# Pregnancy and repeat breeding rate of high producing dairy cows for morning, evening or night artificial insemination

Taxa de prenhez e de repetição de estro em vacas leiteiras de elevada produção inseminadas pela manhã, tarde ou noite

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#### Abstract

The study aimed (1) to verify if artificial insemination (AI), performed in the morning, evening or nighttime has influence on the pregnancy rate on high production dairy cows, and (2) to determine the repeat breeding rate after the AI at intervals of 01-21, 22-43 and 44-65 days. We analyzed 1980 AI data from Holstein Friesian cows from years 2004 to 2008. The average milk production on this period was 39 kg/milk/day/ cow (305 days of lactation). The AI was performed 12 hours after the first detection of estrus. For the data analysis one AI was computed. The AI was done based on conventional method. The incidence of the AI in the morning, evening and night was 29.8; 50.4 and 19.8% respectively; and the pregnancy rate 45.7; 44.9 and 48.4%. The frequency of the repeat breeding rate at the interval of 1-21 days on morning, evening and night after failed AI was 28.0; 22.3 and 19.8% respectively; at the interval of 22-43 days was 52.0; 52.2 and 55.2%, respectively and at interval of 44-65 days was respectively 20.0; 25.5 and 25.0%. Comparing the three times of the day each other, the cows showed greater repeat breeding on 22-43 days (P<0.05) after the AI. It was concluded that times of the day has no influence on the on pregnancy rate; the highest repeat breeding rate took place between 22 and 43 days after AI, resulting significant delaying of the open period.

Keywords: Artificial insemination. Dairy cows. Pregnancy rate. Repeat breeding.

#### Resumo

O estudo objetivou (1) determinar se as inseminações artificiais (IA) executadas pela manhã, tarde ou noite influenciam a taxa de prenhez (TP) em vacas leiteiras de elevada produção, e (2) verificar a taxa de repetição de estro (TRE) pós IA, segundo os intervalos de 01-21, 22-43 e 44-65 dias. Foram analisados 1980 dados de IA (anos de 2004 a 2008) de vacas da raça Holandesa Preta e Branca, com produção média de 39 kg/leite/dia/vaca em 305 dias de lactação. Doze horas após a detecção visual do estro os animais eram submetidos à IA com sêmen congelado. A incidência de IAs executadas nos períodos da manhã, tarde e noite foi de 29,8; 50,4 e de 19,8% e a TP foi de 45,7; 44,9 e 48,4%, respectivamente. A TRE no intervalo de 01-21 dias foi de 28,0; 22,3 e 19,8% respectivamente para as IA feitas pela manhã, tarde e noite; no intervalo de 22-43 dias foi de 52,0; 52,2 e 55,2% e no intervalo de 44-65 dias após o insucesso na IA foi, respectivamente, de 20,0; 25,5 e 25,0% para manhã, tarde e noite. Comparando-se os três intervalos entre si, as vacas manifestaram mais elevada TRE no período de 22-43 dias (P<0,05) após as IA. Concluiu-se que o período do dia não interferiu significativamente sobre a TP; as mais elevadas TREs após as IAs ocorreram entre os dias 22 e 43, acarretando significativo alongamento do tempo de espera.

**Palavras-chave:** Inseminação artificial. Período do dia. Repetição de estro. Taxa de prenhez. Vacas leiteiras.

### Introduction

The artificial insemination (AI) has been established as one of the most important assisted reproductive technologies in the recent decades (BARUSELLI; GIMENES; SALES, 2007), a tool for genetic improvement of the livestock. According to Overton and Sischo (2005), significant improvements in the overall reproductive performance of the herd is achieved when the AI is associated to the use of high performance breeding animals. Several factors interfere on the best performance of this technique. For Moreira et al. (2000), the body condition score affects pregnancy rates increasing the cost per cow/year being a relevant factor to be analyzed on the use of AI.

One of the main difficulties for the success of AI is the estrus detection mainly by working on high production dairy cattle. Baruselli, Gimenes and Sales (2007) report that dairy cows show a short duration of estrus (30 minutes to 36 hours). The length and intensity of the estrus in dairy cattle is directly related to the animal category (heifer or cows) and the level of milk production. Some others variables can set influence on AI success like the postpartum loss of body condition (negative energy balance – NEB), uterine infections, poor uterine

involution along the puerperal time, the return to ovarian cyclicity, heat stress and the hormonal manipulation of the estrous cycle (SARTORI, 2007). The postpartum NEB affects the expression of the estrus manifestation (SARTORI; MOLLO, 2007). Cows in NEB increase the blood concentrations of non-esterified fatty acids while the concentration of the insulin-like growth factor (IGF-1), glucose and insulin decrease. Consequently, the changes in the serum levels of these metabolites compromise the ovarian function and fertility. NEB influences the postpartum ovulation and the more intense, the higher will be the delaying of the first postpartum ovulation. According to Wathes et al. (2007), the NEB affects the ovarian function and it is associated to metabolic disorders as well as produces effects on the estrous cycle, resulting in biochemical and morphological changes caused by alterations in the genes expression, which are involved in lipid and neo-glycogen catabolism, and synthesis and stability of IGF-1.

López-Gatius et al. (2005a) observed that the intensity of movement in the time of estrus displays a relationship with the animal fertility. Haugan et al. (2005) did map a seasonal peak for the performance of AIs. High temperatures, mainly in the summer, are adverse to the conception rate in cattle, evidencing modifications in the parameters of follicular development and hormone concentrations in the follicular fluid (LEW; MEIDAN; WOLFENSON, 2006). Putney, Drost and Thatcher (1989) evaluated the quality of embryos in cows subjected to heat stress, verifying that the embryo is sensitive to maternal thermal stress in the first seven days after estrus.

Dalton et al. (2001) checked the effect of the number of hours from onset of estrus to AI on fertility rates in dairy cows. They concluded that the AIs performed within 12 hours showed the best results. In another study, Foote (1979) reported that AI carried out in the morning, afternoon or evening did not differ on the pregnancy rate. Nebel, Walker and McGilliard (1994) stated that the rate of no return to estrus in cows were similar when the AIs were done in different times of the day, noting, however, that the AIs performed between 08:00 and 11:00 were more efficient.

The aim of the study was (1) verify if the artificial insemination performed in morning, evening or nighttime has influence on the pregnancy rate on high producing dairy cows and (2) verify in which period after the AI the repeat breeding rate is higher.

#### Material and methods

Data from 1980 artificial inseminations (AIs) performed in high producing dairy cows from Friesian Breed, 2 to 4 calvings, between 2004 and 2008 were evaluated. The animals were kept in a free stall system (walls free, natural ventilation). Corn silage, hay oat, concentrated and mineral salt ad libitum constituted the base of the feeding. The annual temperature was around 18 °C; latitude 25° 25' 40" S; longitude 49° 16' 23" W and altitude 934 m. The milk production was 39 kg/milk/day/ cow (305 lactation days) and body condition score 2.7 to 3.4 (1=thin; 5=obese). Data from AI were divided according to the time of the day: morning, evening and night. It was established the intervals of the repeat breeding from 01 to 21 days, 22 to 43 days and 44 to 65 days after the AI. It were used visual signs for observations of estrus and AI was done 12 hours after the first detection of estrus. Semen was thawed in water bath at 35 degrees for 30 seconds and the AI was carried out based on conventional method (*via transcervicalis*) placing the semen in the uterine body. Cows were not subjected to any hormonal treatment. Only cows clinically healthy were submitted to AI. The AIs were performed between 50 and 70 days post partum. Around 50 days after AI, the diagnosis of pregnancy was checked by ultrasonography (Aloka 500, 5MHz, Japan).

#### Statistical analysis

Data were submitted to ANOVA and Chi-square test to analyze the relations between the results of the AI on the morning, afternoon and evening, considering the pregnancy rates. The Fisher exact test was used to evaluate the estrus frequency distribution within the intervals (days) of the repeat breeding after the AI. The analysis was performed by the statistical Software GraphPad Prism version 3 for Windows, San Diego, California, USA. The significance level was considered on probability of 5%.

#### **Results and discussion**

The obtained results are expressed in Table 1.

**Table 1** – Incidence of artificial insemination (AI), pregnancy rates/time of the day and repeat breeding rate/intervals after AI failure at the range of 01-21, 22-42, 43-64 days in Holstein Friesian dairy cows (n=1980)

Time of the Day	AI incidence per time	Pregnancy rate/time	Repeat breeding Intervals after AI failures		
	(%)	(%)	01-21 (%)	22-43	44-65 days
Morning	29.8ª (590/1980)	45.7ª (270/590)	28.0ª	52.0 <sup>⊾</sup>	20.0 <sup>c</sup>
Evening	50.4 <sup>b</sup> (998/1980)	44.9ª (448/998)	22.3ª	52.2⁵	25.5ª
Night	19.8° (392/1980)	48.4ª (190/392)	19.8ª	55.2⁵	25.0ª

Note: Different letters in the same column indicate significance (P<0.05) for the variables: Al incidence per period of the day and pregnancy rates. Different letters on the same line indicate significance (P<0.05) for the variable intervals of repeat breeding after Als (days). Source: Research data.

Some factors may interfere on dairy cattle reproductive performance, emphasizing the poor body condition score (MOREIRA et al., 2000); uterine infections (GRUNERT; BIRGEL; VALE, 2005); poor uterine involution in puerperium (KOZICKI, 1998); failure in the ovarian cyclicity (GRUNERT; BIRGEL; VALE, 2005; LÓPEZ-GATIUS et al., 2005a); high milk production (LÓPEZ-GATIUS et al., 2005b); hormonal manipulations (SARTORI, 2007) and metabolic diseases (WATHES et al., 2007). Stressful high temperature (HAUGAN et al., 2005; PIRES et al., 2002; CHEBEL et al., 2004; DEMETRIO et al., 2007) changes the physiological parameters of follicular growth and hormone concentrations in follicular fluid (LEW; MEIDAN; WOLFENSON, 2006).

In the present study, it was observed no significant differences regarding the pregnancy rates between the AI performed in the morning, evening or night (Table 1). The pregnancy rate (one AI service considered) was 45.7, 44.9 and 48.4 in the morning, evening and at nighttime, respectively (P>0.05). The greatest number of AI were performed during the evening time (n=998) (P<0.05). Our pregnancy rate results are quite similar to those reported by Etherington, Kinsel and Marsh (1996), which found 46.7% of conception rate in herds producing 21.0 to 24.0 Kg/milk/day, but they did not discuss about time of the day of the AI.

Foote (1979), studying 44.707 dairy cows in an AI program (commercial herd), performed AIs in the morning (until 12:00 pm), afternoon (12:00 to 18:00) and after 18:00 h, also did not found differences in pregnancy rate. This fact corroborates the data of this this study, since no difference in pregnancy rate was detected in the AI made in different periods of the day, although they have got pregnancy rate higher (67.1 to 69.9%) than in the present study (44.9 to 48.4%). Probably the difference can be attributed to the use of unfrozen semen reported by Foote (1979). Another hypothesis is that the location of dairy farms are in a milder temperature than those of the present study. Nebel, Walker and McGilliard (1994) also found pregnancy rate similar in dairy cows in performing AI in different periods of the day (no significance), noting however that the AI in the morning (between 8:00 and 11:00) may have some advantage.

Causes of repeat breeding in dairy cattle have been the focus of many publications in recent years and pregnancy maintenance during embryonic phase and early fetal periods was discussed as a critical period by López-Gatius (2012). Our data showed a repeat breeding after the AI failure in all the intervals of this study, however, it was notably higher in the period of 22-43 days (52.0% in the morning to 55.2% at night), than 01-21 and 44-65 days (P<0.05) reaching almost the double (Table 1). According to Grunert, Birgel and Vale (2005), there are many factors that can cause repeat breeding in cows such an unfavorable uterine environment, infections, delayed ovulation, ovarian cysts, problems at oviducts, aged gametes, embryonic mortality and failure of ovulation (LÓPEZ-GATIUS et al., 2005a) among others. The embryo mortality can happen in the first three weeks of gestation, causing estrus return before or more frequently after the third week. Vandeplassche (1988) and López-Gatius (2012) states that high milk production and climatic factors (high environmental temperature) are among the main causes of embryonic mortality in cattle, making irregular repeat of estrus after the 3rd week.

Significant embryonic losses occur between 8-17 days of pregnancy (PETERS, 1996; HANZEN et al., 1999; INSKEEP; DAILEY, 2005). However, it appears that there are a growing number of pregnancy losses of non-infectious origin during the late embryonic period (LÓPEZ-GATIUS, 2012), especially in systems with high milk production (CARTMILL et al., 2001a; CARTMILL et al., 2001b; GRIMARD et al., 2006). López-Gatius et al. (1996) report losses in dairy cows with previous postpartum disorders, warm temperatures (LÓPEZ-GATIUS et al., 2004; GARCÍA-ISPIERTO et al., 2006; GARCÍA-ISPIERTO et al., 2007; SANTOLARIA et al., 2010) and bull among others (LÓPEZ-GATIUS, 2012). Losses in the late embryonic/early fetal period reach just before day 50 of gestation because the placenta is not yet fully consolidated (SANTOS et al., 2004). It was just at this phase that the cows of the present study had the greatest embryo/fetal losses following repeat breeding. We sustain that caution should be undertaken in the first 50 days after AI to minimize the losses on this critical period of gestation.

# Conclusions

This study concluded that the AIs performed in the morning, evening or nighttime did not interfere significantly on the pregnancy rates; high percentage of the repeat breeding occurs between 22 to 43 days after AI, leading a significant delaying of the estrous cycle, mainly due the embryonic mortality in the first three weeks of pregnancy.

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