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Research Article

Prevalence of subclinical endometritis in two dairy farms in Iran and consequences for further fertility

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Abstract

A high level of reproductive efficiency requires each cow to be bred successfully and calve with a calving interval that maximizes the output of milk within the herd. Subclinical endometritis (SCE) has been defined as the presence of an elevated number of polymorphonuclear cells (PMNs) within the uterine lumen, without any clinical signs. This condition is relatively prevalent in postpartum dairy cows and results in substantial economic losses due to decreases in fertility and on longer-term milk production. In literature, different prevalence numbers have been described for this postpartum condition, although it is currently not clear what the prevalence of this condition is in modern dairy herds in Iran. Therefore, Holstein cows (N=150) from 2 commercial dairy herds that had calved without any difficulty and had passed a normal puerperal period, were sampled at 30±3 days post partum by the low-volume uterine lavage method. Samples were centrifuged at 700 *g* for 5 minutes and one drop of sediment was subsequently streaked on a glass slide. Samples were stained with Diff-Quick so that present cells could be counted and percentages of PMNs calculated. In the present study, a threshold of 18% was used to diagnose the disease. Due to low sample quality, 42 cows were excluded from the study. Also 4 cows were culled before the end of the study. Mean prevalence of subclinical endometritis at day 30 was 38.5%, while in farm 1 this prevalence was 27% and in farm 2, 47%. Cows, in which SCE was diagnosed, showed an increase in calving to first service (73 vs. 66 days; P=0.097) and calving to conception interval (118 vs. 105 days; P=0.3 (positive vs. negative, respectively). In conclusion, the results of the current study show a high prevalence of uterine inflammation in clinically normal cows in two dairy herds in Iran. Negative impacts on fertility were demonstrated by the increase in the time to first service and the time to conception.

Keywords: Subclinical endometritis; uterine inflammation; dairy cow

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Introduction

In the current intensive dairy industry, cows should be ready to be inseminated by the end of the voluntary waiting period (VWP) and get pregnant soon after inseminations have been started. Many factors however delay the interval calving-conception, among which a local inflammation of the endometrium, currently defined as subclinical endometritis (SCE), seems to become more and more important. Subclinical endometritis is based on the concept that cows can have uterine disease without any observable signs at clinical examination. It is defined as the presence of uterine inflammation, having a negative impact on reproductive performance, in cows unaffected by clinical endometritis (Kasimanickam et al., 2004). Subclinical endometritis has been also defined as the presence of an elevated number of inflammatory cells mostly polymorphonuclear cells (PMNs) within the uterine lumen, but without signs of clinical endometritis (Földi et al., 2006; Sheldon et al., 2006a; Chapwanya, 2008). According to Sheldon et al. (2006a) diagnose should be made >26 days postpartum in order to avoid confusion with normal uterine involution. Furthermore, Gilbert et al. (2005) found that the proportion of cows with uterine inflammation decreases with time, from 100% at 2 weeks postpartum to 89, 58, and 41% at 4, 6, and 8 weeks postpartum, respectively.

Understanding of uterine diseases improved in the last 10 years by the development of novel diagnostic techniques. Among these, endometrial cytology and determination of the relative number of PMNs has been shown to be of extreme value. Samples can be taken by a brushing device, a low-volume lavage, or a biopsy (Bonnett et al., 1991; Gilbert et al., 1998; Kasimanickam et al., 2004). Endometrial cytology has been used as a diagnostic tool in horses since long time, but Kasimanickam et al. (2004) and Barlund et al. (2008) used a modified cytobrush to collect endometrial cytology samples in cows. Furthermore, low volume uterine lavage (20 ml of sterile sodium chloride 0.9% or PBS) has been also described as a tool for cytological examination of the uterus in dairy cows (Gilbert et al., 1998; Barlund et al., 2008). Barlund et al. (2008) reported that lavage cytology and cytobrush cytology were essentially the same tests. However, endometrial biopsy is currently rarely used in practice because it is considered time consuming and potentially harmful to subsequent fertility and it can't be done as a cow side test (Etherington et al., 1988; Sheldon et al., 2006b). Diagnosis of SCE based on endometrial cytology is currently considered as the gold standard technique (Kasimanickam et al., 2004; Gilbert et al., 2005; Barlund et al., 2008). Recent data furthermore suggest that concomitant cervical sampling may be useful (Ahmadi et al., 2005& 2006).

Diagnosis of SCE is based on evaluation of the cytological smear and calculation of the ratio of PMNs to the sum of endometrial epithelial cell plus PMNs (PMNs/ PMNs + endometrial cells). Various threshold proportions of PMNs have been reported mainly in relation to the time post partum samples are taken. Although thresholds vary between different authors, there is a general agreement that earlier in the postpartum period greater proportions of PMNs are indicative for disease. Furthermore, in all studies, cows with a high percentage of PMNs experienced more problems to conceive than those with low PMN numbers (McDougall, 2001a; Gilbert et al., 2005; Barlund et al., 2008). Kasimanickam et al. (2004) defined SCE by cytobrush derived cytology sample containing >18% PMNs at 21-33 days postpartum, or >10% PMNs at 34 and 47 days postpartum. Gilbert et al. (2005) characterized SCE by >5% PMNs visible on a cytosmear obtained by low volume lavage at 40 to 60 days postpartum. However, other studies found other thresholds (Hammon et al., 2006; Barlund et al., 2008; Kaufmann et al., 2009), mainly due to the evaluation of different cows population, regardless of their clinical endometritis status.

Currently, there are no data available concerning the prevalence of SCE and its effects on further fertility on dairy herds in Iran. Therefore, it was the aim of the present study to harvest data in this respect based on a field trial carried out on two representative herds.

Materials and Methods

The present study was conducted from September 2012 until June 2013, on two commercial dairy herds (Herd 1: 2,100 dairy cows; Herd 2: 800 dairy cows) located in the Tehran Province, Iran (35°39′35″N 51°03′33″E and 35°56′13″N 50°38′38″E respectively).

Total 150 cows (Holstein Friesian) (N=97 in second lactation, N=53 in third and fourth lactation) were enrolled in the study. Cows were selected based on absence of calving difficulties and puerperal disorders. Moreover, no ecbolic drugs or hormones had been administered to the cows since calving. Cows from both herds were milked 3 times daily and herds' average 305d milk production was 10,223 and 9,562 kg for herd 1 and 2 respectively.

At 30 DIM, a low-volume (30 ml) uterine lavage as described by Gilbert et al. (2005) and Kasimanickam et al. (2005a) was performed to harvest samples for endometrial cytology.

Briefly, 30 ml of a sterile 0.9% saline solution (Daropakhsh Co. Tehran, Iran) was infused into the uterus using a sterile, 18 Fr Foley catheter (Rusch Co., Waiblingen, Germany), 30cc balloon and 40 cm long with protection sheet. To minimize contamination,

the vulva and perineum were thoroughly cleansed with water. Subsequently, the catheter supported by a stain steel stylet and protected by an external sheet was introduced into the vagina and further manipulated through the cervix. Once the catheter had passed the cervix and was placed in the body of the uterus, the balloon was inflated. After catheter fixation, 30 ml of a sterile 0.9% saline solution (35-40°C) was infused into the uterus using a 60 ml syringe. Subsequently, the uterus was gently massaged for about 10 seconds, whereupon some of the infused fluid was harvested in a 15 ml falcon tube by means of gravity. The samples were placed in a cooled box and transported to the laboratory within 4 hours.

In the laboratory, samples were centrifuged for 5 min at 700 *g*, after which a drop of the sediment was placed on a glass slide and streaked with the tip of sampler onto a glass slide. Air-dried cytology smears were stained with the modified Wright-Giemsa dye (Diff-Quick; Dade Behring, Dugen, Switzerland) (Kasimanickam et al., 2005a). All slides were examined using light microscopy at magnification 400X and 1,000X to identify individual cell types, including endometrial epithelial cells and PMNs (Gilbert et al., 2005). PMN cell counts were expressed as the proportion of PMNs counted out of the combined number of PMNs plus epithelial cells. Per slide, a total of 300 cells were counted.

At the end of the voluntary waiting period (60 and 55 DIM in farm 1 and 2 respectively), cows were inseminated based on expressed heat signs making use of the am-pm rule. Expert resident technicians in each farm performed all inseminations. Reproductive data of all cows were collected until at least 200 days after parturition or until the date of culling if earlier. Pregnancy diagnosis was performed by transrectal ultrasound examination from day 35 after insemination.

Individual cow data were exported from the farming software program to Microsoft Excel (Microsoft Corporation, Seattle, Washington, USA). All statistical analyses were performed using the SAS software package (Statistical Analyses System Institute, Inc., Cary, NC, USA, 2010). Descriptive statistics were done using PROC MEANS and PROC FREQ of SAS (SAS Institute, Inc., Cary, NC, USA, 2010). The distribution of all variables was checked to approximate the normal Gaussian distribution. To evaluate the days in milk to first AI or conception, a Kaplan-Meier survival graph was constructed using the LIFETEST procedure in SAS version 9.2 for Windows (SAS Institute, Inc., Cary, NC, USA, 2010). To test equality over MD strata, the Peto and Wilcoxon test was used to evaluate difference in the beginning of the survival curves, whereas the log-rank test was used to evaluate difference in the tail of the curves (Hosmer and Lemeshow, 2008). Survival rates are visualized with their 95% Hall-Wellner confidence interval for the Kaplan-Meierestimates.

Results

Of the 150 cows that were originally included in the study, 4 were culled before first insemination due to problems other than reproductive disorders. Also, data of 42 (28%) cows were excluded due to insufficient quality of the cytology slides (Table 1).

Prevalence of SCE at day 30 post partum was 38.5% using the threshold of 18%, showing about 27% and 47% prevalence in farm 1and 2 respectively (Table 2).

Mean calving to 1st service and calving to conception interval for SCE positive and negative cows are 73 vs. 66 and 118 vs. 105 days respectively (Table 3, Fig. 1&2). Hence, SCE increases both intervals although not significantly (Test of equality: Log-Rank P=0.13 and 0.27; Wilcoxon P=0.13 and 0.27 for DIM first service and DIM conception respectively).

Discussion

The results of the present study show a mean prevalence of 38.5% of cows suffering from SCE at day 30 post partum, when a threshold of 18% of PMNs was applied, which is in the range of prevalences reported in other studies (Kasimanickam et al., 2004; Gilbert et al., 2005; Barlund et al., 2008). The chosen threshold of 18% PMNs per 300 counted cells was according to Barański et al. (2012).

Several factors are known to affect the prevalence of SCE, for instance the time of sampling relative to calving and the applied threshold to define SCE. It has been shown that by increasing DIM, the inflammatory reaction of the endometrium decreases (Gilbert et al., 2005), which causes that different thresholds have been proposed for these different time points (Kaufmann et al., 2009; Salasel et al., 2010). It has been shown that the cytological threshold that has been used to diagnose SCE is a critical factor to determine the percentage of affected animals in a herd (Barański et al., 2012). Another factor that might affect the concentration of PMNs in the uterus is the sampling time during the oestrous cycle. Subandrio et al. (2000) postulated that the number of intrauterine PMNs elevate near the time of oestrus. They concluded therefore that the threshold used to diagnose SCE should vary according to the time during the cycle the sample is taken. However, in contrast to Madoz et al. (2013) in grazing dairy cows the percentage of PMNs does not vary in different stages of the oestrous cycle. Further studies based on a larger number of animals are needed to clarify this.

Data of 42 (28%) samples were excluded for further analysis because of the poor visibility. The bad

Table 1: Polymorphonuclear cell counts (in %) in two dairy farms in Iran.

PMN%	Mean	Median	Min	Max
Farm 1 (n=47)	16	10	0	70
Farm 2 (n=57)	25	15	0	70

Table 2: Prevalence of SCE at 30 DIM (18% PMNs as threshold)

	Negative	Positive	Total	Prevalence
Farm 1	34	13	47	27.6%
Farm 2	30	27	57	47.3%

Table 3: Mean and median calving to first service in each farm according to the SCE status

SCE	Positive (n=40)		Negative (n=64)	
Calving to 1st Service	Mean	Median	Mean	Median
Farm 1 (n=47)	61.0	59.0	61.11	61.0
Farm 2 (n=57)	80.03	76.0	72.02	65.5

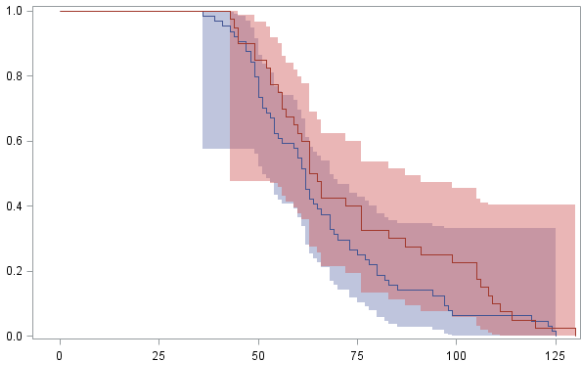

Fig. 1: Survival curves with 95 % Hall-Wellner confidence bands for days to first insemination stratified by subclinical endometritis status (blue=negative, red =positive)

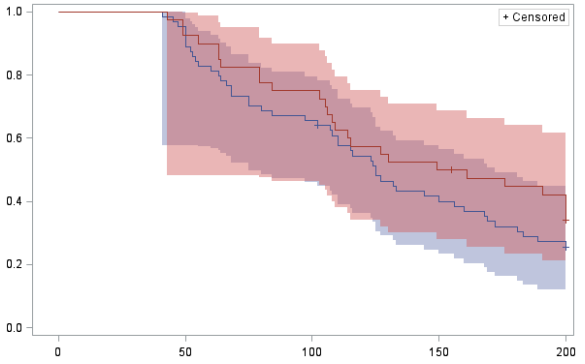

Fig. 2: Survival curves with 95 % Hall-Wellner confidence bands for days to conception stratified by subclinical endometritis status (blue=negative, red=positive). All cows were sensored on 200 days in milk

quality cannot be attributed to the excessive presence of mucus and debris in the smear, which made it difficult to visualize the cells present in the slide, and therefore significantly impaired correct interpretation. Mucus is usually present on cytological smears as relatively thin streaks that stain light blue (LeBlanc, 2011). Chenong et al. (2011) proposed that mucus resulted in a poor adhesion of cells to the slides resulting in an insufficient number of cells to accurately diagnose SCE.

In the present study, cytological samples were taken at 30 days post partum when cows were examined to control the uterine involution process and the presence of puerperal diseases. Samples were furthermore only taken from cows that were diagnosed to be clinically normal and having a clean uterus based on rectal palpation and ultrasound examination, and no purulent vaginal discharge when examined by Metricheck®. Also, sampled cows had not experienced any calving difficulty or any puerperal disorder and had not been treated with any drugs that potentially could influence further fertility. Salasel et al. (2010) concluded that elevated numbers of PMNs in uterine low volume lavage samples during late postpartum (190 ± 40 DIM), are mainly associated with dystocia, retained foetal membranes and postpartum uterine infection. However, in the current study, as previously mentioned, samples were only taken from cows that had not experienced any of these conditions. Therefore other risk factors might also exist. According to Cheong et al. (2011) the most significant cow-level risk factors for SCE are acute metritis, ketosis and the level of milk production interacting with parity. Moreover, SCE is also known as a management related condition (Gilbert et al., 2005; Hammon et al., 2006). In the present study, however, the prevalence of SCE was not significantly different between the two herds. Further studies based on a larger number of herds with a varying herd management are, however, warranted to further elucidate the effect of herd management on the prevalence of SCE.

Despite the variation in prevalence of SCE in different studies, there is a striking similarity in most of the studies regarding the association of SCE with an extended calving to pregnancy interval (Kasimanickam et al., 2004; Gilbert et al., 2005; Galvão et al., 2009). Gilbert et al. (2005) postulated that the validity of endometrial cytology as a tool to examine postpartum uterine health, is confirmed by the fact that cows that are cytologically positive suffer from impaired reproductive performance. Different statistical analyses such as survival analysis can be used to unravel such effects. Using survival analysis, the time to an event per individual subject is measured including those subjects that do not experience the event of interest or are lost during the study (Dohoo et al., 2003). Therefore, survival analysis reduces the bias of losing information from cows that were culled or were not pregnant at the end of the study (Morton, 2006). In the present study,

main contributors to impaired reproduction were increased calving to first service and calving to conception interval (Table 3 and Fig. 1). This is similar to the findings of LeBlanc et al. (2002a) who reported, by means of survival analysis, a slight (3 days) delay in days to first insemination and days to conception in SCE positive cows. Due to the low number of samples in the present study, observed differences were not statistically significant.

Current treatment strategies for endometritis are based upon two different protocols: intrauterine infusion (IU) of antibiotics and administration of prostaglandin F2α or one of its analogues (Kasimanickam et al., 2005b). There is little evidence that intrauterine antibiotic infusions are beneficial, except for cephapirin which had a consistent positive impact for mitigating the effect of endometritis on subsequent reproductive performance (McDougall, 2001b; LeBlanc et al., 2002a; McDougall, 2003; Kasimanickam et al., 2005b; Runciman et al., 2008). In a recent study, the efficacy of a homeopathic treatment for endometritis was examined although no positive effect could be demonstrated (Arlt et al., 2009). Likewise, administration of prostaglandins did not show any preventive effect on the prevalence of SCE, however, this treatment had a positive impact on reproductive performances, such as median days to pregnancy and pregnancy risk at first service (LeBlanc et al., 2002b; Kasimanickam et al., 2005b; Hendricks et al., 2006; Galvão et al., 2009).

In conclusion, based on the present study, we demonstrated a relatively high incidence of SCE in modern dairy cows in Iran. This high prevalence in healthy cows that had not suffered from any other puerperal disease necessitates the search for both preventive and curative treatment strategies.

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