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Conception rate of artificially inseminated Holstein cows affected by cloudy vaginal mucus, under intense heat conditions

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Abstract – The objective of this work was to obtain prevalence estimates of cloudy vaginal mucus in artificially inseminated Holstein cows raised under intense heat, in order to assess the effect of meteorological conditions on its occurrence during estrus and to determine its effect on conception rate. In a first study, an association was established between the occurrence of cloudy vaginal mucus during estrus and the conception rate of inseminated cows (18,620 services), raised under intense heat (mean annual temperature of 22°C), at highly technified farms, in the arid region of northern Mexico. In a second study, data from these large dairy operations were used to assess the effect of meteorological conditions throughout the year on the occurrence of cloudy vaginal mucus during artificial insemination (76,899 estruses). The overall rate of estruses with cloudy vaginal mucus was 21.4% (16,470/76,899; 95% confidence interval = 21.1–21.7%). The conception rate of cows with clean vaginal mucus was higher than that of cows with abnormal mucus (30.6 vs. 22%). Prevalence of estruses with cloudy vaginal mucus was strongly dependent on high ambient temperature and markedly higher in May and June. Acceptable conception rates in high milk-yielding Holstein cows can only be obtained with cows showing clear and translucid mucus at artificial insemination.

Index terms: dairy cattle, heat stress, reproductive efficiency, seasonal pattern, subclinical endometritis, uterine infection.

Taxa de concepção de vacas Holandesas inseminadas artificialmente e afetadas por muco vaginal turvo, sob condições de intenso calor

Resumo – O objetivo deste trabalho foi obter estimativas de prevalência de muco vaginal turvo durante o estro em vacas Holandesas criadas sob intenso calor, para avaliar o efeito das condições climáticas sobre sua ocorrência durante o estro e determinar seu efeito sobre a taxa de concepção. Em um primeiro estudo, foi estabelecida a associação entre a ocorrência de muco vaginal turvo durante o estro e a taxa de concepção de vacas inseminadas (18.620 serviços), criadas sob intenso calor (temperatura média anual de 22°C), em fazendas altamente tecnificadas, na região árida do norte do México. Em um segundo estudo, os dados desses grandes estábulos leiteiros foram utilizados para avaliar o efeito das condições climáticas, ao longo do ano, na ocorrência de muco vaginal turvo durante a inseminação artificial (76.899 cios). A taxa global de cios com muco vaginal turvo foi de 21,4% (16.470/76.899; intervalo de confiança de 95% = 21,1–21,7%). A taxa de concepção das vacas com muco vaginal límpido foi maior que a de vacas com muco anormal (30,6 vs. 22%). A prevalência de estros com muco vaginal turvo foi fortemente dependente de elevada temperatura ambiente, e significativamente maior em maio e junho. Taxas de concepção aceitáveis de vacas Holandesas com elevada produção de leite somente são obtidas em vacas que apresentem muco translúcido durante a inseminação artificial.

Termos para indexação: gado leiteiro, estresse por calor, eficiência reprodutiva, padrão sazonal, endometrite subclínica, infecção uterina.

Introduction

Microbial invasion of the uterine lumen has great impact on dairy cattle health and productivity (Amiridis et al., 2003; Sheldon, 2004). Such a condition may cause cervicitis or endometritis, which, in turn, may lead to embryonic losses (Vanroose et al., 2000), reduced pregnancy rate (Gautam et al., 2009), or impairment of sperm transport (Rutllant et al., 2005).

The natural course of uterine repair (involution), with all the physiological mechanisms associated to it, is typically very effective in decreasing the population

of bacteria and the inflammation in the uterus, mostly due to the innate immune system (Sheldon & Dobson, 2004). Therefore, the uterus is commonly free of infection by the time of insemination (Gautam et al., 2010). However, these infections may persist in some cows several weeks postpartum (LeBlanc et al., 2011). In fact, some kind of uterine infection is still present in up to 40% of cows three weeks after calving (Gilbert et al., 2005; Sheldon et al., 2008). Endometritis occurs when uterine infection persists beyond four weeks postcalving, with prevalence of polymorphonuclear cells higher than 6% and mucopurulent vaginal discharge (Dubuc et al., 2010). The prevalence of this condition in dairy cows ranges from 12% (Barlund et al., 2008) to more than 50% (Hammon et al., 2006; Galvão et al., 2009).

Subclinical endometritis has been identified as a disease that reduces reproductive performance of cows and herds (Sheldon et al., 2006; Barlund et al., 2008; Salasel et al., 2010), but some authors have not found negative effects of this uterine disease on reproductive performance (Kasimanickam et al., 2005; Plöntzke et al., 2010). In this "silent" disease (Gilbert et al., 2005), flakes of exudate in otherwise clear estrous mucus are observed. There is no general consensus among researchers and technicians, in charge of artificial insemination (AI) in dairy farms, about the convenience of inseminating cows with cloudy estrous vaginal discharge. This is particularly true in large commercial dairy operations, where cytological examination of the uterus to diagnose uterine or cervical inflammation is costly and time consuming.

The incidence of subclinical endometritis has not been extensively studied at herd level, especially in environments with intense heat. In addition, the impact of this reproductive disorder on conception rates in large dairy operations has not been thoroughly researched in regions of intense heat during the most part of the year. The effect of high ambient temperatures on the occurrence of cloudy estrous vaginal discharges after 50 days postpartum is also poorly studied.

The objective of this work was to obtain prevalence estimates of cloudy vaginal mucus in artificially inseminated Holstein cows raised under intense heat, in order to assess the effect of meteorological conditions on its occurrence during estrus and to determine its effect on conception rate.

Materials and Methods

This study followed institutional guidelines approved by the Animal Care Committee of Universidad Autónoma Agraria Antonio Narro, in Saltillo, Mexico. The study was carried out in six large highly-technified dairy farms, in northern Mexico (25.0–25.8°N, 99.6–103.4W, at 1,120 m above sea level), with mean annual temperature of 22°C and mean annual precipitation of 230 mm. The farms were located close to each other, in a radius of 30 km. Herd size ranged from 1,200 to 4,000 lactating Holstein-Friesian cows.

The cows were housed in open-lot, dirt-floor pens with ample shade structures in each pen, which had a centralized feed alley. In all farms, cows were fed diets very similar in nutrient content, with approximately 50% concentrate and 50% forage, formulated to provide nutrients for maximum milk yield. The rations consisted of corn silage and alfalfa hay, as roughage components, supplemented with concentrate feed (corn grain and soybean meal) to fulfill energy and protein requirements (National Research Council, 2001). Concentrates were supplied according to production level. Cows were fed and milked thrice a day, and the rolling-herd average ranged from 10,600 to 11,900 kg per cow per year.

The herds were vaccinated against diseases that interfere with reproductive functions, such as infectious bovine rhinotracheitis, bovine viral diarrhea, bovine respiratory syncytial virus, parainfluenza, and leptospirosis (5-varieties). They were also vaccinated against brucellosis and were annually tested for bovine tuberculosis. Cows were weekly examined for postpartum reproductive disorders, such as retained placenta, metritis, and endometritis. Only cows without postpartum reproductive disorders and with body condition score higher than 2.5 were included in the study. Cytology of the uterine lumen was not carried out to assess subclinical endometritis, nor was endocervical inflammation assessed; however, during rectal palpation, any increase in size and thickness of the uterine wall was recorded.

The voluntary waiting period was 50 days postpartum, regardless of peak milk production, after which cows were subjected to AI when in estrus. Estrus was assessed twice daily by visual observation, and inseminations were carried out using the a.m/p.m rule. Timed breeding protocols, such as Ovsynch and timed AI, were occasionally performed. Service sires were chosen by

the herd manager as part of the routine reproduction and genetic program. Pregnancy was diagnosed by palpation per rectum, between 42 and 50 days after AI. When nonpregnant, cows were re-inseminated.

The data were screened to include only cows with at least one service, in lactation, and with an interval from first service greater than 45 days and less than 150 days. The conception rate was defined as the number of cows that conceived, out of the number of cows detected in estrus and inseminated during the first 200 days of lactation. This wide breeding window was used because prolonged lactations (>15 months) were common in these dairy operations.

Maximum daily ambient temperature and humidity, on the day of insemination, were obtained from a weather station located approximately within 20 km from the dairy farms. This information was used to calculate the temperature-humidity index (THI) for each day, according to the following equation (Mader et al., 2006): THI = 0.8 × temperature + RH/100 × (temperature - 14.4) + 46.4, in which RH is the relative humidity (%).

Two studies were carried out from January 2012 to December 2013. In the first study, 1,976 estruses from primiparous lactating Holstein cows and 16,644 from multiparous ones, from a single large dairy operation, were considered (n=18,620). Cows were divided into two groups: control (n=13,706), whose estruses were accompanied by clear and translucent vaginal mucus, with healthy viscosity and without bad odor; and a group (n=4,914) whose estruses were accompanied by atypical vaginal mucus, with the presence of slight cloudiness to opaque mucus, containing gray flecks. Several technicians were involved in AI in the evaluated farms, and all of them received training to detect cloudy vaginal mucus. Observation of mucus was made during spontaneous discharges at estrus or by the scrutiny of the mucus adhered to the insemination pipette after AI.

In the second study, the occurrence of cloudy vaginal mucus at AI was recorded from January 2012 to December 2013, in primiparous and multiparous lactating cows from all the six large commercial dairy operations studied (n=62,075 estruses). Seasonal occurrence of cloudy vaginal mucus at AI was evaluated, and the association between the occurrence of estruses with cloudy vaginal mucus and meteorological conditions was determined.

Conception rate was analyzed using the Proc GenMod of SAS, version 9.4 (SAS Institute, Cary, NC, USA). The model included group (estruses with cloudy vs. translucent vaginal mucus), parity (first vs. multiple), and number of services postpartum (first vs. two or more) as explanatory variables, as well as the interactions between group and parity, and between group and service number. Additional statistical analyses were performed with the Proc GenMod of SAS, version 9.4 (SAS Institute, Cary, NC, USA) to determine the effects of month and THI at breeding on the occurrence of estruses with cloudy vaginal mucus (yes/no). The covariables considered included the year of breeding and the farm. Monthly means were compared using the probability of a statistical difference (PDIFF option of SAS). Statistical differences were considered significant at 5% probability.

Parity was categorized into less than two, and two or more calvings. Number of services was divided into two classes: lesser or greater than two services. THI was grouped into six categories: <65, 65–70, 70–75, 75–80, 80–85, and >85 units. Linear regression and Pearson's correlation were used to determine the association between THI and the occurrence of estruses with cloudy vaginal mucus discharges (yes/no) during AI.

Results and Discussion

The overall prevalence of cloudy vaginal mucus at AI on cows from the six commercial dairy farms evaluated was 21.4% (16,470/76,899; 95% confidence interval = 21.1–21.7%). Conception rate was affected ($p<0.01$) by the presence of cloudy mucus, being 8.6% lower at this circumstance, regardless of parity (Table 1). Regardless of the number of services, cows showing clear vaginal mucus at AI had greater

Table 1. Conception rate (%) of high milk-yielding Holstein cows, categorized by parity and the occurrence of estruses with cloudy vaginal mucus during artificial insemination[(1)].

Vaginal mucus	Parity category		Total
	Primiparous	Multiparous	
Clear	32.5 (358/1,103)	30.5 (3,838/12,603)	30.6 (4,196/13,706)
Cloudy	23.7 (207/873)	21.6 (873/4,041)	22.0 (1,080/4,914)
Significance (p)	<0.001	<0.001	<0.001

[(1)]There was no significant interaction between factors, and parity category did not differ at 5% probability.

(p<0.01) conception rate (Table 2). The interactions group x parity and group x number of services were not significant.

Furthermore, the occurrence of estruses accompanied by cloudy vaginal mucus was highest (p<0.01) in the warmest period of the year, i.e., in May and June, and decreased in months with less intense heat, with prevalence values similar to those of the other months (Figure 1). Seasonal high THI was linearly associated with high occurrence of estruses with cloudy vaginal mucus after 50 days postpartum (Figure 2).

Since only characteristics of the vaginal discharges were evaluated, there was not enough evidence linking cloudy vaginal mucus to endometritis. However, opaque vaginal discharges correspond to grade 1 for clinical endometritis (Sheldon et al., 2009), and large number of leucocytes and pathogenic bacteria are associated with change in the appearance of cervicovaginal mucus (Williams et al., 2005). Dubuc et al. (2010) indicate that purulent vaginal discharge may be more descriptive of clinical endometritis than cytological (cytobrush) diagnostic criteria. In fact, these authors claim that cytological and clinical findings for diagnosis of endometritis may reflect different conditions.

The prevalence of cloudy vaginal mucus at AI (21.4%) was very close to that found for uterine infections based on endometrial cytology, 4 to 8 weeks postpartum (Potter et al., 2010; Cheong et al., 2011; Baranski et al., 2012; Lima et al., 2013). The prevalence of estruses with cloudy vaginal mucus in the present study was lower than some of the incidence rates previously reported for uterine infections – cytologically-diagnosed endometritis or vaginal purulent discharges (LeBlanc et al., 2002; Gilbert et al., 2005; Hendricks et al., 2006) – and higher than others observed by clinical examination and uterine cytology (Knutti et al., 2000; Green et al., 2011).

The prevalence of estruses accompanied by cloudy vaginal mucus did not differ between cows with one or multiple lactations, which is not totally in line with data of Cheong et al. (2011), who found that primiparous cows producing more milk were at higher risk of subclinical endometritis, whereas multiparous cows producing more milk had lower risk of this reproductive disorder. Potter et al. (2010) also found that primiparous cows had higher risk of presenting clinical endometritis. Galvão et al. (2010), however, detected that the prevalence of subclinical endometritis tended to be greater in multiparous cows compared

Table 2. Conception rate of high milk-yielding Holstein cows, categorized by number of services, and occurrence of estruses with cloudy vaginal mucus during artificial insemination[1].

Vaginal mucus	Number of services		Total
	First service	Two or more services	
Clear	31.9 (1,565/4,905)	29.9 (2,631/8,801)	30.6 (4,196/13,706)
Cloudy	22.3 (181/813)	21.9 (899/4,101)	22.0 (1,080/4,914)
Significance (p)	<0.001	<0.001	<0.001

[1]There was no significant interaction between factors, and the number of services category did not differ at 5% probability.

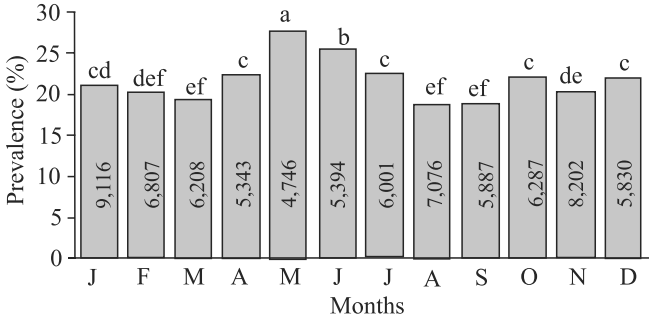

Figure 1. Monthly occurrence of estruses with cloudy vaginal mucus during artificial insemination, in high milk-yielding Holstein cow farms, in a region with hot desert climate (25°N). Numbers inside the bars are monthly observations. Bars followed by equal letters do not differ at 5% probability.

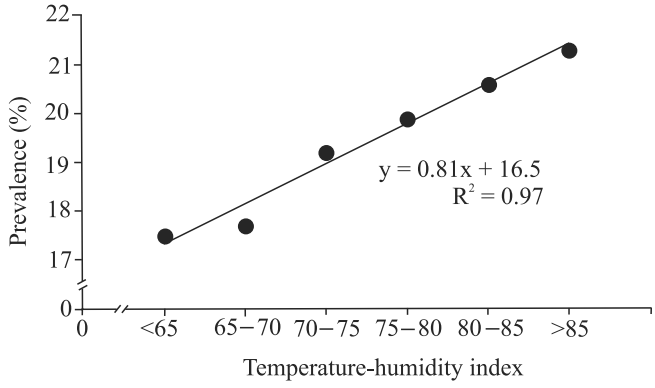

Figure 2. Association between temperature-humidity index at the day of calving and the occurrence of estruses with cloudy vaginal mucus at artificial insemination, in high milk-yielding Holstein cow farms, in a region with hot desert climate (25°N).

with primiparous cows, with similar values to the ones reported here (19 to 38%).

In the present study, prevalence of estruses accompanied by cloudy vaginal mucus was similar between cows at the first service and cows with repeated services. It has been stated that most cows with puerperal purulent discharges are able to get rid of it within a few weeks postpartum, particularly when these cows return to estrus, which helps to flow out the infections from the uterus (McDougall et al., 2007; Gautam et al., 2010, Green et al., 2011). Moreover, the endometrium has an important role in innate immunity, which allows clearance of bacterial infection and epithelial repair, after which the inflammatory response is reduced or switched off (Williams, 2013). Therefore, it is evident that repeated estrus cycles not always lead to the total recovery of cows with cloudy vaginal mucus, and that uterine alterations causing opaque vaginal mucus can often go undiagnosed and may develop into a chronic condition with few outward signs (Gautam et al., 2010; Lima et al., 2013).

The present study is the largest observational investigation to specifically evaluate the effect of cloudy vaginal mucus during AI on the fertility of dairy cows. All cows enrolled in the study did not present thick uterine wall, with doughy feel on rectal examination. Therefore, it is believed that the turbidity or little white flakes in their estrous mucus was the result of a mild chronic uterine contamination. Endometritis is a rather common reproductive disorder in dairy cows, which reduces pregnancy rates (Amiridis et al., 2003; Hammon et al., 2006; Lima et al., 2013). The marked decrease on conception rate reported here confirms these observations. This reduction in the reproductive efficiency of cows is associated with uterine tissue damage, delayed uterine involution (Sheldon et al., 2003; Shrestha et al., 2004), disruption of endometrial function, reduction of steroid concentrations of ovarian follicles (Green et al., 2011), and perturbation of ovarian cycles (Opsomer et al., 2000; Sheldon et al., 2002; Herath et al., 2009).

The fact that cows showing cloudy vaginal mucus at AI had their conception rate reduced, both with one, or with two or more services, is explained by the clinical endometritis persistence beyond 60 days post-partum in 25% of cows that previously had purulent vaginal discharge, and in around 10% of cows previously found to be healthy (Gautam et al., 2010).

The detection of a clear seasonal pattern for the occurrence of estruses with cloudy vaginal mucus agrees with observations of Reyes & Mellado (1994) and Gautam et al. (2009), who found higher rates of uterine infections with purulent cervicovaginal discharge in summer. However, Erb & Martin (1980) registered the peak incidence of this disorder during fall-winter. Contrarily, studies in colder areas have not shown a clear pattern of association of uterine infections along the season (Bartlett et al., 1986).

The mechanisms responsible for seasonal disease incidence are poorly understood. In the present study, the high ambient temperatures possibly enhanced the growth of opportunistic and pathogenic bacteria (Tummaruk et al., 2010), which resulted in a greater survival of pathogens outside the cows (Gautam et al., 2011). Another possibility is a reduction in host immune competence due to severe heat stress during summer in this particular environment (Do Amaral et al., 2011; Tao et al., 2012). The latter seems to be supported by the fact that prevalence of estruses with cloudy vaginal mucus increased linearly with increasing THI at the day of AI. This finding is important for designing programs to prevent or decrease uterine infections when the hot weather can exert severe stress in Holstein cows. The obtained results also indicate that the increment in the prevalence of cloudy vaginal mucus during AI in high milk-yielding Holstein cows is one additional factor that causes the drastic reduction in pregnancy rate reported by Mellado et al. (2013) for cows subjected to high heat load.

Conclusions

1. Acceptable conception rates in high milk-yielding Holstein cows can only be obtained with cows showing clear and translucid mucus at artificial insemination.

2. Several estrus cycles may occur before the uterus clears the persistent cloudy vaginal mucus.

3. Cloudy vaginal mucus discharges have higher incidence during summer, under intense-heat rearing conditions.

4. Dairy farms may benefit from augmenting efforts to detect and treat uterine infections during the warm season of the year, by implementing stricter uterus health control programs, reducing heat stress via ration balancing, and providing extra air movement in the stables.

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