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Causes and effect of endocervical inflammation on calving to conception in Holstein Friesian cows

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Abstract: *The aim of this study was to evaluate the causes and the effect of endocervical inflammation on calving to conception interval in subsequent lactation of Holstein Friesian cows. A total of 162 cows (58; 35.8% primiparous and 104; 64.2% multiparous) were sampled at 29-35 days postpartum (mean 32.5 ± 0.14 days) for diagnosis of endocervical inflammation using and adapted cytobrush technique. A total of 162 slides were obtained and were evaluated for the presence of neutrophil cells. A threshold of ≥ 5% neutrophils was used to select cows as positive for endocervical inflammation. The main events recorded at calving and in early part of postpartum period were assisted calvings, retained fetal membranes (RFM) within 12 hours post calving and puerperal metritis within 18 days postpartum (pp). The overall incidence of endocervical inflammation was 11.1% (n 18/162) and was higher in cows with puerperal metritis (29.41%) and in cows with RFMs (28.12%). The probability (odds) of developing a endocervical inflammation at 29-35 days in milk (DIM) was higher in cows with puerperal metritis (odds ratio 6.25, 95% CI 2.23-17.46; p value 0.0002) and in cows with RFMs (odds ratio; 95% CI 1.88-14.67, p value 0.0007). Endocervical inflammation with more or equal to 5% neutrophils at 29-35 days pp was associated with decreased hazard of pregnancy within 140 DIM (hazard ratio 0.38 95% CI 0.29-0.53). The mean days from calving to conception for all artificial insemination until 140 DIM was 106.6 ± 20.9 days in cows without endocervical inflammation and 122 ± 17.9 days in cows with endocervical inflammation. Days open was increased with 15.4 days (SE 5.1, 95% CI 5.15-25.57, p value 0.0034). The findings of this study indicate which causal factors of endocervical inflammation should be monitored in pp dairy cows to limit the effects on consecutive reproductive performance.*

Keywords: cytology, endocervical inflammation, fertility.

• Introduction

After parturition the dairy cows face notable challenges. These include: calving, the initiation of lactation, the transition from dry to lactating cow management, decreased dry matter intake (DMI), high nutrient demand, body reserve mobilization, uterine involution and resumption of ovarian cyclicity. All these confrontations after calving make the cows vulnerable to develop uterine diseases such as vulva vaginitis cervicitis, endometritis, metritis and salpingitis [1].
Infectious pustular vulvovaginitis of cows is caused by bovine herpes virus 1 and is transmitted via natural insemination, nasogenital contact of by insect vectors [2]. The sick cows show signs of vaginal discomfort (e.g. raised tail, frequent urination) and have round, white, raised lesions of the vestibular mucosa. These lesions progress to pustules and erosions or ulcers. Mucopurulent vaginal discharge may be present. Cervicitis is another component of postpartum reproductive disease in dairy cattle. Cervicitis is defined as inflammation of the cervix and can be associated with: calving injury (dystocia), trauma, retained fetal membranes (RFM), urovagina, endometritis, metritis and pyometra.
The cervix forms the anatomical and functional barrier between vagina and the uterus, and is formed from a series of mucosal – lined collagenous rings. The bovine cervix when is palpated is described as having a „turkey neck". It has thick walls and a small opening, or cervical as [3] but it is susceptible to inflammation, infection and trauma. The cervicitis severity is classified into 3 stages: C0 = normal cervix, C1 = cervicitis with a swelled and prolapsed 2nd cervical fold, and C2 = cervicitis with a swollen, reddened and prolapsed 2nd cervical fold [4]. Cervicitis is frequently caused as a result of exposure to inflammation provoked by luminal debris from the uterus metritis or endometritis or from the vagina (urine in urovagina condition) [4, 5].
Also, cervicitis is caused by trauma during calving (calving injury) or due to inadequate artificial insemination procedure.
Cervicitis can be the cause of subsequent inflammation within the uterus (endometritis) [4].
After calving uterine diseases affect fertility of dairy cows [6, 7, 8] and generate economic losses [9, 10].
The effect of uterine disease on reproductive parameters is not the same for all categories of cows. Multiparous cows are more susceptible to metritis than primiparous cows [11].
Also, cows with uterine disease have greater pregnancy losses [12]. Thera are several techniques for the diagnosis of subclinical uterine diseases and some of these can be put in application for collection of endometrial or endocervical and inflammatory cells defined as uterine cytology [13, 14].
For subclinical uterine inflammations, methods such as uterine lavage, intrauterine cotton swab, and cytobrush or cytotape sampling and endometrial biopsy are necessary to confirm the diagnosis.
Endometrial and endocervical cytology in cows is not a complicated technique and an easier procedure could be implemented. These methods involve a transvaginal instrument being inserted through cervix using a rectal manipulation or into uterine body and into uterine horns to collect the sample required.
Hartman et al. (2016) [15] determined that cervicitis in dairy cows is an independent disease or occurs concomitantly with inflammation of the uterus and a higher degree of cervicitis is associated with poor reproductive performance.
The principal objectives of this study were to evaluate the prevalence of endocervical inflammation using cytological diagnosis, to identify principal causal factors for developing endocervical inflammation at 29-35 DIM and to calculate its effect on days open in dairy cows.

• Material and method

This experiment was conducted at Agricultural Research and Development Station (ARDS) Șimnic-Craiova, Romania (182 m above sea level, 44°19' N, 23°8' E) in the Research Dairy Farm January 2021 to December 2022. The experiment was performed in compliance with European Union Directive 86/609 Ec and National legislation on Holstein Friesian cows with high-milk-production (9410 ± 330 kg mean and standard deviation). The dairy farm has 305 Holstein Friesian cows with all-round calving. Dairy cows and heifers are housed in barns with access to dry lot prepartum and postpartum.
Nutritional and management strategies for dairy cattle are used to prepare the animals for lactation and to minimize the incidence of disease disorders after calving. Forage a small amount of concentrate, and a trace mineral salt represent the dry ration during early period. Subsequently a close-up ration is fed which provides more nutrients. Postpartum primiparous and multiparous cows are fed in the morning and afternoon with diets formulated to provide recommended nutrients for lactating period. All feeds consisting of corn silage, haylage, chopped green alfalfa, fodder beet, and grains are produced locally. Additionally, all cows and heifers have access to posture (alfalfa or ryegrass).
All lactating cows were milked two times a day (05:00 am and 17:00 pm). The cows had ad libitum access to fresh water. At 3 weeks before due date cows were moved into the close-up pen, where the farm or research personnel monitor the calving process. The lactating cows were milked in a classic DeLaval milking system (2x5).
Research design and selection of data
Data were analyzed from 180 Holstein Friesian cows. After parturition the cows were divided into 2 groups: Group 1 primiparous cows and group 2 multiparous cows. Also, calving difficulty score (CDS) was estimated using a 5-pointscale (each number corresponds with the level of difficulty needed for expulsion of calf): 1 = easy birth (no assistance); 2 = over 2 hours in labor (no assistance); 3 = minimum assistance (not calving difficulty); 4 = use of obstetrical chains and 5 = extremely difficulty using mechanical puller. The 1, 2 and 3 points scale were categorized unassisted calvings and 4 and 5 points scale as assisted calvings.
Retained fetal membranes (RFM) – failure to expel fetal membranes within 12 hours after calving was recorded. All cows between 5 to 10 DIM were evaluated for postpartum disease screening. Animals with watery, foetid, red-brown uterine discharge, rectal temperature ≥39.2°C and clinical signs indicative of systemic illness were recorded as puerperal metritis. All diseases were treated by from veterinarian in accordance with standard treatments defined in the farm's herd health program. At 29-35 days postpartum (mean 32.52 ± 0.14 days) al apparently healthy cows (n = 162) were evaluated for diagnosis of endocervical inflammation using and adopted cytobrush technique as described be [16] with minor modifications. On short: sterile endocervical cytobrush used for preparation of cervical smear for diagnosing cervical cancer in women was modified as endocervical cytobrush in cows. This cytobrush was cut to o length of 6 centimeters and heat fixed on to a previously sharpened stylet of an artificial insemination gun. This device was inserted into an AI sheath (5 mm) covered by sanitary plastic sleeve for protection from vaginal contamination and sterilized by ultraviolet irradiation for 15 minutes. The cow was restrained, and the external genitalia was cleaned and the instrument lubricated with paraffin oil was inserted through the vagina into the external cervical OS, then pushed at second cervical ring. The sanitary sleeve was pushed forward to expose the cytobrush. The endocervical samples were collected by rotating the cytobrush in a clockwise direction and with gentle pressure applied on the cervix transrectally. After rotating the cytobrush on the mucosa, it was pulled back into the sanitary plastic sleeve, and the instrument was removed from the genital tract. The cytology slide was prepared by rolling the cytobrush onto a sterile microscope slide and fixed with 95% methanol for 2-3 minutes. The slides were stained using May-Grünvald-Giemsa standard procedure and were evaluated for the presence of PMN cells. The threshold of PMN cells to classify cows as positive for endocervical inflammation the detection of more than or equal to five percent neutrophilic cells in cytosmear [17, 18]. This limit is directly related to losses in conception rate [18].
All cows (n = 162) were artificial inseminated after 50 days in milk based on natural estrus detection using visual observation by farm and search staff for 30 minutes, three times daily. Between 40-50 days after AI the pregnancy diagnosis was made by transrectal palpation. All cows not pregnant were re-inseminated.
Statistical analysis
All data were entered, organized and summarized in Microsoft Excel program and used for analysis. Parity was categorized into primiparous and multiparous cows. Retained fetal membranes and puerperal metritis cases were divided as yes, and no. all statistical analysis were conducted using SAS version 9.4 (SAS Institute Inc.). Multivariable logistic regression modeling was used to asses' principal causal factors of developing endocervical inflammation in dairy cows and Kaplam-Meier curves were used to assess the number of days from calving to conception. Differences were considered statistically significant when p values were less than or equal to 0.05.

• Results and discussions

In total 162 cows were sampled. Average DIM at sampling was 32.52 ± 0.14 days and 58 (35.8%) cows were primiparous and 104 (64.2%) cows were multiparous. The main events recorded at calving and post calving in sampled cows were assisted calvings (37%), RFMs (failure to expel fetal membranes within 12 hours post calving) (19.7%) and puerperal metritis (within 18 days after calving) (21%).

Table 1. Descriptive statistic of data of endocervical inflammation in relation to some casual factors

	Healthy	Endocervical inflammation			
	n (%)	n (%)	Odds ratio	95% CI	p
Parity					
Primiparous (n=58)	58 (89.66)	6 (10.34)	Reference		
Multiparous (n=104)	92 (88.46)	12 (11.54)	1.13	0.4-3.19	0.40
Calving difficulty					
unassisted (n=102)	95 (93.14)	7 (6.86)	Reference		
assisted (n=60)	49 (81.67)	11 (18.33)	3.04	1.11-8.35	0.015
Retained fetal membranes					
no (n=130)	121 (93.08)	9 (6.92)	Reference		
yes (n=32)	23 (71.88)	9 (28.12)	5.26	1.88-14.67	0.0007
Puerperal metritis					
no (n=128)	120 (93.75)	8 (6.25)	6.25	2.23-17.46	0.0002
yes (n=34)	24 (70.59)	10 (29.41)			

Data were analysed from162 dairy cows (Holstein Friesian cows). The overall incidence of endocervical inflammation was 11.11% (18/162), and was higher in cows with puerperal metritis (29.41%; 10/34) and in cows with RFM (28.12%; 9/32). Numerically a higher development of endocervical inflammation was in multiparous cows than in primiparous cows (12 vs. 6), and in cows with assisted calvings than in cows without assisted calvings (11 vs. 7) (table 1).
The probability (odds) of developing a endocervical inflammation 29-35 days postpartum was higher in cows with puerperal metritis (odds ratio 6.25, 95% CI 2.23-17.46, p – value 0.0002) and in cows RFM (odds ratio 5.26, 95% CI 1.88-14.67, p value 0.0007). Also cows with assisted calvings has 3.04 (95% CI 1.11-8.35, p-value 0.015) higher odds to develop endocervical inflammation (table 1).
The prevalence of endocervical inflammation in primiparous cows (10.34%) was close to that in multiparous cows (11.54%).
For the analysis of the effect of endocervical inflammation on subsequent reproductive performances (only days to conception interval are presented in this report) a total of 162 cows were enrolled. The mean days open in cows without endocervical inflammation at 140 DIM was 106.6 ± 20.9 days and in cows with endocervical inflammation 122 ± 17.9 days (a prolonged of days open with 15.4 days; SE 5.1, 95% CI, 5.15-25.57, p value 0.0034) (table 2).

Table 2. Average survival rates by parity subgroups (a and b), and the difference between them.

	Days open		Hazard		Difference between means	SE	95% CI	p - value
	mean	±SD	Ratio	95%CI				
Cows healthy	106.5	20.9	Reference	-	-	-	-	-
Cows with endocervical inflammation	122	17.94	0.38	0.29-0.53	+15.4	5.1	5.15-25.97	0.0034

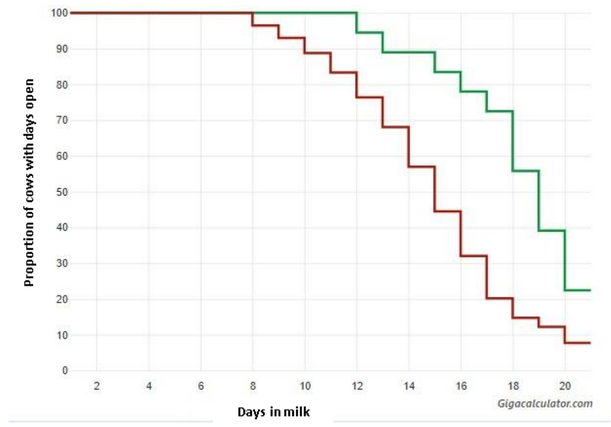

Figure 1. Time to event (conception) curves

Cumulative conception rates were platted and showed in figure 1. Cows with endocervical inflammation had more days open. A straight line seen in early part of the curves represented voluntary waiting period (vwp) for the cows without endocervical inflammation (50 days postpartum) and for cows with endocervical inflammation represented 50 days vwp and poor fertility in the period from 8 to 12 weeks after calving. After that time the slopes decreased but the number of cows with endocervical inflammation that remain open is increased as a results of poor fertility of these cows. Subtracting the proportion of pregnant cows from 100 results proportion of pregnant cows at any time p.p.
Discussion
In the present study we included cytological examination for the specific estimation of endocervical inflammatory condition in dairy Holstein Friesian cows. Our results show that the number of days open of dairy cow is affected by endocervical inflammation diagnosed by cytology method. In another study [18] showed that cytobrush sampling (3 or 4 for 30 days) did not induce any increase in neutrophil infiltration in cervix.
The neutrophil cells represent the primary leukocyte subpopulation in endocervical samples and are predictive of hazard to conception.
The threshold in this study supports the literature references with reported thresholds of more than or equal to 5% neutrophils [18, 15]. Using this threshold (≥5% neutrophils) we identified 11.1% of all cows with more or equal to 5% PMN (n18/162) and 88.9 (114/162) with less than 5% in the cervix at 20-35 days pp. The same prevalence of cervicitis (11% and 11.4%) was reported by Deguillaume et al. (2012) and Hartmann et al. (2016) [18 and 15]. In one study [19] prevalence of inflammatory processes in reproductive treats of crossbred dairy cow was 6% for cervicitis diagnosed by cytology technique.
Puerperal metritis and RFMs were associated with increased odds of endometrial inflammation. A previous study showed that uterine disease after pp period was a major causal factor for developing reproductive tract diseases [20].
It is generally accepted that inflammation of the endometrium has negative impacts on reproductive parameters, but little is known about cervicitis.
Hartman et al. (2016) [15] in their analysis revealed that a significantly high total conception rate was found in cows with C0 (normal cervix) and C1 (cervicitis with a swollen and prolapsed 2nd cervical fold) compared with cows with C2 (cervicitis with a swollen, reddened and prolapsed 2nd cervical fold) and the difference was independent of inflammation of the uterus [15].
Our results are in accordance with [18] which found negative effects of endocervical inflammation on the days open.

• Conclusions

The inflammation of cervix must be evaluated during postpartum period for prediction of subsequent reproductive performance.
The cytobrush sampling is necessary to confirm the diagnosis and a threshold of ≥5% of neutrophil cells can be used to separate cows with endocervical inflammation condition and cows without this condition.
The mechanism underlying why some cows with or without clinical evidence of disease in similar environment condition develop endocervical inflammation and other do not need to be established.
The findings of this study indicate which causal factors of endocervical inflammation should be monitored in pp dairy cows to limit the effects on consecutive reproductive performance.

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