Conjecturas

DOI: 10.53660/CONJ-2153-2Z57

Bioclimatic interference in fertility in cows in the Amazon biome

Interferencia bioclimática en la fertilidad en vacas del bioma amazónico

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ABSTRACT

The objective was to evaluate the correlation between environmental temperature-humidity index (THI), rectal temperature (RT), plasma cortisol concentration and pregnancy rate in embryo recipient cows in the Amazon biome. Embryo recipients (n=235) underwent estrus synchronization via simplification of the P36 protocol for Fixed-Time Embryo Transfer (TETF). On days zero (D0), eight (D8) and day 16 (D16), the rectal temperature of the recipient cows was measured, as well as the ambient temperature and relative humidity. On the 16th day after the start of the synchronization protocol (D16), each recipient received an embryo transferred after being diagnosed with a corpus luteum (CL) in one of the ovaries by ultrasonography (US) and, from each of them, a blood sample was collected by venipuncture of the coccygeal vein, in tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant to obtain blood plasma, where cortisol was measured by radioimmunoassay (RIA) in solid phase, using commercial kits. It was found that in the group of pregnant cows, the mean values of the parameters RT, plasma cortisol and THI ($P < 0.05$) were lower than those presented in the group of non-pregnant cows. Moreover, between the two groups there were positive and significant correlations between RT and THI ($P < 0.05$), as well as in relation to plasma cortisol parameters and THI ($P < 0.05$). It is concluded that it is possible to validate the use of RT and THI as an auxiliary tool for estimating plasma cortisol concentrations.

Keywords: Stress; Embryo; In vitro production; Thermoregulation.

RESUMEN

El objetivo de este estudio fue evaluar la correlación entre el índice de temperatura-humedad ambiental (THI), la temperatura rectal (RT), la concentración de cortisol en plasma y la tasa de preñez en vacas receptoras de embriones en el bioma amazónico. Los receptores de embriones (n = 235) se sometieron a sincronización de estro mediante la simplificación del Protocolo P36 para la transferencia de embriones de tiempo fijo (TETF). En los días cero (D0), ocho (D8) y el día 16 (D16) se midió la temperatura rectal de las vacas receptoras, así como la temperatura ambiente y la humedad relativa. En el día 16 después del inicio del protocolo de sincronización (D16), cada receptor recibió un embrión transferido después de que se diagnosticara un cuerpo lúteo (CL) en uno de los ovarios mediante ultrasonografía (US) y de cada uno de ellos se extrajo una muestra de sangre por punción venosa de la vena coccígea en tubos con etilendiamina tetraacético ácido (EDTA) como anticoagulante para obtener plasma sanguíneo, donde el cortisol se midió mediante radioinmunoensayo (RIA) en fase sólida, utilizando kits comerciales. Se verificó que en el grupo de vacas con embarazo, los valores medios de los parámetros RT, cortisol plasmático y THI ($P <0.05$) fueron menores que los presentados en el grupo de vacas no embarazadas. Además, hubo correlaciones positivas y significativas entre la RT y la THI entre los dos grupos ($P <0,05$), así como el cortisol plasmático y la THI ($P <0,05$). Se concluye que es posible validar el uso de RT y THI como herramienta auxiliar para estimar las concentraciones de cortisol en plasma.

Palabras clave: Estrés; Embrión; Producción in vitro; Termorregulación.

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INTRODUCTION

Brazil's participation in the scenario of in vitro production (IVP) of embryos, in the last 12 years, is around 85% of the world production and this is mainly due to the work with Zebu breeds with which better results are obtained in IVP (VIANA *et al*., 2010). However, the climate in our country is predominantly tropical, with high temperatures throughout the year, causing thermal stress to production animals and causing physiological imbalances. This scenario increases the net energy requirements for maintenance, and consequently reduces the energy available for the production processes (SILVA, 2000), thus limiting the greater effectiveness of IVP in Brazilian cattle herds.

To predict the level of thermal comfort of the environment in which the animal is inserted, it is not recommended that the climatic variables be evaluated separately. Therefore, there is a need to determine the combined effects of these variables, which can be obtained by calculating thermal comfort indices, such as the Temperature-Humidity Index (THI), which considers the associated effect of temperature and relative humidity (SOUZA *et al*., 2014).

The stressor agent, through the pre-optic area of the central nervous system, acts on the neurosecretory cells of the paraventricular nucleus of the hypothalamus and, from this stimulation, these cells will produce corticotropin-releasing hormone (CRH), which will promote the secretion of adrenocorticotrophic hormone (ACTH) by the adenohypophysis (MINTON, 1994). ACTH, in turn, will act on the adrenal glands, stimulating the secretion of corticosteroids, such as the hormone cortisol (BREEN *et al*., 2005).

Thus, cows with an aggressive temperament more intensely stimulate the hypothalamic-pituitary-adrenal axis, resulting in neuro-endocrine stress characterized by increased concentrations of ACTH and cortisol (COOKE *et al*., 2009). This hormone interferes with mechanisms related to fertility, such as the resumption of the estrous cycle, ovulation of a competent oocyte and establishment of pregnancy (DOBSON *et al*., 2001).

However, for some time, concerned with overcoming barriers, support was sought from reproductive biotechnologies with a view to increasing production, achieved mainly with the mass use of artificial insemination (AI), later practiced in the modality of artificial insemination in fixed time (FTAI) (BARUSELLI *et al*., 2004). And later,

through other biotechniques applied to animal reproduction, such as semen sexing, in vitro fertilization, cloning, transgenics, the use of molecular markers and in vitro production (IVP) of embryos (VIEIRA, 2012).

In this sense, the objective of the research was to evaluate, through the parameters rectal temperature (RT), THI and plasma cortisol concentration, the physiological responses, in the pregnancy rate, of cows receiving embryos created in the Amazonian biome.

MATERIAL AND METHODS

The study was prepared in accordance with the Ethics Committee of the Faculty of Veterinary Medicine and Animal Science - Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP) - protocol number 227/2011, and was conducted between June 2017 and September 2018 (15 months). 235 crossbred cows (Bos taurus taurus x Bos taurus indicus) autochthonous from Acre, single or multiparous, aged between three and six years, non-lactating, reared in an extensive regime, with water and mineral salt ad libitum and with a body condition score between 3 and 4, on a scale of 1 to 5 (FERREIRA; TORRES, 1993). These animals came from 11 rural properties, distributed in six municipalities (Rio Branco, Xapuri, Porto Acre, Acrelândia, Senador Guiomard and Plácido de Castro) in the state of Acre (9°6′36″ S, 70°31′12″ W).

Embryos were obtained by the in vitro production technique (IVP), with oocytes obtained by follicular aspiration from donors belonging to the Animal Genetics Improvement and Diffusion Station (EMDGA), in whose laboratory all procedures were performed. The donors and the semen of the bulls used in the IVP were from the dairy Gir breed.

All recipients were submitted to the same estrus induction/synchronization protocol, in batches with 10 to 12 animals at a time, at intervals appropriate to the routine of procedures, and those that were able on the pre-established day, 16˚ day after the beginning of the protocol (D16), received an embryo transferred by the transcervical technique; concomitantly, blood samples were collected by venipuncture of the coccygeal vein to obtain blood plasma, in 5 mL tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. After collection, the tubes were kept inclined at room temperature for 10 minutes and then centrifuged at 900G for 10 minutes in a non-refrigerated

centrifuge. The blood plasma obtained was fractionated into 1.5 mL microtubes, duly identified with the respective number of animals and stored at -20℃ until they could be sent, packed in thermal boxes with ice, to the destination laboratories. The recipient who presented, on that date, a corpus luteum (CL) in one of the ovaries was considered fit; it should be noted that for standardization purposes, the same veterinarian performed all transfers.

Based on the simplification of the P36 protocol for fixed-time embryo transfer (TETF) (BARROS *et al*., 2001), the protocols were developed as follows: on a random day of the estrous cycle (D0), each of the recipients received 1g of P4 through an intravaginal device to release P4 and 2.5 mg Estradiol Benzoate (EB) intramuscularly (IM). On D8, the device was removed from P4 and 150μg of D-cloprostenol (PGF2α), 400 IU of eCG and 1mg of EB were administered IM. On D16, each recipient received a transferred embryo (blastocyst, grade 1 or 2) after previously being diagnosed by ultrasonography (US) (Aloka SSD 500, Aloka, Japan) a CL in one of the ovaries. On D41, the diagnosis of pregnancy was made. Plasma cortisol dosages were performed by radioimmunoassay (RIA) in solid phase, using commercial kits (Coat A – Count DPC, USA).

Sample processing was performed by the Endocrinology Laboratory of the Department of Animal Reproduction and Veterinary Radiology at FMVZ-UNESP, Botucatu Campus, SP. It was found that the interassay coefficient of variation was 6.31% and 6.50% for cortisol.

On D0, D8 and D16, the rectal temperature of each of the recipients submitted to the protocol was measured, using a digital clinical thermometer (G-TECH, model: TH-169), as well as the ambient temperature and relative humidity of the air, both with the aid of their own digital equipment (Thermo - portable digital hygrometer, model HT-260, Instrutherm, São Paulo, Brazil). To calculate the Dry Bulb temperature (Tbs) the column of Analog Thermo-Hygrometer Dry and Wet Bulb -10+50 x 1°C Liquid Plastic Base 190 X 106mm was used. The Temperature-Humidity Index (THI) proposed by Pires *et al*. (2002), using the formula:

$$THI = 0{,}8\,Tbs + RH\frac{Tbs - 14{,}3}{100} + 46{,}3$$

Where:

Tbs: Dry Bulb Temperature (ºC)

RH: Relative humidity (%)

For statistical analysis, Pearson's correlation and Student's “t” test were used at a significance level of 5%.

RESULTS

The yield rates of embryo and pregnancy recipients after protocol application were 67.23% (158/235) and 34.18% (54/158), respectively. Regarding ambient temperature, relative humidity (RH) and rectal temperature (RT) recorded during the 15 months of the research, the mean values were 30.07ºC, 69.23% and 39.28ºC, respectively.

It was found that there was a significant difference ($P < 0.05$) in THI and RT between the two established groups: pregnant cows (GP) and non-pregnant cows (GNP), where the lowest mean values were found in the GP group. It was also verified that both groups presented a significant and positive correlation between the THI and RT parameters (Table 1).

Table 1 - Correlation between THI and RT (Mean ± Standard Error) between non-pregnant and pregnant groups

Parameters	GNP	GP
THI	81,83 ± 0,03[a]	70,50 ± 0,10[b]
RT (˚C)	39,52 ± 0,24[a]	38,66 ± 0,28[b]
Pearson correlation between THI and RT	(r= 0,73; p<0,05)	(r= 0,85; p<0,05)

GNP: non-pregnant group; GP: pregnancy group; THI: temperature-humidity index; RT: rectal temperature. Distinct lowercase letters on the same line indicate significant differences by Student's t test at the 5% significance level.

It is important to point out that the climatic variables in this study refer to typical days in the Amazon region, collected on non-consecutive dates. Thus, it was verified that in the GNP group the THI parameter (81.83 ± 0.03) presented values above the care range,

while the GP group presented values below the safety interval. It was also verified that the THI values (70.50 ± 0.10) of the GP group were below the established values of 72 to 78.

In the analysis of variance, it was observed that there was a significant difference in plasma cortisol and THI between the groups, where lower mean values were found in the GP group. It was also evident that both groups showed a significant and positive correlation between cortisol and THI parameters (Table 2).

Table 2 - Correlation between plasma cortisol concentrations and THI (Mean ± standard error) between non-pregnancy and pregnancy groups

Parameters	GNP	GP
Cortisol (ng/ml)	17,78±5,54[a]	13,78±4,74[b]
THI	81,83 ± 0,03[a]	70,50 ± 0,10[b]
Pearson correlation between Cortisol and THI	(r = 0,45; P < 0,05)	(r = 0,76; P < 0,05)

GNP: non-pregnant group; GP: pregnancy group; THI: temperature-humidity index. Distinct lowercase letters on the same line indicate significant differences by Student's t test at the 5% significance level.

RESULTS

In the performance of protocols, similar results were reported by Barreiros *et al.* (2006) and Rodrigues *et al*. (2010), with rates of 72.9% and 65%, respectively. The results for pregnancy rate were also close to those described by Baruselli *et al*. (2010), which reached 36.60% (108/295), using eCG on D8.

The average value of the rectal temperature (RT) registered was attested as greater than the maximum of 39.1°C, as mentioned by Dukes (2006), also remaining outside the normality limits described by Robinson (1999). On the other hand, McDowell *et al*. (1954) state that cattle of all breeds have an average rectal temperature of 38.3°C with some variations. However, the RT remained within the physiological values that according to Martello *et al*. (2004) for adult cattle is between 37.5°C and 39.3°C. Therefore, the RT of the GNP group exceeded the thermoneutral zone in which the maintenance of homeothermy occurs with maximum mobilization of the heat dissipation mechanisms, responsible for thermoregulation, and this energy deviation may be responsible for a decrease in reproductive performance.

The comfort zone for cattle is relatively small, while for European breeds it is between -1˚C and 16˚C and for Zebu breeds between 10˚C and 27˚C (SANTOS *et al*., 2005), with a critical limit of 35°C (FURTADO *et al*., 2012). In other words, the high temperature indices of the Amazon region present a great challenge for the animals, forcing them to develop adaptive mechanisms of heat dissipation. However, the temperature range that provides thermal comfort, in which there is minimal energy expenditure to maintain homeothermy, also depends on the relative humidity of the air (SILVA, 2000).

The animals in this experiment were inserted in the Amazon biome characterized by high temperature and humidity (AZEVEDO *et al*., 2005). Thus, when air humidity is low, evaporation is facilitated, otherwise the evaporation process becomes slow or even null, making it difficult to maintain homeothermy (WILSON *et al*., 2007). Thus, these conditions of heat and relative humidity are almost always above the thermal comfort zone for the animals, requiring energy expenditure in terms of physiological thermoregulatory mechanisms in an attempt to dissipate heat (BAÊTA; SOUZA, 1997). However, there are occasions when the loss of body heat becomes ineffective, and then there is thermal stress in the animal, which is often a limiting factor for development, production and reproduction (SILVA; GAUDIOSI, 1995).

The reproductive efficiency of dairy cattle exposed to adverse weather conditions is compromised when temperature and humidity are high and solar radiation is intense during most of the year (KAMAL *et al*., 2018), a recurrent situation in the Amazon biome. In the case of the use of IVP, embryonic development becomes impaired when cows suffer from heat stress on the day of artificial insemination (AI) or up to seven days after the procedure, which leads to lower embryonic viability (MACEDO *et al*., 2014), which may render the technique unfeasible.

Martello *et al*. (2004) in a study with Holstein cows, THI values up to 74 correspond to the safety range, and from 74 to 78 the care range. Igono *et al*. (1992), however, considers THI above 76 in any environment to be stressful for cows with high milk production. The climatic variables presented in this study indicate mild thermal stress, thus, the highest THI values found in the GNP group indicate an environmental situation favorable to stress for the animals, where the thermal condition was above the considered comfort. These processes can cause suboptimal reproductive performance, such as a decrease in conception rate during the warm season by 20% to 30% (DE

RENSIS *et al*., 2002) and significant economic losses (COLLIER *et al*., 2006). Weather conditions can be an important contributor to low fertility in dairy cows during the summer months, especially in high-yielding cows (KADZERE *et al*., 2002).

Plasma cortisol concentrations, above 10 ng/mL for the Zebu breeds, were similar to those reported by Yoshida and Nakao (2005) for both groups. In this context, as it is considered the stress hormone, its evaluation, although expensive, has become of great value for establishing the condition of animal welfare (BENATTI, 2010). Increased production of cortisol causes negative feedback in the hypothalamus, decreasing the synthesis of gonadotropin-releasing hormone (GnRH), and consequently reduces the release of gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), facts that, in the end, are responsible for the low production of gonadal hormones (MENEZES, 2010). The reduction of the latter, in turn, causes less manifestation of heat, low conception rate, embryonic mortality and abortions (FERRO *et al*., 2010).

The lower plasma cortisol concentration in the GP group possibly favored embryonic quality and maintenance of pregnancy in relation to the GNP group. Garcia-Ispierto *et al*. (2007) found that the probability of pregnancy loss increases by 1.05 times for each unit increase in THI between days 21-30 of gestation. Due to the results obtained, it is possible to observe that the animals in the GNP group were in a situation of thermal stress, due to the reported increase in plasma cortisol and THI that can negatively act on the luteal function, and consequently, on the maintenance and recognition of pregnancy (REIS *et al*., 2006).

It is noteworthy that embryonic development is very susceptible to heat stress, especially on the third day of development, reducing the proportion of embryos that continue to evolve (SARTORI *et al*., 2006).

Additionally, reduced embryonic growth is associated with lower levels of interferon-tau, which act to inhibit the pulsatile secretion of prostaglandin F2α, i.e., low levels of interferon-tau cannot block corpus luteum regression, making it difficult to maintain pregnancy. (GARCIA-ISPIERTO *et al*., 2007). Thus, animals that are under thermal stress tend to present alterations in the embryonic production of interferon-tau, which causes losses in the maintenance of pregnancy. Therefore, the techniques used in genetic improvement must be associated with measures that reduce the effects of the thermal environment on production animals (HABEED *et al*., 2018).

CONCLUSION

The Amazonian climatic environment induces heat stress with the ability to determine abnormalities in estrous cycles, changes in the synthesis of sex hormones and in embryonic development. As a result, consequently, it negatively affects the pregnancy rate of cows, as demonstrated by the study of RT, THI and plasma cortisol parameters. Thus, it becomes necessary to develop environmental conditions to facilitate the body heat dissipation capacity of these production animals.

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Recebido em: 10/11/2022

Aprovado em: 15/12/2022

Publicado em: 26/12/2022