

The prevalence of clinical metritis and fertility indices in cows treated with low-intensity laser irradiation and antibiotics in the postpartum period

Vytuolis Žilaitis^{1*}, Jūrate Rudejevienė¹, Vida Juozaitienė¹,
Vaidas Krištaponis¹, Giedrius Palubinskas¹, and Edward Malinowski²

¹Department of Non-infectious Diseases, Veterinary Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

²Department of Pathophysiology of Reproduction and Mammary Gland, National Veterinary Research Institute of Poland, Poland

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ABSTRACT

The aim of the study was to compare the efficiency of low-intensity laser (LIL) irradiation, antibiotic injections and a combination of both methods in the prophylaxis and treatment of metritis in cows. Examinations were carried out on 290 freshly calved cows with a rectal temperature on the second day after parturition above 38.9 °C. Cows from group I (n = 95) were given an intramuscular injection of ceftiofur hydrochloride (600 mg) for three days, commenced from the second day after parturition. Group II consisted of 55 cows, the sacroiliac area of which was irradiated for 10 days consecutively with LIL. Group III (n = 65) were given an intramuscular 600mg injection of ceftiofur for three days and the sacroiliac area was irradiated 10 times consecutively, once a day with LIL. The control group IV (n = 55) were not given antibiotics or laser irradiation. A number of cows, ranging from 57.1% (group III a) to 65% (group IV), developed clinical metritis, which was treated with antiseptics. The longest recovery time from metritis (21 ± 6.12 d) was found in the IV group. Cows from group III recovered from metritis 11.5 days more quickly than the cows from the IV (negative control) group (P<0.05) and 4 days more quickly than cows from the I (positive control) group. Fertility indices such as: calving-first insemination, service period, conception rate, insemination index and the calving-pregnancy period, were better in cows treated prophylactically with the combined method (antibiotic and LIL physiotherapy) in comparison to the other groups.

Key words: cows, postpartum period, antibiotic, laser irradiation

*Corresponding author:

Vytuolis Žilaitis, Department of Non-infectious Diseases, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes 18, Kaunas, Lithuania, Fax: +370 37 362 417; E-mail: vytuolis@lva.lt

Introduction

In the treatment of metritis/endometritis antibiotics (GOSHEN and SHPIGEL, 2006), enzymes (DRILLICH et al., 2001), hormones (SHELDON et al., 2006) and an immunomodulator (KACZMAROWSKI et al., 2004), in addition to other medicines (PAISLEY et al., 1986; MALINOWSKI, 1995) have been used. A rectal temperature (RT) above 39 °C is considered to be a valid indication for the start of uterus inflammation (PALENIK et al., 2009). KRISTULA et al. (2001) reported that cows experiencing no clinical problems at calving or during early postpartum had an average RT below 38.9 °C for each day during the first 10 days postpartum. However, cows with metritis may have rectal temperatures within the normal range and may not necessarily develop a fever. BENZAQUEN et al. (2004) found that over half of the cows diagnosed with metritis during the first week postpartum did not have a fever. Programs for the prevention or reduction of uterine infections included the local or systemic administration of antibiotics and/or the application of hormones (DOBSON and NOAKES 1990; CAIROLI et al., 1993; LEWIS 1997; ARLT et al., 2009). In recent years, while searching for new, more efficient and organic methods for treatment and prophylaxis, light therapy and the promising method of low-intensity laser irradiation have been widely discussed. Low-intensity laser irradiation affects cell metabolism, stimulates regeneration, and reduces pain and inflammation (HUANG et al., 2009). The objective of the study was to compare the efficiency of low-intensity laser (LIL) irradiation versus antibiotics and a combination of them in the prophylaxis and treatment of metritis in cows.

Materials and methods

Examinations were carried out on 290 cows calved physiologically in the period between January -June of 2008, without obvious clinical traits of disorders immediately after parturition, in which the RT was higher than 38.9 °C on the second day after parturition. The cows belonged to the Lithuanian Black-and-White, Danish Black-and-White, and German Black-and-White cattle breeds. The age of the cows ranged from two to eight years, and the average milk yield amounted to 6850 kg per lactation. The animals were allocated into 4 groups (Table 1). The first group of 95 fresh dairy cows received prophylactically an intramuscular injection of 12 mL of Excenel RTU® (ceftiofur hydrochloride -50 mg, Pfizer animal health, Belgium) for three consecutive days, from the second day after parturition. The second group comprised 55 cows. Their sacroiliac area was irradiated for 10 days consecutively with LIL STP-99 as the only prophylactic action. The exposure to LIL therapy amounted to three minutes per day. The third group comprised 65 fresh dairy cows which were given an intramuscular injection of 1mL of Excenel RTU® for three consecutive days (as in group I). Their sacroiliac area was irradiated 10 times consecutively, once a day, with LIL. For the control (group IV) 55

similar cows were selected. This group did not receive antibiotic or laser irradiation, but were i.m. injected with 12 mL of saline for three days consecutively.

Table 1. The structure of examinations

Group	Name of group	Number of cows	Method of treatment
I	Positive control	95	Excenel RTU® 12 mL*; 1 × d for 3d
II	Experimental	55	Laser STP-99; 1 × d for 10 d
III	Experimental	65	Excenel RTU® 12 mL; 1 × d for 3d + Laser STP-99; 1x d for 10 d
IV	Negative control	55	12 mL of saline; 1 × d for 3d

*ceftiofur hydrochloride 50 mg/1 mL

The morbidity from metritis and other diseases of cows from each group were recorded. Animals that are not systemically sick but have an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days postpartum, may be classified as having clinical metritis (SHELDON et al., 2006). The cows that showed signs of metritis 10-15 days after parturition were treated with antimicrobials Jodofoam® (iodine 0.2 g; potassium iodine 0.4 g; solvent and gas carrier ad 45.2 g, Biofactor, Poland), Jodouter® (Povidonum iodinum 10%) -10 g in 100 mL of carrier substance (Bioveta, the Czech Republic), Fatroximin® (rifaksimini 300 mg, Fatro, Italy), Pharmasin® (tylosin 5 g Actavis AB, Sweden), Clamoxyl metritis® (amoxicillin 0.84 g, Pfizer animal health, Belgium) intrauterinely once a week. Intramuscular injections of oestrophan and oxytocin were administered between intrauterine applications. These therapies were continued with the changing of the antimicrobial agent every seven days until the uterus recovered its tone and pathological discharges had ceased. The recovery time from metritis (the time until the eradication of the signs of metritis; or the duration of treatment) was recorded.

For all groups the fertility indices as: days open (time from calving to the first insemination), service period (time from the first to the effective insemination), conception rate (percentage of pregnancies as an effect of the first insemination), insemination index (number of inseminations for each pregnancy), calving-pregnancy period (time from the last to the subsequent pregnancy), pregnancy rate (percentage of cows that became pregnant) and culling rate from infertility were calculated.

The test data was processed using the SPSS statistical package, (Chicago, IL, USA, 2006). Data are considered to be statistically reliable when $P < 0.05$.

Results

From Table 2 it can be observed that a number of cows from each group developed clinical metritis. The highest percentage of cows (over 65%) treated for metritis was noted in the negative control group (IV), and the lowest in group III. The results demonstrate

that the group of cows did not have a significant influence on the morbidity from metritis ($\chi^2 = 1.401$, DF = 3, P = 0.705).

Table 2. Percentages of metritis and recoveries following combined treatment with regard to the prophylactic methods applied after calving

Groups of cows	Morbidity of metritis		Time to recovery (days)		
	n	%	\bar{x}	σ	m_x
I ^a (n = 95)	57	60.0	14.0 ^{a,b}	5.23	1.67
II ^b (n = 55)	34	61.8	19.4	7.46	2.33
III ^c (n = 65)	38	58.4	12.3 ^{c,d}	2.40	0.75
IV ^d (n = 55)	36	65.45	20.0	5.31	1.64

* P<0.05 (a,b, c:d)

The time of recovery from inflammation was slightly shorter in the group treated with LIL than in the negative control group. The recovery time from metritis for the cows treated with antibiotics (group I) was 5.4 days (27.8%) less than the cows from group II which were treated with LIL alone (P<0.05). The duration of recovery from metritis of the cows that were treated with LIL and antibiotics after parturition (group III) was 7.1 days less than the group treated solely with laser irradiation (P<0.05). The recovery time of the cows treated with ceftiofur (group I) was indicated to be 6 days (30.0%) less than the cows from group IV. The shortest recovery time from metritis was noted in group III. This was 7.7 days (38.5%) less than cows from the negative control (IV) group and by 2 days (on average) in comparison to the first group.

Table 3 presents the fertility indicators of the experimental and control cows. The time from calving to the first insemination (days open) was the longest in cows from the negative control group and from the positive control group, and the shortest in cows treated with the antibiotic and LIL. Compared to the negative control, it was, on average, 16.0 days shorter (P<0.05). Laser alone decreased this period by 7.6 days (P>0.05) when compared to the negative control group. There was a common trend observed in the groups that were only treated with the LIL therapy, in that the period from the first to effective insemination had decreased, while the conception rate at the first insemination had increased. This longer time of LIL therapy did not have any significant impact on the fertility indices. LIL treatment had a positive, however statistically insignificant, effect on conception rate and the insemination index. A significant effect in the use of laser therapy was found regarding the calving-pregnancy period. The maximal change in this rate was observed under the treatment using both antibiotics and LIL irradiation. Compared to the negative control, the calving-pregnancy period was reduced by about 31.6 days (P<0.05). The group treated with LIL and antibiotics included the highest number of cows for which the calving-pregnancy period was between 80 and 100 days. This group did not contain a

significant number of cows for which the calving-pregnancy period was longer than 160 days. From Table 3 it is also discernible that pregnancy rates were almost identical in all groups. The culling rate from infertility was the highest in the negative control group. However, the differences between the groups were not statistically significant.

Table 3. Fertility indicators of cows treated prophylactically with different methods after calving

Fertility indicators	Groups			
	I	II	III	IV
	$\bar{x} \pm \sigma, m_x$	$\bar{x} \pm \sigma, m_x$	$\bar{x} \pm \sigma, m_x$	$\bar{x} \pm \sigma, m_x$
Calving-first insemination (d)	63.1 ± 8.3; 2.59	54.5 ± 9.2; 2.92	46.1 ± 9.6; 3.01	62.1* ± 10.4; 3.27
Service period (d)	60.2 ± 10.2; 3.17	47.2 ± 8.2; 2.53	42.6 ± 7.3; 2.33	61.5 ± 10.2; 3.02
Conception rate (%)	46.0	46.0	51.0	42.0
Insemination index	2.4 ± 1.13; 0.35	2.2 ± 1.2; 0.37	2.0 ± 1.2; 0.33	2.6 ± 1.51; 0.30
Calving-pregn. period (d)	123.3 ± 28.1; 9.00	102.1 ± 10.7; 3.44	91.9 ± 17.4; 5.04	123.5* ± 23.2; 7.29
Pregnancy rate (%)	90.5	88.0	90.4	87.5
Culling from infertility (%)	4.2	4.0	3.6	7.5

* P<0.05 (a;b;c;d)

The research showed that there was no significant relationship between the group of cows and conception rate ($\chi^2 = 0.98$, DF = 3, P = 0.806), pregnancy rate ($\chi^2 = 0.567$, DF = 3, P = 0.904) and culling for infertility of cows ($\chi^2 = 1.419$, DF = 3, P = 0.701).

In addition, the irradiation of cows with LIL, as well as a combination of antibiotic and laser treatment affected other cases of pathology (such as uterine atony, mammary gland inflammations, and digestive system disorders). Compared to the negative control, the necessity for treatment of these pathologies was reduced by 21.1% with regard to earlier LIL therapy alone, and by 27.5% (P<0.05) with regard to LIL treatment combined with antibiotics.

Discussion

According to the data found in various literatures, the treatment of metritis is controversial (LEWIS, 1997; DRILLICH et al., 2001; LeBLANC et al., 2002) and prevention is difficult because the causes cannot be clearly defined (LEWIS, 1997). The species of bacteria found in the uterus after parturition are similar in healthy and sick cows (KACZMAROWSKI et al., 2004). However, it has been noted that early intrauterine

antibiotic treatment significantly reduces the complications caused by *Actinomyces pyogenes* (DOBSON and NOAKES, 1990). Thus, the demand for alternative therapies has been increasing in the last several years (ARLT, 2006). For the treatment of septic inflammation it is appropriate to use antibiotics. A rectal temperature above 39 °C is considered to be a valid indication of the start of uterus inflammation (PALENIK et al., 2009). In this study cows with a rectal temperature above 38.9 °C were selected for the prophylaxis of clinical metritis. KRISTULA et al. (2001) recorded that cows experiencing no clinical problems at calving or during early postpartum had an average rectal temperature below 38.9 °C each day throughout the first 10 days postpartum. Daily average temperatures of 38.9 °C or higher in cows with purulent lochia occurred only on Day 4, while these temperatures persisted from Day 1 to Day 7 postpartum in cows with putrid lochia (PALENIK et al., 2009). KRISTULA et al. (2001) and BENZAQUEN et al. (2004), observed the highest occurrence of fever in cows with puerperal metritis between Day 3 and 6 postpartum. Independently of the method used for prophylaxis (antibiotics, laser irradiation or a combination of both these methods) the following indicators usually changed: the duration of recovery from metritis, and the calving-pregnancy period. The duration of treatment of metritis that developed in cows prophylactically treated with LIL and antibiotic was less than of those treated with antibiotics alone. The period from parturition to the successful insemination of cows is an objective indicator of reproductive function and it is considered to be the key indicator in assessing fertilization (KUHN et al., 2004). It was noted that the calving-pregnancy period of cows treated prophylactically with LIL immediately after calving was shorter in comparison to the negative control cows. This indicator was also better in the cows treated prophylactically with antibiotic injections in conjunction with LIL irradiations than in cows treated with antibiotics alone. In comparing fertility indicators a direct relationship was obtained: the most effective prevention method was found to be laser treatment and antibiotics, antibiotics alone were moderately effective, and the least effective was laser treatment alone. The use of low levels of visible or near infrared light for reducing pain, inflammation and oedema, promoting the healing of wounds, deeper tissues and nerves, and preventing cell death and tissue damage has been reviewed by HUANG et al. (2009). The positive influence of laser irradiation on the uterus in cows immediately after parturition is probably connected with the regulatory effect on pro-and anti-inflammatory human cytokines *in vivo* and *in vitro* (ZHEVAGO and SAMOILOVA, 2006), and with the stimulation of the immunological system *in vivo* (NOVOSELOVA et al., 2006). This kind of light stimulates the proliferation of different kinds of cells (PINHEIRO et al., 2003; SHANYFELT et al., 2008), increases cell growth for cells stressed by nutritional deficits *in vitro* (EDUARDO et al., 2007) as well as the inhibition of apoptosis in cells participating in the process of skin regeneration (CHYCZEWSKI et al., 2010). The bactericidal effect of LIL irradiation (ŽILAITIS et al., 2006) can also be considered.

In conclusion, prophylactic LIL physiotherapy shortly after parturition reduces the need for metritis treatment and improves some fertility indices in dairy cows. LIL physiotherapy in conjunction with injections of cephalosporin is more prophylactically effective than cephalosporin applied alone. The most clinically efficient method of LIL therapy is one session per day for 10 consecutive days, immediately after parturition. This method of treatment is suitable for cows with a wide range of productivity and ages.

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SAŽETAK

U radu se uspoređuje učinkovitost slabijeg laserskog zračenja, primjene antibiotika i kombinacije objiju metoda u profilaksi i liječenju metritisa u krava. Istraživanje je bilo provedeno na 290 netom oteljenih krava s rektalnom temperaturom višom od 38,9 °C drugog dana nakon teljenja. Kravama I. skupine (n = 95) intramuskularno je bio primijenjen ceftiofur hidroklorid (600 mg) tijekom tri dana, počevši drugog dana nakon teljenja. U II. skupini bilo je 55 krava koje su bile zračene laserskim zračenjem u sakroilijačnom području tijekom 10 uzastopnih dana. Kravama III. skupine (n = 65) intramuskularno je bio primijenjen ceftiofur hidroklorid (600 mg) tijekom tri dana, a bile su zračene laserskim zračenjem u sakroilijačnom području tijekom 10 uzastopnih dana jedanput dnevno. Kontrolna skupina IV (n = 55) nije dobivala antibiotik niti je bila zračena. U određenog broja krava, od 57,1% (skupina IIIa) do 65% (skupina IV), razvio se klinički metritis, koji je bio liječen antisepticima. Najduže vrijeme oporavka od metritisa ($21 \pm 6,12$ d) ustanovljeno je u skupini IV. Krave skupine III oporavile su se od metritisa za 11,5 dana brže od krava IV. (negativne kontrolne) skupine ($P < 0,05$) i četiri dana brže od krava I. (pozitivne kontrolne) skupine. Pokazatelji plodnosti poput prvoga osjemenjivanja nakon teljenja, servisnog perioda, stope začeća, indeksa osjemenjivanja i razdoblja od teljenja do bređosti bili su bolji u krava kojima je bio profilaktički primijenjen antibiotik u kombinaciji s laserskim zračenjem u usporedbi s drugim skupinama.

Ključne riječi: krave, postpartalno razdoblje, antibiotik, lasersko zračenje
