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Influence of seasonality, timing of insemination and rectal temperature on conception rate of crossbred dairy cows

Influência da estação do ano, do momento da inseminação e da temperatura retal na taxa de concepção de vacas leiteiras mestiças

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Abstract

This study aimed to evaluate the effects of rectal temperature (RT) on conception rate (CR), as well as the effects of seasonality (spring-summer vs. autumn-winter) and timing of artificial insemination (AI) (morning vs. afternoon) on RT and CR in crossbred dairy cows (Holstein x Gyr). The experiment was conducted on a dairy farm in Centralina, MG, where 1.219 conventional and fixed-time inseminations were analyzed. The RT was measured immediately before AI using a digital thermometer. Pregnancy diagnosis was performed using ultrasonography between 28 and 60 days after AI. T The effects of seasonality and timing of AI on RT were analyzed with a Mann-Whitney U test and the effects of RT (above or below the average), seasonality and timing of AI on CR were analyzed with a Chi-squared test, both using the SAS program. The RT average was 39.4°C. Cows with RT ≥ 39.4°C had lower CR than cows with RT < 39.4°C (25.78% vs. 32.54%; $P = 0.0096$). During spring-summer, cows had higher RT (39.44°C ± 0.025 vs. 39.27°C ± 0.022; $P < 0.0001$) and lower CR (25.49% vs. 31.75%; $P = 0.0146$) compared with autumn-winter. Cows inseminated in the morning had lower RT (38.96°C ± 0.022 vs. 39.60°C ± 0.018; $P < 0.0001$) and higher CR (32.86% vs. 26.06%; $P = 0.0102$) than cows inseminated in the afternoon. In conclusion, crossbred dairy cows with rectal temperature equal to or greater than 39.4°C had lower conception rate. Moreover, rectal temperature and conception rate were affected by seasonality and insemination time.

Key words: Reproduction efficiency, heat stress, artificial insemination, crossbred cows

Resumo

Objetivou-se avaliar o efeito da temperatura retal (TR) sobre a taxa de concepção (TC), bem como os efeitos da estação do ano (primavera-verão vs. outono-inverno) e do momento da inseminação artificial (IA) (manhã vs. tarde) sobre a TR e a TC de vacas leiteiras mestiças (Holandês x Gir). O experimento foi realizado em uma fazenda leiteira em Centralina, MG, onde foram analisadas 1219 inseminações, tanto convencionais quanto em tempo fixo. A TR das vacas foi medida imediatamente antes da IA usando termômetro digital. O diagnóstico de gestação foi realizado por ultrassonografia entre 28 e 60 dias após a IA.Os efeitos da sazonalidade e do momento da IA sobre a TR foram analisados pelo teste de Mann-Whitney U e os efeitos da TR (acima e abaixo da média), sazonalidade e momento da IA sobre a TC foram analisados pelo teste de Qui-quadrado, ambos no programa SAS. A TR média encontrada foi de 39,4°C. Vacas com TR ≥ 39,4°C tiveram TC inferior à de vacas com TR < 39,4°C (25,78% vs. 32,54%;

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P = 0,0096). Na primavera-verão as vacas apresentaram TR superior (39,44°C ± 0,025 vs. 39,27°C ± 0,022; *P* < 0,0001) e TC inferior (25,49% vs. 31,75%; *P* = 0,0146) em relação ao outono-inverno. Vacas inseminadas durante o período da manhã tiveram menor TR (38,96°C ± 0,022 vs. 39,60°C ± 0,018; *P* < 0,0001) e maior TC (32,86% vs. 26,06%; *P* = 0,0102) do que as vacas inseminadas à tarde. Conclui-se que vacas leiteiras mestiças com temperatura retal igual ou superior a 39,4°C apresentam menor taxa de concepção. Além disso, a temperatura retal e a taxa de concepção são afetadas pela estação do ano e pelo momento da inseminação.
Palavras-chave: Eficiência reprodutiva, estresse térmico, inseminação artificial, vacas mestiças

Introduction

Milk production around the world has increased due to a combination of management improvement, better nutrition and intense genetic selection. However, this progress negatively affects the reproductive efficiency of lactating cows (LUCY, 2001; WASHBURN et al., 2002). In addition, the negative effects caused by heat stress also contribute to reduced reproductive performance in dairy cows.

There is great variation in the temperatures that establish the thermoneutrality zone for bovines. Nãas (1989) showed that temperatures between 13ºC and 18ºC were comfortable for most of the ruminants. Baeta and Souza (1997) mentioned that the comfort zone for adult bovines from European breeds was between –1ºC and 16ºC. Thermal comfort depends on many factors, especially relative humidity, Temperature and Humidity Index calculation was created to evaluate the risk of heat stress according to the USDC-ESSA's (1970) classification, which measures situations from stress absence, slight, moderate, severe stress, or until animal death.

Heat stress is defined as any combination of environmental conditions that leads the temperature to rise above the animal's thermoneutrality zone (THATCHER et al., 2010). Dairy cows' tolerance to high temperatures is reduced during lactation due to the increase in body temperature as a metabolic response to high feed intake and milk synthesis, which decreases the animal's capacity to lose excess heat (KADZERE et al., 2002). In accordance with these authors, less attention has been given to the thermoregulation ability of modern dairy cows selected to increase their milk production capacity.

Most crossbred dairy cows managed in Brazil were originated by mating *Bos taurus* and *Bos indicus* animals with the aim of minimizing the detrimental effects of heat stress. This blend combines the high productive potential of *Bos taurus* with the elevated environment temperature resistance of *Bos indicus*. However, crossbred cows selected for high levels of milk production had an increased metabolic response due to greater feed intake and heat production. This can lead to a difficult thermal balance maintenance, showing that these cows are also susceptible to heat stress (AZEVEDO et al., 2005).

Rectal temperature (RT) may be used as a physiologic parameter related to thermal comfort and adaptability of environmental conditions (HEMSWORTH et al., 1995). Dunlap and Vincent (1971) reported a high significant negative correlation between RT and conception rate (CR); thus, the increase in body temperature compromises fertility in cows.

Heat stress negatively affects many physiological processes until pregnancy establishment, such as follicular and early embryonic development (AL-KATANANI et al., 1999), which increases the proportion of failure embryos and, thus, the CR of dairy cows (HANSEN; ARECHIGA, 1999). Moreover, heat stress influences many milk productive parameters, including milk yield, quality and composition, leading to economic losses. It is necessary to implement strategies to reduce the effects of heat stress to minimize these losses, as the stress factors are directly related to the reproductive efficiency of dairy herds.

The aim of this study was to evaluate the effect of RT on CR, as well as the effects of seasonality (spring-summer vs. autumn-winter) and artificial insemination (AI) timing (morning vs. afternoon) on RT and CR in crossbred dairy cows.

Materials and Methods

This study was conducted on a commercial dairy farm located in Centralina, Minas Gerais, Brazil (latitude 18°34'02" S and longitude 49°11'52" W). The weather in this area is classified as tropical, with temperatures varying between 18°C and 38°C, with an annual average of 23ºC. The rain period usually occurs from October to March, with an annual average pluviometric index of 1,473 mm. During the study period, which occurred between July 2011 and June 2012, 1.219 inseminations were evaluated .

The herd, composed of crossbred dairy cows (Holstein x Gyr), had an average of 480 lactating cows mechanically milked two times per day with an average milk production of 18.75 liters per cow per day. The farm adopted a vaccination calendar, which included vaccines for foot-and-mouth disease, brucellosis, bovine viral diarrhea virus, infectious bovine rinothracheitis and leptospirosis. The animals were also wormed twice a year, with an alternation of the used drugs.

During the rainy season (spring-summer), the animals were kept in rotational grazing patterns on paddocks of Tifton-85 (*Cynodon sp.*) and supplemented with a concentrate made at the farm. Pasture shading was provided by *Eucalyptus* trees planted parallel with fences, which delimitate each paddock. During the dry period (autumn-winter), the cows were kept in a "loosing house" confinement, receiving a total mixed ration (TMR) composed of corn or sorghum silage, which varied by farm availability; the TMR was supplemented with concentrate and minerals. All cows had *ad libitum* access to water and all diets were formulated in accordance with the National Research Council's recommendations (NRC, 2001).

Reproductive farm management was conducted monthly using ultrasonography equipped with a rectal linear transductor of 7.5 MHz (DP3300vet®, Mindray) to evaluate the uterine and ovarian conditions of the cows after 30 days post partum (DPP). During the study, the same veterinarian did all gynecologic evaluations, including the pregnancy diagnosis.

Primiparous and multiparous cows greater than 30 DPP with body conditional scores greater than 2.5 according to the scale proposed by Edmonson et al. (1989) (1 = very skinny and 5 = obese), with good uterine condition and healthy, considering cows without clinical mastitis, lameness and/or digestive disturbances were selected by the same evaluator, to be submitted to one of the two established reproductive management options, according to their ovarian condition.

The first reproductive management option for cows with the presence of a corpus luteum (CL) was an intramuscular (IM) injection of 25 mg (5.0 ml) of $PGF_{2\alpha}$ (Lutalyse®, Dinoprost Tromethamine, Zoetis). After this injection, trained employees observed the cows for estrus behavior twice daily for seven days; cows who were in estrus were bred conventionally ±12 hours after estrus detection.

As a second reproductive management option, the cows without a CL were submitted to the following timed artificial insemination (TAI) protocol: Day zero (D0) – insertion of a progesterone slow-release intravaginal device (CIDR®, Zoetis) containing 1.9 grams of progesterone and an IM injection of 2.0 mg (2.0 ml) of estradiol benzoate (Estrogin®, Farmavet); Day 7 – an IM injection of 12.5 mg (2.5 ml) of $PGF_{2\alpha}$ (Lutalyse®, Zoetis); Day 9 – intravaginal device withdrawal plus an IM injection of 1.0 mg (0.5 ml) of estradiol cypionate (ECP®, Zoetis); Day 11 – TAI. Cows who showed estrus behavior between days 9 and 11 of the protocol were bred ±12 hours after estrus detection.

The cows greater than 30 DPP, who exhibited estrus signs before being evaluated with an

ultrasonography exam or submitted to one of two reproductive managements, as well as the cows who returned in estrus before the expected date of pregnancy diagnosis, were also bred conventionally.

Estrus behavior observation was conducted twice daily, in the morning and in the afternoon for approximately 30 minutes, by two farm workers who were also responsible for the inseminations. RT was measured immediately before each AI using a digital thermometer (G-Tech®). Date and time (morning or afternoon) were recorded for each AI, and the inseminations were done between 7:00 and 9:00 in the morning and between 17:00 and 19:00 at the afternoon. Pregnancy diagnosis was performed between 28 and 60 days post-AI by ultrasonography exam; cows were considered pregnant if the fetus had a heartbeat.

For statistical analysis, RT of cows were numerically evaluated and classified as above or below the RT average of the study. As the RT did not meet the assumptions of normality and homogeneity, the effects of seasonality and timing of AI on RT were analyzed with a Mann-Whitney U test. The effects of RT (above or below the average), seasonality (spring-summer vs. autumn-winter) and timing of AI (morning or afternoon) on CR were analyzed with a Chi-squared test using the SAS program (version 8.2, SAS/STAT, SAS Institute Inc., Cary, NC). Statistical significance was defined as $P \leq 0.05$.

Results and Discussion

The average RT was 39.4°C. Cows with RT greater than 39.4°C had a 25.78% of CR, and cows with RT lower than 39.4°C had 32.54% of CR, showing a significant effect ($P = 0.0096$) for the increase of body temperature on the fertility of crossbred dairy cows. A possible explanation for this is that many processes of the reproductive tract, such as the oocyte, CL and early embryonic developments, as well as the endometrium and hypothalamic-pituitary axis functionality, are sensitive to hyperthermia caused by heat stress (WOLFENSON et al., 2000). Embryo development also is compromised when the cow experiences hyperthermia during the estrus day (PUTNEY et al., 1989) or on the day after estrus (EALY et al., 1993).

The mechanism by which heat stress reduces dairy cows' fertility is multifactorial and varies with the intensity (HANSEN; ARECHIGA, 1999). This reduction may be associated with endocrine changes and the follicular microenvironment where the oocytes are exposed, leading to a lower development competence, which denotes the complexity of these mechanisms (ROTH, 2012). As an alternative, to solve the negative effects of high temperatures on reproductive performance, cooling methods should be used, such as sprinkling and ventilation, which can reduce RT of Holstein cows and, thus, improve CR (WOLFENSON et al., 1988).

The CR average was 28.79% (351/1219). The RT ($P < 0.0001$) and CR ($P = 0.0146$) were affected by seasonality; during the hottest months of the year (spring-summer) crossbred dairy cows had higher RT and lower CR than the autumn-winter months (Table 1).

The increase of RT due to the temperature elevation during summer compromises the reproductive efficiency of dairy cows, especially when the animals are from European breeds (*Bos taurus*). Vasconcelos et al. (2011) evaluated RT associated with seasonality seven days after TAI or during fixed-time bovine embryo transfer (ET) in Holstein dairy cows and reported that the highest average value of RT occurred during summer. Demétrio et al. (2007) concluded that a high RT measured seven days after AI or during ET had a negative effect on the conception of Holstein dairy cows. Pires et al. (2002) showed higher CR during winter when compared with summer (71.2% vs. 45.7%). However, Campos and Santos (2015) did not find an influence of seasonality on CR of Holstein dairy cows submitted to TAI, probably because this author reported a low CR (24.87%) during the entire year.

Table 1. Seasonality effect on rectal temperature and conception rate of crossbred dairy cows.

Season of the year (n)	Rectal temperature (°C)	Conception rate (%)
Autumn-winter (652)	39.27 ± 0.022	31.75
Spring-summer (567)	39.44 ± 0.025	25.49
P-value	< 0.0001	0.0146

High environment temperatures, above the bovine thermoneutrality zone, can drastically reduce CR and increase embryonic losses (CAVESTANY et al., 1985). Many studies, also conducted in the Triângulo Mineiro region, discovered that seasonality influenced CR of crossbred dairy cows. According to the results obtained by Barbosa et al. (2011), the CR was higher during autumn-winter when compared to spring-summer (42,5% vs. 25.0%). Ayres et al. (2014) also reported that CR of crossbred dairy cows submitted to TAI was greater during winter than summer (43.7% vs. 26.9%). Thus, even though *Bos indicus* animals have a significantly higher resistance to heat stress, crossbred cows (Holstein x Gyr) also suffer from the negative effects of the elevated environmental temperature.

The time when the inseminations were performed affected both RT ($P < 0.0001$) and CR ($P = 0.0102$) of crossbred dairy cows. Cows inseminated during the morning showed lower RT and higher CR when compared with the cows bred during the afternoon (Table 2). This may have occurred due to the RT being greater at the afternoon, which compromises conception because of the increase in cows' body temperature. Dunlap and Vicent (1971) concluded that the elevated RT in response to the high temperature might decrease conception in bovine females. In a study performed in Florida, USA, Thatcher (1974) showed that, when temperature increased from 21 to 35°C, the CR decrease from 40 to 31% in Holstein dairy cows.

Table 2. Timing of artificial insemination (AI) on rectal temperature and conception rate of crossbred dairy cows.

Timing of AI (n)	Rectal temperature (°C)	Conception rate (%)
Morning (490)	38.96 ± 0.022	32.86
Afternoon (729)	39.60 ± 0.018	26.06
P –value	< 0.0001	0.0102

With the aim to improve the milk production of dairy cows in tropical regions, animals that are well-adapted to the environmental conditions should be selected, and cooling tools should be used to ensure thermal comfort. These changes could reduce the magnitude of heat stress and allow cows to produce as expected according to their genetic potential. Another option could be to avoid inseminations during the hottest periods of the year; however, the reproduction seasonal schemes that are usual practices in some regions of the world could lead to economic losses because of milk production scarcity during specific periods of the year (HANSEN; ARECHIGA, 1999).

Conclusions

Crossbred dairy cows with rectal temperature equal to or above 39.4°C had lower conception rates. Moreover, rectal temperature and conception rate were affected by seasonality and insemination time, which denotes the importance of ensuring thermal comfort to crossbred dairy cows.

Ethical committee approval

This research was conducted according to the Ethical Principles in Animal Experimentation, approved by the Committee of Ethics in the Use of Animals (CEUA) of the Federal University of Uberlândia (UFU), protocol number 033/11.

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