Impact of fetal vs. maternal contributions of *Bos indicus* and *Bos taurus* genetics on embryonic and fetal development¹

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ABSTRACT: To evaluate how the inclusion of *Bos indicus* genotype influences early fetal development in cattle, a reciprocal embryo transfer approach was used in a completely randomized design with a 2 × 2 × 2 factorial arrangement of treatments to generate 55 pregnancies over 2 consecutive years ($n = 55$). Recipient cows were randomly assigned to (i) a diet that met daily energy maintenance requirements (**MAINT**) or (ii) a diet that restricted intake to 70% of the energy maintenance requirements (**RESTR**). Angus (**AN**) and Brangus (**BN**) embryo donors were superovulated and artificially inseminated with female sexed-sorted semen from the same breed. Embryos were then randomly transferred to either AN or BN recipients fed their respective diets for 28 d. Recipients remained on the dietary scheme until day 91 of gestation and were then comingled and fed a common diet that met their energy requirements until calving. Measurements included pregnancy establishment at day 28 of gestation, interferon-stimulated genes (**ISG**) expression in peripheral blood leukocytes, pregnancy-associated glycoproteins (**PAG**; using two commercial [A1 and A2] and one in-house

assay), and fetal crown-to-rump length (**CRL**). Recipients in the **RESTR** diet had lower BWs and BCS (diet × day; $P < 0.01$) than **MAINT** recipients. Energy-restricted AN recipients experienced greater (recipient breed × diet, $P < 0.01$) pregnancy failure by day 28 than the other recipient breed × diet combinations. Restricted recipients that received AN embryos experienced greater pregnancy failure than **RESTR** recipients receiving BN embryos (embryo breed × diet; $P = 0.03$). No relevant differences were observed in ISG expression ($P > 0.10$). Recipients that received BN embryos had greater plasma concentrations of PAG in both A1 (embryo breed × day, $P < 0.01$) and A2 (embryo breed; $P < 0.01$). Alternatively, recipients that received AN embryos had greater plasma concentrations of PAG for the in-house assay (embryo breed × day; $P < 0.01$). In addition, fetuses from AN recipients had greater CRL on day 91 (breed × day, $P < 0.01$). In summary, *Bos taurus* cows experienced greater pregnancy failure when nutrient restricted. Furthermore, fetal size and the profile of PAG production during early gestation differed between *B. indicus*-influenced and *B. taurus* cattle.

Key words: *Bos indicus*, *Bos taurus*, energy restriction, fetal development, pregnancy-associated glycoproteins, pregnancy failure

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INTRODUCTION

As a consequence of hundreds of thousands years of separate evolution, *Bos indicus* cattle have acquired genes that bestow a greater heat tolerance in comparison to *Bos taurus* cattle (Hansen, 2004). When Zebu cattle are exposed to warmer conditions, they experience less reduction of feed intake (Allen et al., 1963; Seif et al., 1979), growth rate (Cartwright, 1955), and reproductive function (Rocha et al., 1998) compared with *B. taurus* cattle exposed to the same conditions. Furthermore, zebu cattle are thought to utilize low-quality feeds more efficiently than *B. taurus* breeds (Turner, 1980), having greater DMI and digestibility than European breeds under these conditions (Moore et al., 1975; Warnick and Cobb, 1976). For these reasons, cattle producers utilize *B. indicus* breeds in crossbreeding systems to optimize cattle performance in tropical/subtropical climates.

Differences between several gestational parameters are also evident between *B. indicus* and *B. taurus* cattle. Both the literature and recent findings demonstrate *B. indicus* fetuses develop at a slower rate during early (Mercadante et al., 2013) and mid-gestation (Ferrell, 1991a; O'Rourke et al., 1991). During late gestation, *B. indicus* fetuses undergo compensatory growth, having similar or even greater birth weights than *B. taurus* counterparts (Mercadante et al., 2013). The plasma concentrations of pregnancy-associated glycoproteins (PAG) during early gestation also differ between the subspecies, where *Bos indicus*-influenced cows have greater PAG concentrations than *B. taurus* (Mercadante et al., 2013).

Therefore, the objective of this study was to determine how maternal and fetal genotypes influence early fetal development in *B. indicus* and *B. taurus* cattle. We hypothesized that early fetal and placental development is modified by the inclusion of *B. indicus* genetics into the maternal genotype. In addition, since nutrient utilization and feed efficiency differ between *B. indicus* and *B. taurus* cattle (Turner, 1980; Elzo et al., 2009), we hypothesized dietary energy restriction during early pregnancy would impair conceptus development in both *B. indicus*-influenced and *B. taurus* cattle; however, we hypothesized the magnitude of the compromised development would be less evident in *B. indicus*-influenced cattle.

MATERIALS AND METHODS

All procedures involving animals were approved by the University of Florida Institutional

Animal Care and Use Committee (protocol number 201408681).

Experimental Design

A reciprocal embryo transfer approach was used in a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement of treatments in order to generate 55 pregnancies over 2 consecutive years ($n = 55$). Initially, recipient cows ($n = 197$) were randomly assigned to one of the two dietary treatments: (i) a diet to meet 100% of daily energy maintenance requirements (MAINT) or (ii) restricted intake of nutrients to 70% of daily energy maintenance requirements (RESTR). Details of the diets are discussed later. Angus (AN) and Brangus (BN) embryo donors were superovulated and artificially inseminated (AI) with female sex-sorted semen from their respective breed. Embryos were then collected and randomly transferred to either AN or BN recipients fed their respective diets; thereby, generating eight treatment combinations (AN $\times$ AN $\times$ RESTR, $n = 14$; AN $\times$ AN $\times$ MAINT, $n = 19$; AN $\times$ BN $\times$ RESTR, $n = 16$; AN $\times$ BN $\times$ MAINT, $n = 17$; BN $\times$ BN $\times$ RESTR, $n = 15$; BN $\times$ BN $\times$ MAINT, $n = 19$; BN $\times$ AN $\times$ RESTR, $n = 15$; BN $\times$ AN $\times$ MAINT, $n = 19$). Female sex-sorted semen from four AN and two BN sires was used to AI donor cows. The resulting embryos were assigned to recipients in order to randomly generate the previously described treatment combinations and to equally distribute the effects of sire, donor cow, and embryo grade score (Bó and Mapletoft, 2013).

Recipient Diets

A total of 197 AN and BN suckled beef cows were enrolled as recipient candidates for this study. All cows were housed at the University of Florida Feed Efficiency Facility at the North Florida Research and Education Center (30°46'35"N, 85°14'17"W), equipped with a GrowSafe System to monitor individual feed intake (GrowSafe System Ltd., Airdrie, AB, Canada). AN and BN cows were randomly assigned to one of two dietary treatments 28 d prior to embryo transfer (day -21): (i) Diet formulated to meet the daily energy requirements of a 550 kg beef cow (MAINT; NASEM, 2016) and (ii) Diet formulated to meet 70% of daily energy requirements (RESTR; Table 1). During the 2 consecutive years, feed samples were collected once every 2 wk throughout the feeding period and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca,

Table 1. Composition and nutrient analysis¹ of dietary treatments

Item	RESTR	MAINT
Ingredient, % (as-fed basis)		
Fiber pallets	70.0	60.0
Soybean hulls	10.0	30.0
Bermudagrass hay	5.0	5.0
Peanut hulls	15.0	5.0
Nutrient profile		
DM, %	92.3	94.8
CP, %	11.2	12.5
NDF, %	69.2	67.9
ADF, %	63.0	56.2
TDN, %	37	46
NE _l , Mcal/kg	0.73	0.97
NE _m , Mcal/kg	0.68	0.98
NE _g , Mcal/kg	0.15	0.43

¹Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).

MAINT: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pallets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls; RESTR: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls.

NY). Recipient cows remained on the described feeding scheme until day 91 of gestation, when all cows were then offered a diet meeting 100% of their energy requirements for the remainder of gestation. Average recipient BW and BCS were obtained from two consecutive measurements 24 h apart at the beginning (days –22 and –21) and at the end (days 90 and 91) of the feeding scheme. Single measurements of BW and BCS were also assessed weekly from days 28 to 91. The RESTR diet was formulated so that overcompensation by changes in feed intake between dietary treatment groups was restricted by gut fill because of the diet's bulkiness. In addition, individual feed intake combined with the nutrient analysis results allowed for calculation of the actual percentage of energy requirement met (NASEM, 2016) by the dietary treatments during the period that the feeding scheme was superimposed.

Donors

Embryo donor cows ($n = 22$ AN and 24 BN) were superovulated and AI with sexed-sorted semen from their respective breed. Donors were maintained in the same pasture and submitted to the same nutritional and environmental conditions during the experimental period. The superovulation protocol consisted of an injection of 100 μ g of GnRH (2 mL Factrel; Zoetis Animal Health,

Parsippany-Troy Hills, NJ) concurrent with the insertion of a new controlled intravaginal drug release (CIDR) insert (EAZI-BREED CIDR, Zoetis Animal Health) containing 1.38 g of progesterone (0700 hours). Starting 4 d (1800 hours) after CIDR insertion, cows were submitted to a 3-d FSH regimen (Folltropin-V; Bioniche Animal Health USA, Borgart, GA), where FSH was administered twice daily with a 12 ± 2 h interval. The CIDR insert was removed 7 d after insertion (1800 hours). On the same day as CIDR removal, donors received two injections of 25 mg of prostaglandin F_{2 α} (5 mL Lutalyse, Zoetis Animal Health) with a 12 ± 2 h (0700 and 1800 hours) interval between injections. Estrus detection patches (Estroject, Rockyway Inc., Spring Valley, WI) were applied at CIDR removal, and assessed for activation twice daily for 3 d. Estrus detection patches were considered activated when at least 50% of the patch was discolored, or when the patch was absent. Donor cows were AI with one straw of sex-sorted semen at onset of estrus, and were concurrently administered 100 μ g of GnRH. Cows were AI a second time 12 ± 2 h after the first insemination with two semen straws, and a third time 24 ± 2 h after first insemination with one straw. Nonsurgical transcervical uterine flushing was performed to recover embryos 7 d after estrus. Embryos were evaluated under a stereomicroscope, and 134 transferable embryos in the morula or blastocyst stages were considered eligible for transfer.

Recipient Estrus Synchronization and Embryo Transfer

All recipients ($n = 96$ in the first year and 101 in the second year) were submitted to the 7 d CO-Synch + CIDR estrus synchronization protocol (Larson et al., 2006) starting 12 d after the feeding scheme was initiated (day –9). Briefly, the protocol consisted of an injection of 100 μ g of GnRH concurrent with the insertion of a new CIDR insert. Seven days after CIDR insertion (day –2), the insert was removed and recipients received a 25-mg injection of prostaglandin F_{2 α} . A second 100- μ g GnRH injection was administered 48 h after CIDR removal (day 0). Transrectal ultrasonography was performed 7 d after the second GnRH injection to verify the presence of a corpus luteum using an Ibex ultrasound equipped with a linear 5 MHz multifrequency transducer (E. I. Medical Imaging, Loveland, CO). A total of 134 recipients were eligible for embryo transfer based on the presence of a corpus luteum at day 7. On the same day, donors

were flushed, and a single fresh embryo was transferred into the recipient's uterine horn ipsilateral to the corpus luteum. Average $\pm$ SEM for days postpartum was 74 ± 12 at the initiation of the estrus synchronization protocol and was not different between treatments.

Pregnancy Diagnosis and Embryo Morphometry

Diagnosis of pregnancy was performed by transrectal ultrasonography on day 28 of gestation (21 d after embryo transfer). A cow was considered pregnant if a visible embryo, together with a corpus luteum and uterine fluid consistent with pregnancy were present. Cows that received an embryo but were not pregnant at day 28 were considered to have experienced pregnancy failure. Additionally, transrectal ultrasonography was performed weekly from days 42 to 91 to assess embryo morphometry. Individual videos were recorded weekly from days 42 to 91 of gestation. The ideal position and orientation of the embryo/fetus to measure crown-to-rump length (CRL) were selected in a frame-by-frame manner, and CRL was measured. Two individuals independently measured the images twice, and the mean of each individual was used to calculate CRL estimates. Therefore, final CRL was the average of the mean obtained by each individual. On days 63, 70, 77, 84, and 91, crown-to-nose length was used to estimate CRL as previously described (Riding et al., 2008). Only recipients that remained pregnant until parturition and gave birth to a female calf were utilized in the embryo measurements analysis. Gestation length and calf birth weight were recorded at calving.

Interferon-Stimulated Genes (ISG) Expression in Peripheral Blood Leukocytes (PBL)

In order to investigate potential differences in early embryo development in the present study, blood samples were collected on days 18 and 21 of gestation during the second year of study for the

isolation of PBL according to Gifford et al. (2007) from cows that were diagnosed as pregnant at day 28 of gestation ($n = 23$). The PBL pellet was washed with ice-cold phosphate-buffered saline, centrifuged at $300 \times g$ for 10 min at 4°C , and the supernatant discarded. The pellet was resuspended with 0.8 mL of Trizol (Molecular Research Center, Inc., Cincinnati, OH), transferred into 1.5 mL microtubes, and stored at -80°C until further analysis.

Total RNA was purified and concentrated (PureLink RNA Mini Kit; Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Total RNA concentration and quality was determined by measuring absorbance at 260 and using a 280 nm NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). All RNA samples contained a 260:280 nm ratio >1.8 . One microgram of total cellular RNA was treated with deoxyribonuclease (DNase I Rnase-free; Invitrogen) before synthesis of cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Inc., Foster City, CA). Complementary DNA (50 ng) was amplified using gene-specific primers (Table 2) and SYBR Green chemistry in a 20- μL reaction (Mastercycler EP Realplex System; Eppendorf AG, Hamburg, Germany) in triplicate. Amplification parameters were 40 cycles of a two-step amplification protocol (15 s at 95°C followed by 60 s at optimized annealing temperature of 57°C and by 15 s at 95°C followed by 15 s at 60°C). Primer efficiencies ranged from 86% to 90%. Reactions were run in triplicate and exposed to a melting curve analysis to ensure amplification of a single product. Samples lacking reverse transcriptase were also included to verify that no genomic contaminants were amplified. Threshold cycle (Ct) values for reference gene, *Peptidylprolyl isomerase A* (*PPIA*; Green et al., 2010), were used for computation of delta cycle threshold (ΔCt) values. This internal control was chosen because its transcript abundance was not altered by breed, parity, time period, or treatment

Table 2. Nucleotide sequence of bovine-specific primers used in the quantitative real-time reverse transcription PCR to determine expression of target genes

Target gene	Gene name	Accession no.	Primer	Primer sequence
<i>ISG15</i>	<i>Interferon-stimulated gene 15</i>	NM_174366	Forward	5'-GGTATCCGAGCTGAAGCAGTT-3'
			Reverse	5'-ACCTCCCTGCTGTCAAGGT-3'
<i>PPIA</i>	<i>Peptidylprolyl isomerase A</i>	BF230516	Forward	5'-GCCATGGAGCGCTTTGG-3'
			Reverse	5'-CCACAGTCAGCAATGGTGATCT-3'
<i>MX2</i>	<i>Myxovirus resistance 2</i>	NM_173941	Forward	5'-CTTCAGAGACGCCTCAGTCG-3'
			Reverse	5'-TGAAGCAGCCAGGAATAGT-3'
<i>OAS1</i>	<i>2'-5'-Oligoadenylate synthetase 1</i>	NM_001040606	Forward	5'-TAGCCTGGAACATCAGGTC-3'
			Reverse	5'-TTTGGTCTGGCTGGATTACC-3'

for the particular tissue of interest. Relative fold change in gene expression was calculated with the formula $2^{-\Delta\Delta C_t}$. Intra-assay variation was assessed based on the mean standard variation of the triplicates according to [Bustin et al. \(2009\)](#), and values were 15% for *PPIA*, 37% for *Myxovirus resistance 2 (MX2)*, 25% for *2'-5'-oligoadenylate synthetase 1 (OAS1)*, and 22% for *interferon-stimulated gene 15 (ISG15)*.

Circulating PAG Concentrations

Blood samples were collected from the jugular vein into 10-mL evacuated vials containing 143 IU of sodium heparin (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) at days 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91 of pregnancy. Blood samples were immediately placed on ice and later centrifuged at $1,500 \times g$ at 4 °C for 15 min. The plasma was transferred into polypropylene tubes and stored at -20 °C until further analysis. Two commercial (A1 and A2) and one in-house ELISA were used to determine circulating concentrations of PAG in plasma. The BioPRYN Quantitative ELISA (A1; BioTracking Inc., Moscow, ID) was used to quantify circulating pregnancy-associated glycoprotein 1 (PAG1; also known as pregnancy-specific protein B). The assay was completed as described by the manufacturer using plasma diluted 1:10 in phosphate-buffered saline (pH 7.2) containing 0.5% (w/v) BSA. All standards, controls, and samples were run in duplicates. Absorbance was read at 650 nm using an Infinite M1000 PRO microplate reader (Tecan, Männedorf, Switzerland). Two plasma controls containing moderate (2.0 ng/mL) and low (1.0 ng/mL) PAG concentrations were included in each plate. Plasma concentrations of PAG were also measured by a monoclonal-based PAG in-house ELISA (in-house) as described by [Green et al. \(2005\)](#) and modified by [Pohler et al. \(2016\)](#) and [Reese et al. \(2018\)](#), as well as a second commercial assay (A2; Idexx, Westbrook, ME). The in-house assay uses a polyclonal antibody raised against first-secreted PAG (Ab 63; [Reese et al., 2018](#)), whereas the antigen used to create a detection antibody for A2 consisted of a mixture of PAG (PAG 4, 6, 9, 16, 18, and 19; US Patent no. 7,604,950B2). Both assays (in-house and A2) were run with a standard curve, pooled samples from a pregnant cow on day 60 of gestation, and pooled samples from a nonpregnant cow. Standards, controls, and samples were run in duplicates. Absorbance was read at 405 nm using an ELx808 microplate reader (Bio-Tek, Winooski,

VT). Intra- and inter-assay coefficients of variations were 2.19% and 3.98% for A1, 6.32% and 7.23% for A2, 6.85% and 6.02% for the in house assay, respectively.

Statistical Analysis

All data were analyzed using the SAS (SAS Inst. Inc., Cary, NC; Version 9.4). The present study was a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement of treatments, where recipient breed (AN or BN), embryo breed (AN or BN), and recipient diet (MAINT and RESTR) represented the three different factors. Recipient cow individual intake was obtained daily during the experiment; therefore, cow was considered the experimental unit. The effects of the explanatory variables on recipient BW, BCS, plasma concentrations of PAG, as well as fetal CRL and ISG expression were analyzed as repeated measures using the MIXED procedures of SAS. The covariance structure for all the analyses was selected based on the smallest Akaike information criterion for each variable analyzed. The models used for BW and BCS included the fixed effects of diet, day, and diet $\times$ day interaction. When analyzing cow BW and BCS, initial BW and BCS were used as covariates for the respective analysis. The models for recipient plasma concentrations of PAG and fetal CRL included the fixed effects of recipient breed, embryo breed, diet, day, and all the interactions that included day. When analyzing ISG expression, data were log transformed and models included the fixed effects of recipient breed, embryo breed, diet, day, and all possible interactions. Recipient BW and BCS loss during the feeding period were also analyzed by the MIXED procedure of SAS. The model included the fixed effect of diet. All models included the random effect of year.

Pregnancy failure was analyzed by ANOVA using the GLIMMIX procedure of SAS. The models included recipient breed, embryo breed, diet, and the respective interactions as fixed effects, and year as a random effect. All embryos flushing, handling, and transfer were performed by the same personnel. Hence, their effects were not included in any of the analysis. In addition, embryos were assigned to recipients to equally distribute the effects of sire and donor across the different recipient breed $\times$ diet combinations; therefore, those variables were not included in the analysis. For a more conservative interpretation of the data and to reduce the chances of type I error, Tukey adjustment was used for all simultaneous pairwise comparisons. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

The aim of the present study was to better understand how the inclusion of *B. indicus* genetics in both maternal and fetal systems alters fetal plane of development during early gestation. In addition, we were interested on the effects of feed restriction during early gestation on embryo and fetal development of both *B. indicus* and *B. taurus* cattle. Therefore, a feeding scheme was applied to recipient cows during the first trimester of gestation in order to create conditions similar to those in the field, when feed intake sometimes fails to meet the energy requirements of the postpartum beef cow. The dietary approach utilized in this experiment induced an energy restriction scenario, as shown by the differences ($P < 0.01$) in the percentage of energy requirements consumed by cows in the different diets (Table 3), along with the impact of diet on the average recipient BW ($P = 0.02$) and BCS change ($P < 0.01$; Table 3). When weekly BW and BCS were evaluated from days 28 to 91 of gestation, a diet $\times$ day interaction was observed for both recipient BW ($P < 0.01$; Figure 1) and BCS ($P < 0.01$; Figure 2). Recipients in the RESTR diet had lesser ($P < 0.05$) BW on days 28, 35, 70, 77, and 84, as well as lesser ($P < 0.05$) BCS at days 70, 77, 84, and 91. When looking only at BW change from the beginning of the feeding scheme (day -21) until the first pregnancy diagnosis (day 28), cows in the RESTR diet were already experiencing greater BW loss ($P = 0.03$; Table 3).

Although fertilization rates in beef females are estimated to be close to 90% (Santos et al., 2004), the average pregnancy rates to fixed-time AI at around day 30 of gestation ranges between 45% and 60% (Lamb et al., 2010). Therefore, the interval from fertilization to the first pregnancy diagnosis is a critical period for the establishment

of pregnancy in cattle (Wiltbank et al., 2016). In the present study, we were able to evaluate whether nutrient restriction during early gestation affects the establishment of pregnancy in *B. indicus*-influenced and *B. taurus* cattle. A recipient breed $\times$ diet interaction was observed on pregnancy failure by day 28 of gestation ($P < 0.01$), where AN-RESTR had increased pregnancy failure compared with AN-MAINT and BN recipients in both RESTR and MAINT diets (Figure 3). In addition, there was an embryo breed $\times$ diet interaction ($P = 0.03$) on pregnancy failure at day 28. Restricted recipients receiving an AN embryo experienced greater pregnancy failure than recipients in the MAINT diet receiving AN embryos, regardless of recipient breed (Figure 4). Undernutrition prior to breeding is known to have deleterious impacts on postpartum resumption of estrous cycles, pregnancy success, and reproductive efficiency of beef cows (Randel, 1990; Diskin et al., 2003). Interestingly, the negative effects of undernutrition are not restricted to the modulation of the hypothalamic-pituitary-gonadal axis and the resumption of postpartum anestrus. Studies restricting intake shortly after AI had a negative impact on reproductive performance of beef heifers (Perry et al., 2015; Kruse et al., 2017), indicating nutrient restriction might also impact early embryonic development. When embryos were recovered from heifers submitted to energy restriction immediately after AI, the collected embryos had decreased quality, decreased number of blastomeres, and tended to have a lower percentage of live cells (Kruse et al., 2017).

ISG are upregulated in PBL in response to interferon- τ production by the developing conceptus (Hansen et al., 2017). Since interferon- τ is required for pregnancy establishment in cattle, and ISG mRNA expression in PBL was positively

Table 3. Effects of dietary treatments¹ on recipient BW and BCS

Item	RESTR	MAINT	SEM	P-value
NE _m requirements met ² , %	83.2	108.7	2.49	<0.01
Initial BW, kg	532.1	514.9	8.11	0.11
BW at day 28, kg	466.2	475.7	3.68	0.08
BW change from days -21 to 28, kg	-57.6	-45.3	3.90	0.03
BW change from days -21 to 91, kg	-47.7	-27.2	6.30	0.02
Initial BCS	5.4	5.2	0.07	0.16
BCS change from days -21 to 90, kg	-1.0	0.4	0.12	<0.01

¹MAINT: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, total digestible nutrients = 46%); RESTR: Diet formulated to meet 70% of daily energy requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, total digestible nutrients = 37%).

²Percentage energy requirements for maintenance (NE_m) that was met by the diet. Values were calculated based on nutrient analysis and individual intake of nutrients, according to the NASEM (2016).

Figure 1. Effects of diet treatments on recipient BW. MAINT: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60% fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%); RESTR: Diet formulated to meet 70% of daily energy requirements, comprised of 70% pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Diet effect: $P < 0.01$; day effect: $P < 0.01$; diet $\times$ day: $P < 0.01$. Error bars represent the SEM. *Least square mean difference: $P < 0.05$.

Figure 2. Effects of dietary scheme on recipient BCS; MAINT: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60% fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and RESTR: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Diet effect: $P < 0.01$; day effect: $P < 0.01$; diet $\times$ day: $P < 0.01$. Error bars represent the SEM. *Least square mean difference: $P < 0.05$.

correlated to the amount of interferon- τ produced by the conceptus (Matsuyama et al., 2012), we were interested in evaluating whether the pregnancy failure observed in this study was associated with impaired conceptus development and interferon- τ production. However, the only effect observed on ISG mRNA expression in PBL was an increase in the mean *ISG15* mRNA expression in AN cows from days 18 to 21 ($P = 0.01$), which provides no insight on the biology controlling the differences in pregnancy failure observed in this study. The fact that ISG mRNA expression was only measured in the second year of the study might have contributed

Figure 3. Effects of recipient breed on pregnancy failure at day 28 of gestation. Explanatory variables: MAINT: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60% fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%); RESTR: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%); AN: Angus recipients; BN: Brangus recipients. Recipient breed $\times$ diet interaction: $P < 0.01$. Error bars represent the SEM. ^{a,b}Significant least square mean difference ($P < 0.05$).

Figure 4. Effects of embryo breed on pregnancy failure at day 28 of gestation. Explanatory variables: MAINT: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60% fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%); RESTR: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%); AN: Recipient cows receiving Angus embryos; BN: Recipient cows receiving Brangus embryos. Embryo breed $\times$ diet interaction: $P = 0.03$. Error bars represent the SEM. ^{a,b}Significant least square mean difference ($P < 0.05$).

to the absence of significant differences, due to the lack of statistical power in the analysis.

Although most experiments evaluating the effects of nutrient restriction on reproductive performance in beef cows have been performed in *B. taurus* cattle (Randel, 1990; Diskin et al., 2003; Perry et al., 2015), data indicate that *B. indicus* females in a poor nutritional status also experience impaired reproductive performance (Baruselli et al., 2004; Sa Filho et al., 2010). Our results, however, indicate that the magnitude of the detrimental effect of nutrient restriction differs between

B. taurus and *B. indicus*-influenced cattle. The *B. taurus* genotype in both maternal and fetal systems decreased pregnancy when nutrient restriction was superimposed, indicating that *B. taurus* cows were more susceptible to experience embryonic loss when exposed to the same negative plane of nutrition. A potential explanation for the greater impact of energy restriction on *B. taurus* cattle could be the different energy requirements between the subspecies. Crossbred cattle consisting of both *B. indicus* and *B. taurus* genetics are thought to have ~5% lesser energy requirements when compared with *B. taurus* animals (Frisch and Vercoe, 1977). Hence, the magnitude of restriction in the scenario proposed by this study could potentially be less for *B. indicus*-influenced cows. In contrast, more recent reports do not support the concept of reduced energy requirements for maintenance in *B. indicus* × *B. taurus* cattle (Chizzotti et al., 2007, 2008), indicating that genetic selection towards greater productivity might have resulted in increased nutrient requirements for *B. indicus*-influenced cattle. The generalization of reduced energy requirements for a specific group of cattle (i.e., *B. indicus*) might not be appropriate since breed genetics have changed over time. Unfortunately, little is known about the differences in physiological adaptation of *B. indicus* vs. *B. taurus* females in the postpartum period under limited energy availability. Therefore, further research is required to better understand how suckled *B. indicus* and *B. taurus* cows differ with regards to homeorhetic adaptations to energy restriction and might provide insights as to why *B. taurus* cattle experience greater pregnancy loss under nutrient restriction.

Previous reports indicate that the pattern of fetal growth during gestation differs between *B. indicus* and *B. taurus* cattle. In a series of experiments, our research group previously showed that *B. indicus*-influenced fetuses grow at a slower rate during early gestation compared with *B. taurus* counterparts (Mercadante et al., 2013). Furthermore, fetuses harvested during mid-gestation had reduced growth rates in *B. indicus* compared with *B. taurus* cattle (Ferrell, 1991a; O'Rourke et al., 1991). In the present study, differences in CRL were also detected; however, only at day 91 of gestation (recipient breed × day; $P < 0.01$). AN recipients had larger fetuses than BN recipients, regardless of the embryo breed or diet (Figure 5). When a similar reciprocal embryo transfer approach was used to compare fetal development of Charolais (*B. taurus*) and Brahman (*B. indicus*) cattle, recipient breed was also associated with fetal size during mid to late-gestation

(Ferrell, 1991a). Similar to the results from our studies, the growth of Charolais fetuses was reduced in Brahman cows compared with Charolais cows, indicating that recipient genotype may restrain fetal development. Intriguingly, calf birth weight can be similar or sometimes greater in *B. indicus* cattle compared with *B. taurus* counterparts (Mercadante et al., 2013), indicating that *B. indicus* cattle undergo compensatory gain during late gestation. In the present study, recipient breed, embryo breed, diet, or any of the respective interactions did not affect calf birth weight ($P > 0.10$). In addition, although there was an interaction between recipient breed and embryo breed on gestation length ($P = 0.05$), the least square means comparisons were not different ($P > 0.10$). Overall, our results corroborate the idea *B. indicus*-influenced cattle grow at a slower rate during early and mid-gestation, and then undergo compensatory growth during the last trimester, resulting in similar birth weights.

The differences in fetal development between cattle subspecies could be associated with different placental function or efficiency (Ferrell, 1991b). The ruminant synepitheliochorial placenta has binucleate cells that arise from mononuclear trophoblast cells through mitotic polyploidy (Wathes and Wooding, 1980; Wooding, 1984). The binucleate cells can migrate through the uterine-placental interface and fuse with uterine luminal epithelial cells. Interestingly, these cells have secretory granules that release their contents via exocytosis into the uterine stroma. PAG are located within these secretory granules, and can make their way into the maternal circulation early in gestation (Wallace et al., 2015). Although the function of PAG is not well understood, changes in plasma

Figure 5. Effects of recipient breed on fetal crown-to-rump length (CRL). Transrectal ultrasonography was performed weekly from days 42 to 91, and fetal CRL was assessed. At days 63, 70, 77, 84, and 91, crown-to-nose length was used to estimate CRL as previously described (Riding et al., 2008). AN: Angus recipient and BN: Brangus recipient. Recipient breed × day; $P < 0.01$. Error bars represent the SEM. *Least square mean difference $P < 0.05$.

concentration of PAG have been observed in physiological scenarios of placental insufficiency, such as somatic cell nuclear transfer cloning (Chavatte-Palmer et al., 2006; Constant et al., 2011) and increased incidence of embryonic mortality (Franco et al., 2018; Gatea et al., 2018). Previous research from our laboratory indicates that *B. indicus*-influenced females (BN) have greater plasma concentrations of PAG when compared with *B. taurus* counterparts (AN; Mercadante et al., 2013). Nevertheless, there are no previous reports of circulating PAG differences between *B. indicus* and *B. taurus* animals using a reciprocal embryo transfer approach. Therefore, the results of the present study are unique and provide a notion on whether the maternal or the fetal genotype is controlling the previously observed differences in circulating PAG. An embryo $\times$ day interaction was observed in A1 ($P < 0.01$) with recipients receiving BN embryos having greater concentrations of PAG at day 91 (Figure 6). Similarly, a main effect of embryo breed ($P < 0.01$) was observed in A2, where cows that received a BN embryo had greater concentrations of PAG compared with cows receiving an AN embryo throughout all measurements (Figure 6). There was also an embryo breed $\times$ day interaction when the in-house assay was used. However, as opposed to the other assays, cows receiving AN embryos had greater concentrations of PAG at days 28 and 35 of gestation than cows receiving BN embryos (Figure 6). Recipient breed or diet did not affect any of the three PAG assays ($P > 0.10$).

The PAG family of trophoblast-expressed genes encodes for several PAG products, and the differences observed between assays in this study are because the assays are probably detecting different PAG. Both A1 and A2 are commercially available pregnancy tests, where A1 is an assay that detects mostly PAG1, and A2 uses a polyclonal antibody that detects different members of the PAG family (PAG 4, 6, 9, 16, 18, and 19; Gatea et al., 2018). Conversely, the in-house assay using Ab 63 is specific to early-secreted PAG, which have been shown to differ in maternal circulation between *B. indicus* and *B. taurus* animals (Franco et al., 2018). When multiparous Nelore (*B. indicus*) cows were AI with Nelore or Angus (*B. taurus*) sires, cows inseminated to AN sires had greater circulating PAG (Franco et al., 2018). These results corroborate our findings, where cows receiving *B. taurus* embryos had greater plasma concentrations of first-secreted PAG and support the idea the profile of PAG production during early gestation differs between *B. indicus* and *B. taurus* conceptuses.

In summary, the *B. taurus* genotype in either maternal or fetal systems decreased the probability

Figure 6. Effects of embryo breed (Angus = AN; Brangus = BN) on recipient plasma concentrations of pregnancy-associated glycoproteins (PAG). Blood samples were collected weekly from days 28 to 91 of gestation and plasma concentrations of PAG were measured using two commercial PAG ELISA antibody combinations (A1 [Panel A] and A2 [Panel B]), and an in-house monoclonal-based PAG 63 ELISA (Panel C) as described by Green et al. (2005) and modified by Reese et al. (2018). A1: embryo breed $\times$ day ($P < 0.01$). A2: Main effect of embryo breed ($P < 0.01$). In house: embryo breed $\times$ day ($P < 0.01$). Error bars represent the SEM. *Least square mean difference $P < 0.05$.

of pregnancy when nutrient restriction was superimposed, indicating that *B. taurus* cattle are more susceptible to experiencing early pregnancy failure when exposed to a negative plane of nutrition. This study also provides additional evidence that differences in fetal growth rate and PAG production between *B. indicus* and *B. taurus* exist. The differences observed between subspecies on plasma concentrations of PAG were always associated with the conceptus breed. Therefore, the previous reports in the literature where *B. indicus* and *B. taurus* females have different plasma concentrations of PAG (Mercadante et al., 2013) are likely associated with the conceptus genotype rather than the maternal genotype. In addition, our results indicate that the maternal environment potentially controls fetal growth rate to some extent, and thus raises questions as to whether these differences in growth rate can program postnatal development.

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