Chapter

Bioclimatic Influence on the Pregnancy Rate in Embryo-Recipient Cows in the Amazonian Biome

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Abstract

The objective of the research was to evaluate the effect of the climate, through the parameters of rectal temperature (RT), temperature and humidity index (THI), and plasma cortisol concentration, on the physiological responses of embryo-recipient cows in the Amazonian biome. For the conduction of the experiment in which 11 rural properties participated, 235 crossbred cows were used as embryo recipients. The embryos were obtained by means of the in vitro production technique (PIV). The recipients were divided into batches and submitted based on the simplification of the P36 protocol for fixed-time embryo transfer (FTET). To the day of embryo transfer, blood samples were collected by venipuncture of the coccygeal vein in tubes with anticoagulant. Plasma cortisol dosages were done by solid-phase radioimmunoassay (RIA) technique using commercial kits. The rectal temperature of each of the recipients submitted to the protocol was verified using a digital clinical thermometer, and the ambient temperature and the relative humidity of the air were evaluated in this moment, both with the aid of own digital equipment. Statistical analysis of the parameters evaluated was Pearson's correlation and Student's t-test at a significance level of 5%. In the analysis of variance, it was observed that there was a significant difference in plasma cortisol and THI among the groups, where lower mean values were found in the GP group. The Amazonian climate is an inducer of thermal stress, which can cause abnormalities in the estral cycle and changes in the synthesis of sex hormones and embryonic development and, consequently, negatively affect the pregnancy rate in embryo-recipient cows, even in races more adapted to the tropics, as demonstrated by the RT, THI, and plasma cortisol parameters.

Keywords: embryo transfer, thermal stress, thermoregulation, THI

1. Introduction

Brazil is a predominantly tropical country, with high average temperatures during the year, generally causing thermal stress to the production animals and causing physiological imbalances that, in turn, cause an increase in the net energy requirements for maintenance, which, consequently, causes a decrease in the available energy for the productive processes [1]. The heat stress condition harms homeostasis and promotes endocrine changes that have negative effects on reproductive events in the bovine female [2].

The stressor agent, through the preoptic area of the central nervous system, acts on the neurosecretory cells of the paraventricular nucleus of the hypothalamus, and, from this stimulation, these cells produce the corticotrophin-releasing hormone (CRH), which promotes the secretion of the adrenocorticotrophic hormone (ACTH) by the adenohypophysis [3]. ACTH acts on the adrenal glands stimulating the secretion of corticosteroids, such as the hormone cortisol [4]. Thus, cows undergoing thermal stress stimulate with greater intensity the hypothalamic-pituitary-adrenal axis, increasing the concentrations of ACTH and cortisol [5]. This hormone interferes with mechanisms related to fertility, such as resumption of estrous cycle, ovulation of a competent oocyte, and establishment of gestation [6].

However, for a long time, in trying to overcome barriers, we have sought the support of reproductive biotechnologies with a view to increasing production, through the use of techniques such as artificial insemination (AI), artificial insemination at fixed time (AIFT), and fertilization in vitro [7, 8]. Brazil has a great representation in the world scenario of in vitro embryo production, and this is mainly due to the work done with zebu breeds with which best results are obtained, probably due to their better adaptation to the tropics [9]. In this sense, the objective of the research was to evaluate the effect of the climate, through the parameters of rectal temperature (RT), temperature and humidity index (THI), and plasma cortisol concentration, on the physiological responses of embryo-recipient cows in the Amazonian biome.

2. Material and methods

The study was elaborated according to the ethics committee of the Faculdade de Medicina Veterinária e Zootecnia—Universidade Estadual Paulista Júlio de Mesquita Filho (case number 227/2011). For the conduction of the experiment in which 11 rural properties participated, distributed in 6 municipalities of the state of Acre (9°6′36″ S, 70°31′12″ W), 235 crossbred cows were used as embryo recipients (*Bos taurus taurus* × *Bos taurus indicus*), native of Acre, uni- or multiparous, aged between 3 and 6 years, non-lactating, raised in an extensive regime, with water and mineral salt ad libitum, and with body condition score between 3 and 4, on a scale of 1–5 [10].

The embryos were obtained by means of the in vitro production technique (PIV), using semen and oocytes from selected Gir dairy cattle. The recipients were divided into batches containing 10–12 animals and submitted to the same estrus induction/synchronization protocol. Based on the simplification of the P36 protocol for fixed-time embryo transfer (FTET) described by [11], the protocols were developed as follows: on a random day of the estrous cycle (D0), each of the recipients received 1 g of P4 by an intravaginal device and 2.5 mg of estradiol benzoate (EB) intramuscularly (IM). On the eighth day (D8), the P4 device was withdrawn, and 150 μg of D-cloprostenol (PGF2α), 400 IU of eCG, and 1 mg of BE were given intramuscularly. On the 16th day (D16), each recipient received a transferred embryo (blastocyst, grade 1 or 2) after being previously diagnosed by ultrasound of a corpus luteum (LC) in one of the ovaries. On the 41st day (D41), the diagnosis of gestation (DG) was made.

Concomitantly to the day of embryo transfer, blood samples were collected by venipuncture of the coccygeal vein in tubes with anticoagulant. The obtained plasma was fractionated in 1.5 mL microtubes duly identified and stored at 20°C negative until sent to the laboratory for analysis. Plasma cortisol dosages were done by solid-phase radioimmunoassay (RIA) technique using commercial kits.

Prior to performing at the D16 ultrasonography evaluation, the rectal temperature of each of the recipients submitted to the protocol was verified using a digital clinical thermometer, and the ambient temperature and the relative humidity of the air were evaluated in this moment, both with the aid of own digital equipment. The temperature and humidity index was calculated according to [12], using the formula

$$THI = 0.8\ Tbs + RH\frac{Tbs - 14.3}{100} + 46.3$$

where Tbs refers to the dry-bulb temperature (°C) and RH to relative humidity (%). Statistical analysis of the parameters evaluated was Pearson's correlation and Student's t-test at a significance level of 5%.

3. Results

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

It was found that there was a significant difference of the THI and RT between the two established groups: pregnant cows (GP) and nonpregnant cows (GNP), where the lowest mean values were found in the GP group. It was also found that both groups had a significant and positive correlation between the THI and RT parameters (**Table 1**).

The climatic variables in this study refer to the typical days of the Amazon region, collected on nonconsecutive dates. It was verified that the GNP group for the THI parameter (81.83 ± 0.03) had values above the care range; the GP group presented values below the safety range. It was also found that the THI values (70.50 ± 0.10) of the GP group were below the established values of 72–78.

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 the THI and RT	($r = 0.73$; $p = 0.0208$)	($r = 0.85$; $p = 0.0126$)

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

Table 1.
Correlation between THI and RT (average ± standard error) in the groups which are not pregnant and pregnant.

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 the cortisol and THI	(r = 0.45; p = 0.0368)	(r = 0.76; p = 0.0186)

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

Table 2.
Correlation between plasma cortisol concentrations and THI (average ± standard error) in the groups which are not pregnant and pregnant.

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

4. Discussion

On the yield of protocols, similar results have been reported by [13, 14], with rates of 72.9 and 65%, respectively. The results for pregnancy rate were also shown to be close to those described by [15], which reached 36.60% (108/295), using eCG in D8.

The mean value of the recorded RT was attested as higher than the maximum of 39.1°C, as mentioned by [16], also remaining outside the limits of normality described by [17]. On the other hand, [18] state that cattle of all races have an average rectal temperature of 38.3°C with some variations. However, the RT remained within the physiological values that according to [19] for adult cattle is between 37.5 and 39.3°C. Therefore, the RT of the NP group exceeded the thermoneutral zone in which the maintenance of homeothermia occurs with a maximum mobilization of the mechanisms of heat dissipation, responsible for thermoregulation, and this deviation of energy may be responsible for a decrease in reproductive performance.

The comfort zone for cattle is relatively small, whereas for the European breeds, it is between −1 and 16°C and for the zebu breeds between 10 and 27°C [20], with limit critical from 35°C [21, 22]. In other words, the high-temperature indices of the Amazon region present a great challenge to the animals, requiring them to develop adaptive mechanisms for heat dissipation. However, the temperature range that confers thermal comfort in which there is minimum energy expenditure to maintain the homeothermia also depends on the relative humidity of the air [1].

The animals of this experiment were found in the Amazon biome characterized by high temperature and humidity [21, 22]. Therefore, when the air humidity is low, evaporation is facilitated; otherwise the evaporation process becomes slow or even null, making maintenance of homeothermia difficult [23]. Thus, these conditions of heat and relative air humidity, almost always, are above the zone of thermal comfort for the animals, demanding, by them, energy expenditure in terms of thermoregulatory physiological mechanisms in the attempt of heat dissipation [24]. However, there are occasions in which the loss of body heat becomes ineffective, and then thermal stress of the animal occurs, which is often a limiting factor for development, production, and reproduction [25].

The reproductive efficiency of dairy cattle exposed to adverse climatic conditions is compromised when temperature and humidity are high and solar radiation is intense for most of the year [26], a recurrent situation in the Amazonian biome.

In the case of the use of PIV, embryo development becomes impaired when cows suffer from heat stress on AI day or up to 7 days after the procedure, which leads to less embryonic viability [2], which may render the results of the technique unfeasible.

In a study with Dutch cows by Martello et al. [19], THI values up to 74 correspond to the safety range and from 74 to 78 care range. Igono et al. [27], however, consider THI above 76 in any environment stressful for cows with high milk yield. The climatic variables presented in this study indicate mild thermal stress; thus, the highest values of THI found in the GNP group indicate an environmental situation favoring stress for the animals, where the thermal condition was above that considered comfort. These processes may cause reproductive performance below ideal, such as a decrease in conception rate during the hot season by 20–30% [28] and significant economic losses [29]. Climatic conditions may be an important contributor to the low fertility of dairy cows during the summer months, especially in high-yielding cows [30].

Plasma concentrations of cortisol, above 10 ng/mL for zebu breeds, were similar to those reported by [31] for both groups. Therefore, because it is considered the stress hormone, its evaluation, although expensive, has become of great value for the establishment of the animal welfare condition [32]. Higher production of cortisol causes negative feedback in the hypothalamus, decreasing the synthesis of gonadotrophin-releasing hormone (GnRH), and consequently reduces the release of gonadotrophins FSH and LH, facts that, in the end, are responsible for the low production of the gonadal hormones [33]. The reduction of the latter, in turn, causes less manifestation of estrus, low conception rate, embryonic mortality, and abortions [34].

The lower plasma cortisol concentration in the GP group possibly favored the embryonic quality and maintenance of gestation in relation to the GNP group. Garcia-Ispierto et al. [35] found that the probability of pregnancy loss increases by 1.05 times each unit increase in THI between days 21 and 30 gestations. Due to the results obtained, it is possible to observe that the animals of the GNP group were in a situation of thermal stress, due to the increase of the reported plasma cortisol and of the THI that can act negatively on the luteal function, consequently, and thus the maintenance and recognition of pregnancy [36].

Embryonic development is very susceptible to thermal stress, especially on the third day of development, reducing the proportion of embryos that continue to evolve [37]. In addition, the reduction of embryonic growth is associated with lower levels of interferon-tau that acts on the inhibition of pulsatile secretion of prostaglandin F2α, that is, low levels of interferon-tau may not block the regression of the corpus luteum, making it difficult to maintain the gestation [35]; thus, animals that are under thermal stress are likely to present alterations in the embryonic production of interferon-tau, which causes losses in the maintenance of gestation. Therefore, the techniques used in breeding should be associated with measures that reduce the effects of the thermal environment on production animals [38].

5. Conclusion

The Amazonian climate is an inducer of thermal stress, which can cause abnormalities in the estral cycle and changes in the synthesis of sex hormones and embryonic development and, consequently, negatively affect the pregnancy rate in embryo-recipient cows, even in races more adapted to the tropics, as demonstrated by the RT, THI, and plasma cortisol parameters. Therefore, it is recommended to adopt measures that may reduce the effect of environmental conditions on the reproductive performance of production animals.

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