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Conception rate and estrous return detection after TAI in Holstein cows

Taxa de concepção e detecção do estro de retorno após a IATF em vacas Holandesas

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Abstract

The aim of this study was to evaluate the influence of season-of-the-year at the moment of calving and insemination, days in milk (DIM) and the presence of corpus luteum (CL) at the onset of hormonal protocol on conception and estrous return detection rates. Multiparous lactating Holstein cows were submitted to the timed artificial insemination (TAI) protocol: Day 0 - insertion of progesterone releasing intravaginal device (CIDR®, Zoetis) and an intramuscular injection of 0.02 mg of gonadorelin (GnRH, Fertagyl®, MSD); Day 7 - 12.5 mg of dinoprost tromethamine ($PGF_{2\alpha}$, Lutalyse®, Zoetis) and removal of the device; Day 8 - 1.0 mg of estradiol cypionate (ECP®, Zoetis); Day 10 - TAI. Pregnancy diagnosis was performed 32 days post-AI using ultrasonography. Cows that had estrous return before pregnancy diagnosis were re-inseminated, conventionally, 12 hours after estrous detection. Trained employees observed cows for signs of estrous twice daily, in the morning and afternoon, for approximately 30 minutes. Cows that did not become pregnant were resynchronized using the same protocol. Data was analyzed by logistic regression in Minitab program. Significance level of $P < 0.05$ was adopted. A total of 406 inseminations were performed, which resulted in 101 pregnancies with a conception rate of 24.87%. Estrous return detection rate was 43.27%. Season of year at calving ($P = 0.930$), DIM ($P = 0.508$) and presence of CL ($P = 0.293$) did not affect conception rate. Season of year at calving ($P = 0.256$), DIM ($P = 0.215$) and presence of CL ($P = 0.076$) also did not influence estrous return detection rate. Season of year at the moment of TAI did not interfere with conception rate ($P = 0.236$); however, there was an affect ($P = 0.012$) on estrous return detection rate; values found in spring-summer were lower than in autumn-winter (38.24% vs. 53.47%).This suggests the detrimental effect of excessive heat on the occurrence of estrous in Holstein cows.

Key words: Cattle, reproductive efficiency, estrous return, synchronization

Resumo

Objetivou-se avaliar a influência da estação do ano no momento do parto e da inseminação, do número de dias pós-parto (DPP) e da presença de corpo lúteo (CL) no início do protocolo hormonal sobre as taxas de concepção e de detecção do estro de retorno em vacas leiteiras Holandesas submetidas à inseminação artificial em tempo fixo (IATF). O protocolo consistiu em: Dia 0 - inserção do dispositivo intravaginal de progesterona (CIDR®, Zoetis) e aplicação via intramuscular de 0,02 mg de gonadorelina (GnRH, Fertagyl®, MSD); Dia 7 - 12,5 mg de dinoprost trometamina ($PGF_{2\alpha}$, Lutalyse®, Zoetis) e remoção do dispositivo; Dia 8 - 1,0 mg de cipionato de estradiol (ECP®, Zoetis); Dia 10 - IATF. O diagnóstico de

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gestação foi realizado 32 dias pós-IA por ultrassonografia. As vacas que retornaram ao estro antes do diagnóstico de gestação foram reinseminadas convencionalmente, 12 horas após a detecção do estro. O manejo de observação de estro era realizado por funcionários treinados, duas vezes ao dia, de manhã e à tarde, por aproximadamente 30 minutos. As vacas vazias foram ressincronizadas utilizando o mesmo protocolo. Os dados foram analisados por Regressão Logística no programa Minitab. Adotou-se nível de significância de $P < 0,05$. Um total de 406 inseminações foram realizadas, que resultaram em 101 gestações com taxa de concepção de 24,87%. A taxa de detecção do estro de retorno foi de 43,27%. A estação do ano ao parto ($P = 0,930$), os DPP ($P = 0,508$) e a presença de CL ($P = 0,293$) não afetaram a taxa de concepção. A estação do ano ao parto ($P = 0,256$), os DPP ($P = 0,215$) e a presença de CL ($P = 0,076$) também não influenciaram a taxa de detecção do estro de retorno. A estação do ano no momento da IATF não interferiu na taxa de concepção ($P = 0,236$), porém afetou ($P = 0,012$) a taxa de detecção do estro de retorno, já que os valores encontrados na primavera-verão foram inferiores aos do outono-inverno (38,24% vs. 53,47%), enfatizando o efeito prejudicial do excesso de calor sobre a expressão e a detecção do estro em vacas leiteiras Holandesas.
Palavras-chave: Bovinos, eficiência reprodutiva, retorno ao estro, sincronização

Introduction

Milk production and reproduction are the main factors that determine the profitability of a herd of dairy cattle. Thus to become profitable, dairy farmers must manage their herds efficiently and productively so that reproductive performance can reach satisfactory economic returns (McMANUS et al., 2008).

Genetic selection for high milk production and nutritive qualities, the complexity of intensive herd handling, and physiologic and hormonal changes that occurs during the transition period highly influence future reproductive efficiency of bovine females. Furthermore, endocrine and metabolic pathways controlling milk production are closely related to reproductive processes and, in dysfunctional cases, compromise the reestablishment of ovarian activity at lactation onset (BUTLER, 2003).

Among challenges associated with dairy cattle reproduction, estrous detection efficiency is an important factor enhancing reproduction. Controlling estrous return brings about suitable reproductive performance. Pharmacological interventions to control the estrous cycle of cows, well-known as Timed Artificial Insemination (TAI), have been largely utilized in the dairy industry over the last few years. The main objective is to eliminate the necessity of estrous observation and thus increase the number of cows submitted for insemination, allowing for the improvement of herd conception rates (NEVES et al., 2010).

Competition within the dairy industry stimulates the need for animals with high milk production potential, leading farmers to select for animals bred especially for this quality, such as the Holstein. However, these animals are originally from temperate environments; due to this, they are less tolerant to tropical conditions. Because environmental factors are not ideal for cows' thermal comfort, they can affect the performance potential of these animals (ROCHA et al., 2012).

Heat stress impairs many physiological processes required for pregnancy establishment, such as follicular and early embryonic development (AL-KATANANI et al., 1999). These impairments may result in an increased proportion of embryos that fail during the developmental stages, compromising the conception rates of dairy cows (HANSEN; ARECHIGA, 1999). Thus, the use of tools that can reduce the effects of heat stress is important to minimize economic losses, since heat stress is directly related to dairy cattle fertility.

We hypothesized that cows that calved during spring-summer would have lower pregnancy rates than cows that calved during autumn-winter, due to the negative effects of heat stress on postpartum reproductive tract recovery, conception and estrous return detection in cows submitted to

TAI. The aim of this study was to evaluate the influence of season-of-the-year at the moment of calving and insemination, days in milk (DIM) and the presence of corpus luteum (CL) at the onset of hormonal protocol on conception and estrous return detection rates of lactating Holstein cows submitted to TAI.

Materials and Methods

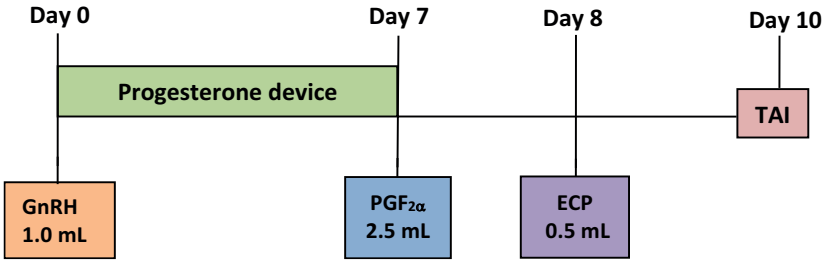
This study was conducted on a commercial dairy farm located in Uberlândia City, Minas Gerais, Brazil, at latitude 48°43'31" longitude 18°86'27" and an altitude of 780 meters. According to bioclimatic zoning done at Triângulo Mineiro Region, which used data collected from the Estação Climatológica do Instituto de Geografia of Federal University of Uberlândia (UFU) between the years 2000 and 2010, the average value of the temperature and humidity index (THI) during the analyzed period for Uberlândia City was 73 (NASCIMENTO et al., 2014).

The herd, composed of Holstein cows, had an average of 500 lactating cows mechanically milked three times per day with an average milk production of 20.5 liters per cow per day. Animals were kept in confinement during the year receiving a total mixed ration (TMR) composed of corn silage, concentrate and minerals with *ad libitum* access to water, in accordance with National Research Council (2001) recommendations. Furthermore, cows received bovine somatotropin (bST - Lactotropin®, Elanco) from 60 DIM until 190 days of gestation with 14 days of interval between each application.

Reproductive management was conducted every 21 days. Multiparous lactating cows, after 45 DIM, were submitted to gynecological examination to evaluate uterine involution and structures present on the ovaries by ultrasonography with rectal linear transductor of 7.5 MHz (DP3300vet®, Mindray). Body condition score (BCS) was also used as criteria to select cows that could be submitted for TAI treatment. During reproductive management, BCS was evaluated following a scale proposed by Edmonson et al. (1989), which considers 1 a very skinny animal and 5 an obese animal; only cows with a BCS equal or higher than 2.5 were included in the trial. Cows in proper condition for reproduction were submitted for TAI. A total of 406 TAI repetitions were performed (each insemination was considered one repetition).

Cows were submitted for the following TAI protocol: Day zero (D0) – insertion of a progesterone slow-release intravaginal device (CIDR®, Zoetis) containing 1.9 grams of progesterone and an intramuscular injection of 0.02 mg (1.0 mL) of gonadorelin (GnRH, Fertagyl®, MSD); Day 7 – intramuscular injection of 12.5 mg (2,5 mL) of dinoprost tromethaminea ($PGF_{2\alpha}$, Lutalyse®, Zoetis) and device removal; Day 8 – intramuscular injection of 1.0 mg (0.5 mL) of estradiol cypionate (ECP®, Zoetis); Day 10 – TAI (Figure 1). All of the injections and inseminations were conducted in the morning.

Figure 1. Schematic representation of the use of Timed Artificial Insemination (TAI) protocol.



After each removal, progesterone intravaginal devices were washed in water, dried and stored in plastic packages protected from sunlight until their next use. Devices were used at least three times and then disposed. Conventional imported semen of Holstein proven bulls acquired from a reputable insemination company was used, following a previously established mating schedule for each cow.

Pregnancy diagnosis was performed 32 days after TAI date by ultrasonography. Cows returning naturally into heat and which showed estrous signs between insemination and pregnancy diagnosis were conventionality re-inseminated 12 hours after estrous detection. Cows diagnosed as non-pregnant and not detected in estrous return were resynchronized using the same protocol until either becoming pregnant, exceeding 300 DIM, or having had more than four inseminations.

Trained employees observed cows for estrous behavior twice daily, in the morning and afternoon for approximately 30 minutes. Two trained and experienced technicians performed both fixed time and conventional insemination procedures and the aforementioned hormone injections.

Conception and estrous return detection rates were analyzed using Minitab's (Minitab, State College, PA) logistic regression. The model took into account the seasonal effects at the moment of calving and insemination (spring-summer vs. autumn-winter), days in milk (DIM) and presence of corpus luteum (CL) at the onset of the hormonal protocol. Statistical differences with levels of $P \leq 0.05$ were considered significant.

Results and Discussion

A total of 406 inseminations were performed which resulted in 101 pregnancies with a conception rate of 24.87% (101/406). Menarin et al. (2005) performed a study in Paraná State, Brazil in which they submitted high producing Holstein cows to the same TAI protocol used in this trial and reported a conception rate of 44%, a value higher than that of this study.

The estrous return detection rate obtained in the current study was 43.27% (132/305). Similar to the current study, Vasconcelos and Garcia (2006) reported an estrous return detection rate of 49.9% (1148/2302) within 28 days after AI for both conventional and fixed time inseminations.

Season of the year at the moment of calving did not influence conception ($P = 0.930$) or estrous return ($P = 0.256$) of lactating Holstein cows (Table 1). Results found in the present study differed from values obtained in a California study (SANTOS et al., 2009), which demonstrated that season of the year effects at calving is a risk factor for conception 30 days after AI. Cows which calved during summer and autumn months (34.7% and 35.2%) had lower conception rate than cows which calved during winter and spring (38.7% and 40.9%, respectively).

Table 1. Effect of season-of-the-year at the moment of calving on conception and estrous return detection rates after TAI in Holstein cows in Uberlândia City, Minas Gerais, Brazil.

Calving season	Conception rate (%)	Estrous return detection rate (%)
Spring-summer	24.65	47.66
Autumn-winter	25.00	40.91
P-value	0.930	0.256

Occurrence of uterine diseases during the post-partum period is a contributing factor to the reduction of conception rate in cows calving during the hottest months of the year (ROCHE, 2006). Heat stress can compromise immune function, leading to late uterine involution and increasing the interval between parturition and first ovulation. Conception rate was not influenced by season-of-the-year effects at calving, probably because the reproductive

efficiency at this farm was low during the entire year. This may indicate that there was no favorable season for reproductive tract recovery in the post-partum period and this probably caused remaining effects on the reproductive performance of Holstein cows. Regarding estrous return detection after TAI, the absence at the moment of calving may be related to the fact that seasonality did not produce impaired cumulative effects on cyclic return after hormonal treatment, showing that the TAI is able to efficiently induce and maintain reproductive activity post-partum.

Season of the year at TAI moment did not interfere (P = 0.236) with conception rate; however, the effect of this variable was observed in the detection rate of estrous return (P = 0.012) of lactating Holstein cows (Table 2). Values obtained in this trial differ from Alnimer et al. (2009) study conducted in Jordan in which they submitted Holstein cows to the Ovsynch protocol with some variations. Independently of treatment they concluded that season of the year had an effect on pregnancy rate, where values obtained during winter were higher than summer (43% vs. 31.5%). Santos et al. (2009) concluded that cows inseminated during summer and autumn had a lower risk of pregnancy than cows bred during winter and spring. In a trial performed in Iran, Ahmadi and Ghaisari (2007) used dairy cows diagnosed with a CL and more than 70 DIM. They compared three different TAI protocols to measure conception rate according to the seasons of the year. These authors demonstrated reduced conception rates during the summer, compared to other seasons of the year.

Table 2. Season-of-the-year effect at the moment of TAI on conception and estrous return detection rates in Holstein cows in Uberlândia City, Minas Gerais, Brazil.

TAI season	Conception rate (%)	Estrous return detection rate (%)
Spring-summer	23.02	38.24
Autumn-winter	28.37	53.47
P-value	0.236	0.012

No detection of seasonal effects on conception rate can be explained by environmental features where the study was carried out. Triângulo Mineiro Region, where Uberlândia City is located, is a tropical environment with high temperatures, predominantly due to intense sun radiation (AZEVEDO et al., 2005). In a trial also conducted in Uberlândia, MG, Ayres et al. (2014) evaluated temperatures between years 2007 to 2010, measured at Estação Meteorológica do Instituto de Geografia of UFU and confirmed similar temperatures between winter and summer in this region. According to the same authors, the average temperature during the winter was 23.55°C, while during the summer the average was 25°C. This region usually has hot and wet springs and summers because both periods have high concentrations of rainfall. However, high temperatures persist during autumn and winter; accentuated relative humidity decreases due to scarce rainfall.

Probably due to the similarity of temperatures between spring-summer and autumn-winter in conjunction with the lack of infrastructure to minimize the negative effects of the hot environment, it was not possible to identify increases in conception rates during autumn-winter months in this study. The majority of reviewed papers published in this field were conducted in subtropical locations, where the four seasons of the year are well-defined with hot summers and mild winters, a situation that is not usually found in most parts of Brazil.

However, some trials conducted in Brazil also detected seasonal influence on the conception rate of dairy cows. Barbosa et al. (2011) reported a seasonal effect on crossbred dairy cows that showed a conception rate of 42.55% in autumn-winter and 25% in spring-summer. Additionally, Pires et al. (2002) found a significant difference on the conception rate of confined Holstein cows after conventional AI in summer and winter, which was confirmed by high variation in THI values occurring in different seasons in the region where the trial was conducted (75.8 in summer vs. 65.3 in winter).

Season-of-the-year at the moment of TAI influenced estrous return detection rate after TAI (Table 2). During the autumn-winter season detection rate reached 53.47%, while during the spring-summer season the rate was 38.24%, demonstrating the negative effect of hot environmental temperature on estrous expression and detection.

Changes in female behaviour caused by heat stress during estrous reduces the probability of estrous being identified by visual observation. The main reason for reduction in estrous expression due to heat stress is physical lethargy (HANSEN; ARECHIGA, 1999). Heat stress can also reduce plasmatic concentrations of estradiol during proestrus and both estrous behaviour expression and its detection in females (GWAZDAUSKAS et al., 1981). As a result, the implementation of TAI programs during critical periods of heat stress can work as a management strategy to compensate for both low estrous expression and the reduction of detection rates observed in dairy herds experiencing hot temperatures (THATCHER, 2010).

The DIM at the onset of the TAI protocol did not affect conception and estrous return detection rates of Holstein dairy cows (Table 3). Cows observed between 101 and 200 DIM were expected to have higher conception rates compared with the other two categories of DIM, because animals at this stage had recovered from a negative energy balance and had passed peak milk production; therefore, reduced milk production level during this period are expected (COBUCI et al., 2003). Conception rates analyzed through the period was lower than expected, independent of DIM. Perhaps cows from this herd may have been suffering from other management problems, which were not detected during the experimental period but that may have contributed to the low conception rates found at all DIM categories.

Table 3. Days-in-milk (DIM) effect on conception and estrous return detection rates after TAI in Holstein cows in Uberlândia City, Minas Gerais, Brazil.

Days in milk (d)	Conception rate (%)	Estrous return detection rate (%)
45 - 100	25.84	39.39
101 - 200	26.20	41.30
> 201	22.31	48.51
P-value	0.508	0.215

In an experiment conducted in Japan with dairy cows submitted to TAI, Yamada (2005) evaluated conception according to DIM between 40 and 221 or more; in this study he also did not identify DIM as an effect on conception rate. Results from the present study were different from the values obtained by Barbosa et al. (2011) in a trial also performed in Uberlândia City using crossbred dairy cows. Researchers reported an effect of DIM on conception rate; cows between 34 and 90 DIM had a conception rate of 43.90% and cows with more than 90 days had a conception rate of 23.94%.

Presence of a CL at the onset of TAI protocol did not interfere with conception or estrous return detection rates in Holstein dairy cows (Table 4). Even without the presence of a CL at the time of the gynecological examination, it is possible that some cows may have recovered ovarian post-partum cyclicity.

Results obtained in the current study were in accordance with those reported by Barbosa et al. (2011), Martel (2008), Souza et al. (2009) and Yamada (2005), which also found no interference of CL presence at the onset of a TAI protocol on conception rate of dairy cows. However, Santos et al. (2009) showed that ovarian cyclicity at 65 DIM affects conception rate in Holstein cows. According to these authors, anestrous cows had a conception rate of 29.0% (419/1445) while cyclic cows had 41.1% (1925/4679). Similar results were

demonstrated by Galvão et al. (2004); comparing the same TAI protocol as this trial, they found a tendency of cyclic cows to have a greater pregnancy rate than anestrous cows (40.3 vs. 29.2%).

Table 4. Effect of corpus luteum (CL) presence at the onset of TAI on detection rates of conception and estrous return in Holstein cows in Uberlândia City, Minas Gerais, Brazil.

Corpus luteum presence	Conception rate (%)	Estrous return detection rate (%)
Yes	26.28	40.28
No	21.00	51.90
P-value	0.293	0.076

The presence of a CL and DIM did not affect estrous return detection rate after TAI in Holstein cows (Tables 3 and 4). There is very little scientific literature available correlating cyclicity and DIM with estrous return detection rate. A probable explanation for the absence of effects for these variables may be that TAI was effective at inducing cyclicity in anestrous animals and in maintaining the regularity of estrous cycles in those already cyclic without, in fact, improving the conception rate of the studied herd.

Estrous return detection rate after TAI is highest during autumn-winter in Holstein lactating dairy cows managed in the Triângulo Mineiro Region. The detection rate obtained in the current study was 43.27%, which may be related to the failure of managing visual estrous observation established at the farm. These failures may be associated with the length of the observation period, frequency of daily observations and the time of day that these were performed (VASCONCELOS, 2000), suggesting that the length and frequency of visual observations should be increased to alleviate these issues.

Ethical Committee Approval

This research was conducted according to Ethical Principles in Animal Experimentation, approved by Committee Ethics in the Use of Animals (CEUA) of the Federal University of Uberlândia (UFU), protocol number 016/13.

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