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Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers[1]

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ABSTRACT: The objective of this study was to evaluate the effects of acclimation to handling on growth, plasma concentrations of progesterone (P4) and cortisol, temperament, and reproductive performance of Brahman-crossbred heifers. Over 2 consecutive years, 37 Braford and 43 Brahman × Angus heifers were initially evaluated, within 30 d after weaning, for BW and puberty status via transrectal ultrasonography and plasma P4 concentrations (d 0 and 10), and for temperament by measurements of chute score, pen score, and exit velocity (d 10 only). On d 11, heifers were stratified by breed, puberty status, temperament score, BW, and age and randomly assigned to receive or not (control) the acclimation treatment. Acclimated heifers were exposed to a handling process 3 times weekly (Mondays, Wednesdays, and Fridays) for 4 wk (d 11 to 39 of the experiment). The acclimation treatment was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. Heifer puberty status, evaluated via plasma P4 concentrations and transrectal ultrasonography, and BW were assessed again on d 40 and 50, d 80 and 90, and d 120 and 130. Blood samples collected before (d 10) and at the end of the acclimation period (d 40) were also analyzed for plasma concentrations of cortisol. Heifer temperament was assessed again on d 40 of the study. No interactions containing the effects of treatment, breed, and year were detected. Acclimated heifers had reduced ($P < 0.01$) ADG compared with control heifers (0.50 vs. 0.58 kg/d, respectively). Attainment of puberty and pregnancy, however, was hastened ($P = 0.02$ and 0.04, respectively) in acclimated heifers compared with control. Acclimated heifers had reduced chute score ($P < 0.01$) and concentrations of cortisol ($P < 0.01$) and P4 ($P = 0.03$; prepubertal heifers only) compared with control heifers after the acclimation period (1.37 vs. 1.84 for chute score; 37.8 vs. 50.5 ng/mL of cortisol; 0.52 vs. 0.78 ng/mL of P4). Results from this study indicated that, although acclimation to handling decreased ADG, it resulted in decreased chute score, reduced plasma concentrations of cortisol and prepubertal P4, and enhanced reproductive performance of Brahman-crossbred heifers.

Key words: acclimation, Brahman, development, handling, heifer, temperament

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INTRODUCTION

Development of replacement heifers is an important component of cow-calf production systems (Bagley, 1993). Management that maximizes number of heifers conceiving by 15 mo of age improves profitability because heifers that calve as 2-yr-olds wean more and heavier calves during their productive lives (Lesmeister et al., 1973). Because conception rates are greater during the third estrus compared with the pubertal estrus (Byerley et al., 1987), replacement heifers should be managed to attain puberty at 12 mo of age so they can conceive by 15 mo of age (Schillo et al., 1992; Bagley, 1993).

Age at puberty is influenced by breed type, and heifers containing Brahman breeding typically reach puberty after 15 mo of age (Plasse et al., 1968; Rodrigues et al., 2002). In addition to this genetic effect, Brahman-crossbred heifers are often described as temperamental (Fordyce et al., 1988; Voisinet et al., 1997), and this trait further negatively influences their reproductive function (Plasse et al., 1970). Cattle with excitable temperament experience stimulated secretion and circulating concentrations of ACTH and cortisol (Cur-

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ley et al., 2008), and these hormones directly impair the mechanisms responsible for puberty establishment and fertility of heifers, such as synthesis and release of GnRH and gonadotropins (Li and Wagner, 1983; Dobson et al., 2000). However, acclimation of beef females to human handling is an alternative to alleviate the negative physiological effects of temperament on reproduction (Echternkamp, 1984). Therefore, we hypothesized that acclimation of Brahman-crossbred heifers to handling procedures would improve temperament, reduce adrenal steroidogenesis, and enhance heifer reproductive performance. Our objectives were to compare growth, temperament, plasma measurements associated with behavioral stress response, puberty attainment, and pregnancy rates of Brahman × Angus and Braford heifers exposed or not to handling acclimation procedures after weaning.

MATERIALS AND METHODS

The animals utilized were cared for in accordance with acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999). This experiment was conducted over 2 yr (2006 and 2007) at the University of Florida, Institute of Food and Agricultural Sciences, Range Cattle Research and Education Center, Ona. Each experimental period was divided into a sampling phase (d 0 to 130) and a breeding phase (d 131 to 191).

Animals

A total of 37 Braford (37.5% Brahman + 62.5% Hereford) and 43 Brahman × Angus (approximately 25% Brahman) heifers weaned at approximately 7 mo of age were assigned to the experiment. Twenty heifers from each breed type were utilized in yr 1, whereas 23 Brahman × Angus and 17 Braford heifers were utilized in yr 2. Heifers were initially evaluated for BW and puberty status (morning of d 0 and 10), and also for temperament score (d 10 only) within 30 d after weaning. Puberty was assessed via plasma progesterone (**P4**) concentrations and transrectal ultrasonography (7.5 MHz transducer, Aloka 500V, Wallingford, CT) to verify ovarian activity. Heifers were considered pubertal if a corpus luteum with volume greater than 2.15 cm^3 and plasma P4 concentrations greater than 1.5 ng/mL (Cooke and Arthington, 2009) were concurrently detected in one or both evaluations. Corpus luteum volume was calculated using the formula for volume of a sphere [$V = 4/3\pi \times (D/2)^3$, where D is the maximum luteal diameter]. On d 11, heifers were stratified by breed, puberty status, temperament score, BW, and age, and randomly assigned to receive or not (control) the acclimation treatment. Heifer was considered the experimental unit. Across years and breeds, heifer mean BW and age (±SD) at the beginning of the experiment were, respectively, 253 ± 27 kg and 269 ± 26 d.

Diets

During sampling and breeding phases, heifers were maintained on separate 6-ha limpograss (*Hemarthria altissima*) pastures according to treatment. Heifers were rotated among pastures every 2 wk. Heifers received a blend of soybean hulls and cottonseed meal (75:25, as-fed basis) 3 times weekly, at a rate to provide a daily amount of 2.7 kg of DM per heifer. Stargrass (*Cynodon nlemfuensis*) hay was offered in amounts to ensure that heifers had ad libitum access when pasture availability was limited. A complete commercial mineral/vitamin mix (14% Ca, 9% P, 24% NaCl, 0.20% K, 0.30% Mg, 0.20% S, 0.005% Co, 0.15% Cu, 0.02% I, 0.05% Mn, 0.004% Se, 0.3% Zn, 0.08% F, and 82 IU/g of vitamin A) and water were offered for ad libitum consumption throughout the experiment. Forage and supplement samples were collected at the beginning and during the experiment and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP, ADF, and NDF, whereas NE_m and NE_g were calculated using the equations proposed by the NRC (1988). Averaged over the 2 yr of study, CP (% of DM), NE_m (Mcal/kg of DM), and NE_g (Mcal/kg of DM) concentrations of dietary components were, respectively, 9.50, 0.99, and 0.44 for pasture, 12.8, 0.92, and 0.37 for hay, 11.5, 1.19, and 0.62 for soybean hull, and 48.2, 1.58, and 0.99 for cottonseed meal.

Acclimation Procedure

Acclimated heifers were exposed to a handling acclimation process 3 times weekly (Mondays, Wednesdays, and Fridays) for 4 wk (d 11 to 39 of the experiment). The acclimation treatment was applied individually to heifers by processing them through a handling facility. During the first week of acclimation, heifers were individually processed through the handling facility but not restrained in the squeeze chute. During wk 2, heifers were individually processed through the handling facility and were restrained in the squeeze chute for approximately 5 s. On wk 3 and 4, heifers were similarly processed as in wk 2 but were restrained in the squeeze chute for 30 s. During the initial 3 wk, heifers were allowed to return to their pasture immediately after processing, whereas during wk 4 heifers remained in the handling facility for 1 h and then returned to pasture. The acclimation treatment had different intensities across weeks so heifers were introduced to the handling procedures gradually and, thus, were not exposed to unfamiliar and stressful procedures, such as prolonged chute restraining immediately on wk 1 of the acclimation period. For each handling acclimation process, acclimated heifers were gathered in the pasture and obliged to walk to the handling facility, whereas control heifers remained undisturbed on pasture. The total distance traveled by acclimated heifers during each of the acclimation events was approximately 2 km (round-trip).

Sampling

During the sampling phase, heifer BW and puberty status were assessed, using the same procedures and criteria as in the initial evaluation (d 0 and 10), in the morning of d 40 and 50, 80 and 90, and 120 and 130. Heifer BW gain during the sampling phase was calculated by averaging the values obtained in both 10-d interval assessments. Further, heifer shrunk (after 16 h of feed and water restriction) BW was collected on d 1 and 192 for calculation of heifer ADG during the study.

Blood samples collected throughout the sampling phase for puberty assessment were also analyzed for plasma IGF-I concentrations to evaluate heifer energy status. Further, on d 10 and 40, 2 extra blood samples were collected from each heifer in addition to the morning collection. Blood samples collected on d 10 and 40 were obtained in 3-h intervals to account for the circadian rhythm of adrenal steroidogenesis (Thun et al., 1981; Arthington et al., 1997) and analyzed for plasma concentrations of cortisol, P4, ceruloplasmin, and haptoglobin. Plasma concentrations of ceruloplasmin and haptoglobin were determined herein to evaluate a potential association between temperament, cortisol concentrations, and the acute-phase response. Further, treatment effects on P4 concentrations were only evaluated within prepubertal heifers, given that circulating P4 concentrations in pubertal *Bos indicus*-influenced heifers varies significantly according to stage of the estrous cycle and consequent corpus luteum size (Figueiredo et al., 1997). Heifers remained in the handling facility during the intervals between blood collections.

Heifer temperament scores were obtained in the morning of d 40, in addition to the initial evaluation (d 10), after blood collection and ultrasonography exam. Heifer temperament was assessed by pen score, chute score, and chute exit velocity, as described by Arthington et al. (2008). Further, within each assessment day (d 10 and 40), heifers were divided in quintiles according to their chute exit velocity and assigned a score from 1 to 5 (exit score; 1 = heifers within the slowest quintile; 5 = heifers within the fastest quintile). Individual temperament scores were calculated by averaging heifer chute score, pen score, and exit score.

During the breeding phase (d 131 to 191), each treatment group was exposed to 1 mature Angus bull (1:20 bull to heifer ratio), and bulls were rotated between groups every other week to account for potential bull effects. Heifer pregnancy status was verified by detecting a fetus with transrectal ultrasonography (5.0 MHz transducer, Aloka 500V) 70 d after the end of the experiment. Date of conception was estimated retrospectively by subtracting gestation length (286 d; Reynolds et al., 1980) from the calving date.

Blood Analysis

Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, placed on ice immediately, and centrifuged at 2,400 × g for 30 min at 4°C for plasma collection. Plasma was frozen at −20°C on the same day of collection.

Concentrations of P4 and cortisol were determined using Coat-A-Count solid phase ^{125}I RIA kits (DPC Diagnostic Products Inc., Los Angeles, CA). A double antibody RIA was used to determine concentrations of IGF-I (Badinga et al., 1991; Cooke et al., 2007). Concentrations of ceruloplasmin were determined according to procedures described by Demetriou et al. (1974). Concentrations of haptoglobin were determined by measuring haptoglobin/hemoglobin complex by the estimation of differences in peroxidase activity (Makimura and Suzuki, 1982), and results are expressed as arbitrary units from the absorption reading at 450 nm × 100. All samples were analyzed in duplicate. Across years, the intra- and interassay CV were, respectively, 9.2 and 6.9% for cortisol, 6.3 and 7.3% for P4, 5.1 and 3.5% for ceruloplasmin, 8.8 and 9.8% for haptoglobin, and 4.5 and 4.6% for IGF-I. Assay sensitivity was 0.1 ng/mL for P4, 5 ng/mL for cortisol, and 10 ng/mL for IGF-I.

Statistical Analysis

Growth, temperament, and physiological data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for analysis of temperament, BW gain, and physiological data contained the effects of treatment, breed, time variables when appropriate, and the resultant interactions. Data were adjusted covariately to measurement obtained before acclimation period (d 10) and were analyzed using heifer (breed × treatment × yr) as random variable. Further, temperament data were tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure of SAS, and results indicated W > 0.80 for all the temperament measurements evaluated. The model statement used for ADG contained the effect of treatment, breed, and year. Data were also analyzed using heifer (breed × treatment × yr) as random variable. Puberty and pregnancy data were analyzed with the GLM and LOGISTIC procedures of SAS. The model statement contained the effects of treatment, breed, time of estimated puberty establishment or conception, year, and the appropriate interactions. Pearson correlations were calculated among ADG, mean concentrations of hormones and metabolites, and mean temperament measurements of heifers during the study with the CORR procedure of SAS. The GLM procedure was utilized

Table 1. Average daily gain and plasma concentrations of ceruloplasmin, haptoglobin, and IGF-I of heifers exposed or not (control) to handling acclimation procedures[1]

Item	Acclimated	Control	SEM	P-value
ADG,[2] kg/d	0.50	0.58	0.016	<0.01
Ceruloplasmin,[3] mg/dL	27.3	25.7	0.69	0.11
Haptoglobin,[3] 450 nm × 100	1.52	1.27	0.169	0.29
IGF-I,[3] ng/mL	216	215	6.2	0.86

[1]Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility. Control heifers remained undisturbed on pasture.

[2]Calculated using initial (d 1) and final (d 192) shrunk BW.

[3]Least squares means adjusted covariately to values obtained before acclimation period (d 10).

to determine effects of treatment, year, and breed on correlation coefficients. No effects or interactions were detected; therefore, correlation coefficients reported herein were determined across treatments, years, and breeds. For all analyses, individual heifer was the experimental unit; results are reported as least squares means and were separated using LSD, significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant or according to the highest order interaction detected.

RESULTS AND DISCUSSION

No interactions containing the effects of treatment, breed, and year were detected for measurements of heifer growth, plasma measurements, temperament, and reproductive performance; therefore, results were combined between breeds and years. Further, no treatment × time(day) interaction was detected for the analysis of plasma measurements on d 10 and 40; therefore, results regarding treatment effects were combined among samples collected within each day.

Acclimated heifers had reduced ($P < 0.01$) ADG compared with control heifers (0.50 vs. 0.58 kg/d, respectively; SEM = 0.016; Table 1), although both treatment groups were provided similar pastures and supplements during the study. Nevertheless, treatment effects on ADG can be attributed to different factors, such as altered grazing behavior and the additional exercise that acclimated heifers were exposed to during the acclimation period. During each acclimation event, assigned heifers had to walk nearly 2 km in addition to the activity inside the handling facility, whereas control heifers remained undisturbed on pasture. According to the Cornell Net Carbohydrate and Protein System for Cattle model (version 5.0.40), at least 0.55 Mcal of ME were utilized by acclimated heifers during each of the 2-km walk and handling acclimation process, what represents approximately 5% of the total ME intake of control heifers. Further, although grazing behavior was not evaluated in the present study, acclimated heifers may have experienced disrupted grazing pattern and consequent reduced forage intake during days when they were exposed to acclimation procedures, what may have contributed to an overall reduced nutrient intake compared with that of control heifers during the acclimation period. Supporting this rationale, heifers from both treatments had similar ($P = 0.92$) BW before the acclimation period (253.0 vs. 253.6 kg of BW for acclimated and control heifers, respectively; SEM = 4.28), but control heifers had numerically greater ($P = 0.18$) BW compared with acclimated heifers (295.2 vs. 287.0 kg of BW, respectively; SEM = 4.29) after the acclimation period (treatment × day interaction; $P < 0.01$; data not shown).

Acclimated heifers had reduced ($P < 0.01$) cortisol concentrations compared with control heifers after the acclimation period (37.8 vs. 50.5 ng/mL; SEM = 0.17; Figure 1). Within prepubertal heifers, P4 concentrations were reduced ($P = 0.03$) in acclimated heifers compared with control heifers after the acclimation period (0.52 vs. 0.78 ng/mL; SEM = 0.08; Figure 2). Previous research indicated that acclimation of cattle to handling procedures was an alternative to prevent elevated concentrations of cortisol in response to handling stress (Crookshank et al., 1979; Andrade et al., 2001; Curley et al., 2006). The same rationale can be extrapolated to treatment effects on prepubertal P4 concentrations. Although the major sources of P4 synthesis in cattle, the corpus luteum and the pregnant placenta (Hoffmann and Schuler, 2002), are absent in prepubertal heifers, their adrenal gland is also capable of producing significant amounts of P4 as an intermediate of cortisol synthesis (Gonzalez-Padilla et al., 1975a; Brown, 1994). Supporting this rationale, concentrations of cortisol and P4 were positively correlated ($P < 0.01$) in prepubertal heifers herein (r = 0.51), as previously reported in Cooke and Arthington (2009), and also in previous research efforts evaluating the effects of ACTH administration to ovariectomized heifers and cows (Båge et al., 2000; Yoshida and Nakao, 2006). In accordance with studies indicating that the adrenal gland synthesizes steroids in a circadian rhythm (Thun et al., 1981; Arthington et al., 1997), a time effect was detected ($P < 0.01$) for the analysis of cortisol and prepubertal P4 across treatments. Concentrations of cortisol and prepubertal P4 were the greatest ($P < 0.01$; data not shown) in the morning collection compared with the 2 subsequent collections (49.1 vs. 37.4

Table 2. Pearson correlation coefficients among ADG, plasma measurements, and temperament of heifers[1]

Item	ADG	Cortisol	Ceruloplasmin	Haptoglobin	IGF-I
Cortisol	−0.14				
	0.23				
Ceruloplasmin	−0.52	0.18			
	<0.01	0.11			
Haptoglobin	−0.15	0.02	0.38		
	0.20	0.88	<0.01		
IGF-I	0.31	−0.17	−0.48	−0.03	
	<0.01	0.15	<0.01	0.77	
Temperament score	−0.43	0.58	0.24	0.06	−0.24
	<0.01	<0.01	0.03	0.59	0.03

[1]Upper row = correlation coefficients. Lower row = P-values.

and 40.6 ng/mL of cortisol, respectively, SEM = 1.58; 1.01 vs. 0.54 and 0.53 ng/mL of P4, respectively, SEM = 0.08).

No treatment effects were detected for concentrations of IGF-I, ceruloplasmin, and haptoglobin (Table 1), although previous studies reported that elevated concentrations of corticoids can stimulate hepatic synthesis of proteins associated with the acute-phase response (Yoshino et al., 1993; Higuchi et al., 1994) and reduce circulating concentrations of IGF-I in cattle (Maciel et al., 2001). Nevertheless, positive correlations were detected ($P < 0.05$; Table 2) between concentrations of IGF-I and heifer ADG, concentrations of ceruloplasmin and haptoglobin, and heifer temperament and concentrations of ceruloplasmin (Table 2). Additionally, heifer ADG and IGF-I concentrations were negatively correlated with heifer temperament and ceruloplasmin concentrations (Table 2). These results support previous studies reporting that IGF-I is a key factor for heifer growth and its synthesis can be reduced during the acute phase response (Elsasser et al., 1997; Cooke et al., 2007) and also suggests that cattle with excitable temperament experience reduced concentrations of IGF-I and stimulated acute phase response, which contributes to their decreased BW gain compared with cattle with calmer temperament (Voisinet et al., 1997; Fell et al., 1999; Qiu et al., 2007).

No treatment effects were detected for temperament scores (Table 3), but acclimated heifers had reduced chute score ($P < 0.01$) compared with control heifers after the acclimation period (1.37 vs. 1.84; SEM = 0.091; Table 3). Further, all measurements of temperament were positively correlated to each other and also to cortisol concentrations ($P < 0.01$; Table 4). Fordyce et al. (1988) indicated that Brahman-crossbred steers frequently exposed to handling procedures exhibited calmer temperament compared with cohorts with no handling experiences. Curley et al. (2006) reported that frequent handling of Brahman bulls decreased their exit velocity, but not their pen and chute score. The positive correlations detected among measurements of temperament and cortisol concentrations reported herein were

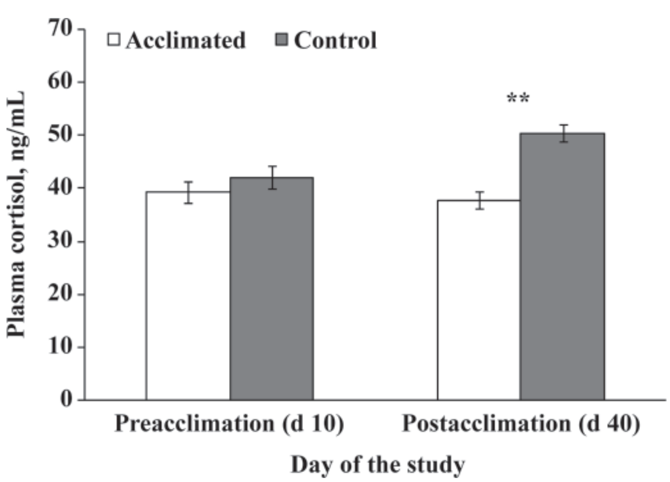

Figure 1. Plasma cortisol concentrations of heifers exposed or not (control) to handling acclimation procedures. Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. Samples collected on d 10 served as covariate; therefore, results reported for d 40 are covariately adjusted least squares means. Treatment comparison within days: **$P < 0.01$.

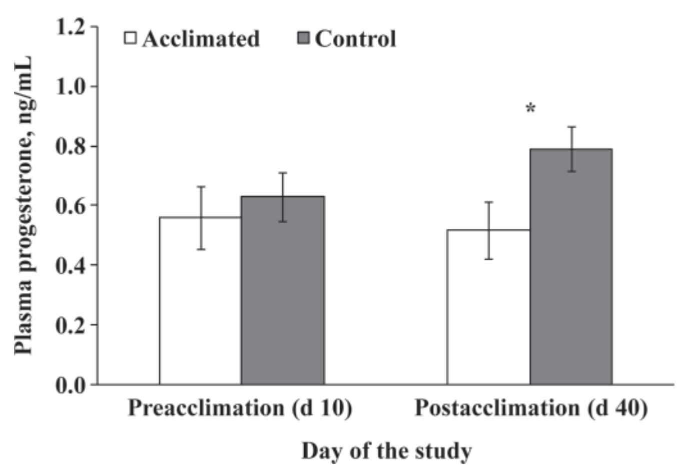

Figure 2. Plasma progesterone concentrations of prepubertal heifers exposed or not (control) to handling acclimation procedures. Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. Samples collected on d 10 served as covariate; therefore, results reported for d 40 are covariately adjusted least squares means. Treatment comparison within days: *$P = 0.03$.

Table 3. Temperament measurements of heifers exposed or not (control) to handling acclimation procedures[1,2,3]

Item	Acclimated	Control	SEM	*P*-value
Chute score	1.37	1.84	0.091	<0.01
Pen score	2.85	2.72	0.137	0.51
Exit velocity, m/s	2.91	2.74	0.148	0.43
Temperament score[4]	2.46	2.48	0.096	0.93

[1]Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility. Control heifers remained undisturbed on pasture.

[2]Least squares means adjusted covariately to values obtained before the acclimation period (d 10).

[3]Chute score, pen score, and exit velocity were assessed according to the techniques described by Arthington et al. (2008).

[4]Calculated by averaging heifer chute score, exit score, and pen score. Exit score was calculated by dividing chute exit velocity results into quintiles and assigning heifers with a score from 1 to 5 (exit score: 1 = slowest heifers; 5 = fastest heifers).

also described by others (Stahringer et al., 1990; Fell et al., 1999; Curley et al., 2006), indicating that these 3 measurements of cattle behavior during handling can be used as indicators of temperament and also denote the amount of stress that the animal is experiencing (Thun et al., 1998; Sapolsky et al., 2000). However, pen score and exit velocity are classified as nonrestrained techniques to evaluate cattle temperament, whereas chute score belongs to the restrained techniques category (Burrow and Corbet, 2000). An important flaw within restrained techniques is that cattle with excitable temperament may "freeze" when restrained, and consequently not express their true behavior during these assessments (Burrow and Corbet, 2000). Therefore, treatment effects detected in the present study for chute score should be interpreted with caution, even though this measurement of temperament was positively correlated with cortisol and nonrestrained assessments (Table 4).

A treatment effect was detected ($P = 0.02$) for puberty attainment. Although age at puberty in cattle is highly associated with BW and growth rate (Schillo et al., 1992; Roberts et al., 2009), acclimated heifers experienced hastened attainment of puberty compared with control heifers despite their reduced ADG (Figure 3). Pregnancy attainment was also hastened ($P = 0.04$) in acclimated heifers compared with control cohorts (Figure 4), and this outcome can be attributed, at least in part, to the earlier attainment of puberty of acclimated heifers. Further assessments of acclimation effects on heifer fertility could not be completed because puberty evaluation was not performed during the breeding season. The main hypothesis of the present experiment was that acclimation to human handling would alleviate the detrimental effects of excitable temperament on reproductive function of Brahman-crossbred heifers and allow acclimated heifers to experience enhanced reproductive performance compared with nonacclimated cohorts. Plasse et al. (1970) reported that excitable temperament influences negatively the reproductive performance of beef females. Cattle with high Brahman influence typically exhibit excitable temperament (Hearnshaw and Morris, 1984; Fordyce et al., 1988; Voisinet et al., 1997) and experience stimulated function of the hypothalamic-pituitary-adrenal axis when exposed to humans and handling procedures, resulting in increased production and circulating concentrations of ACTH and consequently cortisol (Stahringer et al., 1990; Curley et al., 2008). These hormones have been shown to directly impair the physiological mechanisms required for attainment of puberty in heifers, particularly synthesis and release of LH by the anterior pituitary gland (Li and Wagner, 1983; Dobson et al., 2000), whereas methods to acclimate cattle to handling procedures were reported to be successful in reducing circulating cortisol concentrations (Crookshank et al., 1979; Andrade et al., 2001) and increasing pulsatility and mean concentrations of LH (Echternkamp, 1984). Supporting this rationale and our hypothesis, acclimated heifers in the present study had reduced cortisol concentrations and hastened onset of puberty compared with nonacclimated cohorts. Nevertheless, the mechanisms by which acclimation procedures hastened puberty attainment in acclimated heifers, regardless of decreased ADG, remain unclear. Exercise regimens were reported to delay onset of puberty in humans and rodents due to suppressive effects on GnRH and gonadotropin synthesis and secretion (Warren, 1980; Manning and Bronson, 1991). Conversely, dairy heifers exposed to prepartum exercise regiments experienced greater reproductive efficiency compared with control cohorts (Lamb et al., 1979, 1981). Circulating concen-

Table 4. Pearson correlation coefficients among measurements of temperament and plasma cortisol concentrations of heifers[1]

Item	Cortisol	Chute score	Exit velocity
Chute score	0.44		
	<0.01		
Exit velocity	0.55	0.46	
	<0.01	<0.01	
Pen score	0.48	0.40	0.69
	<0.01	<0.01	<0.01

[1]Upper row = correlation coefficients. Lower row = *P*-values.

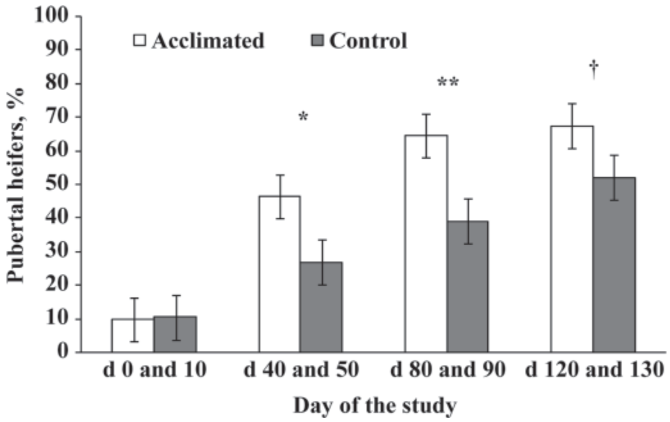

Figure 3. Puberty attainment, according to 10-d interval evaluations, of heifers exposed or not (control) to handling acclimation procedures. Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. Heifers were considered pubertal once corpus luteum and plasma progesterone concentrations greater than 1.5 ng/mL were concurrently detected in one or both evaluations performed on a 10-d interval. A treatment effect was detected ($P = 0.02$). Treatment comparison within sampling dates: $^{**}P < 0.01$, $^{*}P = 0.03$, $^{\dagger}P = 0.10$.

trations of endogenous opioids are altered in response to exercise stimuli (Harber and Sutton, 1984), whereas these substances may modulate gonadotropin secretion and consequent onset of puberty, cyclicity, and fertility of cattle (Mahmoud et al., 1989). Therefore, the additional exercise that acclimated heifers were exposed to may have influenced, negatively or positively, their reproductive performance, whereas further research is required to address this matter.

Based on our hypothesis, it can also be speculated that reduced cortisol concentrations in acclimated heifers facilitated the initiation of the physiological events required for attainment of puberty, particularly increased LH pulse frequency and consequent first ovulatory LH surge (Smith and Dobson, 2002). Although concentrations of cortisol were only evaluated when heifers were handled and restrained for blood collection, one can speculate that acclimated heifers also had reduced cortisol concentrations compared with control heifers on a daily basis given that heifers from both groups were often exposed to brief human interaction, particularly because of feeding and traffic of personnel/vehicles within the research station.

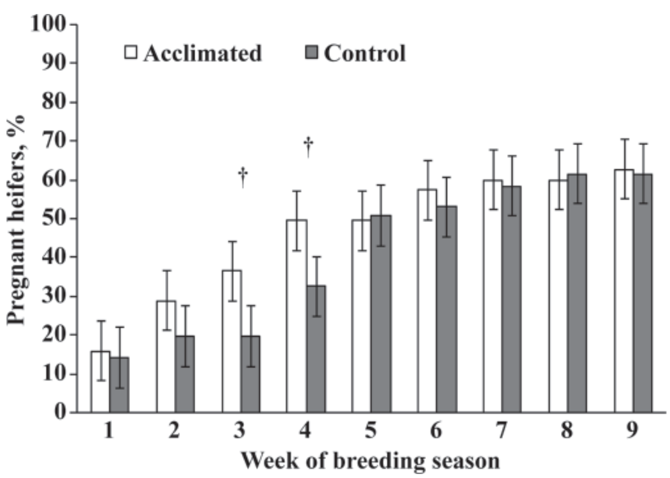

Figure 4. Pregnancy rates during the breeding season (d 131 to 191 of the study) of heifers exposed or not (control) to handling acclimation procedures. Acclimated heifers were exposed to a handling process 3 times weekly for 4 wk (d 11 to 39), which was applied individually to heifers by processing them through a handling facility, whereas control heifers remained undisturbed on pasture. Date of conception was estimated retrospectively by subtracting gestation length (286 d; Reynolds et al., 1980) from the calving date. A treatment effect was detected ($P = 0.04$). Treatment comparison within weeks: $^{\dagger}P = 0.10$.

Prepubertal synthesis of P4 by the adrenal gland may be another mechanism responsible for treatments effects on puberty attainment. As previously reported (Cooke and Arthington, 2009), approximately 55% of the Brahman × Angus and 86% of the Braford heifers from the present study experienced P4 concentrations above 1.0 ng/mL during the handling procedures before reaching puberty, whereas the greatest prepubertal P4 value detected for both breeds was 2.8 ng/mL. These results indicate that elevated production of P4 by the adrenal gland is a common occurrence in prepubertal Brahman-crossbred heifers during handling practices. However, one could theorize that the elevated prepubertal P4 concentrations detected herein were originated from a short-lived corpus luteum, which was not detected between the 10-d interval assessments, whereas that particular heifer would be incorrectly classified as prepubertal. Still, large follicles with potential to ovulate, and consequently their disappearance, regression, or both, were closely monitored during the subsequent ovarian ultrasonography exam (10 d later). Further, as previously reported (Cooke and Arthington, 2009) positive correlations ($P < 0.01$) were detected between prepubertal concentrations of P4 and temperament score ($r = 0.45$), and prepubertal concentrations of P4 and cortisol ($r = 0.51$), suggesting that the elevated prepubertal P4 concentrations detected herein were, at least partially, from adrenal origin and modulated by heifer temperament.

Progesterone appears to be a required stimulus for puberty establishment in heifers given that it suppresses the number of estradiol receptors in the hypothalamus and primes the hypothalamic-pituitary-ovarian axis toward enhanced synthesis and pulsatile secretion of LH (Anderson et al., 1996; Looper et al., 2003). Transient increases in circulating concentrations of P4 were reported in beef heifers 2 wk before the onset of puberty (Gonzalez-Padilla et al., 1975a), and the origin of this hormone was attributed to the adrenal gland or luteal structures found within the ovary, or both (Gonzalez-Padilla et al., 1975a; Berardinelli et al., 1979). Based on this information, it can be theorized that acclimated heifers experienced transient increases in adrenal steroidogenesis and, thus, P4 synthesis during the acclimation process, particularly during the initial weeks when heifers were still unfamiliar with the acclimation events. Transient increases in adrenal P4 synthesis dur-

Table 5. Effects of breed on performance, temperament, and physiologic variables of replacement heifers[1]

Item	Brahman × Angus	Braford	SEM	P-value
ADG,[2] kg/d	0.55	0.49	0.016	0.02
Puberty rate,[3] %	81	39	6.9	<0.01
Pregnancy rate,[4] %	71	67	7.8	0.39
Temperament score[5]	2.47	2.62	0.121	0.50
Cortisol, ng/mL	40.1	44.5	2.00	0.12
IGF-I, ng/mL	200	206	6.4	0.48
Ceruloplasmin, mg/dL	25.2	26.5	0.67	0.18
Haptoglobin, 450 nm × 100	2.14	1.74	0.315	0.35

[1]Values reported are least squares means from all samples/assessments obtained throughout the study.
[2]Calculated using initial (d 1) and final (d 192) shrunk BW.
[3]Estrous cycling heifers/total heifers during the sampling phase of the study (d 0 to 130).
[4]Pregnant heifers/total heifers during the breeding phase of the study (d 131 to 191).
[5]Calculated by averaging heifer chute score, exit score, and pen score. Exit score was calculated by dividing exit velocity results into quintiles and assigning heifers with a score from 1 to 5 (exit score: 1 = slowest heifers; 5 = fastest heifers). Chute score, pen score, and exit velocity were assessed according to the techniques described by Arthington et al. (2008).

ing the early phases of the acclimation process, combined with reduced cortisol synthesis as acclimation advanced, may have contributed to hastened puberty attainment of acclimated heifers compared with control cohorts. Nevertheless, previous research evaluating the stimulatory effects of P4 on puberty attainment of heifers utilized P4 or progestin implants that maintained or mimicked elevated P4 concentrations for several days (Gonzalez-Padilla et al., 1975b; Short et al., 1976; Anderson et al., 1996), whereas in the present study, the proposed increases in prepubertal P4 concentrations were only temporary. Therefore, further research is required to properly address our assumptions.

Breed effects were detected ($P < 0.05$; Table 5) for ADG and attainment of puberty. Brahman × Angus heifers had greater ($P = 0.02$) ADG compared with Braford heifers during the study (0.55 vs. 0.49 kg/d, respectively; SEM = 0.02). A greater ($P < 0.01$) number of Brahman × Angus heifers attained puberty during the sampling phase compared with Braford heifers (80.6 vs. 38.8% pubertal heifers, respectively; SEM = 6.9). No further breed effects were detected (Table 5). These results support previous data from our research group (Arthington et al., 2004) reporting inferior ADG and reproductive performance of Braford heifers compared with Brahman × Angus heifers. The Braford heifers utilized herein and by Arthington et al. (2004) were originated from a straightbred Braford cowherd that has been sharing similar genetics for more than 20 yr, which likely resulted in accumulative inbreeding. Martin et al. (1992) indicated that inbreeding can be detrimental to cattle growth and age at puberty. Therefore, breed differences in ADG and puberty attainment reported herein can likely be attributed, at least in part, to accumulative inbreeding effects within Braford heifers, and also to the retained heterosis of Brahman × Angus heifers (Koger, 1980).

In conclusion, results from this experiment indicate that acclimation of Brahman-crossbred heifers to handling procedures and human interaction reduced ADG, but decreased adrenal steroidogenesis, and hastened attainment of puberty and pregnancy. Therefore, acclimation of Brahman × Angus and Braford replacement heifers to human handling after weaning may be an alternative to enhance their reproductive development and increase the efficiency of heifer development programs in cow-calf operations containing Brahman-influenced cattle.

LITERATURE CITED

Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progestin-induced puberty and secretion of luteinizing hormone in heifers. Biol. Reprod. 54:1025–1031.

Andrade, O., A. Orihuela, J. Solano, and C. S. Galina. 2001. Some effects of repeated handling and the use of a mask on stress responses in zebu cattle during restraint. Appl. Anim. Behav. Sci. 71:175–181.

Arthington, J. D., L. R. Corah, J. E. Minton, T. H. Elsasser, and F. Blecha. 1997. Supplemental dietary chromium does not influence ACTH, cortisol, or immune responses in young calves inoculated with bovine herpesvirus-1. J. Anim. Sci. 75:217–223.

Arthington, J. D., G. C. Lamb, and F. M. Pate. 2004. Effects of supplement type on growth and pregnancy rate of yearling Brahman-crossbred heifers. Prof. Anim. Sci. 20:282–285.

Arthington, J. D., X. Qiu, R. F. Cooke, J. M. B. Vendramini, D. B. Araujo, C. C. Chase Jr., and S. W. Coleman. 2008. Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. J. Anim. Sci. 86:2016–2023.

Badinga, L., R. J. Collier, W. W. Thatcher, C. J. Wilcox, H. H. Head, and F. W. Bazer. 1991. Ontogeny of hepatic bovine growth hormone receptors in cattle. J. Anim. Sci. 69:1925–1934.

Båge, R., M. Forsberg, H. Gustafsson, B. Larsson, and H. Rodríguez-Martínez. 2000. Effect of ACTH-challenge on progesterone and cortisol levels in ovariectomised repeat breeder heifers. Anim. Reprod. Sci. 63:65–76.

Bagley, C. P. 1993. Nutritional management of replacement beef heifers: A review. J. Anim. Sci. 71:3155–3163.

Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276–1280.

Brown, R. E. 1994. An Introduction to Neuroendocrinology. Cambridge Univ. Press, Cambridge, UK.

Burrow, H. M., and N. J. Corbet. 2000. Genetic and environmental factors affecting temperament of zebu and zebu-derived beef cattle grazed at pasture in the tropics. Aust. J. Agric. Res. 51:155–162.

Byerley, D. J., R. B. Staigmiller, J. B. Berardinelli, and R. E. Short. 1987. Pregnancy rates of beef heifers bred either on puberal or third estrus. J. Anim. Sci. 65:645–650.

Cooke, R. F., and J. D. Arthington. 2009. Plasma progesterone concentrations determined by commercial radioimmunoassay kit as puberty criteria for Brahman-crossbred heifers. Livest. Sci. 123:101–105.

Cooke, R. F., J. D. Arthington, C. R. Staples, W. W. Thatcher, and G. C. Lamb. 2007. Effects of supplement type on performance, reproductive, and physiological responses of Brahman-crossbred females. J. Anim. Sci. 85:2564–2574.

Crookshank, H. R., M. H. Elissalde, R. G. White, D. C. Clanton, and H. E. Smalley. 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48:430–435.

Curley, K. O. Jr., D. A. Neuendorff, A. W. Lewis, J. J. Cleere, T. H. Welsh Jr., and R. D. Randel. 2008. Functional characteristics of the bovine hypothalamic–pituitary–adrenal axis vary with temperament. Horm. Behav. 53:20–27.

Curley, K. O. Jr., J. C. Paschal, T. H. Welsh Jr., and R. D. Randel. 2006. Technical note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100–3103.

Demetriou, J. A., P. A. Drewes, and J. B. Gin. 1974. Ceruloplasmin. Pages 857–864 in Clinical Chemistry. D. C. Cannon and J. W. Winkelman, ed. Harper and Row, Hagerstown, MD.

Dobson, H., A. Y. Ribadu, K. M. Noble, J. E. Tebble, and W. R. Ward. 2000. Ultrasonography and hormone profiles of adrenocorticotrophic hormone (ACTH)-induced persistent ovarian follicles (cysts) in cattle. J. Reprod. Fertil. 120:405–410.

Echternkamp, S. E. 1984. Relationship between LH and cortisol in acutely stressed beef cows. Theriogenology 22:305–311.

Elsasser, T. H., S. Kahl, N. C. Steele, and T. S. Rumsey. 1997. Nutritional modulation of somatotropic axis-cytokine relationships in cattle: A brief review. Comp. Biochem. Physiol. 116A:209–221.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.

Fell, L. R., I. G. Colditz, K. H. Walker, and D. L. Watson. 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. Aust. J. Exp. Agric. 39:795–802.

Figueiredo, R. A., C. M. Barros, O. L. Pinheiro, and J. M. P. Soler. 1997. Ovarian follicular dynamics in Nelore breed (*Bos indicus*) cattle. Theriogenology 47:1489–1505.

Fordyce, G. E., R. M. Dodt, and J. R. Wythes. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. Aust. J. Exp. Agric. 28:683–687.

Gonzalez-Padilla, E., R. Ruiz, D. LeFever, A. Denham, and J. N. Wiltbank. 1975b. Puberty in beef heifers. III. Induction of fertile estrus. J. Anim. Sci. 40:1110–1118.

Gonzalez-Padilla, E., J. N. Wiltbank, and G. D. Niswender. 1975a. Puberty in beef heifers. I. The interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091–1104.

Harber, V. J., and J. R. Sutton. 1984. Endorphins and exercise. Sports Med. 1:154–171.

Hearnshaw, H., and C. A. Morris. 1984. Genetic and environmental effects on a temperament score in beef cattle. Aust. J. Agric. Res. 35:723–733.

Higuchi, H., N. Katoh, T. Miyamoto, E. Uchida, A. Yuasa, and K. Takahashi. 1994. Dexamethasone-induced haptoglobin release by calf liver parenchymal cells. Am. J. Vet. Res. 55:1080–1085.

Hoffmann, B., and G. Schuler. 2002. The bovine placenta; a source and target of steroid hormones: Observations during the second half of gestation. Domest. Anim. Endocrinol. 23:309–320.

Koger, M. 1980. Effective crossbreeding systems utilizing zebu cattle. J. Anim. Sci. 50:1215–1220.

Lamb, R. C., M. J. Anderson, and J. L. Walters. 1981. Forced walking prepartum for dairy cows of different ages. J. Dairy Sci. 64:2017–2024.

Lamb, R. C., B. O. Barker, M. J. Anderson, and J. L. Walters. 1979. Effects of forced exercise on two-year-old Holstein heifers. J. Dairy Sci. 62:1791–1797.

Lesmeister, J. L., P. J. Burfening, and R. L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J. Anim. Sci. 36:1–6.

Li, P. S., and W. C. Wagner. 1983. In vivo and in vitro studies on the effect of adrenocorticotropic hormone or cortisol on the pituitary response to gonadotropin releasing hormone. Biol. Reprod. 29:25–37.

Looper, M. L., C. A. Lents, and R. P. Wettemann. 2003. Body condition at parturition and postpartum weight changes do not influence the incidence of short-lived corpora lutea in postpartum beef cows. J. Anim. Sci. 81:2390–2394.

Maciel, S. M., C. S. Chamberlain, R. P. Wettemann, and L. J. Spicer. 2001. Dexamethasone influences endocrine and ovarian function in dairy cattle. J. Dairy Sci. 84:1998–2009.

Mahmoud, A. I., F. N. Thompson, D. D. Peck, K. M. Mizinga, L. S. Leshin, L. A. Rund, J. A. Stuedemann, and T. E. Kiser. 1989. Difference in luteinizing hormone response to an opioid antagonist in beef heifers and cows. Biol. Reprod. 41:431–437.

Makimura, S., and N. Suzuki. 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory disease. Jpn. J. Vet. Sci. 44:15–21.

Manning, J. M., and F. H. Bronson. 1991. Suppression of puberty in rats by exercise: Effects on hormone levels and reversal with GnRH infusion. Am. J. Physiol. 260:717–723.

Martin, L. C., J. S. Brinks, R. M. Bourdon, and L. V. Cundiff. 1992. Genetic effects on beef heifer puberty and subsequent reproduction. J. Anim. Sci. 70:4006–4017.

NRC. 1988. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Plasse, D., A. C. Warnick, and M. Koger. 1968. Reproductive behavior of *Bos indicus* females in a subtropical environment. I. Puberty and ovulation frequency in Brahman and Brahman × British heifers. J. Anim. Sci. 27:94–100.

Plasse, D., A. C. Warnick, and M. Koger. 1970. Reproductive behavior of *Bos indicus* females in a subtropical environment. IV. Length of estrous cycle, duration of estrus, time of ovulation, fertilization and embryo survival in grade Brahman heifers. J. Anim. Sci. 30:63–72.

Qiu, X., J. D. Arthington, D. G. Riley, C. C. Chase Jr., W. A. Phillips, S. W. Coleman, and T. A. Olson. 2007. Genetic effects on acute phase protein response to the stresses of weaning and transportation in beef calves. J. Anim. Sci. 85:2367–2374.

Reynolds, W. L., T. M. DeRouen, S. Moin, and K. L. Koonce. 1980. Factors influencing gestation length, birth weight and calf survival of Angus, Zebu and Zebu cross beef cattle. J. Anim. Sci. 51:860–867.

Roberts, A. J., T. W. Geary, E. E. Grings, R. C. Waterman, and M. D. MacNeil. 2009. Reproductive performance of heifers offered ad libitum or restricted access to feed for a 140-d period after weaning. J. Anim. Sci. 87:3043–3052.

Rodrigues, H. D., J. E. Kinder, and L. A. Fitzpatrick. 2002. Estradiol regulation of luteinizing hormone secretion in heifers of two breed types that reach puberty at different ages. Biol. Reprod. 66:603–609.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21:55–89.

Schillo, K. K., J. B. Hall, and S. M. Hileman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. 70:3994–4005.

Short, R. E., R. A. Bellows, J. B. Carr, R. B. Staigmiller, and R. D. Randel. 1976. Induced or synchronized puberty in heifers. J. Anim. Sci. 43:1254–1258.

Smith, R. F., and H. Dobson. 2002. Hormonal interactions within the hypothalamus and pituitary with respect to stress and reproduction in sheep. Domest. Anim. Endocrinol. 23:75–85.

Stahringer, R. C., R. D. Randel, and D. A. Neuendorff. 1990. Effects of naloxone and animal temperament on serum luteinizing-hormone and cortisol concentrations in seasonally anestrous Brahman heifers. Theriogenology 34:393–406.

Thun, R., E. Eggenberger, K. Zerobin, T. Luscher, and W. Vetter. 1981. Twenty-four-hour secretory pattern of cortisol in the bull: Evidence of episodic secretion and circadian rhythm. Endocrinology 109:2208–2212.

Thun, R., C. Kaufmann, and F. Janett. 1998. The influence of restraint stress on reproductive hormones in the cow. Reprod. Domest. Anim. 33:255–260.

Voisinet, B. D., T. Grandin, J. D. Tatum, S. F. O'Connor, and J. J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75:892–896.

Warren, M. P. 1980. The effects of exercise on pubertal progression and reproductive function in girls. J. Clin. Endocrinol. Metab. 51:1150–1157.

Yoshida, C., and T. Nakao. 2006. Plasma cortisol and progesterone responses to low dosed of adrenocorticotropic hormone in ovariectomized lactating cows. J. Reprod. Dev. 52:797–803.

Yoshino, K., N. Katoh, K. Takahashi, and A. Yuasa. 1993. Possible involvement of protein kinase C with induction of haptoglobin in cows by treatment with dexamethasone and by starvation. Am. J. Vet. Res. 54:689–694.