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Endometritis in Simmental cows: incidence, causes, and therapy options

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Abstract: The objectives of this study were to evaluate endometritis incidence and possible causes, and to assess the different therapy options. From a total of 1300 cows, endometritis was diagnosed in 23.07%, with clinical endometritis in 15.31% and subclinical endometritis in 7.77%. Previous dystocia resulted in 40.59% of the clinical endometritis and 47.52% of the subclinical endometritis ($P < 0.05$). Placental retention caused clinical and subclinical endometritis in 43.18% and 37.50% of the cows, respectively ($P < 0.05$). Body condition score 2 resulted in the highest endometritis rate and body condition score 3–4 resulted in the lowest endometritis rate ($P < 0.05$). Corpus luteum was found in 9.67% of the cows suffering from endometritis, while the rest developed various types of subfertility ($P < 0.05$). Three groups of cows (A, B, and C) with an almost equal number of clinical and subclinical endometritis diagnoses were formed. All of the groups received prostaglandin analogues (PGF Veyx Forte®, Veyx Pharma) intramuscularly 3 times at 11 day intervals. In group A, only prostaglandins were used; in group B, cephapirin (Metricure®, Intervet Schering-Plough) was administered into the corpus uteri simultaneously with the first 2 doses of prostaglandins; and in group C, the first 2 doses of prostaglandins were followed by the ozone product Riger Spray® (Novagen, Italy) applied into the uterus. The criteria applied for the therapy success were the number of cows conceived after the first artificial insemination and the number of days open. The best therapy results were achieved in groups C and B ($P < 0.05$), with the advantage of no milk and meat withdrawing period in the animals of group C.

Key words: Cattle, causes, cephapirin, endometritis, incidence, ozone

1. Introduction

Parkinson (1) reported that one of the most important causes of subfertility in dairy cows is endometritis (E). LeBlanc et al. (2) and Lewis (3) described E as inflammation of the uterus involving the endometrium. Bonnett et al. (4) and Bretzlaff (5) reported that E involves only the endometrium without any systemic signs. According to Kasimanickam et al. (6), E is inflammation of the endometrium associated with delayed uterine involution and poor reproductive performance classified into 2 categories: clinical (CE) and subclinical (SE). LeBlanc et al. (2) defined CE as the presence of purulent or mucopurulent uterine discharge detectable externally or in the anterior vagina and/or with a cervical diameter greater than 7.5 cm and a uterine-horn diameter of >8 cm after 30 days postpartum. According to Kasimanickam et al. (6) and LeBlanc et al. (2), SE can be defined as the presence of fluid (using ultrasonography) in the uterine lumen at 31 days postpartum or later, with a cervical diameter of between 5 and 7 cm and a uterine diameter of between 5 and 8 cm.

Bruun et al. (7) reported that problems such as dystocia and retained placenta (or retained fetal membranes; RFM) greatly increased the odds of metritis. According to Sheldon et al. (8) and Opsomer et al. (9), animals with greater bacterial growth in the uterus tend to develop different types of subfertility when compared to normal postpartum cows.

LeBlanc et al. (10), Kasimanickam et al. (11), Heuwieser et al. (12), and Bretzlaff (5) reported that various treatment approaches have been used, including parenteral administration or intrauterine infusion of antibiotics and intramuscular administration of prostaglandin analogues. A variety of antibiotics have been infused into the uterus of cows in attempts to treat postpartum infections. Kasimanickam et al. (11) reported that ceftiofur is suggested as an alternative if the patient does not appear to respond to penicillin administration. According to Adams (13), cephapirin is resistant to the action of penicillinase and is active in an anaerobic environment as present in an infected uterus.

Ozone (O_3) is unstable gas with a tendency of quickly transforming into oxygen and its antibacterial activity can be more effective than iodine and chlorine, as stated by Silva et al. (14). Data reported by Bocci (15) showed that bacteria, spores, and viruses are inactivated by O_3 after only few minutes of exposure. Ozone may act through

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mechanisms other than oxidation, including the activation of erythrocyte metabolism and immune cells, as reported by Zimran et al. (16), and as a disinfectant, especially for anaerobic bacteria. With bactericidal, as reported by Silva et al. (14), immune-stimulating, as reported by Zimran et al. (16), and antiinflammatory properties, as reported by Gretchkanev et al. (17), O_3 therapy has the potential to alleviate the clinical signs of E. Ogata and Nagahata (18) also reported that local administration of O_3 was successful in treating mastitis in dairy cows.

In the presented study, an attempt was made to establish the incidence of CE and SE and to evaluate the influence of the body condition score (BCS), previous dystocia, and RFM on their incidence. At the same time, different therapy option successes were compared using parental prostaglandin analogues (PGF Veyx Forte®, Veyx Pharma) with intrauterine cephapirin (Metricure®, Intervet Schering-Plough) or the O_3 product Riger Spray® (Novagen) in cows suffering from CE and SE, starting from day 30 to day 35 postpartum.

2. Materials and methods

2.1. Animals

A total of 1300 cows were enrolled in the study, conducted from 1 February 2007 to 1 June 2010 in central Croatia. The animals were kept at 43 commercial dairy farms with 65 ± 26 cows per farm, an average of 5150 ± 590 kg of milk, and similar management. The average age of the involved cows was 5.3 ± 2.7 years.

Exclusion criteria included receiving systemic antibiotic therapy within 7 days prior to involvement in the study, intrauterine therapy or reproductive hormone administration in the current lactation, abnormal internal genitalia (including adhesions), previous cesarean section, a BCS of <2, fever of any origin, and systemic diseases.

2.2. Examination protocol

The first postpartum examination was carried out between days 30 and 35 postpartum by vaginoscopy, transrectal palpation, and ultrasonography with a rectal linear probe (7 MHz, Draminski, PROFI L, Poland). During vaginoscopy, the vaginal content and cervical morphology were monitored. Following vaginoscopy, transrectal palpation of the reproductive tract was performed to determine the diameter, location, consistency, and symmetry of uterine horns; the presence of fluid in the uterus; and the presence of structures on the ovaries (follicle, luteinized follicle, corpus luteum (CL), cyst, and scars). In addition, ultrasonography was used for the measurement of the cervical size and diameter of the uterine horns with imaging of abnormal fluid in the uterine lumen. Normal fluid was considered as a clear fluid at the anterior vagina (using vaginoscopy), or in the uterine lumen with a heterogenic appearance, using ultrasonography. Based on the reproductive examination, the cows were grouped into 3 categories: CE, SE, and sound cows (noE). Cows with a cervical size of >7 cm, uterine diameter of >8 cm, and a purulent or mucopurulent discharge visible through the speculum were classified as having CE. Cows with a cervical size of <7 cm, uterine horns with diameter of 5–8 cm, and abnormal fluid in the uterus visible at ultrasonography were classified as having SE. Cows classified as sound had a cervical size of <7 cm, uterine diameter of <5 cm, and no abnormal discharge externally or in the uterus based on ultrasonographic findings.

Anovulatory estrus was confirmed by ultrasonography and transrectal palpation after finding the follicle of unchanged morphology on a given ovary 7 days after the onset of estrus, as described by Watson and Harwood (19).

Cystic ovarian disease (COD) was diagnosed when one or more fluid-filled structures were found on the ovaries, unilaterally or bilaterally, measuring more than 3 cm in diameter and persisting for 10 or more days with the absence of CL, according to the criteria previously established by Hanzen et al. (20). COD was diagnosed by transrectal palpation and confirmed by transrectal ultrasonography. In these cases, the difference between luteal and follicular cysts was not considered an important criterion for the diagnosis of COD.

According to previous reports that provided descriptive guidelines for diagnosing anestrus given by Peter (21), anestrus was confirmed for all of the cows/heifers presented with smooth, round to oval, and small ovaries (size of a pea) that failed to come into heat by 100 days postpartum.

The CL finding was established according to the report by Parkinson (1) using transrectal palpation and ultrasonography.

Each herd was visited once weekly for regular reproductive examinations, which included the detection and treatment of reproductive disorders, pregnancy diagnosis, artificial insemination (AI), and recording of the BCS at a scale of 1 to 5 with 0.5 increment points, according to Edmonson et al. (22). Cows that were 30 to 35 days postpartum were included in the study and examined until the confirmation of E was diagnosed, or AI or confirmed pregnancy. All data were recorded entering the study regarding the date of calving, calving condition (normal or dystocia: any difficulty in calving that required assistance, RFM), and age.

2.3. Group formation

Cows with confirmed E were classified into those with CE or SE based on the findings. After that, the animals were randomly divided into 3 groups (A, B, and C) based on the last 2 ear-tag numbers. The cows were examined and the groups were formed until there was almost an equal proportion of cows with 2 different stages of E in each group.

2.4. Treatment protocol

All of the groups received an intramuscular (IM) injection of 0.05 mg of cloprostenol (2 mL of PGF Veyx Forte) 3 times at 11 day intervals. The first injection was administered on the same day that the E diagnosis was established. The cows were artificially inseminated 3 days following the third injection of cloprostenol. Insemination was followed by the IM injection of 0.05 mg of gonadorelin (1 mL of Depherilin Gonavet Veyx®, Veyx Pharma). The cows were inseminated daily until transrectal confirmation of ovulation (disappearance of dominant follicle).

The cows in group A (n = 101) received only cloprostenol.

In group B (n = 96), 500 mg of cephapirin (Metricure) was administered into the uterus at the same time as the first and the second dose of cloprostenol.

The cows in group C (n = 103) received 20 mL of O_3 product (Riger Spray) into the uterus at the same time as the first and the second dose of cloprostenol.

Pregnancy was diagnosed 40 days following AI using ultrasonography. The number of pregnant cows following the first AI and number of days open in all of the groups were recorded for further data analysis.

2.5. Statistical analyses

Data were analyzed with the Statistica 8 statistical software program. Models were created with a multivariate logistic regression. The outcome measures were the number of animals conceived following the first AI and the number of days open. Variables included in the model were the E status (CE, SE, and noE), presence of CL, anovulatory estrus, anestrus, COD, BCS, previous dystocia, and RFM. Relevant interaction terms were also tested. Results were significant when P < 0.05.

3. Results

3.1. Endometritis incidence

Out of the 1300 observed cows, E was diagnosed in 300 (23.07%), with CE in 199 (15.31%) and SE in 101 (7.77%). As presented in the Figure, CE was almost 2-fold more frequent than SE.

3.2. Dystocia and endometritis incidence

Out of a total of 1300 cows, 101 (7.77%) suffered from dystocia at birth. Dystocia resulted in CE in 41 (40.59%) and SE in 48 (47.52%) cows (P > 0.05), while in 12 (11.88%) cows, E was not observed (P < 0.05). The results are presented in Table 1.

3.3. Placental retention and endometritis incidence

As presented in Table 1, out of the 1300 observed cows, 88 cows (6.77%) suffered from RFM during the previous birth. Consequently, only 19.3% of the cows suffering from RFM did not develop E (P < 0.05).

3.4. Body condition score and endometritis incidence

The highest CE and SE rate was noticed in cows having a BCS of 2, 2.5, 4.5, and 5 (P < 0.05), while the lowest CE and SE rate was recorded in cows having a BCS of 3 to 4 (P < 0.05). The results are presented in Table 1.

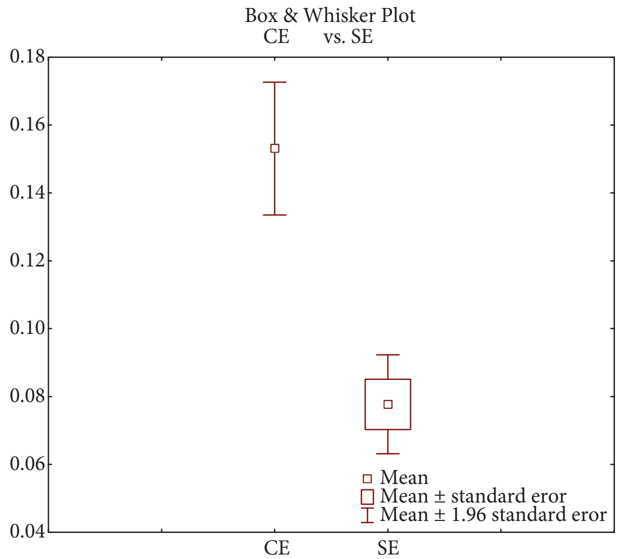

Figure. Clinical and subclinical endometritis (E) ratio over a 3-year period (n = 1300). Total sample n = 1300; CE, clinical E (n = 199); and SE, subclinical E (n = 101).

3.5. Endometritis and ovarian function

As shown in Table 2, the highest impact of CE and SE diagnosis on the ovarian function manifested with anestrus (P < 0.05). In cows with an E diagnosis, CL was found in only 9.67% of the animals (P < 0.05). When compared to the noE group, CL was found in almost half of the examined animals (P < 0.05).

3.6. Therapy success

As presented in Table 3, the most efficient therapy was achieved in groups B and C (P < 0.05), where 1.7 times more cows conceived following the first posttreatment AI and 1.5 times fewer days open were recorded when compared to group A.

4. Discussion

The objectives of this study were to evaluate the incidence of CE and SE, together with the influence of dystocia, BCS, and RFM on the E rate. Different treatment options were compared, including the parenteral application of hormones and intrauterine application of antibiotic (cephapirin) versus O_3 product on the outcome of E, measured by the first successful posttreatment AI and the number of days open.

The incidence of CE in the study conducted by LeBlanc et al. (2) was 16.9% and is similar to presented results (15.3%).

Increased incidence of postpartum E following abnormal calving (dystocia) is consistent with the results of Gautam et al. (23) and Markusfeld (24), but in contrast to those of Kim and Kang (25). Here, dystocia increased

Table 1. Influence of dystocia, retention of fetal membranes, and body condition score on the clinical and subclinical E incidence.

D

(Av ± SD)	%	CE (Av ± SD)	%	SE (Av ± SD)	%	noE (Av ± SD)	%
0.08 ± 0.27	7.77 (101/1300)	0.4 ± 0.49[a]	40.59 (41/101)	0.47 ± 0.5[a]	47.52 (48/101)	0.12 ± 0.32[b]	11.88 (12/101)

RFM

(Av ± SD)	%	CE (Av ± SD)	%	SE (Av ± SD)	%	no E (Av ± SD)	%
0.07 ± 0.25	6.77 (88/1300)	0.43 ± 0.5[a]	43.18 (38/88)	0.38 ± 0.49[a]	37.50 (33/88)	0.19 ± 0.4[b]	19.32 (17/88)

BCS

	%	CE (Av ± SD)	%	SE (Av ± SD)	%	no E (Av ± SD)	%
2	6.77[1] (88/1300)	0.58 ± 0.5[1]	57.95 (51/88)	0.26 ± 0.44[1]	26.13 (23/88)	0.16 ± 0.37[1]	15.91 (14/88)
2.5	9.07[1] (118/1300)	0.32 ± 0.47[2]	32.2 (38/118)	0.19 ± 0.39[2]	18.64 (22/118)	0.49 ± 0.5[2]	49.15 (58/118)
3	21.23[2] 276/1300	0.04 ± 0.19[3]	3.98 (11/276)	0.02 ± 0.13[3]	1.81 (5/276)	0.94 ± 0.23[3]	94.2 (260/276)
3.5	22.38[2] 291/1300	0.03 ± 0.17[3]	3.09 (9/291)	0.01 ± 0.12[3]	1.37 (4/291)	0.96 ± 0.21[3]	95.53 (278/291)
4	22.77[2] 296/1300	0.06 ± 0.24[3]	6.08 (18/296)	0.02 ± 0.14[3]	2.02 (6/296)	0.92 ± 0.27[3]	91.89 (272/296)
4.5	9.15[2] 119/1300	0.25 ± 0.44[4]	25.21 (30/119)	0.16 ± 0.37[2]	15.97 (19/119)	0.59 ± 0.49[2]	58.82 (70/119)
5	8.61[2] 112/1300	0.37 ± 0.49[2]	37.50 (42/112)	0.2 ± 0.4[2]	19.64 (22/112)	0.43 ± 0.5[2]	42.86 (48/112)

D, dystocia; RFM, retention of fetal membranes; BCS, body condition score; CE, average number of clinical E cases expressed with standard deviation (±SD); SE, average number of subclinical E cases expressed with ±SD; noE, average number of cows without E diagnosis (sound cows) expressed with ±SD; Av, average; SD, standard deviation; and %, percent of animals expressed as total number of animals in the respective subgroup in brackets.

[a, b]Values in each row marked with a different letter in superscript differ significantly ($P < 0.05$).

[1,2,3]Values in each column marked with a different number in superscript differ significantly ($P < 0.05$).

the odds of E. It can be presumed that dystocia can increase the risk of trauma to the uterine wall, thus increasing the odds of E. Moreover, calving assistance increases the risk of introducing infection. The presented results are also supported by the findings of Gröhn et al. (26).

E developed in 80% of the cows suffering from RFM. These study results are supported by the reports of Gautam et al. (23) that increased risk of E is connected with RFM because RFM may serve as a perfect medium for bacterial growth and increase the susceptibility for E. The presented findings are also supported by Bruun et al. (7), who reported that cows with RFM had higher odds of E irrespective of the breed.

The highest CE and SE rates were observed in the skinny as well as in the fat cows (having a BCS of 2 and 5). At the same time, the lowest CE and SE rates were recorded in cows having a BCS of 3 to 4. It can be speculated that the BCS had a significant influence on the E development, probably caused by different metabolic disorders, both in the skinny as well as in the fat cows.

E showed significant impact on the ovarian function. In the present study, less than 10% of the cows had normal cycles resulting in a CL finding, while the rest developed various types of subfertility. The presented results are supported by those of Sheldon et al. (8) and Opsomer et al. (9).

Table 2. Influence of clinical and subclinical E on the ovarian function and observed subfertility diagnosis.

Subfertility	CE (n = 199)	SE (n = 101)	E (CE + SE) (n = 300)	noE (n = 1000)
COD	0.23 ± 0.42[a]	0.26 ± 0.44[a]	0.16 ± 0.37[1]	0.05 ± 0.21[b1]
%	22.61 (45/199)	25.74 (26/101)	16.33 (49/300)	5.00 (50/1000)
AE	0.28 ± 0.45[a]	0.22 ± 0.42[b]	0.26 ± 0.44[2]	0.21 ± 0.41[b1]
%	27.63 (55/199)	21.78 (22/101)	25.66 (77/300)	21.00 (210/1000)
A	0.41 ± 0.49[a]	0.33 ± 0.47[b]	0.25 ± 0.43[2]	0.27 ± 0.44[c2]
%	40.70 (81/199)	32.67 (33/101)	24.67 (74/300)	27.00 (270/1000)
CL	0.09 ± 0.29[a]	0.2 ± 0.4[b]	0.09 ± 0.3[3]	0.47 ± 0.5[c3]
%	9.04 (18/199)	19.80 (20/101)	9.67 (29/300)	47.00 (470/1000)

CE, clinical E; SE, subclinical E; COD, cystic ovarian disease; AE, anovulatory estrus; A, anestrus; CL, corpus luteum; and E (CE + SE), sum of subfertility diagnoses expressed by the average and SD with percent in brackets.
[a, b, c]Values in each row marked with a different letter in superscript differ significantly (P < 0.05).
[1,2,3]Values in each column marked with a different number in superscript differ significantly (P < 0.05).

Table 3. Therapy success measured by the number of cows pregnant following the first posttreatment AI and the number of days open.

	Group A (n =101)	Group B (n = 96)	Group C (n =103)
Therapy success			
CP	51[a]	85[b]	91[b]
%	50.49	88.54	88.35
Av ± SD	0.4 ± 0.5	0.88 ± 0.32	0.88 ± 0.32
DO	108[a]	85[b]	80[b]
Av ±	108.25 ± 57.1	85 ± 32.78	79.91 ± 29.26

DO, days open; CP, cows pregnant following the first posttreatment AI; %, percent of pregnant animals in the respective group; Av ± SD, average with SD; Av ±, average expressed with ± (min. and max. values) in the respective group.
[a, b]Values in each row marked with a different letter in superscript differ significantly (P < 0.05).

The best therapy results were achieved in group C, where the animals were treated with prostaglandin analogues parenterally and O_3 product (as a novel treatment option) applied into the uterus (group C). This treatment option resulted in the highest number of cows conceived following the first posttreatment AI and the lowest number of days open. Slightly less effective, although not significantly, was the treatment combining prostaglandin analogues and cephapirin (group B). The lowest therapy success was achieved in group A. The results of the present study are supported by those of Hendricks et al. (27), who reported that treatment with prostaglandin analogues has no effect on the incidence of CE. The poor therapy result in group A could be the consequence of the ovarian status with the small number of cows having CL on their ovaries, and thus ineffective prostaglandin treatment. Obviously, some kind of treatment of the endometrium should be used in the treatment of E, coupled with hormone administration. As cows treated with O_3 showed an increase in the number of cows conceived following the first AI and decreased number of days open, this is likely due to its disinfecting effect coupled with an immunomodulative capacity of the O_3 at the level of contact with the uterine mucosa, as reported by Jakab et al. (28). Therefore, O_3 is likely to provide a more favorable uterine environment for insemination and fertilization by reducing the spermicidal effect and E-related inflammation. Ozone products, combined with prostaglandins, are slightly more effective than cephapirin in postpartum E treatment with the advantage of no milk (and meat) withdrawing period.

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