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## Prelliminary Study on Ozone Therapy in Postpartum Endometritis of Dairy Cows

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### Abstract

The aim of research was to evaluate the effects of intrauterine foam ozone application in parturient endometritis on first service conception rate (FSCR), the average of straws until pregnancy (ASUP), the average of days not pregnant to first service (ADOFS) and the average of days not pregnant to pregnancy (ADOP). Another objective of this study was to observe the influence of ozone against uterine bacteria. The study involved 24 Holstein and Cross Breed Holstein cows, divided into two groups (Ozone O n=12, and Control C n=12) based on the uterine findings and diagnosis (puerperal endometritis and control). In cases when endometritis was diagnosed, we used ozone foam (RIGER SPRAY®, Novagen, Italy). Ozone was applied by means of a vial containing ozone foam under pressure for 10 seconds. Bacterial and fungal samples were taken at the first uterine examination and the second probe, at the moment of artificial insemination. After the days open period, all animals were put on hormonal therapy to induce the oestrus and then were inseminated. The next step was the ultrasonographic pregnancy diagnosis after 29-32 days. Bacteriological examination of uterine aspirate samples from the animals revealed single type of bacteria in 7 cases (29.16%) and mixed bacterial isolates in 17 cases (70.84%). The most isolated bacteria from group O was *Escherichia coli* (91%) followed by *Staphylococcus hyicus* (33%), *Staphylococcus bovis* (25%) and *Arcanobacterium pyogenes* (25%) and from group C *Escherichia coli* (66%), then *Streptococcus bovis* (16%). No fungal species were found. The median days open to first service and the median days open to pregnancy were similar in both groups: group O (65.4 days) and group C (65.1 days) for ADOFS, and for ADOP 112.3 days respectively 108.9 days. The significant difference between the two groups was observed for the FSCR and for the ASUP where half of the cows from the group O were pregnant at first service.

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## 1. Introduction

Regarding high-yielding dairy cow's management, the transition period (3 weeks before and 3 weeks after parturition) is particularly at risk because animals undergo pronounced physiological changes that can cause suppression of immunological mechanisms (Islam et al., 2014).

Relaxation of the vulva and cervical dilatation during and after the onset of parturition allows the entry of bacteria into the uterus, causing infection in 80-100% of cows by 14-21 days post-partum (Sheldon et al., 2006; Islam et al., 2013). The most common bacteria are represented by *Escherichia coli*, *Arcanobacterium pyogenes*, *Pseudomonas aeruginosa*, *Pasteurella multocida*, *Staphylococcus aureus*, *Streptococcus uberis*, *Clostridium* spp., *Prevotella* spp. and *Fusobacterium* spp. (Singh et al., 2008; Kaçar and Kaya, 2014).

These bacteria compromise animal welfare, as well as causing subfertility and infertility because of uterine inflammation and/or infection (Zobel et al., 2014) represented by metritis, clinical and subclinical endometritis.

Endometritis may prolong the days open until first service, days open until pregnancy, the intercalving period, the conception rate and the risk of cows being culled due to infertility (Đuričić et al., 2015).

The most common way to treat endometritis is either intrauterine or systemic antibiotic administration (Zobel et al., 2012; Zobel et al., 2014). Other scientific works revealed the use of non-antibiotic substances such as: garlic extract (Sarkar et al., 2006), dextrose (Brick et al., 2012), neem - *Azadirachta indica* (Kumar et al., 2013), non-steroidal anti-inflammatory drugs,  $\text{PgF}_{2\alpha}$ , antiseptic solutions (Troitzky, 2010) and others.

A novel antibacterial, antifungal and immunostimulant therapy in animals is represented by different pharmaceutical forms of ozone (cream, gas, injections, paillettes, foam, pearls, boluses) (Djuricic et al., 2012 a; Zobel et al., 2012; Zobel and Tkalčić, 2013; Zobel et al., 2014; Đuričić et al., 2015). Ozone ( $\text{O}_3$ ) is a gaseous and unstable molecule composed of three oxygen atoms, colorless, with a specific smell and a tendency for quickly transforming into the oxygen (Djuricic et al., 2012 b; Đuričić et al., 2015; Ersoz et al., 2015; Polat et al., 2015). Some of these forms can be used in postpartum endometritis because the most important ozone derivatives are lipohydroperoxides, which act directly on pathogens and activate erythrocytes and immune cells (Zobel et al., 2014; Đuričić et al., 2015; Enginler et al., 2015; Ersoz et al., 2015; Calderon et al., 2016).

The aim of this study was to evaluate the effects of intrauterine foam ozone application in parturient endometritis on first service conception rate (FSCR), the average of straws until pregnancy (ASUP), the average of days not pregnant to first service (ADOFS) and the average of days not pregnant to pregnancy (ADOP). Another objective of this study was to observe the influence of ozone against uterine bacteria.

## 2. Materials and Methods

### 2.1. Animals

The study involved 24 Holstein and Cross Breed Holstein cows, divided into two groups (Ozone O  $n=12$ , and Control C  $n=12$ ) based on the uterine findings and diagnosis (puerperal endometritis and control), from one commercial dairy farm in the south part of Romania (Ploiești County) with temperate-continental climate over the period of 5 months (Sept.-Feb.).

The cows were housed in free-stall barns and mattress bedding. Of crucial importance was the lying/resting area. All the females spent up to 14 h per day lying with about half of the resting period ruminating (Winckler, 2014). Cows were milked three times per day (morning, noon and evening milking schedule). All cows were fed with a unique nutritional ratio twice per day. The animals from the two groups had a physiological puerperium but the exclusion criteria included birth assistance, receiving systemic antibiotic therapy within 60 days prior to calving, abnormal internal genitalia (including adhesions),  $\text{BCS} < 2.5$ , systemic diseases, retention of foetal membranes, any kind of dystocia, including caesarean section, lameness, puerperal mastitis. Only cows following the second to the fourth partus were involved in the study. The groups' average milk yield during the study was 43.95 kg per day for C group and 40.84 kg per day for O group.

## 2.2. Experimental protocol and clinical evaluations

Veterinary farm visits took place on a weekly basis with scheduled reproductive examinations. Cows 7-10 days post-partum were included in the study and examined weekly until the first service took place. The first post-partum examination was performed between 7 and 10 days after calving and included clinical, vaginal and transrectal examination, bacterial and fungal culture. The first group comprised cows suffering from puerperal endometritis diagnosed on days 7 and/or 10 following parturition. The second group included animals without clinical signs of uterine inflammation. In cases when endometritis was diagnosed, ozone foam (RIGER SPRAY<sup>®</sup>, Novagen, Italy) was applied into the uterus weekly for four times under epidural anesthesia. Caudal epidural anesthesia was made at the sacrococcygeal space (S5–Co1) using an 18-gauge, 3.8-cm needle (with no syringe attached). After a local iodine asepsia, the needle was directed perpendicular to the skin surface. Once the skin was penetrated, a drop of local anesthetic solution was placed in the hub of the needle (hanging drop technique). The needle was advanced slowly until the anesthetic solution was drawn into the epidural space by negative pressure. The syringe was attached to the needle, and the anesthetic solution slowly injected with no resistance. The dose of local anesthetic used was 0.5 ml per 45 kg (99 lb) of body weight (Lin and Walz, 2014).

Ozone was applied by means of a vial containing ozone foam under pressure for 10 seconds. All cows underwent vaginal and transrectal examination weekly for four times following parturition. After the days open period, all animals were put on hormonal therapy to induce oestrus and then there were inseminated with frozen-thawed sex-sorted semen (0.25 ml straw) by experienced inseminators. The pregnancy diagnosis was taken 29-32 days post artificial insemination by transrectal ultrasonographic control.

## 2.3. Clinical examination

Gynaecological and clinical examinations were performed by one veterinarian and included vaginoscopy and transrectal palpation. All cows appeared healthy at the time of examination of eight areas of the cow's body and had body condition scores > 2.5 (scale 1 to 5, with 0.25 unit increments); score 1 indicated emaciated condition and score 5 an obese condition.

The hygiene of perineum (HPS) of cows after calving was scored using a 1-3 scale (1 = free of dirt-manure and completely dry; 2 = slightly wet, dirt-manure in 1-10% of the surface; 3 = moderately wet, covered with dirt-manure in >10% of the surface) (Schuenemann et al., 2011). The angle of the vulva was recorded as abnormal if less than 70° to the horizontal axis judged using a protractor.

Evaluation of cervix and uterus trans-rectum involves estimation of size (> 7.5 cm), symmetry and consistency.

The vaginal examination is a standard part to evaluate a suitable cow or whenever discharge is noted at the vulvar lips. The vaginal speculum examination may aid in determination of the origin of the exudates which may be from the uterus, cervix or vagina. The character, color and odor (score 0 for no odor and score 1 for a fetid odor) of vaginal discharge were scored. If the vaginal discharge shows clear translucent mucus character, the score is 0 and when it shows clear mucus without pus, containing flecks of white pus character, the score is 1 and when it contains ≤ 50% white or yellow pus character, the score is 2, and when it contains ≥ 50% white, yellow or bloody pus character, the score is 3. The character score reflects the presence and semi-quantitative load of certain bacteria in the uterus.

## 2.4. Bacterial and fungal culture

A uterine culture is an essential tool to determine the etiology of uterine infection. Briefly, the vulva was cleaned thoroughly with a dry paper towel. A true uterine culture was aspirated from uterine lumen using sterile uterine catheter, transferred into sterile tubes and carefully transported at 4°C to the Faculty of Veterinary Medicine, Bucharest for further bacteriology and fungal testing. First bacterial and fungal samples were taken at the first uterine examination and the second sample at the moment of artificial insemination.

## 2.5. Farm, clinical, and fertility data

Farm and fertility data were recorded by farm staff and entered into a herd health monitoring computer programme (Dairy Plan C 21, GEA Farm Technologies). Information regarded to the animals included parity, milk yield, and month of calving. Information on the periparturient period was recorded, including cases of hypocalcaemia, retained fetal membranes, calving assistance, and other disease conditions: lameness, mastitis, and metabolic disorders.

## 2.6. Statistical analysis and modeling

First service conception rate (FSCR), the average of straws until pregnancy (ASUP), the average of days not pregnant to first service (ADOFS) and the average of days not pregnant to pregnancy (ADOP) were calculated using Microsoft Excel 2003.

## 3. Results and Discussions

The results of bacteriological and fungal examination of 24 uterine samples from the two groups are presented in Table 1. Bacteriological examination of uterine aspirate samples from the animals revealed a single type of bacteria in 7 cases (29.16%) and mixed bacterial isolates in 17 cases (70.84%) of samples. The group O frequencies of bacteria isolated were *Escherichia coli* (91%) followed by *Staphylococcus hyicus* (33%), *Staphylococcus bovis* (25%), *Arcanobacterium pyogenes* (25%), *Enterococcus hirae* (16%), *Fusobacterium necrophorum* (16%), *Streptococcus zooepidemicus* (16%), *Enterococcus faecium* (16%), *Proteus vulgaris* (8%), *Streptococcus agalactiae* (8%), *Staphylococcus chromogenes* (8%), *Pseudomonas aeruginosa* (8%) and *Enterococcus faecalis* (8%). In the group C, *Escherichia coli* was the most frequent isolated bacteria (66%), then *Streptococcus bovis* (16%), *Staphylococcus hyicus* (8%), and *Proteus vulgaris* (8%). No fungal species were found.

**Table 1. Bacteria isolated from the two groups**

Bacteria isolates	Endometritis 7-10 days in milk	Control	Endometritis At the moment of artificial insemination	Control
<i>Escherichia coli</i>	11	8	9	9
<i>Staphylococcus hyicus</i>	4	1	3	0
<i>Staphylococcus bovis</i>	3	2	4	3
<i>Staphylococcus chromogenes</i>	1	0	0	0
<i>Enterococcus hirae</i>	2	0	0	1
<i>Enterococcus faecium</i>	2	0	0	0
<i>Enterococcus faecalis</i>	1	0	0	0
<i>Streptococcus zooepidemicus</i>	2	0	3	0
<i>Streptococcus agalactiae</i>	1	0	2	1
<i>Arcanobacterium pyogenes</i>	3	0	0	0
<i>Pseudomonas aeruginosa</i>	1	0	0	0
<i>Proteus vulgaris</i>	1	1	0	1
<i>Fusobacterium necrophorum</i>	2	0	1	0

Coliform bacteria producing endotoxins are almost always found in lochia in cows following dystocia and fetal membrane retention. These endotoxins may directly affect bovine neutrophils with their cytotoxic effect and this

facilitates infection with bacterial species such as *A. pyogenes*, *Escherichia coli*, streptococci and staphylococci, which are very frequent during early puerperium (Đuričić et al., 2015).

Table 2 describes the reproductive performance of group O and C of cows. The average of days open to first service and the average of days open to pregnancy were similar in both groups: group O (65.4 days) and group C (65.1 days) for ADOFS, and for ADOP 112.3 days respectively 108.9 days. The significant difference between the two groups was observed for the FSCR and for the ASUP, where half of the cows from group O were pregnant at first service.

**Table 2. Reproductive performance of the two groups**

Reproductive performances	Group O	Group C
The average of days open to first service	65.4	65.1
The average of days open to pregnancy	112.3	108.9
First service conception rate (%)	50 %	0 %
Average of straws until pregnancy	1.75	3.25

The objective of this trial was to validate the use of intrauterine ozone foam in puerperal endometritis and shortening some reproductive parameters. However, this is the first time that foam ozone was used in dairy cows endometritis in Romania. The most recent use of ozone as a preventive and therapeutic agent for the improvement of reproductive efficiency has been published by Đuričić et al., 2014, Đuričić et al., 2015, Enginler et al., 2015, Polat et al., 2015 and by Calderon et al., 2016.

In a study (current knowledge) from 2015, Đuričić et al. presented the importance of preventive intrauterine ozone application. The cows treated with either ozone foaming spray or pearls 24–48 hours after calving in comparison to the non-treated control cows became pregnant earlier (110.5 days or 105 days vs. 112 days, respectively) and had better first service conception rate (FSCR 20.4% or 27.8% vs. 14.6%, respectively). Our results are similar with their work.

#### 4. Conclusions

Even if the average of days open to first service (ADOFS) and average of days open to pregnancy (ADOP) from group O were slightly higher regarding the same reproductive parameters from group C, we can say that the use of foam ozone as intrauterine treatment of the puerperal endometritis improved the first service conception rate (FSCR) and the average of straws until pregnancy (ASUP) with 50%. We could not observe a noticeable effect of foam ozone against uterine bacteria.

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