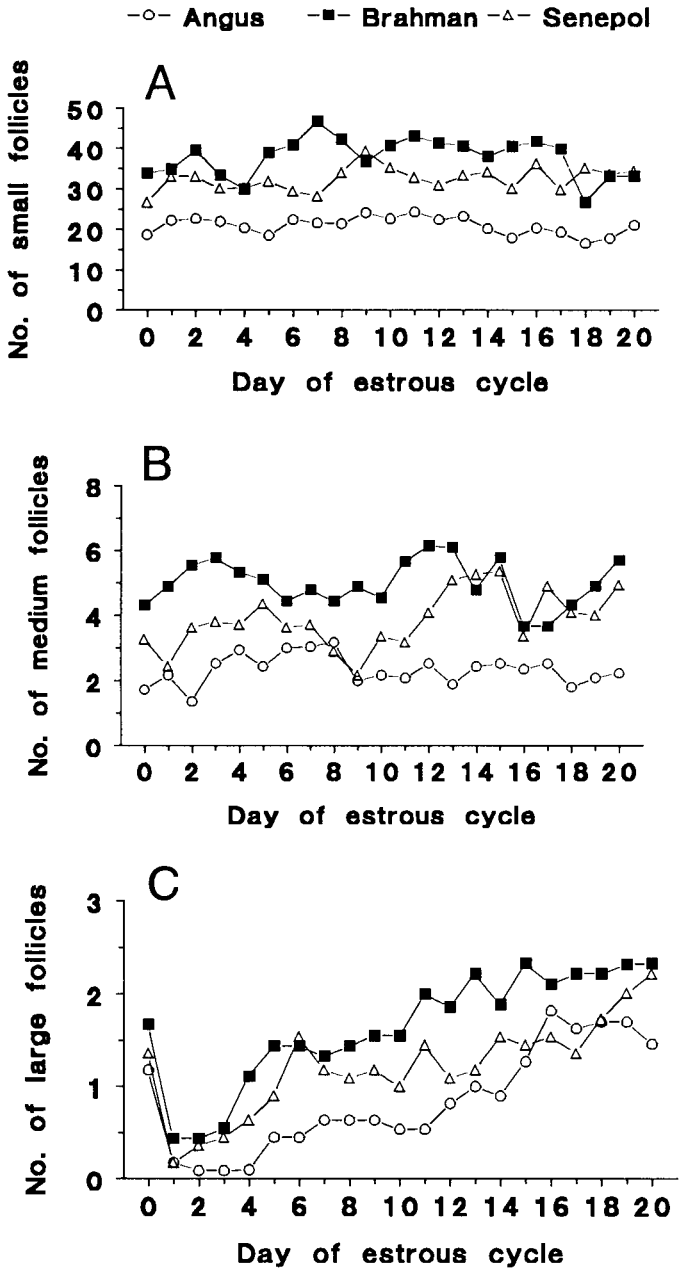

Figure 1. Number of small (2 to 5 mm; Panel A), medium (6 to 8 mm; Panel B), and large (≥ 9 mm; Panel C) follicles in Angus, Brahman, and Senepol cows during an estrous cycle as determined by daily rectal ultrasonography. Standard errors averaged over days were 3.9, .7, and .2 for small, medium, and large follicles, respectively.

Results

Follicular Dynamics and Function. Number of small (2 to 5 mm) follicles was greater ($P < .05$) in Brahman (39 ± 4) than in Angus (21 ± 4) or Senepol (33 ± 4) cows and was greater ($P < .001$) in Senepol than in Angus cows (Figure 1A); there was neither a day effect nor breed × day interaction ($P > .10$). Number of medium (6 to 8 mm) follicles was less ($P < .05$) in Angus (2.3 ± .7) than in Brahman (5.0 ± .7) or Senepol (3.9 ± .7) cows (Figure 1B); there was neither a day effect nor breed × day interaction ($P > .10$). The number of large (≥ 9 mm) follicles was greater ($P < .05$) in Brahman (1.6 ± .2) than in Angus (.9 ± .2) or Senepol (1.2 ± .2) cows (Figure 1C), and there was no breed × day interaction ($P > .10$). There was a day effect ($P < .001$) on number of large follicles. Number of large follicles in all breeds increased ($P < .05$) from d 1 (day of ovulation) to d 20 of the estrous cycle.

Maximum diameter of the dominant follicle of the first wave of follicular development was less ($P < .01$) in Angus cows than in Brahman or Senepol cows (Table 1). Growth rate of the first dominant follicle tended to be greater ($P < .10$) in Brahman cows than in Angus and Senepol cows (Table 1). Maximum diameter of the ovulatory follicle was greater ($P < .01$) in Brahman than in Angus or Senepol cows (Table 1). No differences ($P > .10$) in growth rate of the ovulatory follicle were found among Angus, Brahman, and Senepol cows (Table 1). Although Angus and Brahman cows had predominantly two waves of follicular development during the estrous cycle (72.7% and 55.6%, respectively) and Senepol cows had mostly (70%) three waves of follicular development during the estrous cycle (Table 2), these breed differences were not significant ($P > .10$). Length of the estrous cycle did not differ ($P > .10$) among Senepol (20.4 ± .6 d), Angus (19.5 ± .6 d), and Brahman (19.7 ± .6 d) cows. Among breeds, length of the estrous cycle was longer ($P < .005$) in cows with three waves of follicular development (20.9 ± .47 d) than in cows with two waves of follicular development (18.7 ± .44 d) during the estrous cycle.

Luteal Development. There was a breed × day ($P < .06$) interaction for CL diameter across d 0 to 20. Regression of the CL occurred earlier in the estrous cycle of Angus and Brahman cows than in Senepol cows (Figure 2A). If CL diameter was analyzed for only d 2 to 14 of the estrous cycle, the breed × day interaction was not significant ($P > .10$). The average diameter of the CL between d 2 to 14 was greater ($P < .001$) in Brahman (16.4 ± .3 mm) and Senepol (16.3 ± .3 mm) than in Angus (15.0 ± .3 mm) cows. The diameter of the CL increased ($P < .001$) from d 2 to 8 in all breeds (Figure 2A). Maximum diameter of the CL was greater ($P < .05$) in Senepol (22.2 ± .3 mm) than in Angus (19.6 ± .2 mm) cows and tended ($P < .10$) to be greater in Brahman (21.3 ± .3 mm) than in Angus cows. Maximum CL diameter was similar ($P > .10$) between Brahman and Senepol cows.

Endocrine Profiles. There was a breed × day ($P < .07$) interaction for plasma concentration of estradiol across d 0 to 20 of the estrous cycle. The preovulatory increase of estradiol was earlier in the estrous cycle of Angus and Brahman cows than Senepol cows (Figure 3). When plasma concentration of estradiol was analyzed for d 2 to 14 of the estrous cycle to assess "basal" estradiol secretion, the breed × day interaction still existed ($P < .06$); this was due to an increase in plasma estradiol observed between d 2 and 5 in Angus and Brahman

Table 1. Characteristics of dominant and ovulatory follicles during an estrous cycle in Angus, Brahman, and Senepol cows as determined by rectal ultrasonography

	First dominant follicle		Ovulatory follicle	
Breed	Maximum diameter, mm	Growth rate, mm/d	Maximum diameter, mm	Growth rate, mm/d
Angus	11.4 ± .6[a]	1.18 ± .12[c]	12.8 ± .4[a]	1.39 ± .10
Brahman	15.3 ± .6[b]	1.55 ± .12[d]	15.6 ± .5[b]	1.35 ± .11
Senepol	13.9 ± .5[b]	1.22 ± .12[c]	13.6 ± .4[a]	1.46 ± .10

[a,b]Within a column, means lacking a common superscript letter differ ($P < .01$).
[c,d]Within a column, means lacking a common superscript letter differ ($P < .10$).

cows but not in Senepol cows (Figure 3). Maximum concentration of estradiol in plasma did not differ ($P > .10$) among Angus (9.1 ± 1.4 pg/mL), Brahman (8.9 ± 1.6 pg/mL), and Senepol (8.7 ± 1.4 pg/mL) cows.

There was a breed × day ($P < .01$) interaction for plasma concentration of progesterone across d 0 to 20. Luteal regression, indicated by a decrease in plasma progesterone, occurred earlier in Angus and Brahman cows than in Senepol cows (Figure 2B). When plasma concentration of progesterone was analyzed for d 2 to 14 of the estrous cycle, there was no significant breed × day interaction and mean plasma concentration of progesterone did not differ among breeds. The concentration of progesterone increased ($P < .001$) in plasma from d 0 to 12 in all three breeds (Figure 2B). Maximum plasma concentration of progesterone did not differ ($P > .10$) among Angus (4.3 ± .33 ng/mL), Brahman (4.4 ± .37 ng/mL), and Senepol (5.2 ± .33 ng/mL) cows.

There was a breed × day interaction ($P < .05$) for plasma concentrations of FSH across d 0 to 20 of the estrous cycle. The increases of FSH between d 1 and 2 of the estrous cycle were more pronounced ($P < .005$) in Angus than in Brahman and Senepol cows (Figure 4A). Maximum plasma FSH concentration achieved between d 0 and 4 averaged 1.18, .61, and .67 ± .10 ng/mL for Angus, Brahman, and Senepol cows, respectively. During d 0 to 20 of the estrous cycle, plasma concentration of FSH averaged .62, .45, and .44 ± .03 ng/mL for Angus, Brahman, and Senepol cows, respectively.

There was a breed × day interaction ($P < .01$) for concentrations of LH in plasma across d 0 to 20 of the estrous cycle (Figure 4B). If data for plasma concentration of LH were analyzed for d 2 to 14 of the estrous cycle to assess "basal" LH secretion, there were no significant effects of breed, day, or breed × day. During d 2 to 14 of the estrous cycle, plasma concentration of LH averaged 4.3, 4.2, and 4.4 ± .5 ng/mL for Angus, Brah-

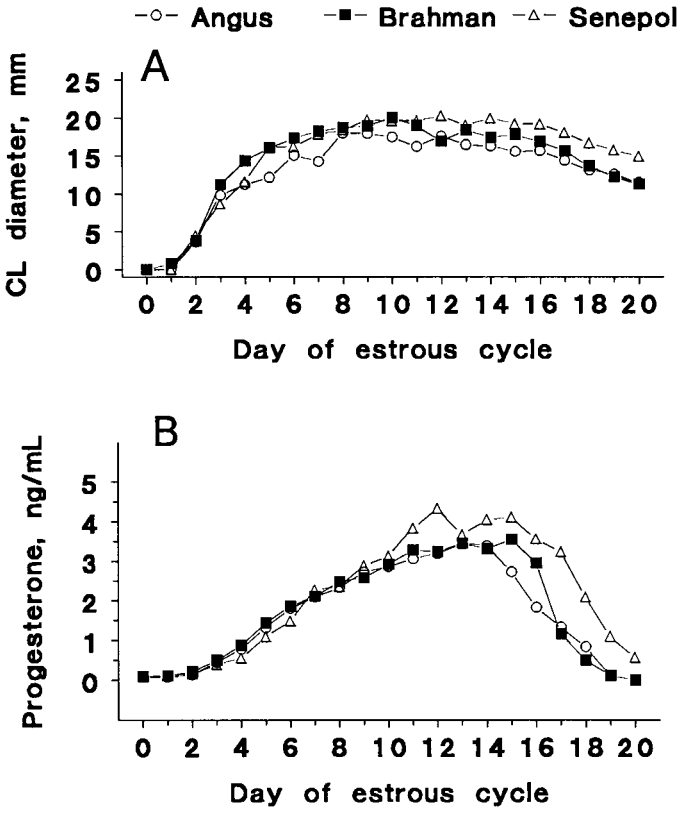

Figure 2. Diameter of the corpus luteum (CL; Panel A) as determined by daily rectal ultrasonography and plasma concentration of progesterone (Panel B) in Angus, Brahman, and Senepol cows during an estrous cycle. Standard errors averaged over days were .9 mm and .2 ng/mL for CL diameter and plasma progesterone, respectively.

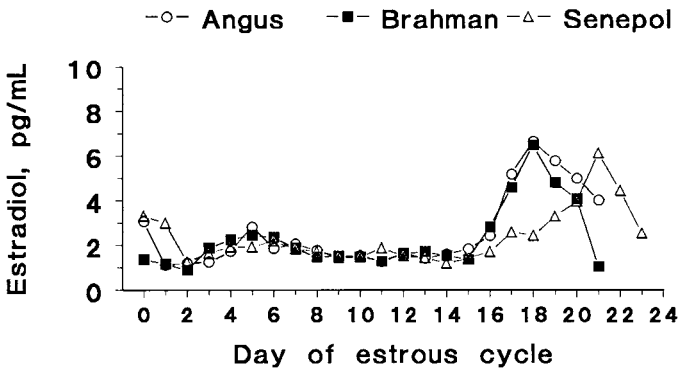

Figure 3. Plasma concentrations of estradiol in Angus, Brahman, and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over days were .4, .4, and .3 pg/mL for Angus, Brahman, and Senepol cows, respectively.

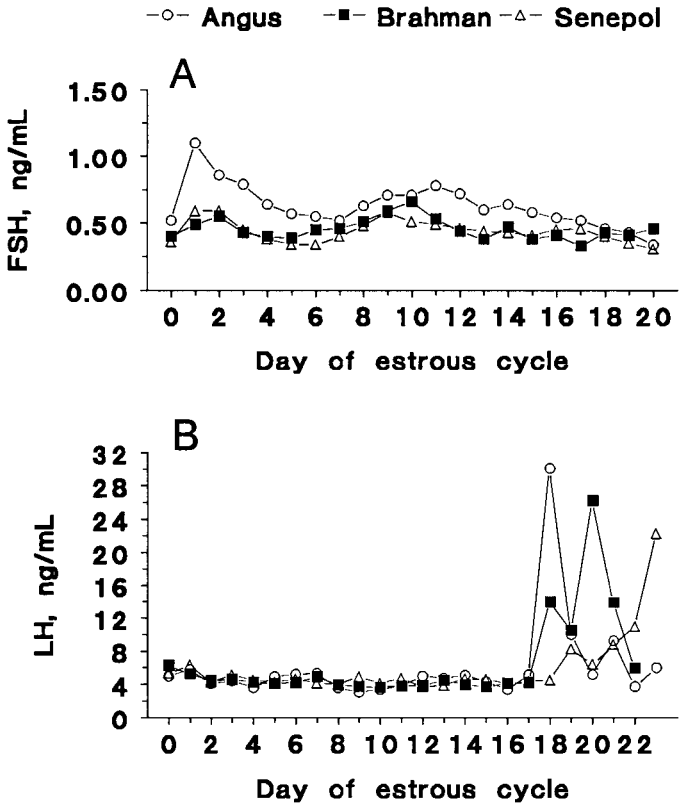

Figure 4. Plasma concentrations of FSH (Panel A) and LH (Panel B) in Angus, Brahman, and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over days were .2 ng/mL and .5 ng/mL for FSH and LH, respectively.

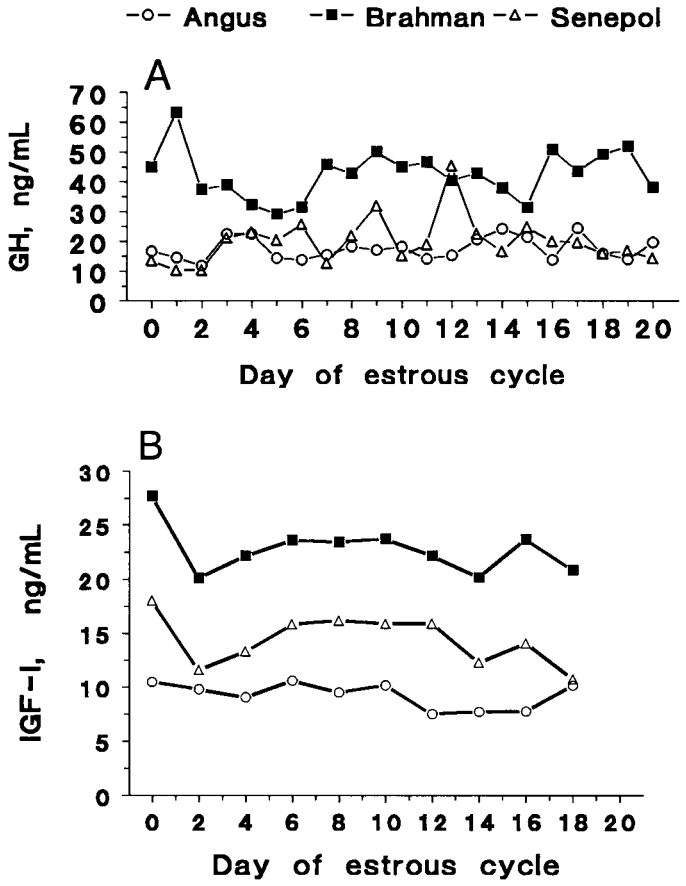

Figure 5. Plasma concentrations of growth hormone (GH; Panel A) and insulin-like growth factor I (IGF-I; Panel B) in Angus, Brahman, and Senepol cows as determined by RIA. Blood samples were collected daily for GH and every other day for IGF-I. Standard errors averaged over days were 6.9 ng/mL and 2.6 ng/mL for GH and IGF-I, respectively.

man, and Senepol cows, respectively. Maximum plasma LH concentration did not differ ($P > .10$) among breeds and averaged 31.7, 30.7, and 22.1 ± 8.15 ng/mL for Angus, Brahman, and Senepol cows, respectively.

Concentration of GH in plasma from d 0 to 20 of the estrous cycle was greater ($P < .001$) in Brahman (42.2 ± 2.2 ng/mL) than in Angus (17.4 ± 2.1 ng/mL) or Senepol (20.1 ± 2.0 ng/mL) cows (Figure 5A). There was no day effect or breed × day interaction ($P > .10$) observed for plasma concentration of GH.

Concentration of IGF-I in plasma samples collected every other day from d 0 to 20 of the estrous cycle was greater ($P < .001$) in Brahman (22.9 ± .9 ng/mL) than in Senepol (14.5 ± .8 ng/mL) and Angus (10.0 ± .8 ng/mL) cows (Figure 5B). Senepol cows also had greater ($P < .001$) plasma concentrations of IGF-I than Angus cows. Neither day nor the breed × day interaction affected plasma concentrations of IGF-I ($P > .10$).

Plasma concentration of IGF-II determined on d 0 and 10 within each breed did not differ ($P > .10$) between d 0 (173 ± 7 ng/mL) and d 10 (169 ± 7 ng/mL). Plasma concentration of IGF-II on d 0 and 10 did not differ ($P > .10$) among breeds and averaged 166, 176, and 171 ± 9 ng/mL for Angus, Brahman, and Senepol cows, respectively. There was no breed × day ($P > .10$) interaction for plasma concentration of [^{125}I]IGF-II.

Table 2. Number of waves of ovarian follicular development and length of the estrous cycle in Angus, Brahman, and Senepol cows as determined by rectal ultrasonography

Breed	Number of cows	2-Wave cycles		3-Wave cycles	
		n (%)	Cycle length, d	n (%)	Cycle length, d
Angus	11	8 (72.7)	18.3 ± .6	3 (27.3)	20.7 ± .9
Brahman	9	5 (55.6)	18.6 ± .7	4 (44.4)	20.8 ± .8
Senepol	10	3 (30.0)	19.3 ± .9	7 (70.0)	21.4 ± .6

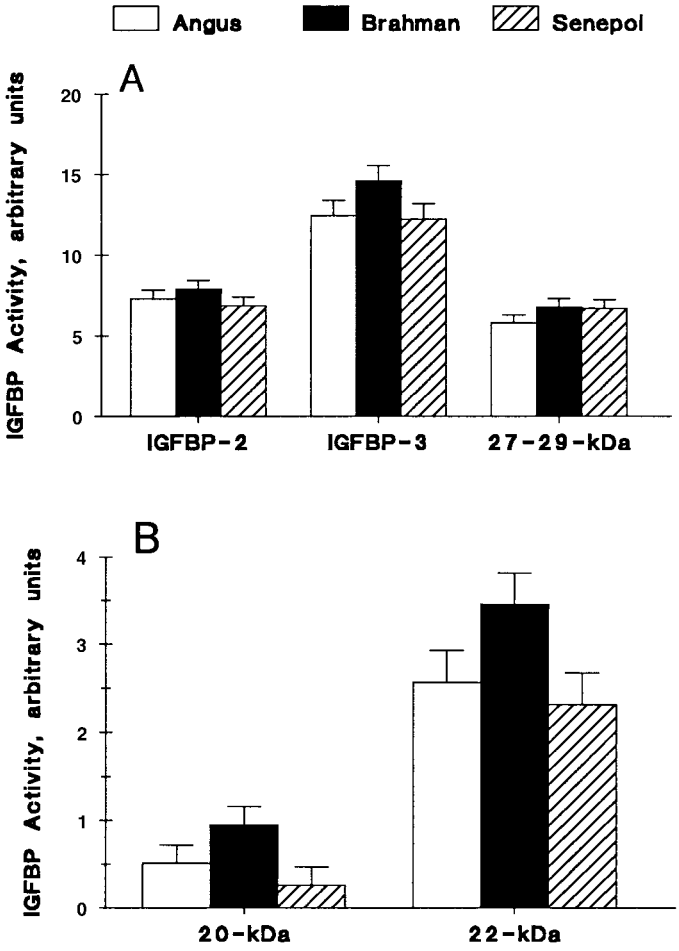

Figure 6. Binding activity of IGFBP-2, IGFBP-3, a 27- to 29-kDa IGFBP, and a 20- and 22-kDa IGFBP in Angus, Brahman, and Senepol cows on d 10 of the estrous cycle as determined by ligand blotting.

Ligand blotting with [^{125}I]IGF-II revealed at least five forms of IGFBP; 40- to 44-kDa (IGFBP-3), 34-kDa (IGFBP-2), 27- to 29-kDa, 22-kDa, and 20-kDa. Binding activities expressed in arbitrary densitometric units of IGFBP-3, IGFBP-2, and the 27- to 29-kDa IGFBP (Figure 6A) did not differ ($P > .10$) among breeds and averaged 13.1 ± 1.0, 7.4 ± .5, and 6.5 ± .5, respectively, on d 10 of the estrous cycle. Brahman cows tended ($P < .10$) to have greater 22-kDa and 20-kDa IGFBP binding activity (3.5 ± .4 and 1.0 ± .2 unit) than Angus (2.6 ± .4 and .5 ± .2 unit) or Senepol (2.3 ± .4 and .3 ± .2 unit) cows (Figure 6B).

Plasma concentration of insulin from d 0 to 20 of the estrous cycle was greater ($P < .001$) in Brahman (.68 ± .05 ng/mL) than in Angus (.44 ± .05 ng/mL) and Senepol (.49 ± .05 ng/mL) cows, but did not differ between Angus and Senepol cows (Figure 7A). There was neither a day nor breed × day effect ($P > .10$) on plasma insulin.

Plasma Metabolites. Concentration of glucose in plasma from d 0 to 20 of the estrous cycle was greater ($P < .001$) in Senepol (76.7 ± .6 mg/dL) and Brahman (74.5 ± .7 mg/dL) than in Angus (68.8 ± .6 mg/dL) cows (Figure 7B); plasma glucose concentrations also differed ($P < .05$) between Brahman and Senepol cows. There was a day effect ($P < .01$), but no breed × day interaction on plasma concentration of glucose, indicating that the decrease in glucose concentration between d 0 and 8 of the estrous cycle was similar among breeds (Figure 7B).

Plasma urea nitrogen concentration from d 0 to 20 of the estrous cycle was greater ($P < .001$) in Brahman cows (15.4 ± .2 mg/dL) than in Angus (11.8 ± .2 mg/dL) or Senepol (14.6 ± .2 mg/dL) cows, and was also greater

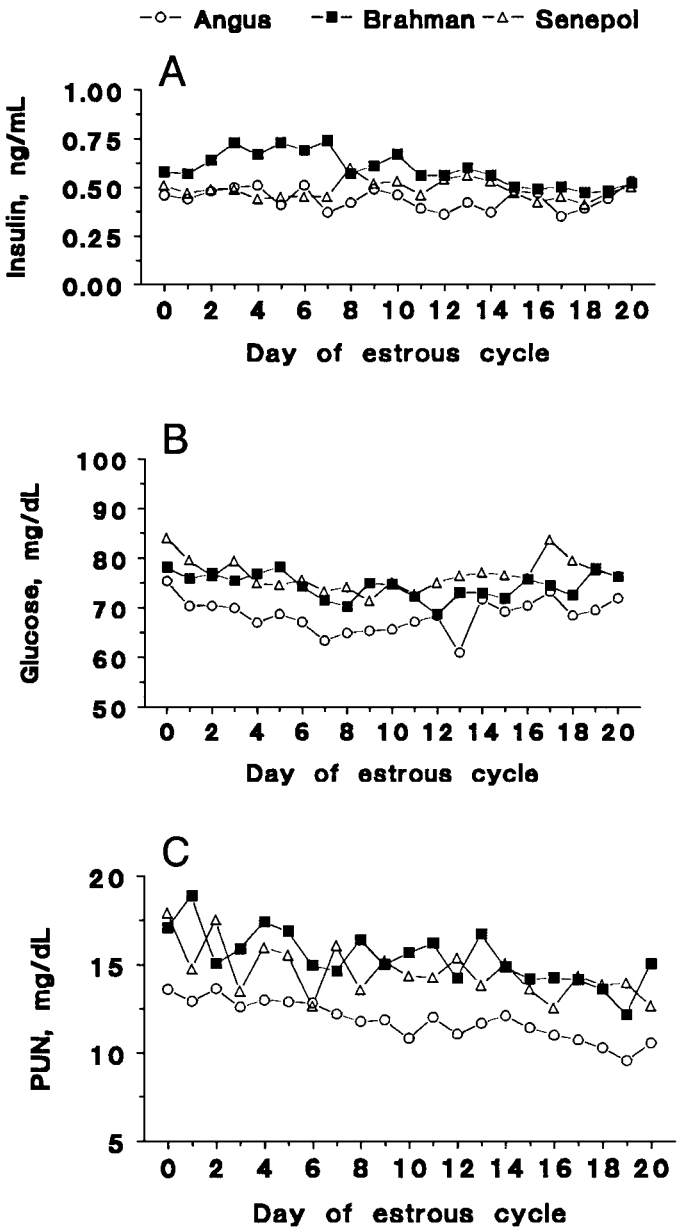

Figure 7. Plasma concentrations of insulin (Panel A), glucose (Panel B), and plasma urea nitrogen (PUN; Panel C) in Angus, Brahman, and Senepol cows. Blood samples were collected daily. Standard errors averaged over days were .2 ng/mL, 3.1 mg/dL and .9 mg/dL for insulin, glucose, and PUN, respectively.

($P < .001$) in Senepol than in Angus cows (Figure 7C). There was a day effect ($P < .001$) but no breed × day interaction ($P > .10$) on PUN concentration, indicating that the decrease in PUN concentration between d 0 and 20 of the estrous cycle was similar among breeds (Figure 7C).

Discussion

Ovarian follicular growth as indicated by number of follicles in all categories, growth rate of the first dominant follicle to develop during the estrous cycle, and maximum diameter of the first dominant and ovulatory follicles was greater in Brahman (*Bos indicus*) than in Angus (temperate *Bos taurus*) cows of the present study. Rather than being Brahman-like, follicular growth in Senepol (tropical *Bos taurus*) cows was intermediate and between that observed for Brahman and Angus cows, and thus we reject our original hypothesis. These results are consistent with previous studies indicating that numbers of antral follicles are greater in Brahman than in Angus cows (Segerson et al., 1984; Simpson et al., 1994). The hormonal stimulus for increased follicular growth in Brahman vs Senepol vs Angus cows is discussed in detail later but is most closely associated with increased secretion of IGF-I.

To our knowledge, this is the first study in which the wave pattern of follicular growth in Senepol cows was determined. Senepol cows had mostly three waves of follicular development during their estrous cycle, whereas the majority of Angus and Brahman cows had two waves. The percentage of Brahman cows with two waves of follicular development (56%) was greater in the present study than the 38% reported by Zeitoun et al. (1996), but less than the 84% reported by Figueiredo et al. (1997). Reasons for these discrepancies are unclear but may be due to the fact that the present study was conducted during summer (July and August) in Florida, whereas Zeitoun et al. (1996) conducted their study during the spring (May) and Fall (October) in Texas, and Figueiredo et al. (1997) conducted their study during winter (July and August) in Brazil. Seasonal differences in the number and size of antral follicles of Brahman cows have been reported (Lammoglia et al., 1996). Differences in nutritional status of the cows between studies could also account for the discrepancies. In the present study, cows were allowed free access to rhizoma perennial peanut hay, whereas Zeitoun et al. (1996) and Figueiredo et al. (1997) supplemented cows with concentrate. The delayed luteal regression of the Senepol cows may account for the predominance of three waves of follicular development during estrous cycles in Senepol cows. Ginther et al. (1989) suggested that the number of waves of follicular development during the estrous cycle in cattle is regulated by the length of the luteal phase. Generally, estrous cycles with three or four waves of follicular development have longer luteal phases than estrous cycles with two waves of follicular development (Sirois and Fortune, 1988; Ginther et al., 1989; Zeitoun et al., 1996), and artificially extending the luteal phase with exogenous progesterone has resulted in estrous cycles with four or five waves of follicular growth (Sirois and Fortune, 1990).

In spite of the dramatic differences in the number and growth patterns of follicles that existed among breeds, no differences in maximum plasma concentration of estradiol were observed. However, the significant breed × day interaction for plasma concentration of estradiol revealed that there was an increase in concentration of estradiol on d 5 of the estrous cycle of Angus and Brahman cows but not Senepol cows; this was likely due to the development of the first dominant follicle. There was an earlier preovulatory increase in concentration of estradiol in Angus and Brahman cows than Senepol cows; however, peak (maximum) concentration of estradiol did not differ among breeds. In contrast, Segerson et al. (1984) found that the concentration of estradiol from d 7 to 17 of the estrous cycle was less in Brahman than in Angus cows. Reasons for this inconsistency are unclear but may be due to 1) the different seasons that samples were collected between studies (i.e., summer in the present study vs fall in the Segerson et al. (1984) study); 2) Angus heifers being less tolerant to heat than Brahman and Senepol heifers (Hammond et al., 1996); and(or) 3) Segerson et al., (1984) including pregnant and nonpregnant cows in their analysis. A greater concentration of estradiol in plasma of Brahman cows than in Angus cows would be expected due to the greater number of follicles in the former. However, Simpson et al., (1994) reported that follicles from Brahman cows treated with gonadotropins to superstimulate follicular development contain less estradiol than those of Angus cows; this could explain why maximum plasma concentration of estradiol did not differ between Brahman and Angus cows in the present study. Why plasma concentration of estradiol was similar between Brahman and Angus cows in spite of a greater number of follicles in Brahman cows is uncertain. Perhaps the two-fold greater concentration of IGF-I in plasma of Brahman than Angus cows in the present study inhibited insulin-stimulated estradiol production by granulosa cells of small and large follicles as previously reported (Spicer et al., 1994). Thus, Brahman cows with greater concentration of IGF-I may have reduced estradiol production from follicles and need more follicles to yield the same plasma concentration of estradiol as Angus cows.

In addition to greater concentration of IGF-I, the greater follicular activity in Brahman cows could be explained by the greater mean concentration of GH and(or) insulin than in Angus cows. The greater secretion of GH and IGF-I in Brahman than in Angus cows agrees with previous studies (Simpson et al., 1994; 1997). Unfortunately, the frequency of blood sampling used in the present study (i.e., daily) did not lend itself to an analysis of GH pulse frequency or amplitude. In vitro, insulin and IGF-I are potent stimulators of bovine

granulosa and thecal cell proliferation, whereas GH, LH, and FSH have little or no effect (Spicer et al., 1993; Stewart et al., 1995; Spicer and Stewart, 1996). However, exogenous insulin had no effect on the number of antral follicles in Angus and Brahman cows treated with gonadotropins to superstimulate ovarian follicular development (Simpson et al., 1994). In heifers and lactating dairy cows treated with bovine GH, an increase in the number of small follicles was correlated with an increase in serum GH and IGF-I (Gong et al., 1992; 1993; Lucy et al., 1992). However, in spite of the increased numbers of follicles after GH treatment, others (Schemm et al., 1990; Gong et al., 1991) did not find any differences between control and GH-treated cows in systemic concentration of estradiol, which is consistent with findings of the present study. Because treatment with GH increases peripheral concentrations of GH, IGF-I, and insulin (Gong et al., 1991; 1993; Stanko et al., 1994), determining the hormonal cause for increased follicular growth and steroidogenesis after GH treatment can be difficult.

To our knowledge this is the first time CL diameter in *Bos taurus* and *Bos indicus* cows have been compared using ultrasonography. We found that luteal growth was greater in Brahman and Senepol than in Angus cows. However, smaller CL may be present in *Bos indicus* than in *Bos taurus* heifers (Irvin et al., 1978; Rhodes et al., 1982; Segerson et al., 1984). These inconsistencies between the results of present and previous studies may be due to differences in timing and frequency of luteal measurements during the estrous cycle, and(or) differences in season in which the studies were conducted.

In spite of 10% greater CL diameter in Brahman and Senepol than in Angus cows, there was no difference in concentration of progesterone among breeds before d 14 of the estrous cycle. This finding agrees with previous studies in which no difference was detected in plasma concentration of progesterone (Rhodes et al., 1982; Diaz et al., 1986), but it contrasts with others studies that found lower concentration of progesterone (Adeyemo and Heath, 1980; Randel, 1984; Segerson et al., 1984) in *Bos indicus* than *Bos taurus* cattle. Differences in the sampling frequency, season and climate at time of sampling, and age and physiological state (e.g., lactating or not) of the animals may account for these inconsistencies. Heat stress affects production of progesterone, because, in Holstein cows, serum concentrations of progesterone between d 6 and 18 of the estrous cycle were less during summer than during spring (Howell et al., 1994). In addition, luteal content of progesterone was greater in Holstein cows exposed than in cows not exposed to forced ventilation (Younas et al., 1993). In agreement with previous studies (Kastelic et al., 1990; Rajamahendran and Taylor, 1990; Ribadu et al., 1994), CL diameter and concentration of progesterone was positively correlated in Angus (r = .70), Brahman (r = .73), and Senepol (r = .75) cows between d 0 and 14 of the estrous cycle of the present study.

The greater luteal growth in Brahman and Senepol cows vs Angus could be explained by the greater concentration of GH and(or) IGF-I. Injections of GH in cattle that increased serum IGF-I concentration also increased size of CL and concentration of progesterone in plasma (Schemm et al., 1990; Gallo and Block, 1991; Lucy et al., 1994). In vitro, IGF-I stimulates progesterone production by cultured bovine luteal cells (Schams et al., 1988; Sauerwein et al., 1992; Chakravorty et al., 1993), and reduced luteal progesterone secretion is associated with decreased plasma IGF-I in dairy cows (Spicer et al., 1990). Luteal tissue in *Bos indicus* (Brahman) and *Bos taurus* (Hereford) cows have similar histological and morphological characteristics, such as organization, apparent population of cells per area, and cell types present (Irvin et al., 1978). In vitro, however, luteal tissue from Brahman cows secretes less progesterone than luteal tissue from Hereford cows (Rhodes et al., 1982). The latter observation could explain why in our study Brahman cows had larger CL than Angus but plasma concentration of progesterone did not differ between these breeds; perhaps Brahman cows needed larger CL to produce the same amount of progesterone because of their larger body mass. In addition to direct stimulatory effects of IGF-I on CL growth and steroidogenesis, direct effect of GH on the CL seems likely, since bovine CL contained more GH receptor mRNA and GH receptor protein than other reproductive tissues (Lucy et al., 1993; Kirby et al., 1996).

Ligand blotting with [^{125}I]IGF-II revealed at least five forms of IGFBP: 40- to 44-kDa (IGFBP-3), 34-kDa (IGFBP-2), 27- to 29-kDa, 22-kDa, and 20-kDa. Breed differences in IGFBP-3 and -2 activity were not found in the present study, and this agrees with the study of Simpson et al. (1997), in which no differences were found in IGFBP-2 and total IGFBP binding activity between ovariectomized Angus and Brahman cows, but it is inconsistent with Simpson et al. (1994), who found 10% greater total plasma IGFBP activity in Angus than in Brahman cows treated with gonadotropins to superstimulate follicular development. Simpson et al. (1997) found greater plasma IGFBP-3 levels in estradiol-treated ovariectomized Brahman than Angus cows. Differences in season, physiological state of animals, and frequency of sampling may account for the inconsistencies of results between studies. Growth hormone has been implicated in the regulation of IGFBP-2 and -3 (Stanko et al., 1994). However, despite the differences in circulating GH, no significant differences in IGFBP-2 or -3 were found among breeds in the present study.

Differences in the hypothalamic-hypophyseal axis exist between *Bos indicus* and *Bos taurus* cattle (Rhodes et al., 1978). Ovariectomized Brahman cows produced less LH in response to GnRH than Hereford cows (Griffin and Randel, 1978). Also, Brahman heifers have less LH during the preovulatory surge of LH, an earlier LH surge, and they have a shorter interval from estrus to ovulation than Hereford cows (Randel, 1984). We found no difference in mean concentration of LH between d

2 and 14 of the estrous cycle or in maximum LH concentration among Angus, Brahman, and Senepol cows, but daily samples taken in the present study were not frequent enough to detect differences in the timing and magnitude of the LH surge. However, in agreement with the present study, Griffin and Randel (1978) did not find differences in mean serum levels of LH in ovariectomized Brahman and Hereford cows. Also, Rhodes et al., (1978) did not find any differences in basal concentrations of LH between ovariectomized Brahman and Hereford cows. Thus, differences in luteal function among breeds of the present study are not likely due to differences in luteal phase LH secretion, but further research will be required to verify this suggestion.

The pattern of FSH secretion appears to differ among the three breeds in the present study. Besides the greater mean plasma concentration of FSH found in Angus cows of the present study, the secondary (postovulatory) peak of FSH was more distinct in Angus than Brahman or Senepol cows. The latter observation likely explains why the breed × day interaction on mean concentration of FSH was significant. The greater concentration of FSH in Angus cows may be due to less ovarian production of inhibin in Angus than in Brahman and Senepol cows, but support for this statement requires further study. Peaks of FSH occur before the rise of a follicular wave, and the number of peaks is related to the number of waves of follicular development in heifers (Adams et al., 1992). Despite the greater plasma concentration of FSH in Angus cows, the number of ovarian follicles was less in Angus than Brahman or Senepol cows, and we suggest that this may be due to the lower IGF-I concentration observed in Angus cows. We hypothesize that with elevated FSH but low IGF-I, dominant follicles are more persistent and, thus, fewer follicular waves develop during an estrous cycle. We further speculate that, with elevated IGF-I but low FSH, dominant follicles are less persistant and, thus, more follicular waves develop during an estrous cycle.

Concentration of glucose in plasma was greater in Brahman than in Senepol or Angus cows. In contrast, Simpson et al. (1994; 1997) did not find any differences in plasma concentration of glucose in Angus and Brahman cows treated to superstimulate follicular development or estrogen-treated ovariectomized Angus and Brahman cows. Reasons for this inconsistency are unclear but may be due to differences in the diets among studies. The greater concentration of plasma insulin observed in the present study also may be due to a different sensitivity to insulin among breeds and it is further emphasized by the finding that Senepol cows had greater plasma glucose than Angus, yet no difference in concentration of plasma insulin between Senepol and Angus cows was observed. Whether the elevated GH in Brahman cows caused insulin resistance in this breed will require further study.

The greater concentration of PUN concomitant with greater concentration of IGF-I observed in Brahman and Senepol vs Angus cows in the present study is consistent with Simpson et al. (1994; 1997), who found greater PUN concentrations in Brahman than in Angus cows despite the greater plasma concentration of IGF-I and GH in Brahman than Angus cows. Increased levels of PUN have been reported to be associated with decreased nitrogen retention and protein accretion in growing cattle (Enright et al., 1990; Hayden et al., 1993). In estradiol-implanted growing steers, treatment with GH increased plasma concentration of IGF-I and decreased level of PUN (Preston et al., 1995). Why greater plasma concentration of IGF-I is associated with increased level of PUN in mature cows whereas, in growing cattle, plasma concentrations of IGF-I and PUN are negatively correlated will require further elucidation.

Implications

Breed differences in ovarian function were found among *Bos indicus* (Brahman) cows, temperate *Bos taurus* (Angus), and tropically adapted *Bos taurus* (Senepol) cows maintained in a subtropical environment. The two breeds adapted to the tropics have greater follicular activity than the unadapted Angus cows, as suggested by the greater number of small and medium follicles observed in the Brahman and Senepol cows. The greater concentration of metabolic hormones in Brahman vs Angus and Senepol cows suggests a greater activity of the somatotropic axis in Brahman cows than in Angus and Senepol cows. However, the activity of the gonadotropic axis is greater in Angus than in Brahman and Senepol cows, as suggested by the greater concentrations of follicle-stimulating hormone found in Angus cows. The relative roles of the somatotropic vs gonadotropic axes in regulating reproductive performance in *Bos taurus* and *Bos indicus* cattle await additional studies.

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