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Effects of temperament on pregnancy rates to fixed-timed AI in *Bos indicus* beef cows ☆

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The objective of the present experiment was to assess the effects of temperament on pregnancy rates to fixed-time AI (FTAI) in *Bos indicus* beef cows. A total of 761 multiparous lactating Nelore cows, originated from 4 different commercial cow–calf ranches, were evaluated for BCS and temperament at the time of FTAI (day 0). Temperament was assessed by chute score and exit velocity. Further, individual exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow). Temperament scores were calculated by averaging cow chute score and exit score. Cows were also classified for temperament type according to temperament score (≤3 = adequate temperament, >3 = excitable temperament). Pregnancy status was verified by detecting a viable conceptus with rectal ultrasonography approximately 40 days after FTAI. Chute score, exit velocity, and temperament score were not correlated to BCS ($P>0.31$). Hence, BCS did not differ ($P=0.30$) according to temperament type (4.13 vs. 4.09 for cows with excitable and adequate temperament, respectively; SEM = 0.070). Pregnancy rates to FTAI tended to be negatively affected by temperament score ($P=0.08$), whereas the probability of cows becoming pregnant to FTAI was negatively associated with temperament score (linear effect, $P<0.01$). Accordingly, pregnancy rates were reduced ($P=0.05$) in cows with excitable temperament compared to cows with adequate temperament (35.3 vs. 42.8% of pregnant cows/total cows, respectively; SEM = 2.85). Pregnancy rates to FTAI were not affected by chute score ($P=0.25$), although the probability of cows becoming pregnant to FTAI tended to be negatively associated with chute score (linear effect, $P=0.07$). Pregnancy rates to FTAI were negatively affected by exit score ($P=0.05$), and the probability of cows becoming pregnant to FTAI was negatively associated with exit score and with actual exit velocity (linear effects, $P<0.01$). Results from this experiment indicate that excitable temperament is detrimental to pregnancy rates of *B. indicus* cows assigned to an estrus synchronization + FTAI protocol.

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1. Introduction

The major objective of cow–calf systems is to produce 1 calf per cow annually. Therefore, reproductive performance of the cowherd is the primary factor in determining the efficiency of cow–calf operations. Recently, our research group reported that behavioral and physiological responses associated with excitable temperament are detrimental to reproductive efficiency of beef cows (Cooke et al., 2009a,

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2010), independently if cows are assigned to natural breeding or estrus synchronization + fixed-time AI (FTAI) protocols. Excitable temperament can impair reproductive function in beef females by several mechanisms, such as decreasing feed intake and nutritional status (Cooke et al., 2009b; Nkrumah et al., 2007), and by stimulating neuroendocrine stress responses that disrupt the physiological processes associated with fertility (Curley et al., 2008; Dobson et al., 2001).

However, the effects of temperament on cattle reproduction were only evaluated in *Bos taurus* and *B. indicus* × *B. taurus* females (Cooke et al., 2009a, 2010). Research has demonstrated that excitable temperament is detected more frequently in *B. indicus* cattle compared to *B. taurus* and *B. taurus*-crosses (Fordyce et al., 1988; Voisinet et al., 1997a). Therefore, cattle temperament might be of even greater importance for reproductive efficiency of cow–calf operations based on *B. indicus* cows, such as the cow–calf industry in Brazil — the second largest beef producer and main exporter of beef in the world (USDA, 2011). Based on this rationale, we hypothesized that excitable temperament is also detrimental to reproductive performance of *B. indicus* beef females, and consequently impact overall efficiency of cow–calf operations based on *B. indicus* cattle. Therefore, the objective of the present experiment was to initially assess the effects of temperament on reproductive performance of *B. indicus* females by associating temperament characteristics and pregnancy rates to FTAI in Nelore (*B. indicus*) beef cows.

2. Materials and methods

2.1. Animals

This experiment was conducted in January 2011 at 4 commercial cow–calf ranches (Catapane, Monte Alto I, Monte Alto II, and Salgueiro) located in the state of Goiás, Brazil. The animals utilized in these experiments 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).

A total of 761 lactating multiparous Nelore cows, approximately 40 to 60 days postpartum, were evaluated for BCS (Wagner et al., 1988) and temperament at the time of FTAI (day 0). Within each ranch, cows were randomly divided into 2 pasture groups. Distribution was based on pasture size and availability. The following distribution of cows within groups was evaluated at each ranch (groups 1 and 2, respectively); Capatane, n = 148 and 65 cows; Monte Alto I, n = 42 and 113 cows; Monte Alto II, n = 116 and 103 cows; and Salgueiro, n = 97 and 77 cows.

2.2. Reproductive management

Cows were assigned to an estrus synchronization + FTAI protocol at the beginning of the breeding season. Only cows receiving the first service of the breeding season were assigned to the experiment. The same estrus synchronization + FTAI protocol was used across all ranches and groups. Cows received a 2 mg injection of estradiol benzoate (Estrogin; Farmavet, São Paulo, SP, Brazil) and a second-use intravaginal progesterone releasing device (CIDR®, originally containing 1.9 g of progesterone; Pfizer Animal Health) on day −11, a 12.5 mg injection of prostaglandin F2α (Lutalyse; Pfizer Animal Health) on day −4, CIDR removal in addition to 0.6 mg of estradiol cypionate (ECP; Pfizer Animal Health) and 300 IU of eCG (Novormon, Schering-Plough Co., São Paulo, Brazil) on day −2, and FTAI on day 0 (Meneghetti et al., 2009). All cows were inseminated by the same technician, and semen from 8 different sires was randomly utilized across all ranches. Pregnancy status to FTAI was verified by detecting a viable conceptus with transrectal ultrasonography (5.0-MHz transducer; 500 V, Aloka, Wallingford, CT, USA) approximately 40 days after FTAI.

2.3. Temperament evaluation

Individual cow temperament was assessed by chute score and exit velocity when cows were processed for FTAI, as previously described by Cooke et al. (2009a) and Cooke et al. (2010). Chute score was assessed by a single technician, immediately before FTAI, based on a 5-point scale where: 1 = calm with no movement, 2 = restless movements, 3 = frequent movement with vocalization, 4 = constant movement, vocalization, shaking of the chute, and 5 = violent and continuous struggling. Exit velocity was assessed immediately after FTAI by determining the speed of the cow exiting the squeeze chute by measuring rate of travel over a 1.9-m distance with an infrared sensor (FarmTek Inc., North Wylie, TX). Further, within each ranch group, cows were divided in quintiles according to their exit velocity, and assigned a score from 1 to 5 (exit score; 1 = cows within the slowest quintile; 5 = cows within the fastest quintile). Individual temperament scores were calculated by averaging cow chute score and exit score. Cows were also classified according to the final temperament score (temperament type) as adequate temperament (temperament score ≤ 3) or excitable temperament (temperament score > 3).

2.4. Statistical analysis

Effects of temperament on pregnancy to FTAI were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) with Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement contained the temperament measurement (temperament score, temperament type, chute score, or exit score), ranch, and the resultant interaction. The random statement contained the effects of group (ranch) and semen sire, which was not distributed equally across ranches, groups, and according to cow temperament. Effects of temperament on cow BCS were analyzed using the MIXED procedure of SAS with Satterthwaite approximation. The model statement contained the temperament type, ranch, and the resultant interaction. The random statement contained the effects of group (ranch). Comparison of ranches according to herd temperament was conducted with the GLIMMIX (proportion of excitable cows) or the MIXED (temperament score, chute score, and exit velocity) procedures of SAS with Satterthwaite approximation. These model statements contained the effects of ranch, whereas group (ranch) was included as random variable. All results are reported as least squares means and were separated by LSD or PDIFF.

The probability of cows to become pregnant to FTAI was evaluated according to temperament score, chute score, exit velocity, and exit score. The GLM procedure of SAS was initially used to determine if each individual measurement influenced pregnancy maintenance linearly, quadratically, or cubically, and also to determine effects of ranch and group (ranch) on the statistical model. The LOGISTIC procedure was used to generate the regression model, determine the intercept and slope(s) values according to maximum likelihood estimates from each significant continuous order effect, and the probability of pregnancy was determined according to the following equation: Probability $= (e^{\text{logistic equation}})/(1+e^{\text{logistic equation}})$. Logistic curves were constructed according to the minimum and maximum values detected for each temperament measurement. Pearson correlations were calculated among exit velocity, chute score, and BCS with the CORR procedure of SAS. The GLM procedure was also utilized to determine effects of ranch and group (ranch) on correlation coefficients.

For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and $P \leq 0.10$. Results are reported according to treatment effects if no interactions were significant, or according to the highest order interaction detected.

3. Results

Distribution of cows according to temperament score or temperament type within and across ranches is reported in Table 1. Overall herd temperament score was similar ($P = 0.40$; Table 2) across all ranches. Accordingly, the incidence of cows classified as excitable was also similar ($P = 0.87$; Table 2) across ranches. However, chute score tended to be greater ($P < 0.10$; Table 2) in the Monte Alto I ranch compared to all other ranches (ranch effect; $P = 0.10$). Exit velocity was reduced ($P < 0.05$; Table 2) in the Monte Alto I and Salgueiro ranches compared to Catapane and Monte Alto II (ranch effect; $P = 0.03$).

Across all ranches, chute score and exit velocity were positively correlated ($P < 0.01$, r = 0.51). However, the correlation coefficient between chute score and exit velocity, although always statistically significant ($P < 0.01$), varied among ranches (r = 0.44, 0.62, 0.53, and 0.65 for Catapane, Monte Alto I, Monte Alto II, and Salgueiro), resulting in a ranch interaction in the correlation analysis ($P < 0.01$). Chute score, exit velocity, and temperament score were not correlated to BCS ($P > 0.31$). Hence, BCS did not differ ($P = 0.30$) according to temperament type (4.13 vs. 4.09 for cows with excitable and adequate temperament, respectively; SEM = 0.070).

Pregnancy rates to FTAI tended to be negatively affected by temperament score ($P = 0.08$; Fig. 1). The probability of cows becoming pregnant to FTAI was negatively associated with temperament score (linear effect, $P < 0.01$; Fig. 1). Accordingly, pregnancy rates were reduced ($P = 0.05$;

Table 1
Distribution of cows according to temperament score and temperament type within the commercial cow–calf ranches where the experiment was conducted.

Item	Ranch				Total
	Catapane	Monte Alto I	Monte Alto II	Salgueiro	
Temperament score [1]					
1.0	13	17	23	24	77
1.5	40	17	29	26	112
2.0	32	25	33	30	120
2.5	32	25	33	28	118
3.0	37	23	39	19	118
3.5	34	15	35	20	104
4.0	21	19	17	16	73
4.5	4	14	10	11	39
5.0	0	0	0	0	0
Temperament type [2]					
Adequate	154	107	157	127	545
Excitable	59	48	62	47	216

[1] Calculated by averaging cow chute score and exit score. Exit score was calculated by dividing chute exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow).
[2] Cows were classified according to the final temperament score as adequate temperament (temperament score ≤ 3) or excitable temperament (temperament score > 3).

Table 2
Ranch effects on overall temperament characteristics of the herd.[1]

Item	Ranch				SEM	P=
	Catapane	Monte Alto I	Monte Alto II	Salgueiro		
Chute score	2.16^x	2.43^y	2.19^x	2.03^x	0.076	0.10
Exit velocity, m/s	2.13^a	1.72^b	2.23^a	1.40^b	0.133	0.03
Temperament score[2]	2.58	2.68	2.59	2.49	0.073	0.40
Excitable cows, %[3]	27.7	30.9	28.3	27.0	0.033	0.86

[1] Values with different superscripts differ at $P < 0.10$ (x vs. y) or $P < 0.05$ (a vs. b).
[2] Calculated by averaging cow chute score and exit score. Exit score was calculated by dividing chute exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow).
[3] Number of excitable cows (temperament score > 3) divided by the total number of cows within each ranch.

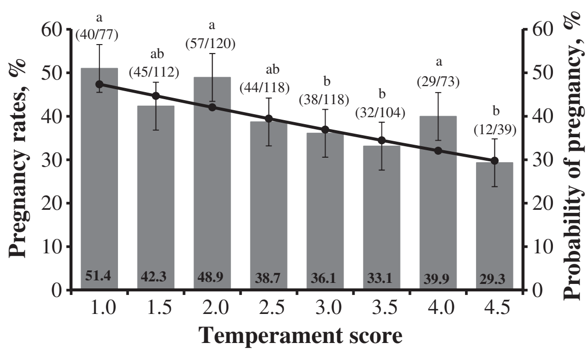

Fig. 1. Pregnancy rates (bars) and probability of pregnancy (line) to fixed-time AI (FTAI) in Nelore (*Bos indicus*) beef cows according to temperament score, which was calculated by averaging cow chute score and exit score at the time FTAI. Exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow). Pregnancy rates tended ($P = 0.08$) to be negatively affected whereas probability of pregnancy was negatively associated (linear effect, $P < 0.01$) with temperament score. Values within bars correspond to LSMEANS. Values in parenthesis correspond to pregnant cows divided by total cows assigned to the estrus synchronization + FTAI protocol. Means with different superscripts (a vs. b) differ at $P < 0.05$.

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Fig. 2) in cows with excitable temperament compared to cows with adequate temperament (35.3 vs. 42.8% of pregnant cows/total cows, respectively; SEM = 2.85).

Pregnancy rates to FTAI were not affected by chute score ($P = 0.25$; Fig. 3), although the probability of cows becoming pregnant to FTAI tended to be negatively associated with chute score (linear effect, $P = 0.07$; Fig. 3). Pregnancy rates to FTAI were negatively affected by exit score ($P = 0.05$; Fig. 4), and the probability of cows becoming pregnant to FTAI was negatively associated with exit score and with actual exit velocity (linear effects, $P < 0.01$; Figs. 4 and 5).

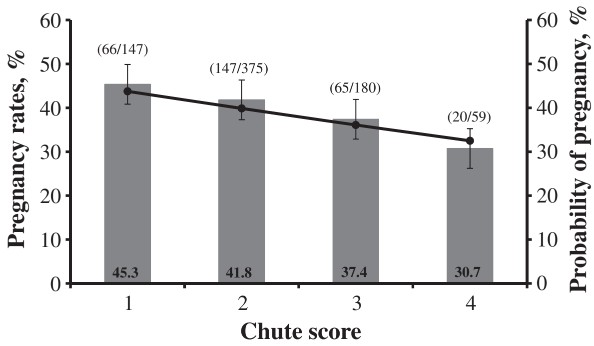

Fig. 3. Pregnancy rates (bars) and probability of pregnancy (line) to fixed-time AI (FTAI) in Nelore (*Bos indicus*) beef cows according to chute score evaluated at the time FTAI. Pregnancy rates were not affected by chute score ($P = 0.25$), whereas the probability of cows becoming pregnant to FTAI tended to be negatively associated with chute score (linear effect, $P = 0.07$). Values within bars correspond to LSMEANS. Values within parenthesis correspond to pregnant cows divided by total cows assigned to the estrus synchronization + FTAI protocol.

4. Discussion

Several research studies reported that, independently of breed type, cattle with excitable temperament have impaired growth (Nkrumah et al., 2007; Voisinet et al., 1997a), health (Burdick et al., 2010; Fell et al., 1999), and carcass quality parameters (Cafe et al., 2011; King et al., 2006; Voisinet et al., 1997b), demonstrating the importance of cattle temperament to beef production systems. Our research group was the first to report that temperament also has direct implications to reproductive performance of beef females (Cooke et al., 2009a, 2009b, 2010). However, these research studies only evaluated *B. taurus*-influenced cattle, and to our knowledge, the present experiment is the first to directly assess the effect of temperament on reproductive performance of *B. indicus* beef females.

In the present study, based on our temperament criteria, approximately 28% of the cows evaluated were classified as excitable (216/761 excitable cows/total cows; Table 1). The incidence of excitable cows was similar among ranches (Table 2), supported by the similar mean temperament score across herds (Table 2). It is important to note that chute score and, consequently, temperament score of 5 were not detected in any of the animals evaluated herein (Table 1). All cows were processed through the chute 3 times within 11 days prior to FTAI for estrus synchronization, which may have ameliorated and prevented extremely aggressive behavior during chute restraining for FTAI (Cooke et al., 2009b). Matsunaga et al. (2002) estimated that the incidence of excitable Nelore cattle in Brazilian beef operations is at 10%, which differs from the results reported herein. This difference can be attributed to several factors, including the number of cattle evaluated, differences in cattle population and production systems, methods of temperament evaluation, and interpretation of temperament measurements. However, the major objective of the present study was to determine if temperament impacts reproduction in *B. indicus* beef females,

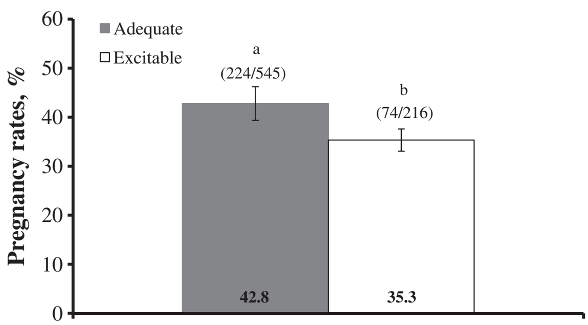

Fig. 2. Pregnancy rates to fixed-time AI (FTAI) in Nelore (*Bos indicus*) beef cows according to temperament type, which was calculated based on cow temperament score (adequate temperament, temperament score ≤ 3; excitable temperament, temperament score > 3). Temperament score was calculated by averaging cow chute score and exit score at the time FTAI. Exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow). Pregnancy rates were reduced ($P = 0.05$) in cows with excitable temperament compared to cows with adequate temperament. Values within bars correspond to LSMEANS. Values within parenthesis correspond to pregnant cows divided by total cows assigned to the estrus synchronization + FTAI protocol. Means with different superscripts (a vs. b) differ at $P = 0.05$.

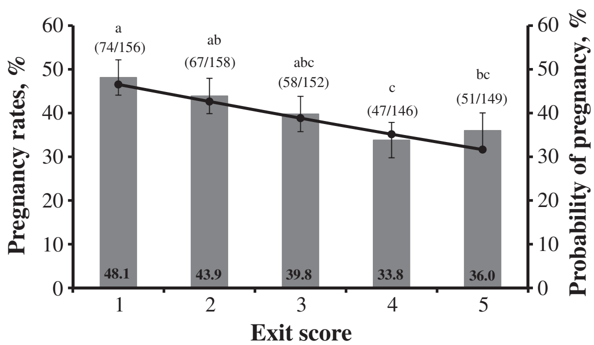

Fig. 4. Pregnancy rates (bars) and probability of pregnancy (line) to fixed-time AI (FTAI) in Nelore (*Bos indicus*) beef cows according to exit score evaluated at the time FTAI. Exit score was calculated by dividing exit velocity results into quintiles and assigning cows with a score from 1 to 5 (exit score: 1 = slowest cows; 5 = fastest cow). Pregnancy rates were negatively affected ($P = 0.05$) whereas probability of pregnancy was negatively associated (linear effect, $P < 0.01$) with exit score. Values within bars correspond to LSMEANS. Values in parenthesis correspond to pregnant cows divided by total cows assigned to the estrus synchronization + FTAI protocol. Means with different superscripts (a, b or c) differ at $P < 0.05$.

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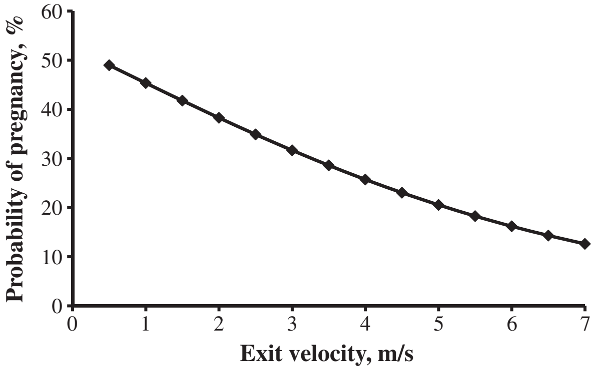

Fig. 5. Probability of pregnancy to fixed-time AI (FTAI) in Nelore (*Bos indicus*) beef cows according to exit velocity evaluated at the time FTAI. A linear effect was detected ($P<0.01$).

and not to determine the incidence of excitable females in Brazilian herds. The methods used herein to evaluate cattle for temperament were similar to our previous research efforts with *B. taurus*-influenced cattle (Cooke et al., 2009a, 2009b, 2010), and have the purpose of classifying cattle according to temperament characteristics by using techniques that can be feasibly completed during routine cattle processing (Cooke, 2009).

Although mean temperament score was similar, ranch effects were detected on mean chute score and exit velocity of the herds (Table 2). Further, the correlation coefficient among chute score and exit velocity differed among ranches. These differences can be attributed to inherent characteristics of each herd and operation, including differences in cattle management and design of the handling facilities (Cafe et al., 2011; Cooke, 2009). Still, exit velocity and chute score were positively correlated in all ranches, and coefficient values were similar to those previously reported by our research group with *B. taurus*×*B. indicus* heifers and cows (Cooke et al., 2009a, 2009b).

Research studies demonstrated that cattle with excitable temperament may have reduced nutritional status compared to cohorts with adequate temperament due to reduced feed intake (Nkrumah et al., 2007) and altered nutrient metabolism to support stress-related physiological and behavioral responses (Carroll and Forsberg, 2007; Elsasser et al., 1997; Maciel et al., 2001). Therefore, excitable temperament may indirectly impair reproductive performance of beef females by reducing their nutritional status (Wettemann and Bossis, 2000). Previous research reported that temperament was negatively associated with BCS and nutritional status in growing cattle (Cooke et al., 2009b; Petherick et al., 2009), but not in mature cows (Cooke et al., 2009a, 2010; Sandelin et al., 2005), and the reason for this age difference is unknown. Nevertheless, cow BCS at FTAI was not affected by temperament in the present experiment, indicating that any effects of temperament on reproductive performance are independent of cow nutritional status.

Supporting our main hypothesis, excitable temperament was detrimental to pregnancy rates to FTAI in *B. indicus* beef cows. In fact, pregnancy rates were reduced by 17% when comparing cows with excitable temperament and cows with adequate temperament (35.3% divided by 42.8%, respectively; Fig. 2), or by 43% when comparing cows with the highest temperament score with those with the lowest temperament score (29.3% divided by 51.4% for temperament score 4.5 and 1, respectively; Fig. 1). When analyzed individually, the measurements of temperament utilized herein also impacted reproductive outcomes. The probability of cows becoming pregnant to FTAI was negatively associated with exit velocity and subsequent exit score (Figs. 4 and 5), whereas pregnancy rates to FTAI were reduced by 25% when comparing cows with the highest exit velocities and cohorts with the lowest exit velocities (36.0% divided by 48.2% for exit score 5 and 1, respectively; Fig. 4). Chute score did not impact pregnancy rates to FTAI, although it was negatively associated with probability of pregnancy to FTAI (Fig. 3). Exit velocity is classified as a nonrestrained technique 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), which may have contributed to the lack of chute score effects on pregnancy rates to FTAI in the present experiment. On the other hand, cattle with excitable temperament may resist to chute restrain to the point of suffocation or lose balance when leaving the squeeze chute, and both instances can distort exit velocity (Cooke, 2009). Therefore, using more than one measurement to evaluate cattle temperament may help preventing incorrect assessments in excitable animals. Accordingly, temperament score was better correlated to plasma cortisol concentrations during processing, the main indicator of the neuroendocrine stress response to handling (Sapolsky et al., 2000; Thun et al., 1998), compared to chute score and exit velocity in *B. indicus*×*B. taurus* heifers and cows (Cooke et al., 2009a, 2009b).

Results from the present experiment, combined with previous research from our group (Cooke et al., 2009a, 2010), demonstrate that excitable temperament is detrimental to reproductive performance of beef cows independently of breed type. Further, cattle temperament might be of even greater importance for reproductive efficiency of cow–calf operations based on *B. indicus* cows because excitable temperament is detected more frequently in *B. indicus* cattle compared to *B. taurus* and *B. taurus*-crosses (Fordyce et al., 1988; Voisinet et al., 1997a). Cattle with excitable temperament have stimulated function of the hypothalamic-pituitary-adrenal axis when exposed to humans and handling procedures, resulting in a neuroendocrine stress response characterized by increased synthesis and circulating concentrations of ACTH and cortisol (Cooke et al., 2009a, 2009b; Curley et al., 2008). These and other stress-related hormones directly impair the physiological mechanisms required for fertility in beef cows, including resumption of estrous cycles, ovulation of a competent oocyte, and establishment of pregnancy (Dobson et al., 2001). More specifically, elevated circulating concentrations of ACTH and cortisol disrupt synthesis and release of gonadotropins (Dobson et al., 2000; Li and Wagner, 1983), reduce the sensitivity of the brain to estrogen (Hein and Allrich, 1992), and impair progesterone production by the corpus luteum (da Rosa and Wagner, 1981; Wagner et al., 1972). In the present experiment, reduced pregnancy rates of cows with excitable temperament can be attributed, at least partially, to neuroendocrine stress responses stimulated during handling for estrus synchronization and FTAI. However, beef cows with excitable temperament and maintained on extensive conditions, with no human interaction or handling, during natural breeding also experienced reduced reproductive performance compared with calmer cohorts (Cooke et al., 2009a, 2010). In addition, one can speculate that the exogenous hormones administered herein to cows during estrus synchronization compensated for the detrimental effects of excitable temperament on synthesis and release of steroids and gonadotropins. Therefore, additional mechanisms associating temperament and reproduction in beef females, including post-conception effects and genetic and innate deficiencies within the reproductive system of excitable cows, warrants further investigation. These warranted research efforts should also evaluate the impacts of temperament on reproductive performance of *B. indicus* cows assigned to natural breeding only, as well as performance of the subsequent calf crop given that temperament is a heritable trait in Nelore (Carneiro et al., 2006) and other *B. indicus* breeds (Hearnshaw and Morris, 1984).

5. Conclusion

Results from this experiment indicate that excitable temperament is detrimental to pregnancy rates of *B. indicus* cows assigned to an estrus synchronization + FTAI protocol. Additional research is still required to fully comprehend the effects of temperament on reproductive performance of *B. indicus* beef cows, as well as its effects on reproductive physiology of beef females. Nevertheless, temperament of the cowherd impacts the reproductive and overall efficiency of cow–calf operations based on *B. indicus, B. taurus,* or *B. indicus* × *B. taurus* cattle.

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