

Intrauterine ozone administration for improving fertility rate in Simmental cattle

Robert Zobel^{1*}, Robert Martinec², Duško Ivanović³, Nikola Rošić⁴, Zdravka Stančić⁵, Ivica Žerjavić⁶, Berislav Flajsig⁷, Helena Plavec⁸, and Ozren Smolec⁹

¹*Sisak-Moslavina County, Department of Agriculture and Forestry, Sisak, Croatia*

²*Veterinary practice Bosgen d.o.o., Zagreb Croatia*

³*Veterinary practice Daruvar, Daruvar, Croatia*

⁴*Veterinary practice Jastrebarsko, Jastrebarsko, Croatia*

⁵*Veterinary practice Grubišno Polje, Grubišno Polje, Croatia*

⁶*Veterinary practice Duga Resa, Duga Resa, Croatia*

⁷*Geo Agro Farm, Novi Grabovac, Croatia*

⁸*Veterinary practice Gejzir, Dubrava, Croatia*

⁹*Clinic for Surgery, Orthopaedics and Ophthalmology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

ZOBEL, R., R. MARTINEC, D. IVANOVIĆ, N. ROŠIĆ, Z. STANČIĆ, I. ŽERJAVIĆ, B. FLAJSIG, H. PLAVEC, O. SMOLEC: Intrauterine ozone administration for improving fertility rate in Simmental cattle. Vet. arhiv 84, 1-8, 2014.

ABSTRACT

The objective of the study was to determine whether fertility in cows can be improved by postpartal intrauterine ozone administration. The cows were divided into two groups - treatment (T) and control (C). The treatment group received 20 mL of ozonated foam into the uterus within 6 hours after calving and 24 hours later. Control group received no treatment. The outcome measurements were the number of days open and the number of artificial inseminations until pregnancy. The ozone was found to be effective treatment modality, resulting in a shorter period of days open (75.47 vs. 98.03, $P < 0.05$), and fewer inseminations until pregnancy (1.68 vs. 2.42, $P < 0.05$) in treated cows compared to the control group. Postpartal intrauterine ozone flush, coupled with intracornual insemination represents an effective treatment option that could lead to an improved fertility rate in Simmental cows.

Key words: cow, ozone, fertility

*Corresponding author:

Robert Zobel, PhD, DVM, Trg grofova Erdödy 19, 44317 Popovača, Croatia, Phone: +385 91 3021 968, E-mail: zobel.robert@gmail.com

Introduction

Before parturition, the uterine lumen is sterile. During parturition, the physical barriers of the cervix, vagina and vulva are compromised, providing an opportunity for bacteria to ascend the genital tract from the environment. Postpartum uterine health is more likely to be compromised in cattle than other ruminants (REGASSA and NOAKES, 1999; SHELDON et al., 2002). The reasons for the species differences are not clear, as sheep, goats and cattle inhabit similar environments, and the progress of uterine involution is similar (REGASSA et al., 2002; SHELDON and DOBSON, 2003).

The innate immune system is responsible for combating bacterial contamination of the uterus (NOAKES et al., 1991). The main phagocytic barrier is provided by the invasion of neutrophils in response to bacterial challenge, and the inflammatory barriers include non-specific defence molecules (i.e. acute phase proteins, defensins, and lactoferrin) (ZERBE et al., 2000; SHELDON et al., 2002). However, the functional capacity of neutrophils is reduced after parturition in many cattle (ZERBE et al., 2000), and may predispose to uterine disease (BEUTLER et al., 2003).

Uterine bacterial infection compromises animal welfare, as well as causing subfertility and infertility (BONNETT et al., 1993). Uterine inflammation and/or infection not only influences ovarian cyclicity, but also disrupts the establishment of pregnancy by the physiological presence of pus, and by altered immune responses that are essential at the interface between the endometrium and the embryo (SHELDON et al., 2002). Also, infections of the uterine mucosa are associated with poor reproductive performance (BONNETT et al., 1993), a 20% lower conception rate, and 30 more days open (LeBLANC et al., 2002). In addition, the subfertility associated with uterine infection involves disruption of ovarian function as a risk factor for delayed ovulation (OPSOMER et al., 2000), slower follicular growth rate (SHELDON et al., 2002), and increased incidence of follicular cysts (PETER et al., 1989).

The most common method of uterine treatment is either intrauterine (LeBLANC et al., 2002; KASIMANICKAM et al., 2005; GALVÃO et al., 2009) or systemic antibiotic administration (LeBLANC et al., 2002). A variety of intrauterine antimicrobial agents and antiseptic chemicals, systemic antibiotics, and hormone therapy have been introduced in the field (SHELDON and NOAKES, 1998; RISCO et al., 2007; ŽILAITIS et al., 2013).

Ozone (O₃) is an unstable gas with a tendency for quickly transforming into the oxygen and its antibacterial activity may be more effective than iodine and chlorine (SILVA et al., 2009). As reported by BOCCI (1996), bacteria, spores, and viruses are inactivated by ozone after only a few minutes' exposure. Ozone acts through various mechanisms including the activation of erythrocyte metabolism and immune cells, and as a disinfectant, especially against the anaerobic bacteria. With bactericidal (SILVA et al., 2009), immune-stimulating (ZIMRAN et al., 2000) and anti-inflammatory properties (GUENNADI et al., 2010), O₃ therapy

has potential to alleviate the clinical signs of endometritis. In addition, as reported by ZOBEL et al. (2012), ZOBEL (2013), and ZOBEL and TKALČIĆ (2013) ozone has been found to be more effective in the treatment of urovagina, endometritis and retained placenta in dairy cows, compared to hormonal and/or antibiotic treatment, with no negative effect on the host regarding residues.

The aim of the present study was to evaluate the effect of intrauterine ozone flush, during the first two days postpartum, on the fertility rate in cattle, measured by the number of days open and the number of artificial inseminations until pregnancy.

Materials and methods

Animals. A total of 400 cows of the Simmental breed were enrolled in the study conducted from February 1, 2010 to June 1, 2012 in central Croatia. The animals were kept on 5 commercial dairy farms, with 65 ± 26 cows per farm, an average of 4850 ± 390 kg of milk and similar management. The average age of the observed cows was 5.3 ± 2.7 years.

Exclusion criteria included receiving systemic antibiotic therapy within 7 days prior to calving, abnormal internal genitalia (including adhesions), BCS <2.5 , measured according to EDMONSON et al. (1989), fever of any origin (rectal temperature >40 °C), systemic diseases, retention of foetal membranes, any kind of dystocia, including caesarean section, puerperal mastitis and/or increased somatic cell count $>200,000/\text{mL}$.

Only cows following the third partus were involved in the study, in order to avoid age and parity as variables in statistical analysis.

Experimental protocol. Two groups (T, $n = 200$ and C, $n = 200$) of animals equally distributed among the farms were created. Cows were randomly assigned to the groups based on the last ear tag number (odd number: group C, even number: group T). Cows in group T received 20 mL of ozonated foam (Riger Spray[®], Novagen, Parendzana, Italy) into the uterus within 6 hours post partum and 24 hours later. Animals in group C received no therapy. The rectal temperature was measured daily, and in cases of fever within seven days after calving, the animals were treated according to findings and excluded from the study.

Artificial insemination. The voluntary waiting period for oestrus to occur was 120 days following calving. Animals that failed to come in heat by that time were transrectally examined and treated according to the diagnosis, using prostaglandin and GnRH analogues in order to stimulate heat, and they were excluded from the study. The onset of oestrus was detected and documented by experienced farm managers, who were instructed to monitor the cows throughout the day (a minimum of three times daily, for 25 minutes each) for changes in behaviour, and characteristic clinical and gynaecological signs of oestrus. Veterinary clinical examination techniques followed and included vaginoscopy

and transrectal palpation. Semen originating from six bulls of the Simmental breed was used. An attempt was made for an equal number of semen doses originating from the same bull to be used in all the involved farms, and also regarding the quartile of the year, in order to avoid season as a factor in data analysis. Artificial inseminations were performed daily using thawed frozen semen (thawed in water at a temperature between 35 °C and 37 °C over 60 seconds). Semen was deposited into the uterine horn ipsilateral to the ovary bearing dominant follicle. Artificial inseminations were performed daily until positive confirmation of ovulation (disappearance of the dominant follicle). Rebreeding cows were reinseminated with the semen originating from the same bull a maximum of the four times, and after that they were excluded from the research and treated or culled, according to the findings and the suggestions of the owner. One of the six experienced veterinarians performed all artificial inseminations and gynaecological examinations.

Statistical analysis. The number of days open and the number of artificial inseminations until pregnancy were analysed using categorical data modelling (STAT SOFT, Tulsa, USA). The Cox proportional-hazards regression model was used to evaluate the relationships between the groups, regarding the number of days open and the number of artificial inseminations until pregnancy. Results were considered significant when $P < 0.05$.

Results

As presented in Table 1, the cows in the treatment group had 22.56 (1.3 times) fewer days open and an average of 0.74 (1.44 times) fewer artificial inseminations until pregnancy compared to the animals in the control group ($P < 0.05$). Furthermore, the cows in the control group were 33% (HR 0.31 vs. 0.64) and 45% (HR 0.23 vs. 0.68) slower to become pregnant compared to those of the treatment group. Postpartum intrauterine ozone treatment was found to be statistically significant for days open and the number of artificial inseminations until pregnancy.

Table 1. The influence of group on the number of days open and the number of artificial inseminations until pregnancy

Group	Days open	HR	AI number	HR
T (n = 200)	75.47 ± 31.15 ^a	0.31 (0.22 - 0.38)	1.68 ± 1.61 ^a	0.23 (0.17 - 0.31)
C (n = 200)	98.03 ± 28.96 ^b	0.64 (0.53 - 0.71)	2.42 ± 1.58 ^b	0.68 (0.44 - 0.60)

T = treatment group; C = control group; days open = days from calving until pregnancy expressed as average and standard deviation (±); nAI = number of artificial inseminations until pregnancy expressed as average and standard deviation (±); HR = hazard ratio. ^{a,b} = values within columns marked with different letter in superscript differ significantly ($P > 0.05$)

Discussion

Fairly recent in being considered as an antiseptic treatment option in medicine, ozone (O_3) was considered for intrauterine application for the treatment of retained placenta, urovagina and endometritis in cattle (ZOBEL et al., 2012; ZOBEL and TKALČIĆ, 2013; ZOBEL, 2013).

The objectives of the present study were to evaluate the influence of postpartal intrauterine ozone administration on fertility in Simmental cattle, measured by the number of days open and number of artificial inseminations until pregnancy. As the cows treated with intrauterine ozone flush within 6 hours after calving and 24 hours later showed significantly higher fertility, namely fewer days open and fewer artificial inseminations until pregnancy compared to the untreated group (controls), the opinion of the authors is that this is the result of the disinfecting effect, coupled with the immunomodulative capacity of the ozone, at the level of contact with vaginal mucosa (JAKAB et al., 1995).

As has been reported in people with in-vitro fertilization failure, subclinical endometrial infection has a role in implantation failure, spontaneous abortion, and preterm birth, due to the effects of bacterial endotoxins and inflammatory mediators produced by the host: cytokines and chemokines (ROMERO et al., 2004). Thus, it may be suggested that the ozone product acted as a non-irritant disinfectant in the uterus in the present study.

Ozone therapy has been reported as a successful treatment option in medicine and dentistry, due to its potent antimicrobial activity for a wide range of micro organisms, and based on its high oxidation potential and its fast transformation into free oxygen (BIALOSZEWSKI et al., 2010). Ozone also stimulates host immunity by activating erythrocyte metabolism and local tissue immunity through an increase in lysozyme activity, IgA and cervical mucus myeloperoxidases levels, with a simultaneous decrease in IgM and IgG, resulting in a balanced coefficient of local immunity factors (GUENNADI et al., 2010). Our results indicate that ozone treatment proved to be effective in providing a microenvironment favourable for successful conception and pregnancy. In this case, O_3 was able to mitigate possibly undiagnosed endometrial inflammation, suggesting that O_3 may also be considered as a treatment option for other diseases that cause subclinical or clinical endometritis.

The low average number of days open (98) and the low number of artificial inseminations until pregnancy (2.42) in controls may be attributed to the above mentioned exclusion criteria, and the fact that artificial inseminations were performed daily until confirmation of ovulation with semen deposited into the uterine horn ipsilateral to the ovary with a dominant follicle. Despite this fact, the cows in the treated group showed significantly higher fertility measured by the same data.

Obviously, some form of local treatment of the postpartal uterine mucosa should be used in cows to prevent mucosal infection and/or inflammation and related subfertility. The ozone product proved to be efficient in improvement of fertility in cows with the advantage of no milk and meat withdrawal period.

References

- BEUTLER, B., K. HOEBE, X. DU, R. J. ULEVITCH (2003): How we detect microbes and respond to them: the Toll-like receptors and their transducers. *J. Leukoc. Biol.* 74, 479-485.
- BIALOSZEWSKI, D., E. BOCIAN, B. BUKOWSKA, M. CZAJKOWSKA, B. SOKÓŁ-LESZCZYŃSKA, S. TYSKI (2010): Antimicrobial activity of ozonated water. *Med. Sci. Monit.* 16, 71-75.
- BOCCI, V. (1996): Ozone as bioregulator: pharmacology and toxicology of ozone therapy today. *J. Biol. Regul. Homeost. Agents.* 10, 31-53.
- BONNETT, B. N., S. W. MARTIN, A. H. MEEK (1993): Associations of clinical findings, bacteriological and histological results of endometrial biopsy with reproductive performance of postpartum dairy cows. *Prev. Vet. Med.* 15, 205-220.
- EDMONSON, A. J., I. J. LEAN, L. D. WEAVER, T. FARVER, G. WEBSTER (1989): A body condition scoring chart for holstein dairy cows. *J. Dairy Sci.* 72, 68-78.
- GALVÃO, K. N., L. F. GRECO, J. M. VILELA, M. F. FILHO, J. E. P. SANTOS (2009): Effect of intrauterine infusion of Ceftiofur on uterine health and fertility in dairy cows. *J. Dairy Sci.* 92, 1532-1542.
- GUENNADI, O. G., O. V. KATCHALINA, H. EL-HASSOUN (2010): The New Method of Treatment of Inflammatory Diseases of Lower Female Genital Organs. *Congreso Mundial Ozono, Londres*, pp. 48-51.
- JAKAB, G. J., E. W. SPANNHAKE, B. J. CANNING, S. R. KLEEBERGER, M. I. GILMOUR (1995): The effects of ozone on immune function. *Environ. Health Perspect.* 103, 77-89.
- KASIMANICKAM, R., T. F. DUFFIELD, R. A. FOSTER, C. J. GARTLEY, K. E. LESLIE, J. S. WALTON, W. H. JOHNSON (2005): The effect of a single administration of cephalixin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. *Theriogenology* 63, 818-830.
- LeBLANC, S. J., T. F. DUFFIELD, K. E. LESLIE, K. G. BATEMAN, G. P. KEEFE, J. S. WALTON, W. H. JOHNSON (2002): Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.* 85, 2223-2236.
- NOAKES, D. E., L. WALLACE, G. R. SMITH (1991): Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms. *Vet. Rec.* 128, 440-442.
- OPSOMER, G., Y. T. GROHN, J. HERTL, M. CORYN, H. DELUYKER, A. DE KRUIF (2000): Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology* 53, 841-857.

- PETER, A. T., W. T. K. BOSU, R. J. DEDECKER (1989): Suppression of preovulatory luteinizing hormone surges in heifers after intrauterine infusions of *Escherichia coli* endotoxin. *Am. J. Vet. Res.* 50, 368-373.
- REGASSA, F., D. E. NOAKES (1999): Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria. *Vet. Rec.* 144, 502-506.
- REGASSA, F., I. M. SHELDON, D. E. NOAKES (2002): Effect of experimentally induced metritis on uterine involution, acute phase protein response and PGFM secretion in the postpartum ewe. *Vet. Rec.* 150, 605-607.
- RISCO, C. A., R. S. YOUNGQUIST, M. D. SHORE (2007): Current therapy in large animal theriogenology. 2nd ed. W.B. Saunders Company, Philadelphia, pp. 339-344.
- ROMERO, R., J. ESPINOZA, M. MAZOR (2004): Can endometrial infection/inflammation explain implantation failure, spontaneous abortion, and preterm birth after in vitro fertilization? *Fertility and Sterility* 82, 779-804.
- SHELDON, I. M., D. E. NOAKES (1998): Comparison of three treatments for bovine endometritis. *Vet. Rec.* 142, 575-579.
- SHELDON, I. M., D. E. NOAKES, A. N. RYCROFT, D. U. PFEIFFER, H. DOBSON (2002): Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction* 123, 837-845.
- SHELDON, I. M., H. DOBSON (2003): Reproductive challenges facing the cattle industry at the beginning of the 21st century. *Reproduction* 61, 1-13.
- SILVA, R. A., J. E. G. GAROTTI, R. S. SILVA, A. NAVARINI, A. JR PACHECO (2009): Analysis of the bactericidal effect of ozone pneumoperitoneum. *Acta Cir. Bras.* 24, 124-127.
- ZERBE, H., N. SCHNEIDER, W. LEIBOLD, T. WENSING, T. A. KRUIP, H. J. SCHUBERTH (2000): Altered functional and immunophenotypical properties of neutrophilic granulocytes in postpartum cows associated with fatty liver. *Theriogenology* 54, 771-786.
- ZIMRAN, A., G. WASSER, L. FORMAN, T. GELBART, E. BEUTLER (2000): Effect of ozone on red blood cell enzymes and intermediates. *Acta. Haematol.* 102, 148-152.
- ZOBEL, R., S. TKALČIĆ, I. ŠTOKOVIĆ, I. PIPAL, V. BUIĆ (2012): Efficacy of ozone as a novel treatment option for urovagina in dairy cows. *Repr. Dom. Anim.* 2, 293-298.
- ZOBEL, R., S. TKALČIĆ (2013): Efficacy of ozone and other treatment modalities for retained placenta in dairy cows. *Repr. Dom. Anim.* 48, 121-125.
- ZOBEL, R. (2013): Endometritis in Simmental cows: incidence, causes and therapy options. *Turk. Anim. Vet. J.* 37, 13-18.
- ŽILAITIS, V., J. RUDEJEVIENĖ, V. JUOZAITIENĖ, V. KRIŠTAPONIS, G. PALUBINSKAS, E. MALINOWSKI (2013): The prevalence of clinical metritis and fertility indices in cows treated with low-intensity laser irradiation and antibiotics in the postpartum period. *Vet. arhiv* 83, 1-9.

Received: 1 December 2012

Accepted: 17 April 2013

ZOBEL, R., R. MARTINEC, D. IVANOVIĆ, N. ROŠIĆ, Z. STANČIĆ, I. ŽERJAVIĆ, B. FLAJSIG, H. PLAVEC, O. SMOLEC: Utjecaj intrauterine primjene ozona na plodnost u krava simentalke pasmine. Vet. arhiv 84, 1-8, 2014.

SAŽETAK

Cilj rada bio je utvrditi utjecaj intrauterine primjene ozona tijekom prva 24 sata nakon porođaja na plodnost krava. Krave su nasumično bile podijeljene u dvije skupine: kontrolnu i skupinu liječenu ozonom. Kravama liječene skupine u maternicu je bio primijenjen pripravak ozona unutar 6 sati poslije porođaja i 24 sata kasnije. Uspjeh liječenja mjeren je brojem međubremenih dana i brojem osjemenjivanja do bređosti. Rezultati pokazuju da je u krava liječenih ozonom utvrđen manji broj međubremenih dana (75,47 prema 98,03, $P < 0,05$) i manji broj umjetnih osjemenjivanja do bređosti (1,68 prema 2,42, $P < 0,05$) u odnosu na krave kontrolne skupine. Rezultati upućuju na zaključak da primjena pripravka ozona u maternicu netom oteljenih krava simentalke pasmine može biti uspješan način povećanja plodnosti.

Ključne riječi: plodnost, ozon, krave
