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Highlights

- Excitable cows tended to lose more body condition score during FTAI period.
- Temperament did not affect body weight, and plasma haptoglobin and ceruloplasmin.
- Excitable cows tended to have a smaller dominant follicle diameter and corpus luteum diameter and volume.
- Excitable cows produced less progesterone.

Effects of temperament on body parameters, ovarian structures and inflammatory response in grazing Nellore cows following fixed-time artificial insemination

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ABSTRACT

This study evaluated the associations of temperament with body weight (BW) and body condition score (BCS) change, ovarian structures, and inflammatory response in grazing Nellore cows assigned to a fixed-time artificial insemination (FTAI) protocol. Forty multiparous cows were kept on pasture and offered free-choice access to mineral/vitamin supplementation and FTAI on d 0. Individual temperament scores (1-5) were assessed in the squeeze chute on d -30, where 1 are animals extremely calm and 5 extremely excitable. Cows with a chute score ≤ 3 were classified as "calm", and > 3 as "excitable". The BW and BCS were evaluated on d -30, 0 and 30, ovarian structures on d 0 and 14, and blood samples collected on d -30, -11, 0, 7, 14, 21 and 30. Excitable animals tended to have less (P = 0.09) BCS and lose more (P = 0.09) BCS from d -30 to 0. Temperament did not affect (P ≥ 0.12) BW and BW change. Excitable animals tended (P ≤ 0.10) to have smaller dominant follicles, corpus luteum (CL) diameters and CL volumes. Temperament did not affect (P ≥ 0.14) plasma concentrations of haptoglobin and ceruloplasmin, but excitable animals had less (P = 0.04) plasma concentrations of progesterone on d 7 and 14. Thus, excitable cows had less plasma progesterone concentrations and tended to have a greater decrease in body condition score and had smaller dominant follicles and corpus luteum size.

Keywords: Behavior; Chute score; Corpus luteum; Excitability; Reproduction

Introduction

Several factors may influence the reproductive performance of beef cows, and one of them is temperament (Cooke et al., 2009a, 2011, 2017; Kasimanickam et al., 2014; Rueda et al., 2015). Animal temperament can be defined as a response to environmental or social stimuli (Haskell et al.,2014). Potential causes of the excitable temperament in cattle include genetics, inadequate facilities, and improper handling.

According to Rueda et al. (2015), handling excitable cows in the squeeze chute increases the incidence of aggression to the handlers, insemination time, likelihood of accidents, contamination in the perineal region during artificial insemination (AI), and reduces the technician AI efficiency. In addition, excitable cows have less pregnancy percentage following fixed-time AI (FTAI; Cooke et al., 2009a, 2011, 2017; Kasimanickam et al., 2014; Rueda et al., 2015). Temperament can negatively affect reproductive performance by several mechanisms, such as decreasing dry matter intake (DMI) and nutritional status (Bruno et al., 2016), and increasing the stimulation of the hypothalamic-pituitary-adrenal axis leading to greater circulating concentrations of cortisol (Curley Jr et al., 2008) and acute phase proteins (Cooke et al., 2009a, 2012; Francisco et al., 2015). Kasimanickam et al. (2014) observed that temperament also impacts ovarian parameters, and is negatively correlated with follicles number and size of the dominant follicle. We are unaware of studies evaluating the associations of temperament with corpus luteum diameters/volumes in purebred *Bos indicus* cows. We hypothesized that the excitable temperament in the squeeze chute can mainly reflect in reduction of the size of the corpus luteum and in its production of progesterone. Thus, the objective of this study was to evaluate the associations of temperament with body weight (BW) and body condition score (BCS) change, ovarian structures and inflammatory response of grazing Nellore cows synchronized to FTAI.

Materials and methods

This study was conducted according to the ethical standards applied to animal research and approved by the Ethics Committee on Animal Use of the Universidade Federal de Mato Grosso do Sul (UFMS) under protocol no. 754/2016.

The experiment was conducted at the School Farm of Faculdade de Medicina Veterinária e Zootecnia of the Universidade Federal de Mato Grosso do Sul, Terenos, Mato Grosso do Sul, Brazil (20°26'50.8"S54°50'21.5"W). A total of 40 Nellore multiparous cows (BCS = 4.75 ± 0.49, scale 1 to 9; BW = 392 ± 30 kg, and 5.12 ± 2.04 yr of age] were selected for the study. Cows were allocated to two paddocks (12 hectares each) of marandu-grass [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu] and had free-choice access to water and a complete trace mineral/vitamin mix (Mega Fós 90 Milk, AgroMega Indústria de Alimentos Animal, Tamboara, PR, Brazil; Ca, 196 g/kg, P, 90 g/kg, Na, 99 g/kg, Mg, 20 g/kg, S, 14 g/kg, Fe, 2400 mg/kg, Zn, 300 mg/kg, Mn, 1670 mg/kg, Se, 40 mg/kg, Cu, 1200 mg/kg, F, 900 mg/kg, Co, 200 mg/kg, I, 180 mg/kg, vitamin A, 150000 UI/kg, vitamin D3, 30000 UI/kg, and vitamin E, 1500 UI/kg; target consumption of 100 g/day). Twenty animals were allocated to each paddock (10 calm and 10 excitable on paddock 1 and 9 calm and 11 excitable on paddock 2 - defined by the chute score on d -30) where they remained throughout the study.

The temperament of the animals was evaluated by a single trained technician on d -30 through subjective scores (1-5) in the squeeze chute adapted from Cooke et al. (2011) and described in Table 1. Animals with scores ≤ 3 were classified as "calm" and with scores > 3 as "excitable". All cows were calmly handled in the corral without the use of electric shock or any other physical contact until they reached the squeeze chute. Throughout the experiment, cows were handled eight times. Four handlings were performed until the day of AI (d -30, -11, -2, and 0) and four after AI (d 7, 14, 21 and 30).

All cows were assigned to a FTAI protocol from d -11 to 0. On d -11, cows received a 2-mg i.m. injection of estradiol benzoate (Gonadiol; Zoetis, São Paulo, Brazil) and inserted with an intravaginal progesterone-releasing device containing 1.9 g of progesterone (CIDR; Zoetis, São Paulo, Brazil). On d −2, CIDR device was removed, and cows received intramuscular (i.m.) injections of $PGF_{2\alpha}$ (12.5 mg; Lutalyse; Zoetis, São Paulo, Brazil), estradiol cypionate (1 mg; ECP; Zoetis, São Paulo, Brazil) and eCG (300 IU; Novormon; Zoetis, São Paulo, Brazil). On d 0 (approximately 48 h after the injection of $PGF_{2\alpha}$), cows were timed-AI by a single technician using semen from a single Nellore bull. Pregnancy status was assessed on d 30 by transrectal ultrasonography (7.5-MHz transducer; Mindray DP 2200 VET, Shenzhen, China).

The ovarian structures were evaluated by transrectal ultrasonography (Mindray DP 2200 VET with transducer of 7.5 MHz, Shenzhen, China). The diameters (mm) of the dominant follicles on the day of AI (d 0) and the diameters of the CL14 d after AI (d 14) were evaluated. The CL volumes (cm^3) were calculated using the formula for volumes of the sphere [$V=4/3\pi(D/2)^3$ where D is the maximum diameters (mm) of the CL (Cooke et al., 2009b)]. Cow BW and BCS were assessed on d -30, 0 and 30. Cow BCS was evaluated by a single trained technician (not the same that evaluated the chute score), before the cows enter in the squeeze chute (Herd and Sprott, 1986), which kept the AI technician blind to temperament classification.

Blood samples were collected from the coccygeal vein on d -30, -11, 0, 7, 14, 21 and 30 into 10-mL blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) with sodium heparin. Immediately after collection, blood samples were stored on ice and then centrifuged at 1200 × g for 30 min for plasma harvest. Plasma samples were stored at -20ºC for further analysis of the plasma concentrations of haptoglobin, ceruloplasmin, and progesterone. Plasma concentrations of progesterone were analyzed on d 0, 7, 14, 21 and 30. Hand plucked samples of pastures were collected on d -30, 0 and

30, and then dried at 60°C for 5 d, ground to 1 mm, composed into a single sample, and analyzed for chemical composition.

Forage samples were analyzed according to AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ethereal extract (EE), method 920.39 and ashes, method 942.05. The concentrations of lignin, neutral detergent fiber (NDF) and acid (ADF) were done according to the methodology of Van Soest et al. (1991). The concentration of DM was 291.5 g/kg, CP, 68.8 g/kg, EE, 20.9 g/kg, ashes, 91.2 g/kg, NDF, 727.7 g/kg, ADF, 420.8 g/kg and lignin, 43.7 g/kg.

Plasma samples were analyzed as follows: haptoglobin was analyzed as described by Cooke and Arthington (2013), and ceruloplasmin as described by Demetriou et al. (1974). The inter- and intra-assay CV were 4.23 and 6.90% for haptoglobin, and 3.43 and 4.86% for ceruloplasmin. Plasma progesterone concentrations were determined using a solid-phase, competitive, chemiluminescent enzyme immunoassay (Immulite 1000, Diagnostics Products Corp.) previously validated for bovine samples (Martin et al., 2007). Detectable range and intra-assay CV for plasma progesterone concentrations were 0.2 to 12.2 ng/mL and 4.53%, respectively.

All dependent variables were tested for normality using Univariate procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) and all were normal distributed. Then, ovarian structures, plasma analyses, BW, BW change, BCS and BCS change were analyzed using MIXED procedure, whereas pregnancy rate was analyzed using GLIMMIX (with the binomial distribution option) procedure of SAS. For both procedures, the Satterthwaite approximation was used to determine the denominator degrees of freedom for the test of fixed effects. The chute score, BCS change, BW change, pregnancy percentages, and ovarian parameters were tested for the fixed effect of temperament, paddock and the interaction, and using cow (temperament) as random effects. The BW, BCS, and plasma analyses were analyzed as repeated measures and tested for fixed effects of temperament, day, paddock and all possible interactions and using cow (temperament) as random variables. For the analysis of plasma concentration of progesterone, the pregnancy status was included as fixed effect (interacting with temperament, day, paddock and temperament × day × paddock) but was excluded from the model because no interactions were detected ($P \geq 0.83$). The autoregressive covariance structure was selected, defined through the lowest Akaike information criterion. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10.

Results

No associations of paddock, or interaction of paddock with temperament, day, or temperament × day were detected ($P \geq 0.34$) for any variable. Associations of temperament were detected ($P < 0.0001$)

with chute score and were greater for excitable vs. calm cows (Table 2). Associations of temperament, but not temperament × day (P = 0.21), tended to be detected (P = 0.09) and association of day was detected with cow BCS (P = 0.05; Table 2). Excitable cows tended (P = 0.09) to have less mean BCS during the experiment compared to calm cows (Table 2). Excitable cows also tended (P = 0.09) to lose more BCS than calm cows from d -30 to 0 (Table 2). Associations of temperament were not detected ($P \geq 0.12$) with BCS change from d 0 to 30 and d -30 to 30 (Table 2). Associations of day, but not temperament × day and temperament ($P \geq 0.31$), were detected ($P < 0.0001$) with cow BW (Table 3). Associations of temperament were not detected ($P \geq 0.39$) with BW change (Table 2) and pregnancy rates (61.90 vs. 57.89 ± 10.96% for calm and excitable cows, respectively). Associations of temperament tended ($P \leq 0.10$) to be detected with dominant follicles, CL diameters, and CL volumes and the larger sizes were observed to calm vs. excitable cows (Table 2). Associations of temperament × day, temperament and day were not detected ($P \geq 0.14$) with plasma haptoglobin (Table 2). Associations of day, but not temperament × day and temperament ($P \geq 0.40$), were detected with plasma ceruloplasmin ($P < 0.0001$; Table 2). The lowest concentration of ceruloplasmin was on d -30 and increased throughout the experiment ($P < 0.0001$; Table 2). Associations of temperament × day and day were detected ($P \leq 0.04$) and associations of temperament tended to be detected (P = 0.06) with plasma concentrations of progesterone (Table 2). Calm cows had greater plasma concentration of progesterone on d 7 and 14 compared to excitable cows (P = 0.04; Fig. 1). Furthermore, the lowest concentration of plasma progesterone was observed on d 0, the highest on d 14, and intermediate values on d 7, 21 and 30 ($P <$ 0.0001; Fig. 1).

Discussion

In the present study, excitable cows had a greater decrease in BCS from d -30 to 0 than calm cows. In growing cattle, excitable temperament has a negative correlation with BCS (Petherick et al., 2009), and mature cows acclimated to human interactions showed a smaller decrease in BCS during the breeding season (Cooke et al., 2009a). Other studies observed no associations of temperament with BCS of mature cows (Cooke et al., 2011, 2017; Kasimanickam et al., 2014). In our study, the greater number of handlings during the experiment (4 in the first month of study) differs from previous studies cited above and may have contributed to the greater BCS loss of excitable cows. It is plausible that the decrease in BCS in excitable cows was caused by a reduced forage intake compared to calm cows as observed in other studies (Nkrumah et al., 2007, Bruno et al., 2016). From d 0 to 30, no associations of temperament were observed with cow BCS change, which may be attributed to the acclimatization of cows to human interaction (Cooke et al., 2009a,b), and thus reducing the intensity of temperament on BCS.

Temperament did not impact cow BW. Excitable animals in the growing phase usually present less feedlot growth performance due to DMI reduction (Bruno et al., 2016). However, in agreement to the present study, Cooke et al. (2017) did not observe temperament association with BW and BCS of mature cows. In our study, the absence of temperament effect on BW associated with a negative effect on BCS may be a result of a change in the body composition of the animals, with a reduction in fat proportion.

Excitable temperament negatively affected the size of the dominant follicles and CL. Kasimanickam et al. (2014) also reported negative association with temperament on follicles number and size of the dominant follicles; however, the present study is the first one to report that more excitable cows tend to have a smaller CL diameters/volumes. This reduction may have happened for three reasons: (1) the fact that excitable cows presented a greater decrease in BCS leading to less energy supply to the ovary and negatively influenced the follicular development. Lents et al. (2008) observed that cows with BCS < 5 had a smaller diameters of the dominant follicles compared to cows with BCS ≥ 5. Cows with higher BCS may have higher circulating concentrations of insulin-like growth factor IGF-1, leptin, insulin, thyroxine, glucose and non-esterified fatty acid (NEFA; Ciccioli et al, 2003) and these changes may affect the follicular development. In addition, (2) excitable cows may have experienced less forage intake compared to calm cows. According to Murphy et al. (1990), the lower nutrient intake leads to higher turnover rate of cells and the increase in turnover rate in follicles, and consequently, decreasing follicles sizes. Additionally, (3) excitable cows present greater stimulation of the hypothalamus-pituitary-adrenal axis when they are handled which increases synthesis and release of circulating cortisol (Cooke et al., 2009b, 2017; Curley Jr et al., 2008; Kasimanickam et al., 2014), prolactin and substance-P, and directly affecting follicular dynamics and ovarian steroidogenesis (Kasimanickam et al., 2014).

Excitable cows had lower plasma progesterone concentrations on d 7 and 14, which may be explained by the smaller CL sizes as CL size is positively correlated with progesterone synthesis (Kastelic et al., 1990). In addition, excitable cows exhibit hyperstimulated hypothalamic-pituitary-adrenal axis and increased synthesis of circulating cortisol and ACTH (Curley Jr et al., 2008). Greater concentrations of peripheral ACTH and cortisol may decrease the production of progesterone by the CL (Rosa and Wagner, 1981). Although temperament did not affect the concentration of the acute phase proteins haptoglobin and ceruloplasmin, some studies have shown that calm or acclimated cattle may present lower concentrations of plasma haptoglobin (Cooke et al., 2012; Francisco et al., 2015) and ceruloplasmin (Cooke et al., 2009a) than excitable or not acclimated cattle. However, serum haptoglobin concentrations peak 24 to 72 h after an acute stressor in cattle (Arthington et al., 2008), and in the current study, haptoglobin and ceruloplasmin concentrations were analyzed at least every 7 d after handling, which may have reduced the likelihood to detect temperament associations. Despite the absence of an association between temperament and plasma ceruloplasmin, the lower plasma concentration of ceruloplasmin occurred at beginning of the experiment (d -30) and increased on d 0, indicating that the handling of the animals in the corral increased the inflammatory response regardless of temperament scores.

Although it was not the objective of our study, casual observations indicated that excitable cows were more difficult to handle than calm cows and required more time to inseminate. Other studies already mentioned that excitable temperament increases the risk of aggressive handling and tends to decrease labor efficiency by increasing the time needed to perform AI and reducing cow body hygiene (Rueda et al., 2015). Thus, further studies exploring the associations between temperament and the quality of the handling may be warranted.

Conclusions

The results of this experiment suggest that excitable behavior of cows in the squeeze chute during and following a FTAI protocol did not change cow body weight and plasma concentration of acute phase proteins, but reduced plasma concentrations of progesterone and tend to led to greater BCS loss, and smaller dominant follicles and corpora lutea. Future experiments evaluating the ovarian structures or progesterone production by beef cows should consider the possibility of including the temperament of the animals in the randomization of treatments.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship statement

The idea for the paper was conceived by Marcelo Vedovatto and Gumercindo Loriano Franco. The experiments were designed by Marcelo Vedovatto and Gumercindo Loriano Franco. The experiments were performed by Marcelo Vedovatto, Deiler Sampaio Costa, Fábio José Carvalho Faria, Rafaela Nunes Coelho and Gumercindo Loriano Franco. The data were analyzed by Marcelo Vedovatto, Reinaldo Fernandes Cooke and Philipe Moriel. The paper was written by Marcelo Vedovatto, Reinaldo Fernandes Cooke, João Marcelo Dalmazo Sanchez, Philipe Moriel, Rafaela Nunes Coelho and Gumercindo Loriano Franco.

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References

AOAC, 2000. Official methods of analysis, 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD.

Arthington, J.D., Qiu, X., Cooke, R.F., Vendramini, J.M.B., Araujo, D.B., Chase Jr, C.C., Coleman, S.W., 2008. Effects of pre-shipping management on measures of stress and performance of beef steers during a feedlot receiving period. J. Anim. Sci. 86, 2016–2023. https://doi.org/10.2527/jas.2008-0968.

Bernabucci, U., Ronchi, B., Lacetera, N., Nardone, A., 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy. Sci. 88, 2017-2016. https://doi.org/10.3168/jds.S0022-0302(05)72878-2.

Bruno, K.A., Vanzant, E.S., Vanzant, K.A., McLeod, K.R., 2016. Relationships of a novel objective chute score and exit velocity with growth performance of receiving cattle. J. Anim. Sci. 94, 4819–4831. https://doi.org/10.2527/jas.2016-0438.

Carter, F., Forde, N., Duffy, P., Wade, M., Fair, T., Crowe, M.A., Evans, A.C., Kenny, D.A., Roche, J.F., Lonergan, P., 2008. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reprod. Fertil. Dev. 20, 368-75. https://doi.org/10.1071/RD07204.

Ciccioli, N.H., Wettemann, R.P., Spicer, L.J., Lents, C.A., White, F.J., Keisler, D.H., 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81, 3107–3120. https://doi.org/10.2527/2003.81123107x.

Cooke, R.F., Arthington, J.D., Araujo, D.B., Lamb, G.C., 2009a. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. J. Anim. Sci. 87, 4125-4132. https://doi.org/10.2527/jas.2009-2021.

Cooke, R.F., Arthington, J.D., Austin, B.R., Yelich, J.V., 2009b. Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. J. Anim. Sci. 87, 3403-3412. https://doi.org/10.2527/jas.2009-1910.

Cooke, R.F., Bohnert, D.W., Meneghetti, M., Losi, T.C., Vasconcelos., J.L.M., 2011. Effects of temperament on pregnancy rates to fixed-timed AI in *Bos indicus* beef cows. Livest. Sci. 142, 108–113. https://doi.org/10.1016/j.livsci.2011.06.024.

Cooke, R.F., Bohnert, D.W., Cappellozza, B.I., Mueller, C.J., DelCurto, T., 2012. Effects of temperament and acclimation to handling on reproductive performance of *Bos taurus* beef females. J. Anim. Sci. 90, 3547–3555. https://doi.org/10.2527/jas.2011-4768.

Cooke, R.F., Arthington, J.D., 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. J. Anim. Physiol. Anim. Nutr. 97, 531–536. https://doi.org/10.1111/j.1439-0396.2012.01298.x.

Cooke, R.F., Schubach, K.M., Marques, R.S., Peres, R.F.G., Silva, L.G.T., Carvalho, R.S., Cipriano, R.S., Bohnert, D.W., Pires, A.V., Vasconcelos, J.L.M., 2017. Effects of temperament on physiological, productive, and reproductive responses in *Bos indicus* beef cows. J. Anim. Sci. 95, 1–8. https://doi.org/10.2527/jas.2016.1098.

Curley Jr, K.O., Neuendorff, D.A., Lewis, A.W., Cleere, J.J., Welsh, T.H. Jr., Randel, R.D., 2008. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Hormon. Behav. 53, 20–27. https://doi.org/10.1016/j.yhbeh.2007.08.005.

Demetriou, J.A., Drewes, P.A., Gin, J.B., 1974. Ceruloplasmin. In: D. C. Cannon and J. W. Winkelman, editors, Clinical chemistry. Harper and Row, Hagerstown, MD. p. 857–864.

Francisco, C.L., Resende, F.D., Benatti, J.M.B., Castilhos, A.M., Cooke, R.F., Jorge, A.M., 2015. Impacts of temperament on Nellore cattle: physiological responses, feedlot performance, and carcass characteristics. J. Anim. Sci. 93, 5419-5429. https://doi.org/10.2527/jas.2015-9411.

Haskell, M.J., Simm, G., Turner, S.P., 2014. Genetic selection for temperament traits in dairy and beef cattle. Front. Genet. 5, 1-18. https://doi.org/10.3389/fgene.2014.00368.

Herd, D.B., Sprott, L.R., 1986. Body condition, nutrition and reproduction of beef cows. Texas Agricultural Extension Service. B-1526.

Inskeep, E.K., Dailey, R.A., 2005. Embryonic death in cattle. Vet. Clin. Food. Anim. 21, 437–461. https://doi.org/10.1016/j.cvfa.2005.02.002.

Kasimanickam, R., Schroeder, S., Assay, M., Kasimanickam, V., Moore, D.A., Gay, J.M., Whittier, W.D., 2014. Influence of temperament score and handling facility on stress, reproductive hormone concentrations, and fixed time AI pregnancy rates in beef heifers. Reprod. Dom. Anim. 49, 775–782. https://doi.org/10.1111/rda.12368.

Kastelic, J. P., Bergfelt, D.R., Ginther, O.J., 1990. Relationship between ultrasonic assessment of the corpus luteum and plasma progesterone concentration in heifers. Theriogenology. 33, 1268-1278. https://doi.org/10.1016/0093-691X(90)90045-U.

Lents, C.A., White, F.J., Ciccioli, N.H., Wettemann, R.P., Spicer, L.J., Lalman, D.L., 2008. Effects of body condition score at parturition and postpartum protein supplementation on estrous behavior and size of the dominant follicle in beef cows. J. Anim. Sci. 86, 2549–2556. https://doi.org/10.2527/jas.2008-1114.

Martin, J.L., Vonnahme, K.A., Adams, D.C., Lardy, G.P., Funston, R.N., 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. J. Anim. Sci. 85, 841–847. https://doi.org/10.2527/jas.2006-337.

Murphy, M.G., Boland, M.P., Roche, J.F., 1990. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J. Reprod. Fertil. 90, 523–533. https://doi.org/10.1530/jrf.0.090052.

Nkrumah, J.D., Crews Jr, D.H., Basarab, J.A., Price, M.A., Okine, E.K., Wang, Z., Li, C., Moore, S.S., 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. J. Anim. Sci. 85, 2382–2390. https://doi.org/10.2527/jas.2006-657.

Petherick, J.C., Doogan, V., Venu, V.K., Holroyd, R.G., Olsson, P., 2009. Quality of handling and holding yard environment, and beef cattle temperament: 2. Consequences for stress and productivity. Appl. Anim. Behav. Sci. 120, 28–38. https://doi.org/10.1016/j.applanim.2009.05.009.

Rosa, G.O., Wagner, W.C., 1981. Adrenal-gonad interactions in cattle. Corpus luteum function in intact and adrenalectomized heifers. J. Anim. Sci. 52, 1098–1105. https://doi.org/10.2527/jas1981.5251098x.

Rueda, P.M., Sant'Anna, A.C., Valente, T.S., Da Costa, M.J.R.P., 2015. Impact of the temperament of Nellore cows on the quality of handling and pregnancy rates in fixed-time artificial insemination. Livest. Sci. 177, 189–195. http://dx.doi.org/10.1016/j.livsci.2015.04.021.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. J. Dairy. Sci. 74, 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Table 1 Criteria used to define the chute score[a] of each cow on d -30.

Chute score	Cow behavior in the squeeze chute	Temperament
1	Calm with no movement	Calm
2	Restless movements	Calm
3	Frequent movement	Calm
4	Constant movement, vocalization, shaking of the chute	Excitable
5	Violent and continuous struggling	Excitable

[a] Adapted from Cooke et al. (2011).

Table 2 Associations of temperament in the squeeze chute with growth and reproductive performance, ovarian structures and plasma acute phase proteins of Nellore cows submitted to a fixed timed-AI protocol (n = 40)[a].

Item	Temperament[b]		SEM	*P*-value[c]		
	Calm	Excitable		Temp	Day	Temp × day
Nº of animals	19	21				
Chute score, 1-5	2.84	4.05	0.122	<0.0001	-	-
Body parameters						
Body condition score, 1-9				0.09	0.05	0.21
d -30	4.81[a]	4.68[a]	0.20			
d 0	4.71[b]	4.16[b]	0.20			
d 30	4.76[b]	4.21[b]	0.20			
Overall	4.76	4.35	0.17			
Body condition score change, 1-9						
d -30 to d 0	-0.09	-0.53	0.19	0.09	-	-
d 0 to d 30	0.05	0.05	0.17	0.98	-	-
d -30 to d 30	-0.05	-0.47	0.19	0.12	-	-
Body weight, kg				0.31	<0.0001	0.93
d -30	397.36[b]	387.47[b]	6.51			
d 0	391.84[b]	383.38[b]	6.93			
d 30	420.74[a]	410.64[a]	7.20			
Body weight change, kg						
d -30 to d 0	-5.53	-4.19	3.58	0.79	-	-
d 0 to d 30	28.89	27.26	3.24	0.72	-	-
d -30 to d 30	23.37	23.07	3.72	0.95	-	-
Ovarian structure[d]						
Dominant follicles, mm	14.79	12.78	0.76	0.06	-	-
Corpus luteum diameters, mm	35.10	31.11	1.70	0.10	-	-
Corpus luteum volumes, cm^3	25.95	17.88	3.38	0.10	-	-
Plasma analyzes						
Haptoglobin, mg/mL				0.43	0.32	0.14
d -30	0.24	0.18	0.03			
d -11	0.18	0.18	0.02			

d 0	0.14	0.18	0.02			
d 7	0.17	0.18	0.02			
d 14	0.12	0.20	0.02			
d 21	0.13	0.18	0.02			
d 30	0.14	0.17	0.02			
Ceruloplasmin, mg/mL				0.40	<0.0001	0.56
d -30	13.83[e]	14.37[c]	1.06			
d -11	18.27[cd]	17.15[b]	0.89			
d 0	19.79[ab]	18.36[a]	0.91			
d 7	19.33[bc]	18.19[ab]	0.91			
d 14	17.86[d]	17.68[ab]	0.89			
d 21	19.98[a]	18.06[ab]	0.90			
d 30	19.11[bc]	18.11[ab]	0.89			

[a] All cows were assigned to a FTAI protocol from d -11 to 0. Cows were timed-AI on d 0.

[b] Calculated based on the temperament score in the squeeze chute (calm, temperament score ≤ 3, excitable, temperament score > 3) as described by Cooke et al. (2011). Superscript within the same column, without a common letter, differ ($P \leq 0.05$).

[c] Temp = temperament.

[d] Dominant follicle size was evaluated by ultrasonography on d 0 and corpus luteum on d 14.

Fig. 1. Associations of temperament in the squeeze chute with plasma concentrations of progesterone of Nellore cows. All cows were assigned to a FTAI protocol from d -11 to 0. Cows were timed-AI on d 0 and pregnancy status assessed on d 30. Effects of temperament × day and day were detected ($P \leq 0.04$) and effects of temperament tended to be detected (P = 0.06). [a-d]Between days, without a common superscript, differ (P ≤ 0.05). *Within day, means without a common superscript differ ($P \leq 0.05$).