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# TEMPERATURE AND HUMIDITY IN THE BRAZILIAN CENTER-EAST AFFECTING THE *IN VIVO* EMBRYO PRODUCTION OF NELORE COWS

Gustavo Guerino Macedo[1], Carmem Estefânia Zúccari[1], Eliane Vianna Costa e Silva[1]

[1] UFMS
Correspondência: Gustavo Guerino Macedo: ggmacedo@gmail.com

**ABSTRACT:** This study used Nelore embryo donors to evaluate the effects of summer temperatures and humidity in a tropical area on embryo recovery. Seventeen cows were randomly assigned to three groups (G1, G2 and G3) as well as 28 heifers into four groups (G4, G5, G6 and G7), and underwent a consecutive 4 days superovulation protocol (day 0 to 3) and two artificial inseminations (AI; day 4 and 5). Cortisol concentrations were determined on day 0, 4 and 5. Temperature and humidity index (THI) was recorded from days 0 to 7. Embryos were recovered on day 11, and evaluated for quality and quantity. Heifers experienced higher thermal load than cows, however, there was no difference in the cortisol and reproductive parameters measured between heifers and cows. The THI measured throughout the experiment oscillated between 65.8 and 85.2 (76.1 ± 3.9), which is a dangerous range for thermal comfort, especially above 78. Animals from G5 were subjected to high THI throughout the experimental protocol, approaching a dangerous condition (78.7 ± 0.9) on day 4 (first AI). The THI for G2 cattle was also high, as in the other groups, but increased to over 80 two days after AI. Animals from group G5 had the lowest viable production being 2.4 ± 0.9 embryos (38% of viability), followed by cattle from group G2 (3.0 ± 0.9 viable, $P<0.05$). Thus, high thermal load in the summer expressed by high THI, on the day and post- insemination days (during embryo cleavage), compromises embryo number, viability and consequently reproductive performance of Nelore females.
**Key Words:** fertility; heat stress; reproductive performance; zebu; bovine; beef cattle

# TEMPERATURA E UMIDADE NO CENTRO-OESTE DO BRASIL AFETANDO A PRODUÇÃO *IN VIVO* DE EMBRIÕES DE VACAS NELORE

**RESUMO:** Este trabalho avaliou o efeito da temperatura e umidade no verão em região tropical, na recuperação de embriões bovinos de fêmeas Nelore (*Bos taurus indicus*). Foram utilizadas 17 vacas, e para facilitar o manejo foram distribuídas aleatoriamente em três grupos (G1, G2 e G3), assim como 28 novilhas em quatro grupos (G4, G5, G6 e G7) e submetidas a um protocolo de superovulação de quatro dias (dia 1 ao 3) e duas inseminações artificiais (IA; dias 4 e 5). Foi determinada a concentração plasmática de cortisol nos dias 0, 4 e 5. O índice de temperatura e umidade (ITU) foi obtido através de registros de temperatura e umidade do dia 0 ao 7. Os embriões foram coletados no dia 11, e avaliados quanto à qualidade e quantidade. Durante o período experimental, as novilhas foram submetidas a uma maior carga térmica ($P<0.05$) que as vacas, entretanto, não houve diferença entre concentração de cortisol e parâmetros reprodutivos de ambas. O ITU mensurado durante o experimento oscilou entre 65,8 e 85,2 (76,1 ± 3,9), estando em um nível crítico para o conforto térmico, principalmente quando acima de 78. Os animais do G5 foram sujeitos à um alto ITU durante o protocolo experimental, sendo expostos à condição de risco (78,7 ± 0,9) no dia 4 (primeira IA). O ITU do G2 também estava alto, assim como em outros grupos, entretanto foi superior a 80dois dias após a IA. Os animais do G5 tiveram a menor produção de embriões viáveis, sendo 2,4 ± 0,9 embriões (38% de viabilidade), seguidos pelos animais do G2 (3,0 ± 0,9 viáveis, $P<0,05$). Estes resultados indicam que uma alta carga térmica no verão, expressa pelo ITU, no dia da IA e nos dias seguintes (durante a clivagem embrionária), compromete a quantidade de embriões, viabilidade e consequentemente, o desempenho reprodutivo de fêmeas Nelore.
**Palavras-chave:** eficiência reprodutiva; estresse por calor; fertilidade; zebu; bovino; gado de corte

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## INTRODUCTION

World cattle population is estimated to be 1.4 billion heads, 65 percent of which are located in tropical areas (FAO, 2008). However, the milk and meat productivity (per ha) of these places are very low compared to developed countries. The Brazilian cattle herd in 2007 totaled 199.8 million head, remaining as the second largest in the world. Sixty-four percent of the Brazilian cattle population is composed by Nelore breed (ACNB, 2007), a number that ranks higher than total cattle herd in China. Nelore cattle are a suitable model to study because they are often exposed to high thermal loads during tropical summer, a situation that may compromise cattle reproduction, including embryo development. Moreover, in contrast to the synthetic Brahman breed, the Nelore is a distinct zebu breed. Thus, any findings in the present study are likely applicable to zebu cattle reared in other tropical areas.

Summer thermal stress is a major factor associated with low fertility in cattle reared in hot climatic zones (García-Ispierto *et al.*, 2007). Heat stress triggers reproductive alterations such as changes in follicle development (Torres-Júnior *et al.*, 2008), poor oocyte competence (Roth & Hansen, 2005), decreased estrus behavior (Chicoteau *et al.*, 1989) and high embryonic loss (Putney *et al.*, 1988; Bridges *et al.*, 2005). Although high temperatures have a minor effect on the survival of many cells, increased thermal load may be lethal to pre-implanted embryos during cleavage stage (Rivera *et al.*, 2003).

Identification of the amount of heat and at which point of the reproductive processes the exposure would be critical to fertility under field conditions, would prove to be beneficial in understanding homeostasis of embryo donors.

Moreover, decreasing on generation interval is aimed by farmers and breeders, but there is little knowledge about the thermal resistance of young animals. Thus, improving fertility of superior performance cattle used in genetic improvement programs could increase economic profits through strategic livestock management. The aim of the present study was to carry out an *in vivo* study to evaluate the effects of summer temperatures and humidity on embryo recovery in a tropical area.

## MATERIAL AND METHODS

This study was carried out between February and March/2008 in a farm in Mato Grosso do Sul, Brazil (20º20’0’’ S, 55º2’52’’ W, 301 m altitude). Rainfall during the experiment was 79 mm, and relative air humidity was 72% in Feb and 68% in Mar.

Forty five embryo donor Nelore females (5.8 ± 3.1 years old) were used in a superovulation protocol. Cows (n=17) were 8.0 ± 0.7 years of age and cycling heifers (n=28), which were at the first embryo collection, 1.6 years old. The animals were raised at pasture system and were maintained during the study period in a paddock (*Panicum maximum* cv. Tanzania) with mineral supplement and water *ad libitum* as well as good natural shading. The body condition of the animals was measured subjectively at the start of the superovulation protocol by one technician; to enter in the embryo transfer program on this farm, all females had to have a body condition score of 6-7 (on a 1-9 scale), which is the midpoint of the range (5 to 8) known to support reproductive function in *Bos indicus* cattle (Wiltbank, 1983). The experimental protocol was approved by the Ethic Committee on Animal Experimentation, Federal University of Mato Grosso do Sul, Brazil.

To standardize the handling, cattle were randomly allotted within age (cows and heifers) into small groups to undergo a superovulation protocol in a corral. Cows were allocated into 3 groups: G1 (n = 4), G2 (n = 6), G3 (n = 7), whereas the heifers were into 4 groups: G4 (n = 7), G5 (n = 7), G6 (n = 7) and G7 (n = 7). For superovulation, the cows and heifers were injected with decreasing doses of FSHp (250 to 150 mg, Folltropin-V®, Bioniche Animal Health, ON, Canada) twice daily, at 12 h intervals, for 4 days, consecutively (Zanenga *et al.*, 2003). The superovulation protocol was initiated on day 0 (AM). On day 2 (AM), all donors were given d-cloprostenol (0,150 mg, Prolise®, Tecnopec, SP, Brazil). On day 4 (AM), donors were injected with 0.01 mg of buserelin acetate (Conceptal®, Intervet Schering-Plough, SP, Brazil). Cattle were inseminated twice; first in the afternoon of the day 4, and again in the morning of day 5.

From day 5 to day 11, females were held in a paddock with shade, water and food available. On day 11, females were restrained and sedated with 1 ml of 1% acepromazine im (Acepran®, Univet, SP, Brazil) followed by administration of low epidural anesthesia using 5 ml of 2% lidocaine (sp) with 2% epinephrine (Anestésico L®, Eurofarma Laboratory, SP, Brazil). Embryo recovery was performed (total of 45) by nonsurgical transcervical flushing of the uterine horns with a Foley catheter. The embryos were counted with a stereomicroscope, graded as viable (excellent, good, fair and poor), degenerated or unfertilized (Lindner & Wright, 1983).

Plasma cortisol was determined from blood collected in the morning on day 0, and before the artificial insemination procedures (day 4-5). Blood samples of the animals restrained in the squeeze chute were collected in heparinized tubes by coccygeal venipuncture, placed on ice and then centrifuged at 4°C and 3000 *g* for 15 min. The tubes containing plasma were then sealed and stored at -20ºC until cortisol determination by a validated assay as previously described (Negrão & Marnet, 2006), using a commercial enzyme immunoassay kit (Diagnostic System Laboratories, Inc., TX, USA). All samples were tested in duplicate and the assay sensitivity was 1 ng/mL. The intra- and interassay CV averaged 3.5% and 2.1%, respectively.

Daily dry and humid bulbs were recorded hourly from 6 AM to 6 PM using a psychrometer from day 0 to 7. Thermal comfort was assessed by the meteorological temperature and humidity index (THI), calculated using the expression (THOM, 1959): $THI = DBT + 0.36\ DPT + 41.5$, where DBT = dry bulb temperature (ºC) and DPT = drew-point temperature (ºC).

Data were submitted to Cramer-von Mises test to verify the normality. Yule's model for frequency distribution was used to define class intervals and the ideal number of classes on the basis of the variable's distribution, as proposed by Sampaio (1998). Hence, THI was divided into classes 1, 2, 3 and 4, which had THI of 69 – 71.99, 72 – 74.99, 75 – 77.99 and above 78, respectively. Data were analyzed by analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS Institute Inc., Version 8.02 TS Level 02M0). The model considered embryo production parameters as dependent variables, whereas group, animal category and THI during superovulation were treated as independent variables. Analysis of variance was used for repeated measures of the proc MIX of SAS to analyze cortisol concentrations over category. The Duncan test was used to compare measurements of each group and to compare THI within each day and within each group during protocol application. Alpha error was set at 0.05.

## RESULTS AND DISCUSSION

Were analyzed a total of 446 embryos. For the total embryos collected, mean ova/embryo and viable production during the experiment was 10.4 ± 1.2 and 6.7 ± 0.9 embryos, respectively (Table 1). Cows and heifers had similar ova/embryo production (9.3 ± 1.7; 11.1 ± 1.6, respectively, P=0.039) and viable embryos (6.3 ± 1.2; 7.0 ± 1.3, respectively, P=0.046). Superovulation efficiency was assessed by number of embryos. The mean of recovered structures of this study was similar for Nelore cattle in Brazil (10.2 ± 7.8), however, in this study, viable embryos seem to be higher than the 5.1 ± 5.3 described by Peixoto *et al.* (2006). This is likely due to the good management practices adopted on the farm, technical efficiency and the genetic merit of the cattle.

Table 1 - Ova/embryo production, viable embryos recovered from Nelore embryo donor (assigned in 7 groups) super stimulated with pFSH during the summertime in a tropical area

| | Ova/embryo production | Viable embryos |
|---|---|---|
| Group 1 | $10.0^{ab} \pm 1.8$ | $7.3 \pm 0.3^{abc}$ |
| Group 2 | $5.5^{b} \pm 1.8$ | $3.0 \pm 0.9^{cd}$ |
| Group 3 | $12.1^{ab} \pm 3.5$ | $8.6 \pm 2.6^{abc}$ |
| Group 4 | $8.2^{ab} \pm 2.0$ | $6.0 \pm 1.7^{abc}$ |
| Group 5 | $6.3^{b} \pm 1.7$ | $2.4 \pm 0.9^{d}$ |
| Group 6 | $13.0^{ab} \pm 2.5$ | $9.3 \pm 2.0^{ab}$ |
| Group 7 | $17.3^{a} \pm 5.0$ | $10.7 \pm 4.0^{a}$ |
| Total | 10.4 ± 1.2 | 6.7 ± 0.9 |

Means (± SEM) within a column, followed by different letters are statistically different at P<0.05 by Duncan test

Mean THI during the experiment (76.1 ± 3.9) was high enough to cause heat stress in European-origin cattle according Hahn (1985), but it seems to be harmless also to Nelore cattle. An upper THI limit that affects homeothermy has not been defined for Zebu cattle. Nelore breed studied maintained their reproductive performance even when exposed to this THI around 76. The good reproductive performance of Nelore cattle under high THI is likely a consequence of zebu cattle adaptation to the tropics (Randel, 1984). Thermal comfort criteria considered was established by Thom (1959) and categorized by Hahn (1985) for *Bos taurus*: THI < 70 = normal, non-stressful; 71 ≤ THI < 78 = critical (close to the critical upper temperature); 78 ≤ THI < 83 = dangerous; 83 < THI = emergency.

The mean cortisol concentration was similar between heifers and cows (16.2 ± 3.62 ng/mL; 14.1 ± 2.0 ng/mL, respectively; P=0.27). Cortisol is an indicative of psychological/physical and thermal stress (Sapolsky *et al.*, 2000). In general, stress response involves the releasing of adrenocorticotropic hormone (ACTH) by the pituitary gland, which stimulates the adrenal cortex to releases cortisol (MINTON, 1994; Aoyama *et al.*, 2003). High cortisol concentrations inhibit the pituitary response to gonadotropin-releasing hormones (GnRH), decreasing gonadal activity by reducing pulse frequency and amplitude of the luteinizing hormone (LH) released by the gonadotrophs (Dobson *et al.*, 2001; Breen & Karsch, 2004). The reduction of LH pulses in the luteal phase may induce follicular atresia. Suppression of LH releasing patterns during the follicular phase delays or inhibits the preovulatory LH surge and consequently disrupts the oocyte maturation and embryo quality (Mihm *et al.*, 1994; Dobson & Smith, 2000). For instance, heifers subjected to transport stress followed by superovulation reached high cortisol concentrations and had decreased corpus luteum production (Edwards *et al.*, 1987). In Nelore cattle, such a decrease can be problematic since corpus luteum production is positively correlated to a viable embryo rate (Peixoto *et al.*, 2002).

Under pasture conditions in tropical area, puberty age of Nelore females is 2.07 ± 0.10 years old with pregnancy rate of 53.4% on AI (Restle *et al.*, 1999). This age is 0.44 year (5.28 months) older than heifers induced in this experiment. Once cow and heifers had similar reproductive and physiologic stress (cortisol) parameters, the present study shows that Nelore heifers of approximately 1.6 years old exposed to higher heat and humid environment during hot season, are more resistant to heat load than adult cows. It is supported by the observation that mean THI during the superovulation protocol was lower for cows (75.87 ± 0.13) than for heifers (76.41 ± 0.05, P=0.016). This demonstrates that Nelore heifers at 1.6 years old can be introduced in embryo transfer programs at this age aiming a decreasing on generation interval and increasing on genetic gains (ΔG/t).

Temperature humidity index for each day of the superovulation protocol by group is presented in Figure 1. Before AI, a THI above 78 (dangerous condition, according to HAHN, 1985) was experienced by the animals from G3 (Day 2; 78.5), G6 (Day 3; 78.6) and G7 (Day2; 78.6). Whereas, after AI this heat exposure was observed in G2 (Day 6 – 7; 78.2 and 80.1) and G5 (Day 4; 78.7). Ova/embryo and the total number of viable embryos were different between the groups (Table 1). Embryo production and viability was compromised in groups G5 and G2. Group G5 was subjected to high THI (78.7 ± 0.9) on day of first AI, and had only 38% of their embryos as viable compared with the 71% from G3 which experienced a lower THI on the AI day and following days. Specific moments of the reproductive process are more sensitive to heat, like on the AI day and early embryo development (Putney *et al.*, 1989). For instance, cattle exposed to cooling system (sprinkler and a fan) at the AI, have higher pregnancy rate during heat stress (Moghaddam *et al.*, 2009). During this period oocyte maturation is accompanied by quantitative and qualitative alterations in the synthesis of RNA (Rodman & Bachvarova, 1976) and proteins (Edwards & Hansen, 1996) essential for the continuation of meiosis and germinal vesicle breakdown (Van Blerkom & Mcgaughey, 1978). In the heat-stressed animals, oocytes were exposed to high thermal load *in vivo* during a period of time when maturation and the reprogramming of obligatory events are critical for successful oocyte fertilization and subsequent embryonic development is reported to occur (Warnes *et al.*, 1977). Moreover, heat stress increases free radicals (Lawrence *et al.*, 2004), and factors involved in apoptosis (Roth & Hansen, 2004). Although, they are capable of being fertilized and undergo initial cleavage, heat stress at this point decreases their susceptibility to future environmental heat stress, reducing transcription and synthesis of intracellular proteins (Edwards *et al.*, 2009). In G5, THI remained high until day 7, a condition that was harmful to early embryo cleavage. Krininger *et al.* (2003) found that embryo from Zebu cattle are intolerant to heat stress on the second day after insemination (four- cell stage), when deleterious effects are unavoidable. In fact, embryo is vulnerable to high temperatures during cleavage until day 7 post-estrus, compromising blastocyst formation (Putney *et al.*, 1988; Hansen, 2007). It is possibly because before reaching the morula/blastocyst stage (5-6 days post fertilization) embryo genes do not activate the production of the heat shock protein -HSP70 (Memili & First, 2000) and antioxidants that protect them against heat damage (Kawarsky & King, 2001). Groups G6 and G7 were also exposed to high THI, possibly leading some blastomeres to apoptosis, an important mechanism for eliminating damaged, nonfunctional, abnormal or malformed cells (Paula-Lopes & Hansen, 2002). Cells that are severely damaged by heat which do not undergo apoptosis always become necrotic

(Paula-Lopes & Hansen, 2002). However, embryo development in groups G6 and G7 recovered, as a consequence of the sudden drop in THI on days 6-7 after fertilization.

The cows from G2 had low viability and were subjected to high THI during the two first days after insemination, as were other groups. However, on the second and third days (day 6 and 7), THI was above 78 and 80, respectively, being a dangerous threat to homeothermy (Hahn, 1985). This can explain the low viability, once THI can be used to assess thermal balance indirectly (Azevedo *et al.*, 2005) indicating thermal comfort, environmental adversity or their effects on reproduction (Hansen & Arechiga, 1999). Although THI is based only on temperature and humidity, it is a good tool for assessing thermal stress condition in animals (McDowelL *et al.*, 1976).

In brief, Nelore heifers exposed to a higher thermal load than adult cows had same reproductive and physiologic patterns. Thus, females at 1.6 years old respond well to superovulation treatment under hot season and can be introduced into the reproductive programs aiming to decrease generation interval, increasing economical profits. Comparing with previously established classification of heat stress (Hahn, 1985), Nelore zebu cattle have high thermal tolerance and a THI classification for thermal comfort for zebu, different that for taurine should be studied. Furthermore, high values of summer THI on AI and following days compromised embryo viability affecting reproductive performance of embryo donors. Thus, strategic management like cooling systems or low handling on hot period could ameliorate thermal stress coinciding with these days. Further investigation of molecular mechanisms underlying thermal tolerance of *Bos taurus indicus* is needed; especially considering the role that Zebu plays in tropical beef cattle business.

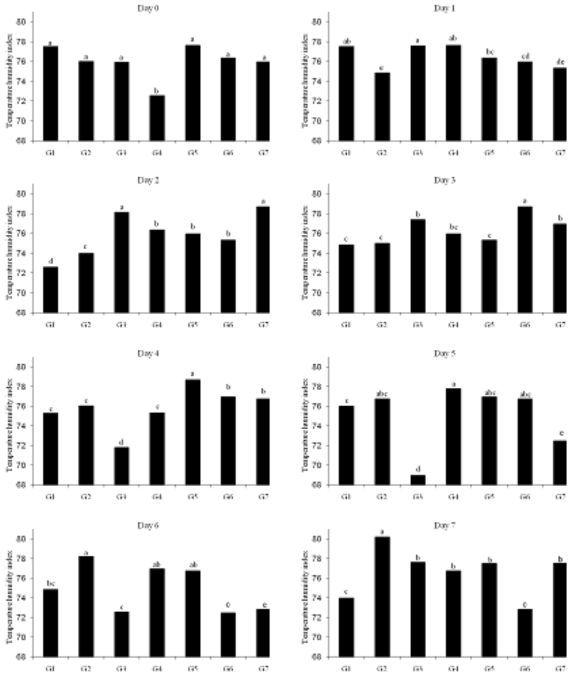

Figure 1 - Temperature and humidity index experienced by Nelore embryo donors allotted in seven groups (G1-G7) during the experiment period were: Day 0 – 3 (superovulation treatment), Day 4 – 5 (artificial inseminations) Days 6 – 7 (days following artificial insemination) Statistical difference among the groups are indicated by different letters (Duncan test (P<0.05)

## CONCLUSION

The present study showed that Nelore heifers are more termotolerant than adult Cows. Moreover, high THI during the tropical summer at the AI time and the following 2 days reduces the viability embryo from zebu females, consequently reducing their fertility.

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