Impact of Subclinical Endometritis on Some Fertility Traits of Dairy Cows

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Abstract
The aim of this of this retrospective study was to determine that most suitable Polymorphonuclear leukocytes (PMNs) threshold for diagnosis of subclinical endometritis (SE), the causes of this disorder and its effect on subsequent reproductive parameters of Holstein Friesian cows. Uterine cytology was executed on 140 Holstein cows at 28-30 days postpartum (pp) to calculate the PMN cells. A threshold of 16% PMNs above which some of the reproductive parameters were significantly affected was used. The retained fetal membranes (Odds ratio; OR = 3.02) and metritis (OR = 4.92) were the causes for SE. subclinical endometritis and metabolic disorders (milk fever and ketosis) affected the resumption of pp cyclicity at 28-30 days in milk. Cows with SE were less likely to conceive after their artificial insemination (OR = 0.44 $p<0.05$) than cows without S.E., and needed more artificial inseminations to conceive (2.4 vs. 2.1.). A PMN threshold of 16% was good enough to detect SE at 28-30 days pp. the RFMs and metritis were causal factors for SE.

Keywords: cytological endometritis, subclinical endometritis, polymorphonuclear cells, reproductive parameters, causal factors.

1. Introduction

Subclinical endometritis (SE) described as an inflammation of the endometrium without clinical signs and often without evidence of infection [1-3]. This uterine disease has been associated with in later time in restarting ovarian activity postpartum, extended intervals from parturition to first AI service, more days open, low conception rates, and high culling rates [4-6].
Subclinical endometritis has also been reported as "cytological endometritis" [7, 8] on the basis of "an elevated ratio of polymorphonuclear cells (PMN)" in endometrial cytology samples. In their report, Dubuc et al., (2010) [8], indicated that cytological endometritis and clinical endometritis may actually represent different manifestations of reproductive tract disease and inflammation, and not a difference in the gravity of the same disease.
The uterine pathogens affect reproduction by direct endometrial injury and by generating endotoxins [9]. The bacterial endotoxins have some effects on cow reproduction [10-12]. But, in the case SE, some authors reported that bacterial populations presented in the uterus of cows with SE did not differ from those presented in the healthy uterus of dairy cows [1, 3].
Some previous reports demonstrated negative impacts of elevated concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), bilirubin, and urea on the function of PMN [13, 14]. Also, in some past reports [15-19] showed that cows with SE had an increased endometrial mRNA expression and elevated serum concentration of pro-inflammatory mediators as compared with healthy cows.
In addition, the type of fatty acids present in cow's ration can affect cellular immune function [20].

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The diagnosis of SE can be due by endometrial cytology [19]. Also, ultrasonography was used, but in some reports ultrasonography method was less sensitive than endometrial cytology [19, 21, 22].

Different thresholds of PMN for diagnosis of SE in cows were accepted [23]. The cutoff PMN% reported has varied between 4 and 25% and has depending on the postpartum period at which the diagnostic was done (18% OMN at 20-33 days postpartum (pp); 10% PMN at 34-47 days pp and 5% PMN between 21-66 days pp [24].

The prevalence of SE reported in the literature in the literature in general, decreases with increasing time pp. Many affected cows recover spontaneously [25-27], and this can happen to four [28] to eight [29] weeks pp as a result pf the estrus. Circulating estradiol concentrations at estrus stimulate uterine motility and PMN function and this is an aid for eliminating abnormal fluid and get control of inflammatory changes [30].

Regarding the effects of SE on reproductive performance some authors [31, 32] did not reported significant effects on reproduction and many others reported a variety of negative effects on fertility [19, 7, 21, 8, 33, 34, 35, 32]. Affected parameters were: Days Open, Pregnancy rate, Postpartum anestrus, First-service pregnancy rate, Services per conception, transferable embryo recovery rate, culling rate, follicle development and ovulation, pregnancy rate at 150 and 250 days pp.

Also, clinical and subclinical endometritis have been associated with a declined milk production [36].

We hypothesized that the PMN threshold used to define SE and the timing of evaluation during pp period vary among herds and the choices of optimal PMN threshold should be calculated for each herd. The aim of this study was to determine the most suitable PMN threshold for diagnosis of SE, the risk factors and its effect on subsequent reproductive parameters of Holstein Friesian cows.

2. Materials and methods

2.1. Animals

Animals involved in this study were from Agricultural Research and Development Station (ARDS) Șimnic-Craiova (182 m above sea level 44°19’ N, 23°48’ E). The dairy farm of ARDS Șimnic-Craiova-Romania has a herd size of 280 Holstein Friesian dairy animals, and milk production of the cows was on average 9440 kg per 305 days of lactation.

All the cows were managed in loose housing with access to dry lot prepartum and postpartum. The cows are milked twice daily at 12 hours interval (morning and evening) in a DeLaval milking parlour.

Cows and heifers are fed in the morning and afternoon with diets formulated to meet or exceed nutritional requirements for dry and lactating dairy cows. The diets consist of high energy ration based on a grain by-products, grain mix and home-grown forages.

Additionally, the cows’ heifers have access to pasture (alfalfa or ryegrass). A prepartum and a postpartum diet is presented in table 1.

Table 1. Prepartum and postpartum diet (dry mother basis)

Item	Prepartum %	Postpartum %
A. Ingredients		
Alfalfa hay	26	29
Grass hay	45	5.5
Corn silage	10	16
Grain mix	16	47
Mineral mix	3	2.5
Total	100	100
B. Nutrient profile		
Net energy lactation	1.28	1.6
Crude protein	12	19
Neutral detergent fiber	46	28
Acid detergent fiber	40	20
Starch	14	27
Calcium	1.4	1.3
Phosphorus	0.3	0.4
Magnesium	0.4	0.4
Kalum	1.5	1.3
Natrium	0.3	0.4
Chloride	1.1	0.4
Sulf	0.4	0.2
DCAD mEq/100 of DM*	5	26.9

*Calculated as DCAD = (mEq of Na + mEq of K) – (mEq of S + mEq of Cl)

Pregnant cows are dried-off 50 ± 10 days before the expected calving date. In the prepartum pen cows are closely monitored by the farm or research personnel for signs of parturition. In the parturition pen all events are recorded.

The experiment was performed in compliance with the European Union Directive 86/609 Ec. and National legislation.
For this study, a total of 162 Holstein Friesian cows' parity 1-6 were enrolled. All this cows with calvings between 1st January 2021 and December 2022 were evaluated for uterine health status between 28 to 30 days in milk (DIM), using rectal palpation, vaginoscopy or transrectal ultrasonography (US). Cows in estrus were not used. Cows with signs of abnormal discharge of uterine fluid, abnormal fluid into the uterus or pathologic enlargement of the uterus were recorded as having clinical endometritis (n=22). Cow with no signs of endometritis were sampled for uterine cytology (n=140). Uterine cytology was performed using a cytobrush modified for use in large animals [37]. On short: The handle of the cytobrush is cut to 3 cm in length, threaded onto a stainless-steel red (65 cm length), and placed in a stainless-steel tube (5 mm in diameter and 50 cm length). The instrument is placed in a sanitary plastic sleeve for protection. The vulva is cleaned and the lubricated instrument is passed through vagina to external cervical OS; the sanitary plastic sleeve is punctured and the instrument is advanced through the cervix into the base of the larger horn, and at this point the stainless-steel tube is retracted to expose the cytobrush. The sample is collected by rotating the cytobrush (clockwise direction) on the uterine wall. Then the cytobrush is retracted into tube, and removed from the uterus.
The instrument can be steam sterilized for 4 minutes between uses. By rolling the cytobrush onto a clean microscope glass slide and allowed to air-dry, all slides are stained. We used a Diff-Quick stain kit, according to the manufacturer's instructions. All slides were examined under microscope (400 X magnification). Up to 200 endometrial cells and PMN were counted and the percentage of PMNs present was calculated per each slide.
Thea health disorders used in this study were: Calving difficulty, Retained fetal membranes, Septicemic metritis, Ketosis and milk fever. The veterinary waiting period from calving to first artificial insemination (AI) was 50 days. All cows were AI at detected estrus. Pregnancy diagnosis was made at 45-50 days after AI, based on transrectal palpation. Reproductive parameters were recorded up to 180 days pp or until pregnancy.

2.2. Study design

Calving difficulty was assessed according to the degree of assistance (1 = no assistance, 2 = easy assistance, 3 = using some force, 4 = using significant force and 5 = caesarian section). Cows with difficulty scores >2 were categorized as having dystocia [38].
Retained fetal membranes was recorded as retention for longer than 12 hours after calving. Metritis was recorded as watery, fetid uterine discharge during 10 days pp and fever (≥39.5°C) ketosis was recorded using clinical signs: depression, anorexia, and odor of acetone on the breath. Milk fever was recorded as recumbency after calving. The research personnel examined ovarian structures follicles, corpus luteum, and uterus by transrectal palpation and ultrasonography. Resumption of cyclicity within 28-30 days pp was recorded by detection of corpus luteum. First, we calculated the % PMN threshold, then prevalence of SE and casual factors for SE.

2.3. Statistical analysis

The data were entered into M. Excel computer program 2007.
Stata version 14 was used to express the results. The causal factors for SE and probabilities of resumption cyclicity, and conception after AI were calculated using logistic regression. Odds ratio, 95% Confidence Interval and p vale were calculated using logistic regression. A p value ≤ 0.05 was considered statistically significant.

3. Results and discussion

The threshold for PMN proportion, above which some of the reproductive parameters were significantly affected was 16%. Using this threshold at 28-30 days after parturition we described SE.
The prevalence of SE was 34.3% (48 from 140 cows). Some of the risk factors for SE are presented in table 2.

Table 2. Odds ratios (OR) of the risk factors for subclinical endometritis

Variables		% of SE incidence (Number of cows)	Odds ratio	95% CI	p value
Retained fetal membranes					
	No	15.21 (14/92)	Reference	1.44-6.34	0.00176
	Yes	54.16 (26/48)	3.020		
Metritis					
	No	10.87 (10/92)	Reference	2.043-11.848	0.0004
	Yes	37.5 (18/48)	4.920		
Dystocia					
	No	13.04 (12/92)	Reference	0.3337-2.7182	0.9273
	Yes	12.5 (6/48)	0.9524		
Metabolic disorders*					
	No	10.87 (10/92)	Reference	0.221 to 2.5150	0.6359
	Yes	8.33 (4/48)	0.7455		

SU = subclinical endometritis
CI = confidence interval
* = metabolic disorders consist of ketosis and milk fever.

The presence of retained fetal membranes (RFM) in cows after parturition and metritis during first 10 days postpartum significantly affected the incidence of SE (table 2). No effect for dystocia and metritis during first 10 days postpartum significantly affected the incidence of SE (table 2). No effect for dystocia and metabolic disorder ($p > 0.1$). Cows with RFM (OR = 3.02) and with metritis during 10 days postpartum (OR = 4.92) had a higher risk of SE than cows without SE (table 2).

Table 3. The probability of resumption of cyclicity within 28-30 days in milk.

Variables		% resumption rate (No. of cows)	Odds ratio	95% CI	p value
SE					
	No	47.82 (44/92)	Reference		
	Yes	20.83 (10/48)	0.3133	0.1396-0.7031	0.0049
RFM					
	No	65 (65/100)	Reference		
	Yes	50 (20/40)	0.5385	0.2560-1.1327	0.10
Metritis					
	Yes	58.03 (65/112)	Reference		
	No	71.42 (20/28)	1.8077	0.7337-4.4539	0.1981
Dystocia					
	No	57.4 (70/122)	Reference		
	Yes	55.55(10/18)	0.9286	0.3428-2.5154	0.8841
Metabolic disorders					
	No	53.96 (68/126)	Reference		
	Yes	71.42 (10/14)	4.6324	1.400-15.325	0.0120

SE = Subclinical endometritis;
RFM = Retained fetal membranes;
CI = Confidence interval.

Subclinical endometritis and metabolic disorders affected the resumption of postpartum cyclicity at 28-30 days in milk. Cows with SE had a decrease probability (OR = 0.31, $P<0.05$) of resumption of cyclicity after parturition than cows without SE (table 3).

Table 4. The probability of conception after the first insemination.

Variable		% conception rate at first insemination	Odds ratio	95% CI	p value
SE					
	No	45.65 (42/92)	Reference		
	Yes	27.1 (13/48)	0.4422	0.2073-0.9430	0.0347
RFM					
	No	46 (46/100)	Reference		
	Yes	30 (10/40)	0.3913	0.1729-0.8855	0.0243
Metritis					
	Yes	44.64 (50/112)	Reference		
	No	28.57 (6/28)	0.3382	0.1274-0.8980	0.0296
Dystocia					
	No	49.2 (60/122)	Reference		
	Yes	27.8 (5/18)	0.3974	0.1335-1.1830	0.0973
Metabolic disorders					
	No	62/126	Reference		
	Yes	4/14	0.3871	0.1151-1.3016	0.1250

SE = Subclinical endometritis;
RFM = Retained fetal membranes;
CI = Confidence interval.

Cows with SE were less likely to conceive after their first artificial insemination OR = 0.44, p < 0.05 than cows without SE (table 4). Also, cows with RFM and metritis had a decreased probability (OR 0.39, 9 < 0.05 and OR 0.33, p< 0.05 respectively) to conceive after first artificial insemination.
Cows with SE needed more artificial inseminations to conceive (2.4 ± 0.3) than cows without SE (2.1 ± 0.2). Also, cows with SE had a median of 41 days longer to conceive until 180 DIM compared with cows without SE.

4. Discussion

Our data show that a threshold ≥16% was valuable for monitoring the uterine health status during voluntary waiting period (VWP).
Kasimanickam et al., (2004) [19] used a threshold level at ≥18% PMN at 20 to 33 days postpartum. Barlund et al., (2008) [21] reported, a threshold at >8% PMN at 28 to 41 days pp. In a recent study Lee et al. (2018) [39] reported an optimal threshold ≥ 14% PMN.
There are diverse factors affecting cow's reproductive performance as nutrition, herd health program, cow production, and the climate or barn condition. In general, most authors used PMN % threshold of 15-18% for SE diagnosis at 21-30 days pp, and values of 4-10% for diagnosis at later periods [24].

The prevalence of SE in this study was 34.3%. The reported prevalence of SE varied between 7 and 53% [24].
In this report the retained fetal membranes and metritis were important factors for SE. The OR of 3.020 in this study, for the risk of a RFM was higher than the OR (1.87) reported by Lee et al. (2018) [38]. Gautam et al., (2009) [25] reported on OR of 4024. The OR (4.92) for metritis in this study was higher than the OR (3.07) reported by Lee et al. (2018) [39].
Retained fetal membranes offer a good natrium or bacterial growth. Also, metritis as chronic infection of the uterus causes uterine inflammation. Dystocia and metabolic disorders do not significantly affect for prevalence of SE.
In our study SE significantly affected some subsequent reproductive parameters. Endocrine disturbances in pp period induced a lower probability of cyclicity resolution within 28-30 days in milk.
Ovulation of dominant follicle can be inhibited as a result of bacterial endotoxins that suppress the release of gonadotrophin – releasing hormone and the luteinizing hormone [40].
The impact of milk fever on the health of dairy cows is noticeable. Martinez et al., (2012) [40] observed that cows with serum Ca < 8.59 mg/dl in at least one of the first 3 days pp had reduced neutrophil phagocytic and killing activities in vitro.

Low probability of conception after first AI, more AIs per conception and longer days to conceive until 180 days in milk in cows with SE in this report are comparable to the results of some previous [21, 7, 19, 39].

Prevalence of SE in a dairy farm may reflect the immune status of dairy cows.

4. Conclusions

The cytobrush technic can provide sufficient uterine cells to calculate % PMN threshold. The method is non-invasive and the samples acquired are representative for endometrial cells involved in the uterine immune defense system.

A PMN threshold of 16% was good to define SE at 28-30 days pp, above which there was a significant effect on some reproductive parameters.

Our results can be useful guidance for monitoring uterine health of dairy cows in the pp period.

The control of RFM and metritis after parturition it is required to prevent SE in dairy herd.

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