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Postpartum uterine diseases and their impacts on conception and days open in dairy herds in Italy

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Abstract

The objective of this study was to describe the incidence and the impact of postpartum uterine diseases in postpartum cows on future uterine status, and reproductive performance in large Italian dairy herds. This study provides an important quantitative estimate of uterine and postpartum diseases incidence that afflict high-producing Italian dairy cows. The total number of cows included in the study was 1498 on three farms; all cows were followed from the dry period until 300 days postpartum. All farms used high-quality data collection systems and standard operating procedures: weekly herd health visits, monthly Dairy Herd Improvement Association (DHIA) visits and - due to cheese-making milk quality requirements - a supplementary milk sample collected at 7 ± 3 days postpartum evaluated for milk components. Clinical metritis in primiparous cows did not change time to first AI or days open; conversely, on multiparous clinical metritis impact on the time to first AI (hazard ratio: 0.66, $P<0.01$) and resulted in a lower conception rate at first insemination and in a increase in days open (odds ratio 0.64, $P<0.05$). Clinical endometritis had a strong deleterious effect on first AI conception rate (odds ratio 0.34, $P<0.05$) and days open across all lactations (hazard ratio 0.68, $P<0.05$). Persistent metritis, defined as the presence of both clinical metritis and clinical endometritis in the same animal in the same lactation, caused low conception rate both in first lactation and in older cows and had a strong negative effect on the proportion of pregnant cows at 300 days ($P<0.05$). In conclusion the impact of endometritis on fertility was true across lactation groups. A good management and precocious diagnosis of the pathologies is not resolutive to restore good fertility parameters and understanding of the immune response in first lactation cows may be of value for developing alternative intervention protocols for older lactation cows.

Key Words: Cows, Metritis, Endometritis, Conception risk, Days open

1. Introduction

As a result of genetic improvement from years of selection, dairy cows have become more efficient in producing milk, but modern cows seem to be more sensitive to environmental changes and less tolerant to stress and disease. Uterine inflammatory diseases (metritis, clinical endometritis and subclinical endometritis) are some of the most important diseases occurring in the postpartum period. Uterine health is often compromised due to postpartum contamination of the uterine lumen by ubiquitous and pathogenic bacteria. Most cows do not develop severe uterine infections, but these infections are frequently persistent clinical diseases that reduce the reproductive efficiency of cows and the profit of dairy farms. Until 2006, the literature showed a disparate classification of uterine pathology. Therefore, some authors [1-3] proposed to categorize postpartum inflammation of the uterus according to the time of onset of the disorder and the characteristics of the uterine discharge. An examination of vaginal discharge in order to detect the presence of pus is the most convenient method for endometritis diagnosis. Characteristics and odor of the vaginal discharge can be scored and this endometritis score is correlated with the growth density of pathogenic bacteria in the uterus. Improvements in the quality of clinical diagnosis may be obtained by the application of an ultrasound examination. Ultrasonography can also help in early detection of silent estrus, anestrous and cystic ovarian conditions and has proven to be useful in reducing days to first service, days open and calving interval [20], [21], [22]. Moreover, precise diagnostic techniques based on routine ultrasound examination in all cows in the herd, along with accurate data on subsequent fertility, would provide accurate information on the impact of uterine disorders on reproductive parameters.

Clinical forms of metritis and endometritis have been reported with an incidence ranging from 14% to greater than 40% in cows [4], [5], [6], [2], [7], [8], [9], [10].. Metritis within a week of parturition was present in 40% of dairy cows [2], with a range from 35% to 50% reported in large surveys [23], [24]. When the status of the reproductive apparatus was assessed at 35 days postpartum, 43% of the cows had subclinical endometritis, and the days open increased by 27 days;

still, quantitative estimates on the incidence of uterine disorders and the impact of these disorders is limited, particularly since more accurate definitions of uterine disorders have been introduced [1]. Uterine disorders were defined as follows: puerperal metritis (PM), clinical metritis (CM), endometritis (E) and subclinical endometritis (SE). Puerperal metritis (abnormally enlarged uterus, fetid watery red–brown uterine discharge, signs of toxemia, and fever >39.5 °C), or clinical metritis (enlarged uterus, purulent discharge in vagina, and no systemic signs) should be diagnosed within 21 days postpartum. Clinical endometritis is characterized by the presence in the vagina of purulent (>50% pus) uterine discharge detectable 21 or more days after parturition, or a mucopurulent discharge after 26 days postpartum. Subclinical endometritis (no detectable clinical changes in the uterus) is diagnosed by the percentage of neutrophils in the postpartum uterine cytology samples.

The objective of this study was therefore to describe the incidence of uterine and postpartum diseases and to evaluate the impact of early uterine diseases in postpartum cows on future uterine health status, time to first artificial insemination (AI), conception risk and time to conception (days open) in large commercial Italian dairy herds.

2. Material and methods

2.1. Herd Descriptions

The data were collected on three large Italian Holstein dairy herds. Two farms (A and C) were using a rotary parlor (40 milking stands), and one farm (B) was milking in a double 12 herringbone. All cows were milked twice a day and dried manure was used as bedding for the lactating cows. Groups of lactating cows were housed in a paddock with cubicles with straw, and dry cows and cows in the calving pen were bedded on a bedded pack with straw. The produced milk was used exclusively for Parmesan cheese production. All farms used standard operating procedures for the most important management processes. High quality data collection systems were present on the farms and included weekly herd health visits reports. All eligible animals were checked weekly using clinical inspection and ultrasound evaluation. Monthly Dairy

Herd Improvement Association (DHIA) visits for recording milk and components and for recording of all disease and reproductive events were entered into a computerized data base. Daily milk production was collected using Delaval® milk meters, and daily milk weight data were transferred from Alpro® to the AfiFarm® data management system. All DHIA and milk production data were imported into the AfiFarm® management system, and reproduction and health data were recorded and managed with the AfiFarm® system. The 305-day milk production was obtained from the records and is defined as the standardized milk production in 305 days of lactation, standardized for the herd, age and season of calving.

2.2. Nutrition

Since the milk was used exclusively for parmesan cheese production, the use of corn silage was precluded from the diet. A total mixed ration (TMR) was fed to the cows twice a day. The diet consisted of 50% concentrates (8 kg of steamed crushed corn, 2 kg of barley, and 3 kg of high protein mix that consisted of soybean and sunflower seeds mixed with a mineral mix) and 50% forages (1/2 grass hay and 1/2 alfalfa, in accordance with the guidelines for the production of parmesan cheese), with 17 kg of water added to the TMR. For dry cows, a dry diet was fed that consisted of ad lib forages (50% hay and 50% alfalfa) with 2 kg of a purchased dry cow concentrate. During the final 14-21 days of the dry period, the diet consisted of 40% of the TMR diet for the lactating cows (see above) and 60% of the diet for early dry cow; during this period, anionic salts were added to the diet.

2.3. Cow Measurements

Disease definition and diagnostic criteria were consistent across farms. Most veterinary work was performed by the first author (FT), and written disease definitions were given to the participating dairy producers. All cows were examined during the routine or during heat detection by manual mucus examination, to assess the healthy or pathological status of the reproductive tract; briefly the vulva was cleaned using a dry paper towel and a clean, lubricated gloved hand was inserted through the vulva into the vagina and the mucus contents was examined: cows were considered as health if they show a clear mucus or pathological if mucus was turbid or contains pus. All the abnormal scored cows were checked by ultrasound, to assess the presence of abnormal fluid in the uterus and confirm endometritis. Retained placenta was defined as the persistence of fetal membranes until at least 24 h after calving, Metritis was defined when a cow presented with a fetid brown vaginal discharge with or without fever, or abnormal vaginal discharge at 3 to 10 days postpartum. Under this definition of metritis, puerperal metritis and clinical metritis are essentially combined into one disease definition. Clinical endometritis (referred to throughout this manuscript as endometritis) was diagnosed from a visual inspection of the cervix when the cow was between 28 and 35 days in milk and was based on the detection of abnormal mucus (turbid, cloudy, purulent or mucopurulent) fluid present in the vagina or cervix [1] and ultrasound detection of abnormal fluid in the uterus. The term persistent metritis was defined as the presence of both clinical metritis and clinical endometritis in the same lactation period in the same cow. Anestrous was defined a cow with no follicular structure on the ovary larger than 5 mm. and no CL as diagnosed in two subsequent visits that were approximately 15 days apart, ovarian cyst or cystic ovarian disease was defined as any follicular structure on the ovary, which is larger than normal follicular size, is present in the absence of luteal tissue and persists for a significant amount of time and affects the estrus cycle of the animal [25]. In this study, we defined an ovarian cyst based on ultrasonography at two time points with a 14 day interval.

The definition of ketosis was defined as the presence of ketone bodies in milk, as measured with a Ketolac® milk ketosis test at 7 (+/- 3 days) post partum, and Displaced abomasum (DA) was defined as an enlarged abomasum filled with fluid and gas, which were mechanically trapped in either the left or right side of the abdominal cavity. Mastitis was defined as any abnormality in milk or udder that resulted in a mastitis treatment by the herd manager.

All culling decisions were made by the herd manager: Paratuberculosis testing was performed during dry off using an ELISA on the serum. When ELISA-positive animals showed clinical symptoms associated with paratuberculosis, the animals were culled. Postpartum culling was intended as culling within 30 days of calving, mostly for leg trauma caused by slipping in the walk ways. Culling for mastitis was performed for both clinical mastitis and/or high somatic cell counts. Both death and culling were the endpoint of observation for cows included in the study. A body condition score (BCS) was performed by the same observer (F.T.) in the week of dry-off, at the time of the first visit (5-12 days after calving), and 50-60 days postpartum when animals were observed to be inseminated. All herds participated in monthly complete herd DHIA testing. In addition, as required for milk quality by Parmigiano-Reggiano cheese makers, a supplementary milk sample, to be defined as an extra test (ET), was collected at approximately 7 days postpartum (+/- 3 days) and evaluated for milk components. This additional milk sample was also evaluated for ketosis using the Ketolac® test. Milk components, measured in the ET sample, were evaluated using a Fossomatic 5000 milk analyzer.

Cows with metritis were treated intramuscularly (IM) with amoxillin or ceftiofur at the label dose [26]; in severe cases with general disease symptoms, anti-inflammatory agents and intravenous fluid therapy were also employed. Metritis cases were followed up by the herd veterinarian (F.T.) on a weekly basis to determine if a second therapy was needed. Endometritis cases were treated with prostaglandin at the label dose, based on the veterinary visit at 28-35 days postpartum. Ketotic cows were treated with glucose-IV and glucocorticoids.

Cows were inseminated based on the data from the activity meter (DeLaval ALPRO™) and visual heat observation, beginning on 45 days after the calving at the end of the Voluntary Waiting Period (VWP). At days 60-67 after calving, cows not yet inseminated were treated with prostaglandin and inseminated at the observed estrus. All the animals negative to an ultrasound pregnancy test were also treated with prostaglandin. Lameness and mastitis were diagnosed by the farm manager on the basis of daily observations.

2.4. Statistical methods

All data were initially managed in the AfiFarm® herd management software. This is a database system that is Microsoft Access-based. Data were exported into spreadsheets and uploaded into statistical software. All statistical analyses were done using the SAS statistical system version 9.2 (SAS Institute, 2009). Descriptive statistics on all variables were performed and outliers investigated. New variables were calculated from the raw data, notably the fat to protein percentage ratio in the early lactation additional test. Fat to protein ratio (FP ratio) was calculated as the simple ratio between the fat percentage in the milk and the protein percentage in the milk. This variable was used as a categorical variable. Based on our previous research, a ratio between 1.0 and 1.5 as the baseline (normal) category was used for comparison [27], [28]. Time to first artificial insemination (AI) and time to positive pregnancy diagnosis (time to first conception) was the outcome of interest in this study, and uterine health was used as the principle predictor, while correcting for possible confounders such as season of calving (summer: April to October and winter: November to March), herd and parity. Parity was categorized into two groups: primiparous and multiparous cows

The relationship between pregnancy at first AI and uterine health was evaluated using a logistic regression analysis (SAS PROC GLIMMIX), where pregnancy (0/1) was used as the outcome variable:

Logit (pregnancy) = intercept + uterine health + confounders + error (1),

where uterine health was metritis, endometritis, or persistent metritis. Confounders were represented by herd, calving season, parity, calving BCS score, FPratio as defined above, and diseases such as DA or mastitis. Retained placenta was also considered as a confounder in the regression models. Time to first AI and time to conception were investigated using survival analysis. The final Cox regression model was defined as follows:

*Hazard pregnancy (or first AI) = baseline hazard * exp (uterine health + confounders)* (2),

Where exp is the exponent function, and uterine health and confounders were as defined above. The baseline hazard is an unspecified hazard in the Cox regression models.

Cows were followed for 300 days into lactation or for fewer days, if culled before the 300 days into lactation. Cows that were not inseminated or did not conceive after insemination and were not culled during 300 days of follow-up were considered censored at 300 days for these analyses. Life tables were constructed using the Kaplan Meier Product Limit estimators for the survival function. Statistical and graphical analysis of the data was performed to evaluate the impact of categorical risk factors. Finally, a Cox

Proportional Hazards survival analysis (PROC PHREG) was performed to relate uterine health to first AI and pregnancy, correcting for potential confounders such as parity, season and herd.

We calculated the Population Attributable Proportion as:

$$[P*(RR-1)] / [1+ P*(RR-1)], \qquad (3)$$

where P is the prevalence of the exposure (uterine disease) in the population and RR is the relative rate (Cox proportional hazard) or odds ratio (logistic regression). In our analysis, uterine diseases were the prevalence of interest. Because uterine diseases were usually associated with a decreased risk of pregnancy, the RR was replaced by 1/RR in the above formula. Direct and total effects for metritis were calculated by either including (direct) or not including (total) endometritis as a covariate in the model. As endometritis occurs at some time after metritis, it may be considered a consequence of metritis; therefore, removing endometritis from the model allows a more proper estimation of metritis impact on the outcome of interest. Similarly, to exclude the impact of a previous metritis case, a second analysis on endometritis was performed in cows that did not have a previous case of metritis (new cases). In all statistical analysis, statistical significance was decided based on a cut-off value of P<0.0.5.

3. Results

Descriptive data of the animals in the study are shown separately for primiparous and multiparous cows. The total number of cows included in the study was 1498 cows that calved between December 1 and October 1 of the subsequent year. Herds were fairly similar in size with 603, 421, and 474 animals for farms A, B and C, respectively. Overall, 35.8% of the animals were primiparous. Average daily milk production was between 33 and 40 kg per day, and the corrected 305 day milk production was 9330 kg, 9746 kg and 9557 kg, respectively for the three herds. Disease incidence for primiparous and multiparous cows is shown in Table 1. Low number of persistent metritis cases are shown in both age groups (Table 1). The impact of disease on days open is shown in Table 2. At first insemination, greater fertility was detected in healthy primiparous compared to multiparous cows (32% versus 22%) (Figure 1). Persistent metritis caused a low conception risk for both primiparos and multiparous cows.

The effect of persistent metritis was particularly strong (DATA??) in primiparous, as shown in Figure 1.

The impacts of uterine health disorders and clinical disease on time to first insemination and conception risk at first insemination are shown in Tables 3 and 4, respectively. Significant factors affecting time to first insemination (Table 3) included anestrous, endometritis in primiparous and multiparous and metritis in multiparous cows. The effect of uterine disease on time to first conception is shown in Figure 2, based on survival curve methodologies.

The most important factors affecting conception risk at first artificial insemination (Table 4) were cystic ovarian disease (COD), endometritis and anestrous, followed by metritis in all lactation groups. Increased milk production showed a negative effect on first conception risk and on days open.

The impact of uterine health disorders on time to pregnancy (days open) is shown in Table 5. The most important factors affecting time to conception in cows include endometritis, metritis and anestrous. The effect of uterine diseases on time to conception is shown in Figure 3. Persistent metritis strongly affected the likelihood of a cow to be pregnant by 300 days in milk. Specifically, persistent metritis, resulted in a low conception risk for both primiparous and multiparous cows and had a strong negative effect on the number of pregnant cows at 300 days (Figure 3). The impact of uterine disorders on the overall population value of the reproduction parameters of interest are shown in Table 6. For time to first AI, endometritis for all cows and metritis for multiparous cows are the most important diseases, as indicated by the population attributable proportion. For conception risk at first AI, the diseases with the largest population attributable proportion is endometritis in all animals and the total effect of metritis in primiparous. For days open, the highest population attributable proportion was endometritis for all lactations and metritis in multiparous cows..

4. Discussion

This field study based on clinical records of postpartum disorders and with ultrasound-based measurements of uterine status, allowed to determine the impact of these disorders in great detail. One of the key findings in our data was the high incidence of clinical metritis in primiparous cows relative to older cows (20% versus 9%). Despite the high incidence of metritis in primiparous cows, the population attributable proportion of days open of metritis was lower in primiparous cows compared to older cows (0.0236 versus 0.051). The interpretation of the population attributable proportion is here that 2.3% of the average days open in the population can be attributed to metritis in primiparous cows while 5.1% of days open can be attributed to metritis in multiparous cows. Additionally, primiparous cows animals with metritis were virtually indistinguishable from healthy animals with regard to time to first AI and time to conception. Primiparous cows animals clearly have a better ability to recover from metritis. Our observations in primiparous cows have been reported by others [29-31]. Our data and these previous reports suggest that metritis had no detrimental effect on reproductive performances in primiparous cows animals and demonstrate that primiparous cows cows had a more rapid recovery in functional local immunity than multiparous cows. A more precise understanding of the immunology and the pathobiology of the response to metritis in primiparous cows animals may provide opportunities for improving immune response to metritis in older cows.

Figure 1Persistent metritis showed a more important impact on multiparous cows compared to primiparous cows cows. Similarly, endometritis had more impact on uterine health in multiparous cows than primiparous cows.

Note that we defined persistent metritis as the presence of both metritis and endometritis in the same cow in the same lactation even though the term persistent implies the continuation of disease from metritis to endometritis. However, we cannot be sure that there was no cure of metritis in early lactation, and subsequently, a new infection resulted in endometritis later in lactation. Hence, persistent metritis, as defined here, is the impact of the combination of metritis and endometritis, whether these diseases are related or not.

The incidence of retained placenta was higher in multiparous cows compared to primiparous cows animals (17% vs. 11%), in contrast, the incidence of metritis was lower in multiparous cows compared to primiparous cows animals (9% vs 20%).. In primiparous cows animals, the risk of metritis was 6.9% and 21.6% for animals with and without RP, respectively, while these risks were 1.8% and 10.3%, respectively, in multiparous cows. Considering the high positive correlation reported in the literature [5], [32], our data showed an unexpected negative correlation between retained placenta and metritis. In contrast, the relationship between RP and endometritis was strong and positive in our data, both in primiparous cows animals and in multiparous cows. In both groups of cows, animals with RP were approximately three times more likely to show endometritis later in lactation compared to animals without RP. Metritis in primiparous cows did not affect time to first AI and days open, but it negatively affected the first conception risk. Metritis in multiparous cows increased time to first AI, decreased first conception risk and increased days open.

Milk production was not significantly different between primiparous and multiparous cows (10,039 Kg vs 9266 Kg). Milk production had very little impact on fertility. Higher producing cows only showed a slightly negative effect on the first conception rate, but no impact was shown on time to first AI or days open. Additionally, the fat to protein ration in primiparous cows did not directly affect fertility parameters. In our previous study, we showed that fat to protein ratio was a strong predictor of uterine disorders [28]. The absence of fat to protein ratio as a predictor of fertility parameters would indicate a perfect causal chain of the fat to protein ratio to uterine disorders to fertility impairment. So, the fat to protein ratio does not have a direct impact on fertility parameters.

As expected, conception risk at first AI in multiparous healthy cows was lower than in primiparous cows. Metritis across lactation groups decreased first insemination conception risk significantly. This effect was present despite the therapeutic protocol that was used on these farms. The apparent cure was better and more effective in primiparous cows, resulting in a better return to a uterine environment conducive to conception. In primiparous cows animals, there was no significant impact of metritis on time to conception. Metritis in older cows resulted in a significant reduction in days open; multiparous cows seem need more time to recover to a healthy uterine condition. This may be due to the high metabolic stress of lactation and a lower immune function in the transition period of these cows [18,24].

Endometritis also had a strong effect on first conception risk, days open (Figure 3) and time to first AI (Figures 3,4 and Tables 2,3,4). The impact of endometritis on fertility was strong in multiparous cows and primiparous cows cows. Persistent metritis also resulted in a low conception risk for all lactation groups and showed a strong negative effect on pregnant cows at 300 days (Figure 3). Decreased fertility due to uterine disorders involves perturbation of the endocrine system and hypothalamus, in addition to the direct effects on the uterus, and appears to persist even after clinical resolution of the disease [33]. This persistence of hormonal disturbance may be part of the effect that we observed in persistent metritis. The therapy applied in our study was considered not fully effective, as a large impact of uterine disorders on fertility parameters remained [34].

In our analyses, we distinguished between direct effect and total effect of metritis on fertility parameters. For the total effect of metritis, the impacts of disorders that are a direct consequence of metritis were included. Hence, the prevention of a case of metritis would also reduce the incidence of subsequent correlated disorders. The total effect, as calculated by the population attributable proportion, is always higher compared to the direct effect. For example the direct effect of metritis in multiparous cows on days open was 3.8%, while the total effect was 5.2% .Therefore, the increased risk of animals with metritis for having a subsequent case of endometritis should be taken into account when judging the impact of metritis on fertility. As might be expected from our data on persistent metritis the impact of new endometritis cases (without a previous metritis) on fertility parameters was always lower compared to all endometritis cases. All these observations clearly show the impact of persistent uterine disorders. Further research into a true cure for primiparous uterine disorders is essential for truly improving fertility in high producing dairy cows.

5. Conclusions

In this study, the incidence of clinical metritis is higher in primiparous cows animals than in multiparous animals. In primiparous cows, the early detection of metritis resulted in a high apparent cure, as indicated by a subsequent good fertility. In multiparous cows, the same diagnostic approach resulted in a lower fertility in metritis-affected animals. Therefore, further investigations are needed to reveal differences in the pathobiology and epidemiology of metritis in the two lactation groups. A good understanding of the immune response in primiparous cows cows may be of value for developing alternative intervention protocols for multiparous cows.

Cows with endometritis, diagnosed by combining inspection and ultrasound examination, treated only with prostaglandin showed significantly lower fertility. The impact of endometritis on fertility was true across lactation groups. Therefore, endometritis in primiparous cows is a much more severe condition with regard to fertility, compared to metritis. More investigations on effective therapy for this disorder are clearly necessary.

Cows with persistent metritis, treated with parenteral antibiotics in early postpartum and with prostaglandin after 30 days, showed low fertility. Further research into the resolution of early lactation uterine disorders is essential for truly improving fertility in high producing dairy cows.

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Table 1. Descriptive statistics of the cows in the three herds.

	Lactation 1			Lactation 2+		
Variable	N	Mean	SD	N	Mean	SD
Parity	536	1	-	962	3,1	1,3
Milk per day (kg)	536	34,5	10,8	962	35,8	32,6
Total milk in lactation (kg)	536	10039	3643	962	9266	4222
Fat to Protein ratio at 7 dim	536	1,79	0,55	962	1,85	0,56
BCS at calving	535	3,13	0,25	962	3,12	0,36
BCS at first AI	527	2,86	0,29	872	2,80	0,33
Days in milk at first AI	522	59,8	20,3	853	60,2	21,9
Pregnant at first AI (%)	522	0,28		853	0,21	
Total nr of inseminations	536	3,3	2,4	962	3,3	2,5
Days open	464	111	64	696	116	59
Uterine health						
Normal	536	0,76		962	0,84	
Metritis	536	0,20		962	0,09	
Endometritis	536	0,07		962	0,09	
Persistent metritis[1]	536	0,03		962	0,02	
Disease incidence (%):						
Retained placenta	536	0,11		962	0,17	

Ketosis (milk based)	536	0.23	962	0.28
Cystic ovarian disease	536	0,10	962	0,15
Anestrus	536	0,06	962	0,02
Displaced abomasum	536	0,14	962	0,06
Mastitis	536	0,34	962	0,40
Lameness	536	0,32	962	0,41

[1] Persistent metritis cases were also included in the metritis and endometritis incidence.

Table 2. Days open descriptive statistics of all cows in the three herds. Data shown are the mean with standard deviation in brackets.

	Primiparous		Multiparous	
Variable	Yes	No	Yes	No
Disease free	104.8 (66.4)		105.6 (74.1)	
Metritis	139.9(84.1)	130.8(84.3)	160.0(92.1)	133.5(85.5)
Endometritis	202.4(83.7)	127.7(82.2)	190.5(82.4)	130.5(85.0)
Persistent metritis	195.1(72.4)	130.9(84.0)	196.2(82.3)	134.7(86.1)
Anestrus	186.5(89.6)	128.9(82.7)	174.3(80.3)	134.9(86.4)
COD	213.7(83.6)	123.7(79.5)	190.3(87.2)	126.6(82.8)
RP	163.2(99.2)	128.9(81.6)	146.3(89.8)	133.7(85.6)
Ketosis	141.0 (85.6)	130.1 (83.8)	128.5 (90.7)	138.8 (84.5)
Lame	144.6(85.1)	127.0(83.4)	149.2(86.8)	126.5(85.0)

Mastitis	139.9(89.9)	128.9(81.6)	141.9(84.1)	131.9(87.7)
DA	150.9(90.2)	129.6(83.0)	129.4(90.7)	136.3(86.2)

Table 3. Cox proportional hazards regression model of time to first artificial insemination.

Parameter	Estimate	SD	P-value	Hazard ratio
Herd A	0,38	0,07	<.0001	1,46
Herd B	0,42	0,08	0,0014	1,52
Herd C	Base			
Primiparous cows yes vs no	-0,06	0,06	0,4955	0,94
Calving season - Spring	-0,01	0,07	0,7956	0,99
Summer	0,17	0,07	0,0055	1,19
Fall	0,19	0,10	0,0274	1,21
Winter	Base			
Metritis – Primiparous cows	-0,11	0,12	0,3444	0,89
Metritis – multiparous cows	-0,41	0,13	0,0021	0,66
Endometritis	-0,55	0,09	<.0001	0,58
Normal uterus	Base			
Milk production per day	0,01	0,00	0,76	1,01
Lameness yes vs no	-0,19	0,06	0,0015	0,83
Retained placenta yes vs no	-0,27	0,08	0,0041	0,76
Cystic Ovarian Disease yes vs no	-0,35	0,08	0,0002	0,70
Anestrus yes vs no	-0,82	0,15	0,00001	0,44

Table 4. Result of Logistic regression analysis of first insemination conception risk.

Parameter	Estimate	SD	P-value	Odds ratio
Intercept	-0,29	0,43	0,3206	
Herd A	-0,06	0,16	0,7325	0,94
Herd B	0,05	0,19	0,7883	1,05
Herd C	Base			
Primiparous cows yes vs no	0,18	0,15	0,2245	1,19
Calving season - Spring	0,14	0,24	0,6934	1,15
Summer	-0,10	0,17	0,4586	0,91
Fall	-0,10	0,23	0,5931	0,91
Winter	Base			
Days in milk at first AI	0,01	0,00	0,03	1,01
Metritis	-0,45	0,22	0,04	0,64
Endometritis	-1,07	0,32	0,001	0,34
Normal uterus	Base			
Milk production per day	-0,03	0,01	0,006	0,98
Lameness yes vs no	-0,62	0,15	<.0001	0,54
Cystic Ovarian Disease yes vs no	-1,36	0,28	<.0001	0,26
Anestrus	-0,98	0,45	0,0253	0,38

Table 5. Cox proportional hazards regression model of time to conception (days open)

Parameter	Estimate	SD	P - value	Hazard ratio
Herd A	0,18	0,07	0,016	1,19
Herd B	0,20	0,08	0,017	1,22
Herd C	Base			
Calving season - Spring	-0,05	0,08	0,532	0,95
Summer	0,17	0,08	0,034	1,19
Fall	0,17	0,11	0,119	1,19
Winter	Base			
Primiparous cows yes vs no	0,21	0,08	0,012	1,23
Metritis primiparous cows	-0,04			0,96
Metritis multiparous cows	-0,37	0,15	0,010	0,68
Endometritis	-0,59	0,14	<.0001	0,55
Normal uterus	Base			
Milk production per day	-0,01	0,00	0,073	0,99
Lameness yes vs no	-0,21	0,06	0,003	0,82
Mastitis yes vs no	-0,13	0,06	0,038	0,88
Retained placenta yes vs no	-0,31	0,09	0,0008	0,73
Anestrus yes vs no	-0,66	0,18	0,0003	0,52

Table 6. Direct and indirect effects of metritis and endometritis. In the Table the rate ratio's and the population attributable proportion are shown.

Uterine disease	Time to first AI		Pregnant at AI 1		Days open	
	RR[1]	PAP[2]	RR	PAP	RR	PAP
Metritis direct effect, primiparous cows	0,90	0,023	0,64	0,102	0,96	0,008
Metritis total effect, primiparous cows	0,84	0,036	0,58	0,125	0,90	0,023
Metritis direct effect, lactation 2+	0,66	0,042	0,64	0,048	0,69	0,038
Metritis total effect, lactation 2+	0,61	0,053	0,58	0,059	0,62	0,051
Endometritis, new cases only	0,55	0,052	0,36	0,105	0,55	0,051
Endometritis, corrected for metritis	0,58	0,055	0,34	0,133	0,55	0,061

[1] Rate ratio

[2] Population attribuable proportion

Figure 1. Impact of uterine health on 1st insemination conception risk (y-axis)

Figure 2. Kaplan-Meier survivor curve of Time to first AI. Animals are categorized in normal uterine health (——) primiparous cows animals with metritis (Δ), lactation 2+ with metritis (○), all cows with endometritis (□) and all cows with persistent metritis (●).

Figure 3. Kaplan-Meier survivor curves of time to conception. Animals are categorized in normal uterine health (——), primiparous cows animals with metritis (Δ), lactation 2+ with metritis (○), all cows with endometritis (□) and all cows with persistent metritis (●).