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Efficacy of Ozone and Other Treatment Modalities for Retained Placenta in Dairy Cows

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Contents

Retained placenta is a worldwide recognized clinical condition in puerperal cows, which can significantly affect their health and fertility. Available treatment modalities are often of questionable efficacy or associated with time constraints, practicality or monetary considerations for their wide application in a routine dairy practice. The objective of this study was to compare and assess the efficacy of different treatment options, including a novel ozone treatment, for the retained placenta. Two hundred cows diagnosed with retained placenta were divided into five treatment groups, each receiving a different treatment option. Group A (n = 40) was given a combination treatment of intrauterine ozone and parenteral cephalexin; group B (n = 40) was given intrauterine ozone; group C (n = 40) was given a combination of parenteral cephalexin and intrauterine antibiotic tablets; group D (n = 40) was given only parenteral cephalexin and group E (n = 40) was given parenteral prostaglandins in 11-day intervals. The control group (group Z, n = 200) included cows that gave birth without assistance and were not diagnosed with a retained placenta. The ozone treatment (groups A and B) was found to be the most effective modality resulting in the shortest period of days open, the smallest number of artificial inseminations until pregnancy, the smallest number of animals diagnosed with fever within 10 days post-calving, the highest percentage of animals pregnant within 200 days after calving and the smallest number of animals culled because of infertility, when compared to the other treatment groups. The intrauterine ozone flush therefore has a potential as an efficacious and cost-effective treatment option for retained placenta, with an overall positive effect on puerperal health and fertility in cows.

Introduction

The definition of a retained placenta, also known as retention of foetal membranes (RFM), a post-partum pathological condition, is based on the physiological process of expulsion of foetal membranes from the cow's uterus. While the majority of cattle will pass the placenta within 6 h after parturition (Van Werven et al. 1992), the definition of a retained placenta varies among different authors, and it can range from the inability to pass the placenta within 8 h (Van Werven et al. 1992), 8–12 h (Paisley et al. 1986; Fourichon et al. 2000; Drillich et al. 2003), 12–24 h (Roberts 1986) or up to 48 h post-partum (Lee et al. 1989). As such, a retained placenta can result in a delayed uterine involution (Holt et al. 1989), endometritis (Laven and Peters 1996), decreased fertility (Curtis et al. 1985; Holt et al. 1989; Stevens and Dinsmore 1997), increased number of services to conception (Holt et al. 1989; McDougall 2001), ketosis (Bruun et al. 2002; Melendez et al. 2003) and potential losses in milk production (Laven and Peters 1996).

Many common therapeutic practices for retained placenta are of relative effectiveness, often with conflicting results reported, or come with a potentially negative impact on future reproduction (Drillich et al. 2007). These include manual removal of retained placenta, intrauterine or systemic application of antibiotics and application of prostaglandins or estradiol, although current evidence does not support their use (Peters and Laven 1996; Stevens and Dinsmore 1997; Drillich et al. 2006, 2007). Drillich et al. (2006), Goshen and Shpigel (2006) and Drillich et al. (2007) suggest that while intrauterine antibiotics (infusion or boluses) can be beneficial in treating endometritis, it is unlikely that they will result in an earlier release of membranes or prevent endometritis in cows with retained placentas. Systemically administered ceftiofur was found to be superior in the treatment of retained placentas when compared to estradiol and prostaglandins, but it did not show a significant effect on overall reproductive performance in dairy cows (Risco and Hernandez 2003; LeBlanc et al. 2005). Also, the immediate post-partum administration of prostaglandins, oxytocin or calcium was not found to be effective in preventing the retained placenta or hastening the separation and expulsion of retained placenta (Peters and Laven 1996; LeBlanc et al. 2005). Another potential treatment option is based on the breakdown of collagen as a key point in placental detachment. From this aspect, the infusion of collagenase-containing products into the umbilical arteries of retained placentas is considered an effective approach in breaking the caruncle–cotyledon bonds and an earlier placental release, although it is not considered practical for a wide-spread use (Eiler and Hopkins 1993; Haffner 1998).

A novel approach using ozone (O_3) containing products may be considered in the treatment of retained placenta as well, because of its reported bactericidal (Bocci 1996; Silva et al. 2009), immune-stimulating (Buckley et al. 1975; Jakab et al. 1995; Zimran et al. 2000; Guennadi et al. 2008) and anti-inflammatory (Jakab et al. 1995; Guennadi et al. 2008) properties that support its current application in medicine and dentistry (Terasaki et al. 2001; Guennadi et al. 2008; Silva et al.

This research was carried out in the central part of Croatia (Moslavina region).
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2009). Ozone was found to be an effective treatment for colpitis in people (Guennadi et al. 2008), as well as mastitis (Ogata and Nagahata 2000; Ohtsuka et al. 2006) and urovagina in dairy cows (Zobel et al. 2012).

The aim of this study was to assess the efficacy of ozone as a novel treatment option for retained placenta and compare it to several other treatment options commonly used by practicing veterinarians in Croatia. The applied criteria considered days open, number of artificial insemination (AI)'s until pregnancy, occurrence of fever, diagnosis of a clinical endometritis (CE) 30–40 days post-calving, percentage of animals pregnant until day 200 post-calving and number of animals culled because of the infertility caused by RFM.

Materials and Methods

Animals

The study involved 200 Simmental dairy cows that gave birth without assistance and were diagnosed with retained placentas 12 h post-partum. As no untreated animals suffering from RFM were available as controls because of ethical reasons, the control (group Z) included 200 clinically healthy cows that gave birth without assistance and with placentas expelled within 8 h post-partum. To reduce the effect of farm in data analysis, animals were housed in two commercial dairy farms with similar management practices. The study was conducted over a course of 5 years (October 2006 to October 2011). The total number of animals in each farm (including heifers and cows) was 165 and 218. To minimize the influence of the first lactation on the treatment outcome, all involved animals were 3–7 years of age (2–5 lactations). In addition, all involved animals contributed just for the one lactation. Cows with confirmed RFM diagnosis were assigned to the different treatment groups based on their last three ear-tag number combinations (group A was composed of cows with three even numbers; group B contained three odd numbers; group C contained even-odd-even number combination; group D contained even-odd-odd number combination; group E contained odd-even-even number combination). Animals were fed hay *ad libitum* and a ration consisting of oat, corn meal, barley and mineral supplements. Additional grass silage, corn silage and glycerol were provided based on age, stage of lactation and milk yield. Cows were milked twice daily, and the average milk yield was 5800 ± 1212 kg/year. Records of the time and type of calving (no assistance or dystocia) were obtained from herd owners. Records of the milk yield, age and parity were obtained from the Croatian Agriculture Agency (HPA).

Experimental protocol

The first physical examination was performed 12–18 h post-calving and included measurement of the body temperature (the elevated body temperature was considered elevated in animals with a rectal temperature above 40°C), manual vaginal exam and transrectal palpation. All animals were examined daily for a total of 10 days post-calving, and after that in 10- to 12-day intervals during the first month. During the second and third month after calving, animals were examined in 20- to 25-day intervals until spontaneous oestrus was detected up to 80 days post-calving. Otherwise, oestrus was induced after 80 days using prostaglandin or GnRH analogues depending on the ovarian status, followed by the AI. Onset of oestrus was detected and documented by an experienced farm manager who was instructed to monitor cows throughout the day (minimum of three times daily, for 25 min each) for changes in behaviour, and characteristic clinical and gynaecological signs of oestrus. Veterinary clinical examination techniques followed and included vaginoscopy, ultrasound and transrectal palpation. The same veterinarian performed all clinical assessments and AI.

Animals with retained placenta were divided into five (A, B, C, D, E) different treatment groups. A group of 200 clinically healthy cows was assigned to a non-treated control group (group Z). Treatment success was evaluated based on the number of days open, number of artificial inseminations until pregnancy (nAI), number of animals with fever (>40°C) within the first 10 days post-calving, number and proportion of animals diagnosed with CE 30–40 days post-calving, percentage of animals pregnant within 200 days post-partum and proportion of animals culled because of the infertility. Diagnosis of CE was based on transrectal palpation, vaginoscopy and ultrasonography with a rectal linear probe 7 MHz (PROFI L; Draminski, Olsztin, Poland). During vaginoscopy, vaginal content and cervix morphology were evaluated. A sterile (autoclaved) metal vaginoscope was inserted into the vagina up to the level of the external cervical orifice. The genital tract was evaluated for the quality of mucus (watery, cloudy and pus) and the morphology of the cervix (open-closed, location, position, colour, size and mucus). Transrectal palpation of the reproductive tract was performed to determine the diameter, location, consistency and symmetry of uterine horns and the presence of the ovarian structures (follicle size and location; *corpus luteum*; palpable structures on the ovaries: cyst formation, luteinized follicles). Ultrasonography included a measurement of cervical size and uterine horn diameter with an image capture of abnormal fluid in the uterine lumen, if present. Uterine fluid was considered normal when a clear fluid was found at the level of the anterior vagina (vaginoscopy), associated with a heterogenic appearance of the uterine lumen by ultrasonography. Based on the reproductive examination, cows were grouped into two categories: cows with CE and clinically healthy cows. Cows between 30 and 40 days in milk with a cervical size >7 cm, uterine diameter >8 cm and with a purulent or mucopurulent discharge visible through the vaginal speculum were diagnosed with CE (LeBlanc 2008). Cows classified as clinically healthy had a cervical size <7 cm, uterine diameter <5 cm and no abnormal discharge externally nor in the uterus based on ultrasonography (Kasimanickam et al. 2004; LeBlanc 2008). Inseminations were performed throughout the year, as requested by the herd manager or during the routine visits to the farms. Based on the previous findings by Looper et al. (1998) and White et al. (2002), AI was performed 24–28 h after the onset of oestrus with semen deposited into the uterine horn ipsilateral to the ovary bearing the dominant follicle, followed by an intramuscular injection of 0.05 mg D-Phe-gonadorelin to facilitate ovulation (Masiulis et al. 2003). Frozen bull semen was thawed in warm water (35–37°C for 30 s), and AI was performed within 15 min after thawing. Twelve to 15 h later, all cows were examined again using an ultrasound to confirm ovulation (disappearance of dominant follicle). The semen originated from five bulls of the Simmental breed. Animals were re-inseminated for a maximum of six times (until day 200 post-partum) and after that excluded from the study and culled because of infertility. In cases with adhesions (*perimetritis fibrosa* and *peribursitis fibrosa*) and/or *salpingitis,* animals were declared infertile and culled.

Exclusion criteria included the administration of systemic antibiotic therapy 20 days prior to calving, manifestation of any systemic disease or fever (>40°C) 10 days prior to calving, Body Condition Score <2 and >4.5 at the time of calving (according to criteria by Edmonson et al. 1989), any kind of dystocia during calving, twinning, post-calving birth canal lesions that required surgical treatment, abnormalities of internal genitalia (including adhesions), previous Caesarean section and mastitis (increased somatic cell count $>200\,000$/ml of milk) 60 days post-calving. In addition, exclusion criteria for the controls (group Z) included manifestation of fever of any origin and use of antimicrobials up to 30 days after calving. The study design involved animals within different treatment groups equally distributed among farms, to avoid farm management as a variable in data analysis.

Treatment protocol

A routine vaginal examination of all animals was performed daily to assess the detachment and expulsion of the retained placenta. External cervical orifice was reached manually, and the placenta was gently pulled out, if possible. The application of a selected intrauterine and/or parenteral therapy regimen was followed. The assigned treatment protocol for each cow diagnosed with a retained placenta was applied daily for 10 days after calving, except for Group E where hormonal therapy was applied in 11-day intervals. Group A cows received 30 ml ozone flush (Riger Spray®; Novagen, Parendzana, Italy) infused directly intrauterine, coupled with 4500 mg of cephalexin (25 ml of Cephalexin®; Veterina, Zagreb, Croatia) intramuscularly. Group B cows received 30 ml intrauterine ozone flush alone (Riger Spray®; Novagen). Group C cows received 4500 mg of cephalexin (25 ml of Cephalexin®; Veterina) intramuscularly, coupled with intrauterine applications of tablets containing 700 000 mg of neomycin and 1000 mg of oxytetracycline (two tablets of Ginobyotic®; Novartis Animal Health, Basel, Switzerland). Group D cows received parenteral applications of 4500 mg of cephalexin (25 ml of Cephalexin®; Veterina). Group E cows received a total of four parenteral applications each of 0.53 mg of cloprostenol sodium (2 ml of PGF Veyx® Forte; Veyx Pharma, Schwarzenborn, Germany) intramuscularly on days 1, 11, 21 and 32 post-calving.

Following treatment, animals were monitored three times daily for signs of oestrus by herd owners, as instructed. When heat was detected, cows were further examined by the first author (for the presence of dominant follicle on one of the ovaries) and artificially inseminated. Pregnancy was confirmed 32–38 days after AI using ultrasonography.

Statistical analysis

The statistical software program ‘Statistica 8’ was used for data analysis. Time to conception (days open), number of AIs until pregnancy (nAI), proportion of animals with fever, proportion of animals with CE, percentage of animals conceived within 200 days post-calving and proportion of animals culled because of infertility were variables across all six groups. Normality of distribution was tested by the Shapiro–Wilk normality test. Differences between treatment groups against all variables were analyzed using a Kruskal–Wallis and a multiple comparison of mean ranks.

Because fertility (a cow is either pregnant or not), fever (a cow has a fever or not), CE (a cow is diagnosed with CE or not) and culling (it is either culled or not) are all binary variables, they were analysed in a categorical mode using a treatment (group) as a factor of variation.

Time to conception (days open) and number of inseminations to pregnancy (nAI) for all groups were used in data analysis. For all six treatment groups, a 6×2 experimental design was used, comparing the two treatment outcomes to the six different treatment options. Differences between the number of culled cow in each treatment group were tested using the chi-square test. To avoid inflated p-values, the Bonferroni method was used. Results were found statistically significant when $p < 0.05$.

Results

As presented in Table 1, the ozone treatment groups (groups B and A) had 5–6 (3–4%) more days open, and 0.3–0.5 (2–3%) more AIs until pregnancy when compared to the controls (group Z) ($p > 0.05$). In the same time, the ozone treatment group resulted in 76 (19%) less days open, 2.25 (8%) less AIs until pregnancy, 17 (42.5%) less animals presented with fever, 17 (42.5%) less animals with CE, 29 (18%) less animals pregnant within 200 days *post-partum* and 5% more animals culled because of infertility when compared to group E (prostaglandin treatment only) ($p < 0.05$). In addition, when compared to the group D (parenteral antibiotics only), the ozone treatment groups resulted in 46 (12%) less days open and 1.7 (5%) less AIs until pregnancy ($p < 0.05$). Although ozone treatment groups reduced the number of animals with clinical illness, resulting in 11 (29%) and 6 (16%) less animals having fever, 11 (25%) and 6 (15%) less animals having CE, 9% and 6% less animals pregnant within 200 days after calving), when compared to the groups D (parenteral antibiotics only) and C (intrauterine and parenteral antibiotics), respectively, the differences were not found to be statistically significant. No statistically significant difference was found between two ozone treatment groups (A

Table 1. Comparison of the ozone to other treatment options for retained placenta and their effects on the common criteria used to assess fertility and puerperal health in dairy cows

Criteria for a successful therapy	Group A (n = 40)	Group B (n = 40)	Group C (n = 40)	Group D (n = 40)	Group E (n = 40)	Group Z (n = 200)
DO	82.8[a]	83[a]	116.8[a]	129[b]	157.6[c]	78.7[a]
nAI	2.1[a]	2.3[a]	3.3[a]	3.9[b]	4.55[c]	1.8[a]
F	2[a] (5%)	3[a] (7.5%)	9[a] (22.5%)	14[a] (35%)	20[b] (50%)	3[a] (1.5%)
CE	4[a] (10%)	6[a] (15%)	12[a] (30%)	16[a] (40%)	22[b] (55%)	6[a] (3%)
% PC	92[a]	91[a]	85[a]	82[a]	73[b]	92[a]
CC	2[a] (5%)	3[a] (7.5%)	3[a] (7.5%)	3[a] (7.5%)	8[b] (20%)	8[a] (4%)

Group A, intrauterine ozone flush and parenteral antibiotics; Group B, intrauterine ozone flush only; Group C, intrauterine and parenteral antibiotics; Group D, parenteral antibiotics only; Group E, hormonal (prostaglandin) treatment; DO, days open expressed as average; nAI, average number of artificial inseminations until pregnancy shown as average; F, number of animals with fever (>40°C) within 10 days post-calving expressed as the total number and percentage of animals within the group; CE, number of animals with clinical endometritis diagnosed 30–40 days post-calving, expressed as the total number and percentage of animals within the group; % PC, percentage of cows pregnant at 200 days in milk expressed as percentage of animals in respective group; CC, number of culled cows because of the infertility expressed with number and percentage of animals within the group.
Values in each row marked with different letter in superscript differ significantly ($p < 0.05$).

and B) regarding the measured criteria. With respect to antibiotic treatments, the combined parenteral and intrauterine antibiotic treatment (group C) showed better results when compared to the group D of animals. The differences between those two antibiotic treatment options were found to be statistically significant for the days open and number of AIs to pregnancy. Group E (prostaglandin analogues alone) showed the least favourable results in measured criteria when compared to other treatment options ($p < 0.05$).

No influence of the bull and parity (lactation number) on the treatment outcome was recorded.

Discussion

Fairly recent in its consideration as an antiseptic treatment option in medicine, ozone (O_3) was not yet considered in intrauterine application for the treatment of retained placenta in cattle. Up until now, broad spectrum antibiotics and hormonal treatments have been commonly used in dairy practices. However, it is speculated that while controlling local bacterial growth, intrauterine antibiotics actually interfere with the necrotizing process at the caruncle–cotyledon interface and further delay in the release of the placenta (Roberts 1986). Tetracyclines are commonly used as intrauterine tablets in cattle, but because of their inhibition of matrix metalloproteinase secretion, they interfere with physiological placental detachment mechanisms (Eiler and Hopkins 1992; Kaitu'u et al. 2005). Our study shows that between the two antibiotic treatment groups, a combined antibiotic treatment (intrauterine and parenteral administration of antibiotics) was more effective when compared to only parenteral antibiotic treatment, yet the difference was not found to be statistically significant, the most probably due to the small sample of animals used. However, these results are in concordance with previous reports by Peters and Laven (1996), Stevens and Dinsmore (1997), Risco and Hernandez (2003), LeBlanc et al. (2006) and Drillich et al. (2007).

Prostaglandin analogues alone (group E) were found to be significantly the least effective in the treatment of retained placenta in all measured treatment criteria, which is in concordance with previous findings of LeBlanc et al. (2005), Drillich et al. (2006, 2007).

While collagenase therapy shows promise in preventing/treating retained placenta in a variety of species (Haffner 1998), this approach is not widely used. Until the time this method could be simplified, made inexpensive, and more appropriate for the daily field practices, alternative methods should be considered.

In this study, the ozone treatment alone and in combination with a parenteral antibiotic treatment was more effective when compared to the antibiotics administered parenterally or the hormone treatment options for retained placenta in some of the criteria employed. At the same time, the differences within the two ozone treatment groups (A and B) were not statistically significant. The ozone therapy has already been considered in intrauterine treatment of urovagina (Zobel et al. 2012), and according to our data, it should be considered as an effective treatment for retained placenta to reduce CE during puerperium. However, owing to the small number of animals involved in this study, statistical significance was inconsistent and these findings are considered preliminary. Therefore, a larger field study should be conducted in the future to test our hypothesis.

Conclusion

In spite of the lack of a consistent statistical significance across all the criteria used in this study, our results overall suggest that intrauterine ozone flush alone or combined with parenteral antibiotics is more efficacious treatment for retained placenta in cows when compared to hormonal and parenteral antibiotic treatment modalities. Owing to its noted advantages, it should be considered as a therapeutic option in routine dairy practice because of its potential to improve overall puerperal health and fertility in cows with placental retention. It offers a practical and relatively inexpensive treatment option for this condition because of its simple administration and lack of residues. Further studies that include a larger population size are needed to establish medical appropriateness of this novel treatment.

Conflict of interest

None of the authors have any conflict of interest to declare.

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Author contributions

Zobel R designed study, analysed data and drafted the paper, and Tkalčić S. drafted the article and gave final approve to be published.

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