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ANIMAL REPRODUTION

Artificial insemination timing on pregnancy rate of Holstein cows using an automated activity monitoring

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ABSTRACT: This study evaluated the probability of pregnancy and associated factors for two times artificial inseminations (AI), 8 or 10 hours after automated activity monitoring (AAM) alarm on the first postpartum AI of 1,054 Holstein dairy cows. The estrus was synchronized by prostaglandin or estradiol-progesterone program. Stepwise logistic regression was performed to analyze the probability of pregnancy, and associated factors (activity, estrus intensity, parity, peripartum health, retained placenta, postpartum vaginal discharge, and season). The highest pregnancy rates were obtained with multiparous animals, inseminated ten hours after the AAM alarm, in the fall or winter season, with a high activity peak and estrus intensity ($P < 0.05$). Peripartum diseases, retained placenta, and postpartum vaginal discharge negatively influenced the pregnancy rate, regardless of parity. Thus, the optimization of AAM models by including on-farm measures like parity, peripartum health history, and environmental conditions may favor the correct identification of estrus and improve the AAM alarm regarding the ideal moment for AI, increasing the reproductive performance in dairy cows.

Key words: peak activity, estrus, peripartum diseases, vaginal discharge, dairy cattle.

Horário da inseminação artificial na taxa de prenhez das vacas Holandesas usando monitoramento automatizado de atividades

RESUMO: O objetivo deste estudo foi avaliar a probabilidade de prenhez e fatores associados para dois horários de inseminação artificial (IA), oito ou 10 horas após o alarme de monitoramento automatizado de atividades (AAM), na primeira IA pós-parto em 1.054 vacas leiteiras da raça Holandês. O estro foi sincronizado por protocolos a base de prostaglandina ou estradiol-progesterona. Regressão logística stepwise foi realizada para analisar a probabilidade de prenhez e fatores associados (atividade, intensidade do estro, paridade, saúde no periparto, placenta retida, descarga vaginal pós-parto e estação do ano). As maiores taxas de prenhez foram obtidas em multíparas, inseminadas 10 horas após o alarme do AAM, no outono ou inverno, com pico de atividade elevado e intensidade de estro ($P < 0,05$). Doenças no periparto, placenta retida e descarga vaginal pós-parto influenciaram negativamente a taxa de prenhez, independentemente da paridade. Assim, a otimização dos modelos de AAM, incluindo medidas rotineiras da fazenda como paridade, histórico de saúde no periparto e condições ambientais, podem favorecer a identificação correta do estro e melhorar o alarme do AAM em relação ao momento ideal para a IA, aumentando o desempenho reprodutivo nas vacas leiteiras.

Palavras-chave: pico de atividade, estro, doenças no periparto, descarga vaginal, gado leiteiro.

The timing of AI is critical for the success of dairy cow breeding programs. Automated activity monitoring systems (AAMS) have revolutionized estrus detection in dairy cows, providing farmers with accurate and real-time information on cow activity, allowing for timely AI (CERRI et al., 2021). Research has shown that inseminating cows within 12-24 hours after an AAMS alarm can increase the chances of successful conception (MAYO et al., 2019), but the exact timing may vary based on several factors such as breed, parity, management practices, and cow reproductive physiology (MARQUES et al., 2020). LEROY et al. (2018), for example, found better conception rates when primiparous cows were inseminated within 0-8 hours compared to 8-12 hours after the AAMS alarm. However, studies investigating AI time to maximize conception rates in dairy cows using AAM systems with a specific focus on the window of the manufactory's recommendation (8-10h after AAM alarm) are scarce. Moreover, customized AI timings based on AAM type, individual cow reproductive physiology, and other factors can further optimize reproductive performance, milk production, and profitability in dairy herds (CERRI et al., 2021; LÓPEZ-GATIUS, 2022).

This study evaluated the probability of pregnancy and associated factors (activity, estrus intensity, parity, peripartum diseases, and season) for two times AI, 8 or 10 hours after AAM alarm, on the first postpartum AI in dairy cows.

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This study used a retrospective observational case-control method with data from a commercial dairy farm in the southwestern state of Goias, Brazil, from January 2018 to December 2020. Data from 1054 Holstein cows with an average 305-day lactation milk yield of 11,154 kg per animal were evaluated. Cows were housed in a free-stall barn with concrete floor, fans over stalls, and sprinklers over the feedline, and milked three times a day. Cows were fed a total mixed ration based on corn silage, soybean meal, ground corn, cottonseed, and a commercial ration, which was offered ad libitum twice a day, following the recommendations of the National Research Council (NRC, 2001). Water was available ad libitum. Peripartum health (21 days before and 21 days after calving) was evaluated (retained placenta, hypocalcemia, ketosis, displacement abomasum, laminitis, foot disorders, pneumonia, and clinical mastitis), body condition score, and locomotion score. Signs of metritis were carried out at 11±4 days postpartum by vaginal discharge analysis using Metricheck® (SimcroTech, Hamilton, New Zealand) and classified according to SHELDON et al. (2006) into A (clear or translucent), B (little purulent material), C (mucopurulent), D (50 % or more pus), and E (fetid watery reddish-brown). Cows classified as D and E were treated with 20 mg ceftiofur hydrochloride (Lactofur®, Ouro Fino, Cravinhos, Brazil) in a single dose.

The daily time (in minutes) of activity and rumination was measured by AAM (SCR, Netanya, Israel). The animal reaching its peak of activity was indicative of estrus and the AAM alarm was activated, with regressive programming of 25 hours to predict ovulation (time zero). The software also generated the estrus intensity to predict fertility, measured on a scale from 0 to 100 (0 = no fertility and 100 = high fertility).

AI was performed with 60±7 DIM in one of two different time periods, at 8 hours (n = 536) or 10 hours (n = 518) after the AAM alarm, with an estrus intensity higher than 30, according to SCR recommendation. Thus, when the cow was inseminated 8 hours after the AMS alarm, there was a greater distance to ovulation (17 hours to ovulation) compared to that from insemination with 10 hours after the AAM alarm (15 hours to ovulation). Cows were bred following hormonal synchronization of estrus using prostaglandin (PGF 0.5 mg cloprostenol, Sincrocio®, Cravinhos, Brazil, n = 558), or synchronization of estrus and ovulation with estradiol and progesterone protocols (n = 496), as described by Pereira et al. (2017).

The seasons were established as defined for the Southern Hemisphere: spring (September 21 to December 20), summer (December 21 to March 20), fall (March 21 to June 20), and winter (June 21 to September 20). Table 1 shows the environmental variables on AI day during the seasons of the year in the evaluated period.

Logistic stepwise regression was performed to analyze the probability of pregnancy, and associated factors (activity and estrus intensity with effect of parity peripartum health, retained placenta, postpartum vaginal discharge, season, and bull). The variable "bull" was not significant and was removed from the models, choosing the best model for each response variable based on the lowest Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). The predictive model for data analysis was made using the packages "car" (FOX & WEISBERG, 2019), "caret" (KUHN, 2020), and "tidyverse" (WICKHAM et al., 2019). Wald's test was used to verify the significance of the effects of the model. The magnitude of the effects was determined via odds ratio analysis using the tidy function. Statistical analyses were performed in R software (R CORE TEAM, 2021).

Model accuracy was 75.6%**.** Regardless of parity and estrus synchronization protocol, cows

Table 1 - Mean and standard error of environmental variables on AI day: environment temperature, relative air humidity (RH), and temperature and humidity index (THI) in the different seasons of the year during the evaluated period.

Variables	Season			
	Spring	Summer	Fall	Winter
Temperature	25.3±0.2[a]	24.7±0.2[a]	22.5±0.1[c]	23.1±0.2[b]
RH	67.5±0.8[c]	75.4±0.9[a]	71.2±0.7[b]	49.4±0.8[d]
THI[1]	73.6±0.2[a]	73.8±0.2[a]	70.2±0.2[b]	68.9±0.2[c]

[1]Temperature and humidity index (THI) according to MADER et al. (2006).
[a,b,c]Means followed by different letters in the same row differed by the Kruskal-Wallis test ($P < 0.05$).

inseminated 10 hours after the AAM alarm had higher pregnancy rates than 8 hours (OR = 1.7, P = 0.0064) when we considered the window of the manufactory's recommendation. High estrus activity and intensity had significant positive effects on pregnancy rate (P < 0.0001, OR=1.1). While results were influenced by factors such as parity (P = 0.0079), season (P < 0.00001), and health disorders (P < 0.0004), as presented in table 2, body score condition and locomotion score were not significant (P > 0.05).

Multiparous had higher pregnancy rates compared to primiparous cows (OR = 1.5), AI performed in seasons with lower THI indexes (fall and winter) than with higher THI (spring and summer) (OR = 2.9 and 2.5, respectively), independently of any other variable.

A lower chance of pregnancy was observed in animals with a retained placenta (OR = 0.3), with some presence of mucopurulent material in the vaginal discharge (mucus B, OR = 0.4; C, D, and E, OR = 0.3) at 11±4 days postpartum, or with some disease (OR = 0.4). However, when we excluded cows with these disorders, the estradiol-progesterone program increased pregnancy rates compared to prostaglandin use, whatever of the season (P = 0.0263).

Cows inseminated 10 hours after the AAM alarm, i.e, closer to ovulation, showed better pregnancy rates, regardless of parity and estrus synchronization protocol, possibly due to the higher number of viable spermatozoa reaching the isthmus and having access to the zona pellucida at the time of AI when performed closer to the time of ovulation (RICHARDSON et al., 2017). LÓPEZ-GATIUS (2022) and COLAZO et al. (2023) suggested that breeding efficiency in dairy herds can be improved by optimizing AI timing closer to the end of estrus behavior once the ovulation typically occurs 10-12 hours after this marker (SUMIYOSHI et al., 2017). Contrarily, LEROY et al. (2018) reported that primiparous cows inseminated within 0-8 hours after estrus identification had a 1.8-times more chance of pregnancy than that when AI was performed between 8-12 hours.

Parous have physiological differences and diverse nutrient requirements (INGVARTSEN & MOYES, 2013). Albeit multiparous higher experience negative energy balance than primiparous, they often have better-developed mammary glands and a more established metabolic adaptation to lactation (OIKONOMOU et al., 2013). In this study, adaptation to lactation may have been more difficult for primiparous to maintain the energy balance needed for successful reproduction, leading to a lower pregnancy rate.

Table 2 - Predicted pregnancy rate at 30 days after the first artificial insemination (AI) in primiparous (n = 393) and multiparous (n = 661) cows estimated by stepwise logistic regression model[1] evaluating AI timing by AAM according to the season and presence of peripartum diseases.

Effects	---Pregnancy rates (%)---								P-value[2]
Season	---------Spring---------		--------Summer--------		-----------Fall----------		--------Winter-------		< 0.0001
Hours after AAM alarm	8h	10h	8h	10h	8h	10h	8h	10h	0.0064
-----------PRIMIPAROUS-----------									
Healthy[2]	24.6	34.9	23.0	32.9	48.3	60.6	45.1	57.4	Reference[4]
Retained placenta	9.3	14.5	8.6	13.4	22.8	32.7	20.6	30.0	< 0.0001
Vaginal discharge[3]	10.0	15.5	9.3	14.3	24.2	34.3	21.9	31.5	< 0.0001
Peripartum disease	11.0	16.8	10.1	15.6	26.1	36.7	23.7	33.8	0.0004
-----------MULTIPAROUS-----------									
Healthy[3]	33.5	45.3	31.5	43.1	59.1	70.4	55.9	67.6	Reference[4]
Retained placenta	13.7	20.7	12.7	19.3	31.4	42.9	28.7	39.8	< 0.0001
Vaginal discharge4	14.7	22.0	13.6	20.5	32.9	44.6	30.2	41.4	< 0.0001
Peripartum disease	16.0	23.8	14.8	22.2	35.3	47.3	32.4	44.1	0.0004

[1]Model accuracy = 75.6 %.

[2]P-value refers to probability of each effect on the model (stepwise logistic regression).

[3]Cows with vaginal discharge score A, with no retained placenta or disease in the peripartum period.

[4]Vaginal discharge: B (little purulent material), C (mucopurulent), D (≥ 50 % pus), and E (fetid watery reddish-brown) scores according to SHELDON et al. (2006).

[5]95 % confidence interval (Retained placenta: 0.5–1.9; Vaginal discharge: 0.2–1.7; Peripartum disease: 0.2–1.9).

AI performed in fall (THI = 70.2±0.2) and winter (THI = 68.9±0.2) presented, respectively, 2.9 and 2.5 higher chance of being pregnant compared to spring (THI = 73.6±0.2). The different seasons of the year in which AI is performed influence animal thermal comfort, interfering with the conception rates of dairy cows (HOOPER et al., 2018). Heat stress causes reduced pregnancy rate by decreasing steroidal hormones impairing follicular and embryonic development; and reduces the estrus expression and duration; and decreased peak decreasing (SCHÜLLER et al., 2017; TIPPENHAUER et al., 2021). Therefore, data found in this research confirmed that animals inseminated with the highest THI, as occurred in spring (THI = 73.6±0.2) and summer (THI = 73.8 ± 0.2) have lower pregnancy rates.

Females with a retained placenta, the presence of some mucopurulent material in the vaginal discharge (Mucus B, C, D, and E) at 11±4 days postpartum, or with some disease presented lower pregnancy chances. MOHTASHAMIPOUR et al. (2020) stated that cows with postpartum health disorders have lower pregnancy rates and higher pregnancy loss rates after a fixed AI time because they have low progesterone (P_4) and high PGF concentrations compared to those that do not have any disorder. Decreased P_4 is related to the presence of cytokines secreted by the infected endometrium (OKUDA & SAKUMOTO, 2003), which activate the COX-2 enzyme and induce the specific production of prostaglandins, causing luteolysis (VAGNONI et al., 2001).

The parameter activity and estrus intensity presented by AAM positively influenced the pregnancy rate. Increased activity and estrus expression measures can be used to predict fertility at the time of AI and be used as a tool to assist breeding program decision-making strategies (MADUREIRA et al., 2019). Similarly, animals with high estrus intensity have a higher occurrence of AI pregnancies (BURNETT et al., 2018) with 1.35 times higher chance of becoming pregnant compared to those cows with a low score (TIPPENHAUER et al., 2021).

In this study, the optimal time for the first insemination postpartum was 10 hours after the automated activity monitoring system alarm. The reproductive efficiency of cows also differed between summer and winter, peripartum diseases, retained placenta, and postpartum vaginal discharge. Therefore, the optimization of automated activity monitoring models by including on-farm measures based on their impact on pregnancy rate may favor the correct identification of estrus and improve the AAM alarm to find the ideal moment for AI, further increasing reproductive performance in dairy cows.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors critically reviewed the manuscript and approved the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This is an observational study. The Instituto Federal Goiano Ethics Committee has confirmed that no ethical approval is required.

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