# Subspecies differences in early fetal development and plasma pregnancy-associated glycoprotein concentrations in cattle[1]

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**ABSTRACT:** Inclusion of *Bos indicus* genetics improves production traits of cattle maintained in hot climates. Limited information exists detailing pregnancy-specific events as influenced by variable amounts of *Bos indicus* genetics. Three experiments were completed to examine the effect of *Bos taurus* and *Bos indicus* genotypes on fetal size and plasma pregnancy-associated glycoprotein (PAG) concentrations. In all experiments, cows were bred by AI after synchronization of ovulation. Fetal measurements were completed by transrectal ultrasonography and plasma PAG concentrations were quantified from plasma harvested the day of each fetal measurement. In Exp. 1, fetal size and plasma PAG concentrations were measured at d 53 of pregnancy in cows composed of various fractions of Angus and Brahman ($n$ = 9 to 21 cows/group). Fetus size was greater in cows containing >80% Angus genetics compared with cows containing <80% Angus influence (3.40 ± 0.28 vs. 2.86 ± 0.28 cm crown-rump length; $P$ < 0.01). Plasma PAG concentrations were reduced ($P$ < 0.01) in cows containing >80% Angus genetics when compared with their contemporaries (6.0 ± 1.5 ng/mL vs. 9.4 ± 1.5 ng/mL). In Exp. 2, fetal measurements and plasma PAG concentrations were determined at d 35 and 62 of pregnancy in Angus and Brangus cows. Breed did not affect fetus size at d 35, but Angus cows contained larger fetuses than Brangus cows at d 62 [3.0 ± 0.03 vs. 2.8 ± 0.03 cm crown-nose length (CNL; $P$ > 0.01)]. Plasma PAG concentrations were not different between breed at d 35 and 62 ($P$ > 0.1). In Exp. 3, fetal measurements and plasma samples were collected at d 33/34, 40/41, 47/48, and 54/55 post-AI in Angus and Brangus cows. Fetus size was not different ($P$ > 0.05) between genotypes on d 33/34, 40/41, and 47/48. Angus fetuses were larger than Brangus fetuses at d 54/55 (2.1 ± 0.03 vs. 1.9 ± 0.03 cm CNL; $P$ = 0.001). Plasma PAG concentrations were less in Angus than Brangus cows at each time point (average 4.9 ± 0.9 vs. 8.2 ± 0.9 ng/mL; $P$ = 0.005). In conclusion, these studies determined that the *Bos taurus* × *Bos indicus* genotype impacts fetal size and rate of fetal development by 7 wk of gestation. Plasma PAG concentrations were increased in cattle with *Bos indicus* genetics in 2 of 3 studies, suggesting that genotype is one of several determinants of PAG production and secretion in cattle.

**Key words:** *Bos indicus*, *Bos taurus*, fetus, placenta, pregnancy



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## INTRODUCTION

Many cow-calf producers located in hot climates throughout the United States use *Bos indicus* genetics to limit the impacts of heat stress, parasites, and low forage quality on productivity (Turner, 1980; Hansen, 2004). Various *Bos taurus* × *Bos indicus* crossbreeding strategies and *Bos indicus*-based cattle breeds (e.g., Brangus,

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Braford) are prevalent in the Gulf Coast region (Gregory and Cundiff, 1980). A predicted rise in global temperatures likely will intensify the incorporation of *Bos indicus* genetics into traditional beef management systems worldwide due to their thermo-tolerant nature.

Several reproductive events are altered in *Bos indicus* cattle by comparison with their *Bos taurus* counterparts. Breeds containing *Bos indicus* genetics exhibit longer gestation lengths (Reynolds et al., 1980; Riley et al., 2007) and divergent fetal growth patterns during mid and late gestation (Ferrell, 1991; O'Rourke et al., 1991). These differences in growth rates may reflect differences in subspecies placental function (Ferrell, 1991). The ontogeny of this protracted growth rate and placental involvement in this process remain undefined.

Circulating pregnancy-associated glycoproteins (**PAG**) are inactive aspartic proteases produced by placental binucleate cells and released into the maternal bloodstream from approximately d 24 to term in cattle (Telugu et al., 2009). The biological functions of PAG are uncertain, but their placental-specific expression permits their usage for diagnosing pregnancy in cattle, sheep, and many other ruminants, including exotic and wild species. They are also being used as early indicators of pregnancy failure in cattle (Gabor et al., 2007). It remains unknown if PAG concentrations are altered in *Bos indicus* cattle. The overall aim of this work was to examine how *Bos indicus* genetics impact fetal development and plasma PAG concentrations during the first 2 mo of pregnancy.

## MATERIALS AND METHODS

All animal use was completed in accordance with and by the approval of the Institute of Food and Agricultural Sciences Animal Care and Use Committee at the University of Florida.

### *Experimental Design*

***Experiment 1.*** This study was completed at the University of Florida Beef Research Unit, Gainesville. Ninety primiparous and multiparous beef cows were used. Genotypes were included Brangus cows ($n$ = 15) and various proportions of Angus and Brahman crossbreds ($n$ = 75; Elzo et al., 2012). Cows were maintained on bahiagrass (*Paspalum notatum*) pasture with coastal bermudagrass (*Cynodon dactylon*) hay, and were supplemented with wet brewers grains, soyhulls, and millet silage from the time estrous synchronization began until pregnancy diagnosis.

Cows were separated into 3 groups according to calving date and began synchronization of estrus an average of 86 d postpartum (range: 55 to 397 d) by administering GnRH (100 μg; Cystorelin, Merial, Duluth, GA) and inserting an intravaginal progesterone device (Eazi-Breed CIDR containing 1.38 g progesterone, Zoetis Animal Health, New York). After 7 d, the insert was removed and $PGF_{2\alpha}$ (25 mg; Lutalyse, Zoetis Animal Health) was administered intramuscularly. Estrus was observed for 3 d. Cows exhibiting estrus were inseminated 12 h later. Cows not exhibiting estrus at 72 h post-CIDR removal were injected intramuscularly with 100 μg GnRH and inseminated. Multiple sires contained varying degrees of Angus and Brahman genetics were used in the multibreed herd.

Transrectal ultrasonography was completed by a single technician with an Ibex portable ultrasound, equipped with a linear 8–5 MHz multifrequency transducer (E.I. Medical Imaging, Loveland, CO), on all cows at a mean gestational stage of 53 d (range 48 to 56 d; d 0 = d of AI). Fetal crown-rump length (**CRL**) was measured and recorded (Chavatte-Palmer et al., 2006). A blood sample (5 mL) was collected at the time of transrectal ultrasonography by coccygeal venipuncture, using EDTA-treated Vacutainers (BD Diagnostics, Franklin Lakes, NJ). Blood was placed on ice until centrifugation at 1500 × $g$ at 4°C for 15 min. Plasma was collected and stored at –20°C until progesterone (**P4**) and PAG analysis. At birth, calving date, calf BW, and gender were recorded, and gestation length was calculated.

***Experiment 2.*** The study was completed at the University of Florida North Florida Research and Education Center, Marianna. Forty-two primiparous and multiparous Angus ($n$ = 17) and Brangus ($n$ = 25) cows were used. Cows were maintained on ryegrass and bahiagrass pasture, with ad libitum access to mineral supplement (Southern States, Marianna, FL) throughout the study period. Cows were separated into 2 groups, based on calving date, and began synchronization of ovulation an average of 67.7 d postpartum (range: 34 to 94 d) by administering 100 μg GnRH and vaginal CIDR insertion. After 7 d, the CIDR was removed and 25 mg $PGF_{2\alpha}$ was injected intramuscularly. After 72 h, 100 μg GnRH was administered and cows were inseminated, using semen from multiple sires within each breed. The fetal genotype was identical to the dam genotype within each breed.

Transrectal ultrasonography was completed at d 35 of pregnancy for CRL measurement and at d 62 for crown-nose length (**CNL**) measurement (Riding et al., 2008). A blood sample (5 mL) was collected at each transrectal ultrasonography event and processed as described previously. At birth, calving date, calf BW, and gender were recorded, and gestation length was calculated.

***Experiment 3.*** This study was completed at the University of Florida Santa Fe River Ranch Unit, Alachua. Seventy-six primiparous and multiparous Angus ($n$ = 43) and Brangus ($n$ = 33) cows were used (34 to 132 d postpartum, average of 81 d postpartum). Cows were provided corn gluten feed and access to bahiagrass pasture,

and either coastal bermudagrass hay or stock-piled forage throughout the study period.

For estrous synchronization, cows received 100 μg GnRH and a CIDR was inserted. The CIDR was removed after 5 or 7 d, depending on parity (5 d for primiparous, 7 d for multiparous cows), and 2 $PGF_{2\alpha}$ injections (25 mg) were administered 8 h apart. Estrous detection was monitored for 72 h after CIDR removal. Cows were inseminated 8 to 12 h after detecting estrus. Multiple sires were used within each breed. The fetal genotype was identical to the dam genotype within each breed.

Transrectal ultrasonography was completed over 2 d each week for 4 wk at d 33/34, 40/41, 47/48, and 54/55 of pregnancy by using an Aloka 500V machine, equipped with a 5.0 MHz transducer (Corometrics Medical Systems, Wallingford, CT). Crown-rump length was measured on d 33/34, 40/41, and 47/48, and CNL was determined on d 54/55 of pregnancy. A blood sample was collected at each transrectal ultrasonography event and processed as described previously. At birth, calving date, calf BW, and gender were recorded, and gestation length was calculated.

### *Progesterone Quantification*

Plasma P4 concentrations were determined by a solid-phase RIA (Coat-A-Count Progesterone kit, DPC Diagnostic Products Corp., Los Angeles, CA; Seals et al., 1998). The standard curve dilution consisted of duplicate uncoated tubes for total counts and nonspecific binding. A 100-μL aliquot of increasing progesterone concentrations (0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 ng/mL) was used to establish the standard curve. The intra-assay CV was 1.3% for samples analyzed in Exp. 1 and 2, and 1.0% for samples analyzed in Exp. 3. All samples within each study were completed in a single assay.

### *Quantification of Pregnancy-associated Glycoproteins*

Plasma PAG concentrations were determined by ELISA as described previously (Green et al., 2005) with slight modifications. A pool of 3 anti-PAG monoclonal antibodies, recognizing different placental binucleate cell-specific PAG (**bPAG**; bPAG4, 6, 7, 16, 20, and 21), was used for antigen absorption. A polyclonal antiserum with broad specificity for PAG was used as the primary antibody in the ELISA. An alkaline phosphatase-conjugated antirabbit antibody was used for detection of immune complexes. Samples were completed in duplicates. Serial dilutions of PAG standards (in nonpregnant heifer serum) were added to duplicate wells for the standard curve. Intra-assay and interassay (between plates within the same experiment) CV were 8.9% and 12.0%, respectively, in Exp. 1; 8.4% and 10.4%, respectively, in Exp. 2; and 8.2% and 18.6%, respectively, in Exp. 3.

### *Statistical Analyses*

Statistical significance was indicated at $P < 0.05$ and a tendency was indicated at $P < 0.1$.

***Experiment 1.*** Data for the Angus-Brahman multibreed cattle were grouped according to the fraction of Angus genetics. Data were examined based on the proportion of Angus genetics for the dam (maternal effect) and the fetus. Brangus cows and fetuses obtained from mating Brangus cattle with Brangus sires were maintained as their own group. The effects of estrous synchronization and timed AI group, genotype, calf gender, and their interactions on CRL, P4 concentrations, PAG concentrations, calf BW, and gestation length were determined with ANOVA, using GLM (SAS Inst. Inc., Cary, NC). The day of pregnancy at transrectal ultrasonography was used as a covariate in CRL, P4, and PAG analyses. Effects of genotype were examined further by completing these orthogonal contrasts: 1) >80% Angus vs. all other groups, 2) Brangus vs. ≤20 to 78% Angus, 3) 65 to 78% vs. ≤20 to 59% of Angus, 4) 40 to 59% vs. ≤20 to 38% Angus, and 5) ≤20% vs. 21 to 38% Angus. Regression analyses were completed to examine associations between genotype, PAG concentrations, P4 concentrations, and fetal measurements. All breed groups, including Brangus, were included in these analyses.

***Experiment 2.*** Effects of estrous synchronization and timed AI group, breed, calf gender, day of gestation, and their interactions on PAG, P4, fetal measurements, calf BW, and gestation length were analyzed using GLM (SAS). Regression analyses were completed to examine whether PAG concentrations were associated with P4 concentrations or fetal measurements, independent of breed.

***Experiment 3.*** The effects of breed, day of gestation, calf gender, and their interactions on PAG, P4, fetal measurements, calf BW, and gestation length were analyzed using a generalized linear mixed model procedure (**GLIMMIX**; SAS). Data with repeated measurements over time within the same experimental unit were analyzed with cow (genotype by day) as the random effect in the model. Several covariate structures were tested and the 1 that resulted in the lowest Akaike information criterion was used (first-order autoregressive). Estrous synchronization protocol (5 d vs. 7 d CIDR exposure) was not included in the model. The GLM was used to examine effects of breed, calf gender, and their interaction on PAG and P4 concentrations that were averaged across week. Also, GLM was used to examine differences in the change in fetal length from the first to the final measurement. Regression analyses were completed to examine if PAG concentrations were associated with P4 concentrations or fetal measurements.

**Table 1.** Effects of maternal genotype on plasma pregnancy-associated glycoprotein (PAG), progesterone (P4) concentrations, and fetal size at d 48 to 56 of pregnancy, and on gestational measurements at term (Exp. 1)

| Maternal Angus enetics,[1] % | No. cows | PAG, ng/mL | P4, ng/mL | CRL,[2] cm (n) | Gestation length, d | Birth weight, kg |
|---|---|---|---|---|---|---|
| ≥80 | 17 | 6.0 | 8.0 | 3.4 (9) | 281.3 | 35.0 |
| 65 to 78 | 15 | 9.7 | 9.2 | 2.8 (9) | 283.1 | 33.4 |
| 40 to 59 | 21 | 8.4 | 10.7 | 3.0 (14) | 287.3 | 35.6 |
| 21 to 38 | 9 | 8.4 | 10.3 | 3.0 (6) | 286.9 | 29.8 |
| ≤20 | 13 | 11.5 | 9.6 | 2.8 (7) | 293.8 | 34.1 |
| Brangus | 15 | 9.9 | 7.2 | 2.6 (7) | 283.6 | 34.3 |
| SE | – | 1.5 | 1.0 | 0.3 | 1.8 | 1.9 |
| Contrast 1[3] | – | <0.01 | 0.2 | <0.01 | <0.01 | 0.44 |
| Contrast 2[3] | – | 0.77 | 0.02 | 0.26 | 0.07 | 0.65 |
| Contrast 3[3] | – | 0.36 | 0.42 | 0.54 | <0.01 | 0.91 |
| Contrast 4[3] | – | 0.54 | 0.56 | 0.72 | 0.22 | 0.14 |
| Contrast 5[3] | – | 0.13 | 0.65 | 0.68 | 0.06 | 0.14 |

[1]Cows were grouped according to the percentage of Angus genetics. Measurements were collected once on a single day (range d 48 to 56 of gestation, mean = d 53 of gestation).

[2]CRL = crown-rump length.

[3]*P*-values for the effects of genotype examined by completing orthogonal contrasts: 1) >80% Angus vs. all other groups, 2) Brangus vs. ≤20 to 78% Angus, 3) 65 to 78% vs. ≤20 to 59% of Angus, 4) 40 to 59% vs. ≤20 to 38% Angus, and 5) ≤20% vs. 21 to 38% Angus.

## RESULTS

### *Experiment 1*

Fetal measurements and blood samples were collected once in early gestation from cows with various degrees of Angus and Brahman fractions, as an initial assessment of whether cow and fetal genotype are associated with PAG and P4 concentrations, and early fetal development. The day of gestation when measurements and blood sampling occurred ranged from d 48 to 56 of gestation (mean = 53 d).

The effects of maternal genotype on plasma PAG and P4 concentrations and CRL, gestation length, and calf BW are presented in Table 1. There was a strong tendency ($P = 0.06$) for an overall effect of genotype on plasma PAG concentrations. By using orthogonal contrasts, plasma PAG concentrations were less ($P < 0.01$) in cows with >80% Angus genetics than other cows (6.0 ± 1.5 vs. 9.6 ± 1.5 ng/mL, respectively). No differences ($P > 0.1$) in PAG concentrations were detected in other contrasts. Correlation analysis detected a linear relationship between plasma PAG concentrations and Angus genotype, with PAG concentrations decreasing as the percentage of Angus genetics increased ($r^2 = 0.07$; $P = 0.01$; Fig. 1).

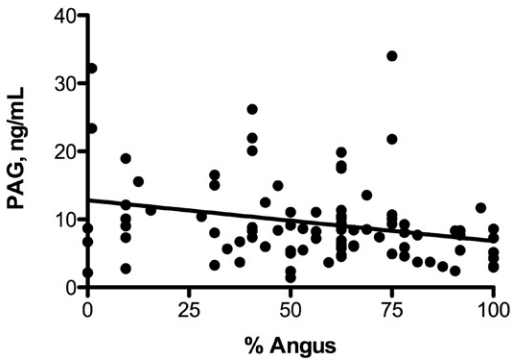


**Figure 1.** The linear relationship between plasma pregnancy-associated glycoprotein (PAG) concentrations and percentage of Angus genetics in cows. The percentage of Angus genetics in each cow was plotted against the corresponding plasma PAG value obtained from a single sample at d 48 to 56 of pregnancy in Exp. 1. $P = 0.01$. CRL = crown-rump length.

Concentrations of P4 were not different ($P > 0.1$) between cows containing >80% Angus genetics and all other cows. However, Brangus cows had lower ($P = 0.02$) plasma P4 concentration than cows containing ≥80% Angus genetics (7.2 ± 1.0 vs. 9.9 ± 1.0 ng/mL, respectively).

An overall effect of maternal genotype on CRL was not observed ($P > 0.1$), but CRL was greater ($P < 0.01$) in cows with >80% Angus genetics than all remaining cows combined (3.4 ± 0.3 vs. 2.8 ± 0.03 cm, respectively). No other differences in CRL were detected among other genotype comparisons.

There was an overall effect of maternal genotype on gestation length ($P < 0.01$). Cows with >80% Angus had reduced ($P < 0.01$) gestation lengths compared with the remaining combined groups (281.3 ± 1.8 vs. 286.8 ± 1.8 d, respectively). Also, several comparisons indicated that cows with greater amounts of Brahman genetics contained prolonged gestation lengths when compared with cohorts containing a greater percentage of Angus genetics. A linear correlation between gestation length and percentage of Angus genetics was detected ($r^2 = 0.29$; $P < 0.01$), where increases in gestation length were associated with cows containing less Angus genetics. Birth weight was not affected by maternal genotype. Also, PAG, P4, and fetal size were not correlated with calf BW or gestation length.

Because fetal genotype was not always identical to maternal genotype, an additional set of analyses was completed to examine how fetus genotype impacted plasma PAG and P4 concentrations, CRL, gestation length, and calf BW (Table 2). No overall effect of fetal genotype on plasma PAG concentrations was detected ($P > 0.1$). Orthogonal contrasts identified differences ($P = 0.05$) in PAG concentrations between fetuses with 20 to 38% Angus composition vs. 40 to 59% Angus genetics of unknown relevance. No correlation was detected between the percentage of Angus genetics in fetuses and PAG concentrations ($P > 0.1$).

No overall effects of fetal genotype on plasma P4 concentrations were detected ($P > 0.1$). However, P4 concentrations were decreased ($P = 0.04$) in fetuses containing >80% Angus genetics when compared with the combined

**Table 2.** Influence of fetal genotype on maternal plasma pregnancy-associated glycoprotein (PAG), progesterone (P4) concentrations, and fetal size at d 48 to 56 of pregnancy, and on gestational measurements at term (Exp. 1)

| Fetal Angus Genetics,[1] % | No. fetuses | PAG, ng/mL | P4, ng/mL | CRL,[2] cm (n) | Gestation length, d | Birth weight, kg |
|---|---|---|---|---|---|---|
| ≥80 | 25 | 8.0 | 7.7 | 3.1 (15) | 281.2 | 34.4 |
| 65 to 78 | 7 | 10.1 | 9.3 | 2.6 (3) | 281.6 | 30.8 |
| 40 to 59 | 16 | 7.0 | 9.7 | 3.2 (9) | 286.5 | 35.4 |
| 21 to 38 | 9 | 10.2 | 11.6 | 2.9 (4) | 288.7 | 35.1 |
| ≤20 | 23 | 11.6 | 10.0 | 2.9 (15) | 290.9 | 32.7 |
| Brangus | 10 | 11.1 | 7.8 | 2.6 (6) | 284.4 | 36.2 |
| SE | – | 1.6 | 1.1 | 0.2 | 2.1 | 2.0 |
| Contrast 1[3] | – | 0.17 | 0.04 | 0.12 | <0.01 | 0.89 |
| Contrast 2[3] | – | 0.53 | 0.09 | 0.24 | 0.42 | 0.32 |
| Contrast 3[3] | – | 0.85 | 0.52 | 0.26 | <0.01 | 0.22 |
| Contrast 4[3] | – | 0.05 | 0.37 | 0.31 | 0.2 | 0.45 |
| Contrast 5[3] | – | 0.57 | 0.31 | 0.97 | 0.56 | 0.45 |

[1]Fetuses were grouped according to the percentage of Angus genetics. Measurements were collected once on a single day (range d 48 to 56 of gestation, mean = d 53 of gestation).

[2]CRL = crown-rump length.

[3]*P*-values for the effects of genotype examined by completing orthogonal contrasts: 1) >80% Angus vs. all other groups, 2) Brangus vs. ≤20 to 78% Angus, 3) 65 to 78% vs. ≤20 to 59 of Angus, 4) 40 to 59% vs. ≤20 to 38% Angus, and 5) ≤20% vs. 21 to 38% Angus.

groups (7.7 ± 1.1 vs. 9.7 ± 1.1 ng/mL, respectively). Also, there was a tendency for decreased ($P = 0.09$) P4 concentrations in Brangus fetuses when compared with fetuses containing <78% Angus genetics (7.8 ± 1.1 vs. 10.1 ± 1.1 ng/mL, respectively). There was no correlation between plasma PAG and P4 concentrations. Fetal genotype did not affect CRL.

An overall effect of fetal genotype on gestation length was observed ($P < 0.01$). Fetuses containing >80% Angus genetics had shorter ($P < 0.01$) gestation lengths than other genotypes (281.2 ± 2.1 vs. 287.4 ± 2.1 d, respectively). Also, fetuses with 65 to 78% Angus genetics had shorter ($P < 0.01$) gestation lengths than fetuses with <60% Angus genetics (280.3 ± 2.1 vs. 288.6 ± 2.1 d, respectively). A linear correlation was observed ($r^2 = 0.29$; $P = 0.05$) between gestation length and fetal genotype, with extended gestation lengths associated with fetuses containing more Brahman genetics. Calf BW was not affected by fetal genotype. Neither PAG nor P4 concentrations were correlated with calf BW or gestation length.

### *Experiment 2*

The study examined PAG and P4 concentrations and fetal measurements at d 35 and 62 of pregnancy between Brangus and Angus cattle located at the North Florida Research and Education Center, Marianna (Table 3). No effects of breed were detected for plasma PAG concentrations ($P > 0.1$), but PAG concentrations were greater at d 35 than at d 62 across both breeds ($P < 0.0001$; 4.7 ± 0.3 vs. 2.3 ± 0.3 ng/mL, respectively). No time by breed interactions were detected.

**Table 3.** Impact of Angus vs. Brangus genetics on plasma pregnancy-associated glycoprotein (PAG), progesterone (P4) concentrations, and fetal size at d 35 and 62 of gestation, and on gestation length and calf birth weight (Exp. 2)

| Parameter[1] | Brangus | Angus | Pooled SE | *P*-value |
|---|---|---|---|---|
| No. cows | 25 | 17 | – | – |
| PAG, ng/mL | | | | |
| d 35 | 4.5 | 5.0 | 0.4 | 0.3 |
| d 62 | 2.5 | 2.0 | 0.3 | 0.3 |
| P4, ng/mL | | | | |
| d 35 | 8.9 | 8.6 | 0.9 | 0.3 |
| d 62 | 7.5 | 7.9 | 0.9 | 0.2 |
| Fetus size, cm | | | | |
| CRL[2] d 35 | 1.5 | 1.5 | 0.03 | 0.9 |
| CNL[2] d 62 | 2.8 | 3.0 | 0.03 | <0.01 |
| Gestation length, d | 283.4 | 279.8 | 1.9 | 0.04 |
| Birth weight, kg | 35.3 | 34.8 | 1.4 | 0.7 |

[1]Blood samples and ultrasonographic data were collected at d 35 and 62 of gestation.

[2]CRL = crown-rump length; CNL = crown-nose length.

No overall effects of breed on P4 concentrations were detected at d 35 or 62 ($P > 0.1$). A linear relationship between PAG and P4 concentrations was detected across both breeds at d 35 ($P = 0.01$), where greater PAG concentrations were associated with greater P4 concentrations ($r^2 = 0.11$). No correlations were detected at d 62.

No overall effect of breed on fetal measurements was detected at d 35 ($P > 0.1$), but Angus fetuses were greater in size than Brangus fetuses at d 62 ($P < 0.01$; Table 3). Gestation length was affected by breed and was shorter ($P < 0.01$) in Angus than Brangus cows. Breed did not affect calf BW ($P > 0.1$). Also, there was no correlation between fetus length at d 35 or 62 on subsequent BW ($P > 0.1$). Neither PAG nor P4 concentrations were correlated with calf BW or gestation length ($P > 0.1$).

### *Experiment 3*

A final study was completed with a large group of Angus and Brangus cattle to further examine the potential influence of *Bos indicus* genetics on PAG and P4 concentrations and early fetal development (Fig. 2). Plasma PAG concentrations were greater ($P < 0.05$) in Brangus than Angus cows at each weekly measurement. The PAG concentrations decreased ($P < 0.01$) in each breed during the 4-wk collection period. Concentrations of PAG were greater ($P < 0.01$) in Brangus than Angus cows after being averaged across the 4-wk collection period (Table 4).

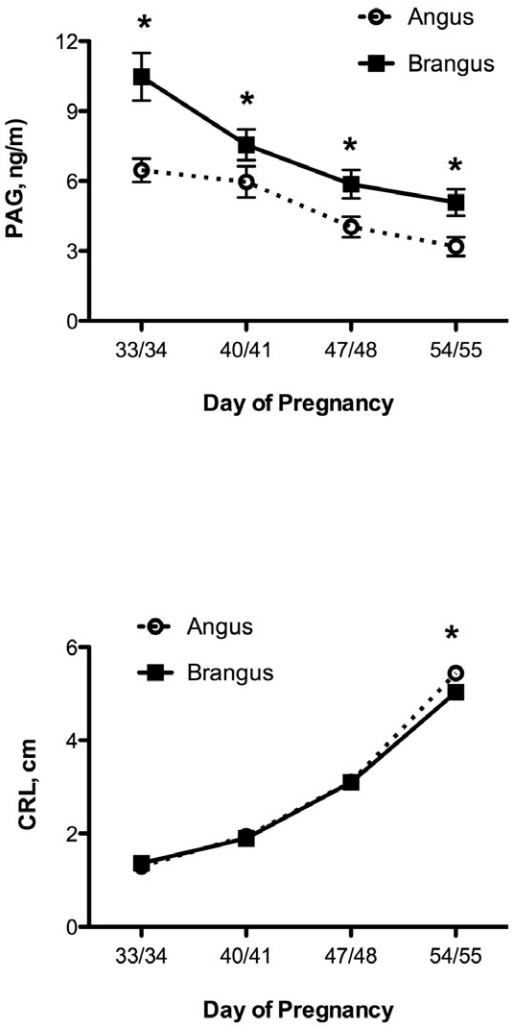


**Figure 2.** Plasma pregnancy-associated glycoprotein (PAG) concentrations and fetal size for Angus and Brangus cows throughout early gestation in Exp. 3. Plasma PAG concentrations and fetus size were determined weekly from d 32/33 to 54/55 of pregnancy. The * indicates differences between breeds ($P < 0.05$). Fetus size at d 54/55 was analyzed by using the original crown-nose length (CNL) data but was graphed after conversion to crown-rump length (CRL), using the equation described by Riding et al. (2008) so that the progression in fetal size could be compared across all weeks.

Plasma P4 concentrations were not affected by breed or week of collection. Pair-wise comparisons detected a tendency for greater ($P = 0.07$) P4 concentrations in Brangus than Angus cows at d 33/34 (9.4 ± 0.4 vs. 8.5 ± 0.4 ng/mL, respectively). No differences ($P > 0.1$) in P4 concentrations were detected at other time points. Regression analysis identified a linear relationship between greater PAG and greater P4 concentrations at d 40/41 ($r^2 = 0.03$; $P < 0.01$) and 54/55 ($r^2 = 0.02$; $P = 0.05$) but not at the other time points. Plasma PAG and P4 concentrations were not associated with eventual calf BW and gestation length at any time point.

Fetus size was not different between breeds at the first 3 measurement dates, but Angus cows contained larger ($P < 0.05$) fetuses than Brangus cows at d 54/55 (Fig. 2). Also, the total change in fetus length between d 33/34 and 54/55 was greater ($P < 0.05$) for Angus than Brangus cows (4.2 ± 0.1 vs. 3.7 ± 0.1 cm, respectively). Angus cows had shorter ($P = 0.01$) gestation lengths than Brangus cows (Table 4). Also, Angus calves weighed less ($P = 0.03$) at birth than Brangus calves (Table 4). There was a tendency ($P < 0.1$) for calf gender to affect overall mean plasma PAG concentrations (5.7 ± 0.8 vs. 7.5 ± 0.8 ng/mL PAG for males vs. females, respectively). This effect was not detected when examining PAG concentrations within day.

**Table 4.** Influence of Angus vs. Brangus breeding on mean plasma pregnancy-associated glycoprotein (PAG) and progesterone (P4) concentrations, across several time points, and on gestation length and calf birth weight (Exp. 3)

| Traits[1] | Angus | Brangus | SE | *P*-value |
|---|---|---|---|---|
| No. cows | 43 | 33 | – | – |
| Pooled PAG, ng/mL | 4.8 | 8.0 | 0.8 | <0.01 |
| Pooled P4, ng/mL | 8.7 | 8.9 | 0.3 | 0.68 |
| Gestation length, d | 280.4 | 282.8 | 0.7 | 0.01 |
| Birth weight, kg | 35.0 | 37.6 | 0.8 | 0.03 |

[1]Mean plasma PAG and P4 concentrations are shown from samples collected at d 33/34, 40/41, 47/48, and 54/55 of pregnancy.

## DISCUSSION

A plethora of physiological factors, such as fetus number, fetus gender, parity, and lactation status, and manipulations, such as in vitro embryo development and nuclear cloning, influence PAG concentrations (Patel et al., 1995; Chavatte-Palmer et al., 2006; Lopez-Gatius et al., 2007a; Constant et al., 2011). There are also indications that PAG concentrations differ between genotypes. For example, serum PAG concentrations differ between Ethiopian Boran and Boran × Holstein-Friesian crossbreds during pregnancy (Lobago et al., 2009). Differences in PAG concentrations also exist between Texel and Suffolk ewes (Vandaele et al., 2005). There are also indications that PAG profiles in Zebu, a *Bos indicus* breed, differ from that of *Bos taurus* cattle (de Sousa et al., 2003).

The present studies provide new insights into the relationship between PAG concentrations and subspecies genotype in early pregnancy. The first experiment used a multibreed Angus-Brahman herd at the University of Florida to examine the impact of genotype on plasma PAG concentrations. Cattle containing ≥20% Brahman genetics contained greater PAG concentrations than those with >80% Angus genetics at a mean gestational age of 53 d. Two subsequent experiments were completed to assess PAG concentrations at multiple time points during early gestation. In Exp. 2, plasma PAG concentrations were not statistically different at d 35 and 62 of pregnancy, whereas in Exp. 3 PAG concentrations were greater in Brangus cows at all days examined (d 33 to 55). The reason for

these disparities between studies is not clear. Any combination of differences in genetic pool, diet, and management may have caused outcomes to change in these experiments. Several noteworthy differences were detected in Exp. 2 and 3. Plasma PAG concentrations of Angus cows were similar between Exp. 2 and 3, but Brangus cows had greater PAG concentrations in Exp. 3 than Exp. 2. Also, Brangus cows in Exp. 3 produced offspring that were heavier than Angus calves at birth, whereas no such differences in birth weight were detected in Exp. 2.

One hallmark feature of PAG profiles in cattle and other ruminants is their substantial increase in plasma concentrations during mid and late gestation (Sasser et al., 1986; Green et al., 2005). However, there is a short-lived period in the second month of gestation when plasma PAG concentrations decrease (Sasser et al., 1986; Green et al., 2005; Thompson et al., 2010). Plasma PAG concentrations peaked in lactating Holstein cows at d 30 to 32, then decreased to approximately half the peak concentrations by d 60, before beginning to increase again (Thompson et. al., 2010). The same reduction in plasma PAG concentrations was detected in these studies during the second month of pregnancy in both *Bos taurus* and *Bos indicus* breeds, and crossbreds. The physiological importance of this biphasic PAG profile in early pregnancy is not known.

There is an ever-growing body of evidence indicating that PAG profiles are a useful indicator of placental fitness and pregnancy success. Several studies detected a relationship between abnormal PAG concentration in early pregnancy (d 30 to 39) and eventual pregnancy loss in lactating dairy cows (Humblot, 2001; Gabor et al., 2007; Lopez-Gatius et al., 2007b; Thompson et al., 2010). In 1 study, pregnancy loss was 10 times greater in cows with low PAG concentrations and 6.8 times greater in cows with high PAG concentrations (Lopez-Gatius et al., 2007b). Too few cows lost their pregnancy after the initial pregnancy diagnosis in these experiments to complete a statistical assessment for a link between PAG and pregnancy failure. No pregnancy losses occurred after ultrasonography at d 53 in Exp. 1 and 2 cows each in Exp. 2 and 3 lost their pregnancies after their first ultrasonography. Each case of pregnancy loss was associated with either reduced ($n$ = 3) or increased ($n$ = 1) PAG concentrations before pregnancy failure. Also, in 2 of the 4 cases, changes in PAG concentrations preceded notable reductions in fetal size.

The PAG ELISA used in these experiments used antisera that reacted against PAG found predominantly during early gestation. These PAG had a shorter half-life in plasma than some of the PAG produced later in gestation, thereby limiting the potential reactivity with PAG that remain from the previous gestation (Green et al., 2005). Indeed, no false pregnancy detections existed in these experiments. Differences in overall PAG concentrations were evident between experiments. Notably, animals from the first experiment had greater overall plasma PAG concentrations than cows sampled at similar times in the other studies. This variation could be attributed to assay-to-assay variation, because samples from Exp. 1 were assayed at a different time than samples from Exp. 2 and 3. The same reference plasma sample was not used across all 3 experiments.

Effects of genotype on plasma P4 concentration were generally not observed. In Exp. 1, an increase in the proportion of Brahman genetics was linked with greater P4 concentrations, but this phenomenon was not detected in the other experiments, except for during 1 of the 4 wk of sample collections in Exp. 3. Previous studies found that *Bos indicus*-based breeds have smaller CL and reduced plasma P4 concentrations (Segerson et al., 1984; Bo et al., 2003). However, this effect of genotype on plasma P4 concentrations is seasonal. *Bos indicus*-based genotypes tend to have greater plasma P4 concentrations in the spring and summer, and reduced plasma P4 concentrations in winter and fall than *Bos taurus* breeds (McNatty et al., 1984; Bo et al., 2003). The 3 experiments described herein were conducted during late spring/early summer. Therefore, *Bos indicus*-based breeds would be expected to contain equivalent or greater plasma P4 concentrations than *Bos taurus* breeds. The mechanisms by which day length and temperature affect reproductive function in cattle are not well established.

Correlations between plasma PAG and P4 concentrations were detected in 2 of the 3 experiments. No such correlation was detected in Exp. 1, possibly because of the large range of genotypes and low animal numbers within genotype groups. In Exp. 2, this correlation was observed at d 35 and not d 62; and in Exp. 3, it was detected at d 40/41 and 54/55, but not at other days. The biological link between PAG and P4 production is tenuous. Studies have detected increases in luteal P4 production after PAG supplementation (Del Vecchio et al., 1996; Weems et al., 2007). However, other studies failed to detect this effect (Del Vecchio et al., 1995). There is evidence that the effects of PAG on luteal function may be indirect. Production of PGE2 was increased in cultured ovine or bovine endometrium, or ovine luteal cells after PAG supplementation (Del Vecchio et al., 1990; Weems et al., 2003, 2007). Based on these observations, it is possible that PAG and P4 production are linked physiologically. However, our inability to consistently detect associations between PAG and P4 concentrations suggests that this link is weak. Regardless of the dependency of PAG and P4 on one another, it is clear that normal production of each factor is requisite for maintenance of pregnancy to term (Gabor et al., 2007).

Another important focus of this work was to examine how *Bos indicus* genetics impact early fetal growth. In each experiment, *Bos taurus* fetuses were larger than *Bos indicus* fetuses at d 53 to 62 of pregnancy but not at earlier time points. Also, in Exp. 3, the change in fetal size between d 33/34 and 54/55 was greater for Angus than

Brangus. This is consistent with previous findings (Ferrell, 1991; O'Rourke et al., 1991). By examining fetal development at slaughter, O'Rourke et al. (1991) identified delays in fetal growth as determined by measuring CRL, heart girth, and foreleg size, when comparing *Bos indicus* breeds (Sahiwal, Africander, Brahman) with *Bos taurus* breeds (Hereford, Simmental). Ferrell (1991) reported that *Bos taurus* fetuses (Charolais) were 70% and 84% greater in weight than *Bos indicus* fetuses (Brahman) after 33 and 38.5 wk of gestation, respectively. Weights of placental membranes were also greater for Charolais than Brahman at these time points, and both breeds contained similar fetal to placental membrane ratios, indicating that placental efficiency is likely similar at each time point. Reciprocal transfers were also examined to establish the relative contribution of fetal and maternal components to fetal growth. At wk 33, weight of Charolais fetuses gestating in Brahman cows was similar to the weight of Charolais fetuses gestating in Charolais cows. Weight of Brahman fetuses was also similar for those gestating in Charolais and Brahman cows. This indicates that fetal genotype is a primary determinant of fetal development during the first 7 mo of gestation. However, the maternal genotype dictates fetal growth in the final 6 to 7 wk of gestation. The rate of fetal growth between wk 33 and 38.5 was 2.4 times greater for Charolais fetuses in Charolais cows than Charolais fetuses in Brahman cows. The growth rate of Brahman fetuses in Charolais cows was also greater than Brahman fetuses in Brahman cows, although the magnitude of this response was less pronounced (1.4-fold effect).

The multibreed Angus-Brahman herd permitted examination of maternal and fetal influences on PAG and P4 concentrations, and fetal measurements. Granted, only a subset of the pregnancies differed based on maternal and fetal genotype, but these differences had important consequences on PAG, P4, and CRL outcomes. Although trends were similar when examining outcomes using maternal and fetal genotype, no changes in PAG concentrations and CRL were detected based on fetal genotype, suggesting that maternal genotype is a determinant of early fetal development. This outcome is contrary to that of Ferrell (1991). However, these studies differ in the period of pregnancy being investigated (early vs. mid/late gestation) and breeds of animals used (Angus/Brangus vs. Charolais/Brahman). Nonetheless, previous observations and present findings indicate that differences in rates of fetal development are evident both early in pregnancy and during the final 6 to 7 wk of gestation. Compensatory fetal growth between early and mid gestation may explain why differences in fetal size were detected at wk 7 to 8 (present work) but not wk 33 of pregnancy. However, this conjecture has yet to be tested.

It appears that *Bos indicus* cattle may compensate for delays in fetal development by extending their gestation length. Traditionally, *Bos indicus*-based breeds have extended gestation lengths and yield calves with similar or greater birth weights than *Bos taurus* breeds (Reynolds et al., 1980; Riley et al., 2007). The present work observed gestation length extensions in cattle with *Bos indicus* genetics. Birth weights were similar between *Bos indicus* and *Bos taurus* in 2 experiments, but an increase in *Bos indicus* birth weight was detected in the last experiment.

It is interesting that *Bos indicus*-based genetics produce smaller fetuses and greater plasma PAG concentrations than complementary *Bos taurus* pregnancies. Elevated plasma PAG concentrations exist in physiological scenarios with placental insufficiencies, including pregnancies generated by nuclear transfer cloning technologies (Chavatte-Palmer et al., 2006; Constant et al., 2011). Too little mechanistic information exists to provide insight into whether a placental insufficiency phenomenon exists in cattle containing *Bos indicus* genetics. A more reasonable presumption is that differences in PAG profiles reflect yet another genotype-dependent alteration in the normal progression of pregnancy between *Bos taurus* and *Bos indicus*.

In summary, *Bos indicus* genetics alter plasma PAG concentrations and fetal size during early pregnancy. Plasma PAG concentrations were greater in cows containing *Bos indicus* genetics between d 32 and 62 of pregnancy in 2 of the 3 experiments. Also, fetus size was similar between *Bos indicus* and *Bos taurus* genotypes in early pregnancy (d 33 to 48) but was reduced in *Bos indicus* cattle thereafter (d 53 to 62). The physiological significance of these findings remains speculative, but these observations indicate that the genetic basis for differences between these subspecies can be detected early during embryonic and fetal development.

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