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## Ozone treatment of metritis and endometritis in Holstein cows

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### ABSTRACT

The aim of research was to investigate the frequency of metritis and endometritis, also the efficacy of ozone spray treatment in Holstein cows. The cows (n = 91) ranged from 2 to 7 years of age and were housed in two commercial dairy farms in the north-western region of Croatia. The study was conducted over the course of 1 year. The cows were divided into the three groups based on uterine findings and diagnosis (metritis, endometritis and control). The first group comprised cows suffering from metritis diagnosed on days 5 and/or 15 following parturition. The second group included cows diagnosed with endometritis on days 25 and/or 45 after parturition. The third group included animals without clinical signs of uterine inflammation. In cases when metritis or endometritis was established, ozone foam (Riger spray G) was inserted into the uterus. Ozone was applied by means of a vial containing ozonated foam under pressure for 5 seconds. The time to the first postpartal insemination was shorter for controls compared to the metritis and endometritis groups (P<0.05). The days open until pregnancy was the longest (133 days) in cows with endometritis (n = 28). In the control group (n = 41) the days open until pregnancy was 125 days and in the group of cows treated for metritis (n = 22) was 120 days. Conception rates for the first, second and third groups were 1.86, 2.21 and 1.90, respectively. The intrauterine ozone flush therefore has potential to alleviate metritis and endometritis as an efficacious and cost-effective treatment option with an overall positive effect on fertility and the host regarding tissues in Holstein cows.

**Key words:** Holstein, ozone, spray, therapy, uterus

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### Introduction

On dairy cows farms a constant effort is being made to increase milk production, however reproduction capabilities are drastically decreasing (DOBRANIĆ et al., 2008;

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SAMARDŽIJA et al., 2008; KOČILA et al., 2013). Diseases that decrease fertility in cows are periparturient diseases such as: puerperal fever, retained fetal membranes, metritis, ketosis, mastitis, lameness and others (MARKUSFELD, 1987; LAVEN and PETERS, 1996; ĐURICIC et al., 2011a; ŽILAITIS et al., 2013). According to GAUTAM et al. (2009) metritis and endometritis are the most significant causes of subfertility in dairy cows. Dystocia (DEMATAWEWA and BERGER, 1997), retained fetal membranes, metabolic disorders, inadequate management and poor hygienic conditions (LEVIS, 1997; SHELDON and DOBSON, 2004; HAN and KIM, 2005; KÖNYVES et al., 2009; ZOBEL et al., 2012) are the most significant predisposing factors for metritis and endometritis in cows. The inflammation of the uterus after parturition is the consequence of dystocia in 10 to 50% cows (LEWIS, 1997), and in approximately 25% of cows with retained fetal membrane (JOOSTEN et al., 1988). Frequently, uterus inflammation decelerates uterus involution and the recurrence of ovarian cyclicity (LAVEN and PETERS, 1996; GRÖHN and RAJALA-SCHULTZ, 2000; MAIZON et al., 2004).

Puerperal metritis (PM) may occur in between four to 21 days after parturition. The uterus is filled with odorous, red-brown content mixed with necrotising putrid tissue (SHELDON and DOBSON, 2004). Endometritis is inflammation of the mucous membrane of the uterus, with mucous, mucoso-purulent to purulent discharge from the vagina, that appears three weeks after parturition or later (TOMAŠKOVIĆ et al., 2007; TURK et al., 2011). The external signs of the disease can be seen during the first ovulation or after ovarian cyclicity recurrence (SHELDON et al., 2004). Clinically, endometritis is observed in 15-20% of cows four to six weeks after parturition and in 30-35% subclinical endometritis is noted between four to nine weeks after parturition (LEBLANC, 2008). Acute endometritis and metritis are caused by coliform G negative anaerobic bacteria, *Arcanobacterium pyogenes* and others (BEKANA et al., 1994; HUSZENICZA et al., 1999; DOHMEN et al., 2000; AZAWI, 2008). Antibacterial broad spectrum agents for topical application (intrauterine), in comparison to the ozone agent, cause loss of activity in the lochia or the presence of pus, residua is retained in the milk and meat, and bacterial resistance occurs (ĐURIČIĆ et al., 2012b). Ozone breaks through the microorganism (bacteria and germs) cell membrane, and also destroys viruses by diffusing through the protein coat in the nucleic acid core, resulting in damage of the viral nucleic acid. The advantage of ozone use is how it blocks microorganism resistance in contrast to antibiotics which may generate resistance (SCROLLAVEZZA et al., 1997). The use of ozone in veterinary medicine is in the form of a foam and pearls in order to treat endometritis and metritis (SCROLLAVEZZA et al., 1997; MARUSI et al., 1999; MARUSI et al., 2000; ĐURIČIĆ et al., 2012a). The puerperal period considerably affects cows' fertility, thus the goal of our research was to determine the effect of ozone treatment on metritis and endometritis.

### Materials and methods

Altogether 91 Holstein dairy cows, at the age of two to seven years were included in this research. The cows were kept on two medium sized dairy farms in the north-western region of Croatia. On the participating farms, 90 and 110 cows respectively were housed in free-stall barns with straw bedding. The cows were fed corn and grass silage, and concentrate on each farm and milked twice daily. The herd's average milk yield was 8900 and 9100 kg per lactation. The research was conducted over the course of one year. The animals were divided into three groups based on the uterine findings and diagnosis (metritis, endometritis and control). The first group comprised cows suffering from metritis diagnosed on days 5 and/or 15 following parturition. The second group included cows diagnosed with endometritis on days 25 and/or 45 after parturition. The third group included animals without clinical signs of uterine inflammation. In cases when metritis or endometritis was diagnosed ozone foam (Riger spray G) was applied into the uterus. Ozone was applied by means of a vial containing ozone foam under pressure for 5 seconds. In the third group there were cows without signs of uterus inflammation. All cows underwent vaginal and transrectal examination on days 5, 15, 25 and/or 45 following parturition. The cows were inseminated with frozen-thawed semen by experienced inseminators at the owner's invitation, and after vaginal examination, transrectal uterine palpation and/or transrectal ultrasonographic controls. All cows were examined gynaecologically when the appearance of the cervical mucus was considered in particular. The animals were examined every 3 weeks and 45 days after insemination by transrectal ultrasonography with a 5 MHz linear-array transducer (SonoVet 2000, Medison, Seoul, South Korea) until confirmation of pregnancy. In terms of management and feeding, both farm were very similar. Animals with postpartal fever (cows with body temperature higher than 39.5 °C) within the first 6 postpartal days were treated with antibiotics (parenteral treatment) consisting of 1 mg of ceftiofur/kg of BW (Excenel RTU, Pfizer Animal Health, Germany).

Data were analysed using the statistical software program StatSoft Tulsa, Statistica, 7.1, using ANOVA and Tukey's test post hoc analysis including days open until pregnancy (DOP) and number of days until the first insemination following partus (DOFS). Statistically significant results were at  $P < 0.05$ .

### Results

Out of 91 cows involved, from both farms, metritis was diagnosed in 22, endometritis in 28, while 7 animals both metritis and endometritis were diagnosed. These 7 cows were classified in the group with metritis. At the same time, in 41 cows no signs of metritis and endometritis were found. Conception rates in the group of cows having metritis, endometritis and controls were 1.86 ( $n = 22$ ), 2.21 ( $n = 28$ ) and 1.90 ( $n = 41$ ), respectively. The highest number of days open until pregnancy was recorded for the group of cows

suffering from endometritis (133.50), followed by controls (125.02) and the group of cows having metritis (120.41) ( $P < 0.05$ ). The longest interval from partus until the first insemination was recorded for the group of cows diagnosed with endometritis (106.86), followed by those suffering from metritis (102.77), while the shortest period was noted for controls (92.85) ( $P < 0.05$ ). The results are presented in Table 1.

Table 1. The time from the first insemination and the days open until pregnancy in Holstein cows with metritis ( $n = 22$ ), endometritis ( $n = 28$ ) and the control group ( $n = 41$ )

		Mean	Min	Max	SD
Time until first insemination	Metritis	102.77 <sup>a</sup>	48	215	43.68
	Endometritis	106.86 <sup>a</sup>	65	163	31.78
	Control	92.85 <sup>b</sup>	45	187	32.19
Days open until pregnancy	Metritis	120.41 <sup>a</sup>	65	245	46.11
	Endometritis	133.50 <sup>b</sup>	89	241	31.54
	Control	125.02 <sup>c</sup>	56	190	38.43

Values within the same column differ significantly with different exponent ( $P < 0.05$ )

## Discussion

There have been a few publications on the topic of using ozone for uterus therapy in cows (SCROLLAVEZZA et al., 1997; MARUSI et al., 1999; MARUSI et al., 2000). Ozone was used in clinically healthy cows with physiological puerperium (ĐURIČIĆ et al., 2011b; ĐURIČIĆ et al., 2012a; ZOBEL et al., 2014), successfully to shorten the days open until pregnancy (DOP) and the time of the first insemination after parturition (DOFS). Intrauterine ozone treatment alone or combined with parenteral antibiotics was a more efficacious treatment for retained placenta in cows when compared to hormonal and parenteral antibiotic treatment modalities (ĐURIČIĆ et al., 2012b; ZOBEL and TKALČIĆ, 2013).

The uterus in most cows after parturition is exposed to bacterial contamination. The frequency of metritis and endometritis is related to the time of occurrence of the disease, examination and the possibility of rectal palpation for inflammation detection (SHELDON et al., 2004; GILBERT et al., 2005). The presence of a muco-purulent discharge after vaginoscopy and the presence of inflammatory cells in flushing fluid from the uterus are the most precise diagnostic methods. In the United States the frequency of clinical and subclinical endometritis is about 53% in the period from the 40<sup>th</sup> to the 60<sup>th</sup> day after parturition (GILBERT et al., 2005). In our research that percentage was lower (about 20%), owing to fewer animals included in the research, and management was adequate on both farms, the individual approach to each animal, and hygiene conditions were mostly good. Of all the cows included in the research, those suffering from metritis were treated with

ozone and the inflammation did not proceed to endometritis and the chronic phase, with the exception of seven cows. In Canada there is a lower frequency of subclinical and clinical endometritis in comparison to our results, it is 17% (n = 1865), whereas in Ireland 24% are affected (n = 6500) (MEE and BUCKLEY, 2003; LEBLANC, 2008). Nine cows were diagnosed with purulent endometritis and in these cases, it would be favourable to retry therapy with ozone (according to the manufacturer's recommendation) at least twice in a three week period in accordance with the oestrus cycle. MAIZON et al. (2004) found no positive correlation between metritis/endometritis and prolonged days until the first post-partum insemination. In contrast to previous authors, EICKER et al. (1996) and SURIYASATHAPORN et al. (1998) reported a significantly prolonged period for the first post-partum insemination in cows with diagnosed endometritis which is in agreement with the results of present study. According to CILEK (2009), in Holstein cows in Turkey had DOFS of  $111.55 \pm 3.73$ , and DOP  $149.60 \pm 4.36$  which is significantly longer compared to our study results. Puerperal metritis may occur four days after parturition until the 21st day, while endometritis may occur only after 21 days of the puerperal period (GILBERT et al., 2005). Cows with metritis were treated earlier than cows in the second group. That is why the first group of cows (cows with metritis) had better results and shorter DOP and DOFS than the second and control groups. ĐURIČIĆ et al. (2012a) established that preventive intrauterine application of a foam ozone (spray) can improve fertility in Holstein (n = 404) and Simmental cows (ĐURIČIĆ et al., 2011b). It may be concluded that intrauterine ozone treatment in cases of metritis and endometritis acts beneficially on overall fertility by shortening days open and days from calving to first post-partum insemination in Holstein cows.

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**ĐURIČIĆ, D., M. LIPAR, M. SAMARDŽIJA: Liječenje metritisa i endometritisa krava ozonom. *Vet. arhiv* 84, 103-110, 2014.**

**SAŽETAK**

Cilj je istraživanja bio ustanoviti učestalost pojavnosti metritisa i endometritisa te učinak liječenja tih upala ozonom u obliku spreja (pjene). Krave holštajnske pasmine (n = 91), starosti od dvije do sedam godina, bile su smještene na dvije farme mliječnih krava na području sjeverozapadne Hrvatske. Istraživanje je trajalo godinu dana. Krave uključene u istraživanje bile su podijeljene u tri skupine na osnovi nalaza u maternici i dijagnoze: metritis, endometritis i kontrola. U prvoj skupini bile su obuhvaćene krave s metritisom koji je dijagnosticiran 5 i/ili 15 dana nakon porođaja. U drugu su skupinu bile uključene krave kojima je dijagnosticiran endometritis 25. i/ili 45. dana nakon porođaja, a u trećoj su skupini bile krave bez kliničkih znakova upale maternice. U slučajevima gdje je dijagnosticiran metritis ili endometritis, prilikom liječenja u maternicu je primijenjen ozonski pripravak u obliku spreja (pjena) (Riger spray G). Ozon u obliku spreja bio je primijenjen u maternicu u trajanju više od pet sekundi. Vrijeme do prvog osjemenjivanja poslije porođaja bilo je kraće u krava kontrolne skupine u usporedbi s kravama koje su imale metritis i endometritis ( $P < 0,05$ ). Najdulje trajanje servis perioda (133 dana) bilo je zabilježeno u krava s endometritisom (n = 28). U kontrolnoj skupini (n = 41) servis period je iznosio 125 dana, a u skupini krava s metritisom (n = 22) 120 dana. Indeks osjemenjivanja u prvoj skupini iznosio je 1,86, u drugoj 2,21 i u trećoj skupini 1,90. Zaključili smo da intrauterina terapija ozonom može imati povoljan utjecaj na smanjenje pojavnosti metritisa i endometritisa, a posljedično tome i na poboljšanje reproduktivne učinkovitosti kao i smanjenje troškova liječenja krava holštajnske pasmine.

**Ključne riječi:** krave holštajnske pasmine, ozon, sprej, liječenje, maternica

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