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Original article

Influence of intra-mammary ozone administration on udder health in herds with contagious mastitis in the context of management practices

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Abstract

This study investigates the effectiveness of intra-mammary ozone administration in the dry period and at the time of delivery for preventing against mastitis in herds with contagious mastitis. The cows were divided into five groups with 10 cows in each. Group 1 was administered an ozone-containing foam preparation via the teat canal into four udder quarters for 5 seconds at the beginning of the dry period; Group 2 was administered ozone at the beginning of the dry period and at the time of delivery; Group 3 was administered ozone at the time of delivery; Group 4 was administered a dry period udder preparation at the beginning of the dry period; and Group 5 was administered only teat seal at the beginning of the dry period. No statistically significant difference was found between the cows with regard to the SCC values at the beginning of the dry period and at the time of delivery (in cows without clinical mastitis, n=25). The SCC values were reported to decrease when the values at the beginning of the dry period and at the time of delivery were compared. All cows except two in Group 1 were detected to have clinical mastitis according to the frequency of microbial isolation in milk at the time of delivery. In conclusion, intra-mammary ozone administration did not prevent mastitis in the dry period or at the time of delivery in herds with contagious mastitis; moreover, it was determined to increase the rate of clinical mastitis in the postpartum period.

Key words: prevention, mastitis, management, microbiological analysis, ozone

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Introduction

Dry period procedures are important among protective measures against mastitis including antibiotic administration, external and internal teat seal procedures, nutritional supplements (vitamins, trace elements) and vaccination for enhancing the defence system of the udder (Blowey and Edmondson 2010, Evink and Endres 2017, Ruegg 2017, Martin et al. 2018).

These methods and tools used to prevent and control mastitis have some disadvantages. For example, antibiotics administered during the dry period remain in the udder as they cannot be released through milk. Moreover, most dry period antibiotics cannot provide prevention at the end of the dry period, which takes 6–8 weeks; they are only protective for the first 4–5 weeks. However, 2–4 weeks before delivery is the period when the risk of intra-mammary infection is the highest. Therefore, the protective effect of antibiotics remains limited to the dry period. Further, the antibiotics used in the dry period are effective for gram-positive bacteria, but not for gram-negative bacteria (Bradley and Green 2001, Lim et al. 2007a, Newton et al. 2008, Petersson-Wolfe et al. 2013).

Teat seal is another method used in the dry period, and this approach can be divided into internal and external (Whist et al. 2006, Lim et al. 2007a, b, Mütze et al. 2012). No significant reduction in new onset intra-mammary infection rates has been reported by studies investigating external teat seal procedures when drying off and during the peripartum period (Edinger et al. 2000, Lim et al. 2007a).

Novel methods are required to prevent and control mastitis in cows, as all dry period methods have some disadvantages. Studies investigating ozone and ozone-containing preparations for intra-mammary use have gradually increased in number in recent years. Ozone is a colourless, tangy gas composed of three oxygen atoms. Medical ozone therapy is a treatment method that includes the administration of an ozone-oxygen mixture obtained through a generator for pure oxygen (0.05–5% O_3; 95–99.95% O_2) (Bocci 2006). It has been used since the first half of the 19th century for disinfection and medical purposes; it shows antioxidant, antimicrobial, immune stimulant and anti-inflammatory effects. Certain amounts of this ozone-oxygen mixture may be administered into body spaces or the circulatory system via the intravascular, intramuscular and subcutaneous routes or topically. This mixture has been used in the treatment of metritis, endometritis, retention secundinarum, uro-vagina and mastitis in gynaecology in the veterinary field. Ozone has been reported to be able to reduce somatic cell count (SCC) in mastitis cases (Onyay et al. 2015).

Ozone therapy can be used to treat mastitis safely and effectively, leading to no residue in milk (Ogata and Nagahata 2000, Duricic et al. 2015, Vural et al. 2016). Ogata and Nagahata (2000) compared intra-mammary ozone administration with antibiotic therapy (kanamycin and benzyl penicillin procaine or cefazolin) in the treatment of mastitis and concluded that ozone therapy did not leave a residue in milk, the procedure was not risky and it was cost-effective and safe.

It has been reported that ozone can be used in acute coliform mastitis and chronic mastitis due to its antimicrobial effect, not inducing endotoxin release and not leading to downregulation in the cytotoxic effect of immunocytes (Liu et al. 2005, Shinozuka et al. 2009).

Staph. aureus and Strep. agalactiae followed by M. bovis are the most common contagious micro-organisms that lead to mastitis. These micro-organisms usually originate from infected udder quarters and infected animals. Contagious micro-organisms rapidly spread to the mammary glands and lead to long-term subclinical mastitis. This condition becomes more prominent in the case of poor hygiene and absence of control. The prevention of contagious micro-organism-related mastitis has been reported to be easier if regular prevention and control programmes are applied (Aarestrup and Jensen 1997, Risvanli and Kalkan 2001, Risvanli and Kalkan 2002).

The present study investigates the effectiveness of intra-mammary ozone administration for the prevention and control of mastitis in herds with contagious mastitis in the dry period in the context of dairy cattle management.

Materials and Methods

The study was conducted at the Aksut Dairy Herd in the Malatya (Turkey) province between May 2017 and December 2018. The herd was composed of 3000 milk Holstein cows aged between 3 and 6 years. The cows are kept in semi-open barns and barns that enable free movement. Barley-containing concentrated food and dry meadow, corn silage, clover and straw are used for rations. The Deleval brand of automated milking machine is used. The mean lactation milk yield was 6500 L and the cows were milked twice daily. Milking hygiene included washing udders before milking and teat dipping. Dry period antibiotic administration is performed at the beginning of the dry period to prevent mastitis. The whole milking system is washed with electrolysed water after each procedure. Contagious subclinical mastitis has long been experienced despite regular screening.

A total of 50 healthy cows without clinical mastitis

aged 3–6 years, with a 3–3.5 body condition score, weighing 450–500 kg were used as the animal material. The animals were divided into five groups with 10 in each. The udder was chosen as a clinical mastitis has swelling, heat, redness, or pain and the milk has a watery appearance with flakes, clots, or pus.

Group 1: Ozone-containing foam preparation (SANOFOAM Agriprom) was applied from the teat canal of four udder quarters for 5 seconds after the last milking at the beginning of the dry period.

Group 2: Ozone-containing foam preparation (SANOFOAM Agriprom) was applied from the teat canal of four udder quarters for 5 seconds after the last milking at the beginning of the dry period and at the time of delivery.

Group 3: Ozone-containing foam preparation (SANOFOAM Agriprom) was applied from the teat canal of four udder quarters for 5 seconds at the time of delivery.

Group 4: 50 mg clavulanate, 200 mg amoxycillin and 10 mg prednisolone-containing dry period udder preparation (BOVIMAST LC Alke) was applied to four udder quarters after the last milking at the beginning of the dry period.

Group 5: Teat seal (ORBESEAL®. Zoetis) was applied to four udder quarters at the beginning of the dry period.

Local ethics committee approval was obtained from the Inonu University Experimental Animal Local Ethics Committee (2017/A-08).

Somatic cell counting

The milk obtained from the four udder quarters of each cow was mixed for somatic cell counting. Samples were placed into 5 mL plastic tubes at the beginning of the dry period, at the time of delivery before applying the procedure, and once a week until the postpartum 6th week. Somatic cells were counted using a DeLaval Cell Counter® (DeLaval International, Sweden) cell counting device (Pyörala 2003).

Microbiological tests

Microbiological tests were performed at the Microbiology laboratory of Melid Park Hospital (Malatya, Turkey). The samples were drawn into 10 mL sterile glass tubes during morning milking at the beginning of the dry period, at the time of delivery and at the postpartum 6th week. They reached the laboratory within 30–40 min in accordance with cold chain (+4°C) rules. The samples were cultured in 5% blood agar and Mac Conkey agar and incubated at 38°C in anaerobic, aerobic and micro-aerophilic environments for microbiological analysis as soon as they reached the laboratory. Afterwards, isolated micro-organisms were reported. The required identifications were performed using conventional methods in the case of isolation (Koneman et al. 1997).

Statistical analysis

Using the data obtained at the end of the study, it was investigated whether the SCC at the beginning of the dry period, at the time of delivery and at the postpartum 6th week, the microbiological analysis results (frequency, colony count and bacteria type) and SCC values at the postpartum 1st, 2nd, 3rd, 4th and 5th weeks provided parametric test assumptions, and descriptive statistics were calculated. The Kruskal–Wallis variance analysis was used to compare the SCC values of the groups at the beginning of the dry period and at the time of delivery. The Wilcoxon test was used to analyse the significance of the variance between the SCC values at the beginning of the dry period and those at the time of delivery. The Kruskal–Wallis variance analysis was also used for the inter-group comparisons of colony counts in microbial isolation-positive milk samples at the beginning of the dry period, and Fisher's exact chi-square test was used for the frequency values. The Mann–Whitney U test was used to analyse the weeks that demonstrated significance. These calculations and analyses were performed using the SPSS 22.0 program (SPSS 2015).

Results

The descriptive statistics of the SCC in milk obtained at the beginning of the dry period and at the time of delivery are presented in Table 1.

The SCC values at the beginning of the dry period were found to be the highest in Group 4 (891.90 ± 143.56 cell/mL×1000) and the lowest in Group 2 (617.40 ± 166.16 cell/mL×1000). The mean SCC value of the cows included in the study was determined as 747.74 ± 81.80 (cell/mL×1000). The minimum SCC was found to be 36, while the maximum SCC was found to be 2019 (cell/mL×1000).

When the SCC values at the time of delivery were evaluated, they were found to be the highest in Group 1 (449.00 ± 205.00 cell/mL×1000) and the lowest in Group 3 (238.29 ± 19.15 cell/mL×1000). The mean SCC value of all groups was determined to be 302.04 ± 27.47 cell/mL×1000.

The differences in the inter-group comparisons of the SCC values at the beginning of the study were not found to be statistically significant ($p>0.05$; Table 1). Similarly, the differences in the inter-group comparisons of the SCC values in animals that did not have

Table 1. Descriptive statistics of the SCC (cell count/mL×1000) in milk obtained at the beginning of the dry period and at the time of delivery.

	Drying off				Time of parturition				
Group	n	$\bar{x} \pm s_{\bar{x}}$	Minimum	Maximum	n	$\bar{x} \pm s_{\bar{x}}$	Minimum	Maximum	p
1	10	789.40±208.17	89	1844	2	449.00±205.00	244	654	0.180
2	10	617.40±166.16	57	1807	2	253.50±11.50	242	265	0.180
3	10	713.50±196.28	81	1963	7	238.29±19.15	176	322	0.128
4	10	891.90±143.56	414	1853	5	320.00±48.86	216	444	**0.043**
5	10	726.50±217.91	36	2019	9	319.78±55.83	165	625	0.110
Total	50	747.74±81.80	36	2019	25	302.04±27.47	165	654	**0.001**
p		**0.625**				**0.628**			

Table 2. Microbiological analysis results of milk at the beginning of the dry period (frequency and colony count).

Group	n	Microbial isolation negative	Microbial isolation positive	Clinical mastitis	Microbial isolation (Colony)		
			Subclinical mastitis		$\bar{x} \pm s_{\bar{x}}$	Minimum	Maximum
1	10	5	5	-	10.00±3.33	0	20
2	10	3	7	-	11.30±6.40	0	65
3	10	6	4	-	3.70±2.14	0	19
4	10	2	8	-	10.80±3.99	0	36
5	10	2	8	-	12.10±4.40	0	43
p			**0.625**		**0.315**		

Table 3. Microbiological analysis results (type of bacteria) of milk at the beginning of the dry period.

Group	Number of microbial isolation positive animals (n)	Type of isolated bacteria				
		Staph. aureus	*Staph. epidermidis*	*Strep. spp*	*Beta hemolytic Strep.*	*E. coli*
1	5	x	x			
2	7	x	x	x	x	
3	4	x	x		x	
4	8	x	x		x	x
5	8	x	x	x	x	

Table 4. Descriptive statistics of the microbiological analysis of milk at the time of delivery (frequency, colony).

Group	n	Microbial isolation negative	Microbial isolation positive	Clinical mastitis	Microbial isolation (Colony)		
			Subclinical mastitis		$\bar{x}$	Minimum	Maximum
1	10	0	2	8	24500	9000	40000
2	10	2	0	8	0	0	0
3	10	5	2	3	3071	0	12500
4	10	3	2	5	7000	0	20000
5	10	7	2	1	4555	0	35000

mastitis at the time of delivery (n=25) were not found to be statistically significant (p>0.05).

When the SCC values at the beginning of the dry period and at the time of delivery were compared, they were determined to decrease in all groups. This decrease was statistically significant (p<0.001). When groups were evaluated separately, only the SCC values in Group 4 were found to decrease statistically significantly.

The descriptive statistics of the microbiological analysis results of the milk at the beginning of the dry period (frequencies and colony counts) are presented in Table 2.

When the microbiological isolation results at the beginning of the dry period were evaluated as frequencies, the maximum isolation was determined in positive (subclinical mastitis) Groups 4 and 5 (8) and the minimum isolation was determined in positive (subclinical mastitis) Group 3 (4). The differences between the groups with regard to the frequency of microbiological

Table 5A. Descriptive statistics of the SCC values in milk at the postpartum 6th week (cell/mL×1000).

	PP 1st week				PP 2nd week				PP 3rd week			
Group	n	$\bar{x} \pm s_{\bar{x}}$	Min	Max	n	$\bar{x} \pm s_{\bar{x}}$	Min	Max	n	$\bar{x} \pm s_{\bar{x}}$	Min	Max
1	2	270.50^{AB}±44.50	226	315	2	258.00±26.00	232	284	2	223.50±18.50	205	242
2	2	210.50^{AB}±5.50	205	216	2	199.00±17.00	182	216	2	192.00±27.00	165	219
3	7	208.57^{AB}±9.37	177	242	7	208.43±12.54	165	264	7	193.86±9.51	165	234
4	5	290.20^{A}±24.72	228	350	5	253.20±19.41	212	314	5	200.40±11.70	180	244
5	9	190.78^{B}±25.00	0	256	9	247.11±50.71	165	646	8	196.38±8.96	165	235
General	25	223.60±13.11	0	350	25	234.52±18.83	165	646	24	198.37±5.16	165	244
p		**0.040**				**0.199**				**0.689**		

A, B: Differences between the mean values of the different letters in the same column are statistically significant ($p<0.05$)

Table 5B. Descriptive statistics of the SCC values in milk at the postpartum 6th week (cell/mL×1000).

	PP 4th week				PP 5th week				PP 6th week			
Group	n	$\bar{x} \pm s_{\bar{x}}$	Min	Max	n	$\bar{x} \pm s_{\bar{x}}$	Min	Max	n	$\bar{x} \pm s_{\bar{x}}$	Min	Max
1	2	212.50±3.50	209	216	2	199.50±4.50	195	204	2	203.00±11.00	192	214
2	2	200.00±4.00	196	204	2	215.00±20.00	195	235	2	211.00±24.00	187	235
3	7	192.43±9.36	165	231	7	193.71±6.71	165	216	7	209.86±6.87	192	244
4	5	199.40±9.05	165	215	5	196.20±11.79	164	235	5	203.80±12.92	164	244
5	8	217.63±5.80	192	246	8	195.75±7.57	172	234	8	217.13±10.89	192	282
General	24	204.58±4.25	165	246	24	197.17±4.14	164	235	2	203.00±11.00	192	214
P		**0.175**				**0.877**				**0.414**		

Table 6. Descriptive statistics of the microbiological analysis results of milk at the postpartum 6th week (frequency and colony count).

Group	n	Microbial isolation negative	Microbial isolation positive	Clinical mastitis	Microbial isolation (Colony)		
			Subclinical mastitis			Minimum	Maximum
1	10	0	2	8	13000	11000	15000
2	10	0	2	8	7000	5000	9000
3	10	4	3	3	13393	0	35000
4	10	3	2	5	8000	0	30000
5	10	4	4	2	14719	0	67500
p					**0.856**		

Table 7. Microbiological analysis results at the beginning of the dry period, at the time of delivery and at the postpartum 6th week (frequency).

Group	n	Microbial isolation negative			Microbial isolation positive Subclinical mastitis			Clinical mastitis		
		Beginning of the dry period	Time of delivery	Postpartum 6th week	Beginning of the dry period	Time of delivery	Postpartum 6th week	Beginning of the dry period	Time of delivery	Postpartum 6th week
1	10	5	0	0	5	2	2	-	8	8
2	10	3	2	0	7	0	2	-	8	8
3	10	6	5	4	4	2	3	-	3	3
4	10	2	3	3	8	2	2	-	5	5
5	10	2	7	4	8	2	4	-	1	2

analysis results at the beginning of the dry period were not found to be statistically significant ($p>0.05$).

When the microbiological isolation results at the beginning of the dry period were evaluated as colony counts, the minimum colony count was determined in Group 3 (3.70 ± 2.14) and the maximum in Group 5 (12.10 ± 4.40). The difference between the groups was not found to be statistically significant. These results were found to be similar to the results of the comparisons made with regard to the frequencies of microbiological isolation ($p>0.05$).

The types of bacteria detected in animals with positive isolation in milk at the beginning of the dry period are presented in Table 3.

Staph. aureus and Staph. epidermidis were detected in all groups. Beta-hemolytic streptococcus was detected in all groups except for Group 1, and E. coli was detected only in Group 4. Strep. spp was detected in Groups 2 and 5.

The descriptive statistics of the microbiological analysis of milk at the time of delivery are presented in Table 4.

When the frequencies of microbiological isolation in milk at the time of delivery were analysed, all cows in Group 1 were determined to have mastitis (subclinical mastitis in two, clinical mastitis in the remaining). The total numbers of cows with subclinical and clinical mastitis were determined as eight, seven, and five in Groups 2, 4, and 3, respectively. The number of cows with mastitis was the least in Group 5 (in three cows: two subclinical, one clinical mastitis).

The descriptive statistics of the SCC values in milk at the postpartum 6th week are presented in Table 5.

The SCC values of the milk showed a regular decrease from the time of delivery to the end of the postpartum 5th week in Groups 1 and 4. A regular tendency was not determined in the other groups with regard to the SCC values.

When the postpartum SCC values were evaluated, the inter-group differences were found to be statistically significant only in the first week after delivery ($p<0.040$). This difference was prominent between Groups 4 and 5.

When the SCC values in milk at the postpartum 6th week were analysed (including milk with and without microbiological isolation), the maximum SCC was in Group 5 (217.13 ± 10.89 cell/mL×1000) and the minimum SCC was in Group 1 (203.00 ± 11.00 cell/mL×1000).

The descriptive statistics of the microbiological analysis results of milk at the postpartum 6th week are presented as frequencies and colony counts in Table 6.

All cows in Groups 1 and 2 were found to have subclinical and clinical mastitis; the number of cows with mastitis was the lowest in Group 3 (subclinical mastitis in three and clinical mastitis in three) and Group 5 (subclinical mastitis in four and clinical mastitis in two).

The microbiological analysis results at the beginning of the dry period, at the time of delivery and at the postpartum 6th week are presented in Table 7.

The numbers of isolation negative cows were determined as five, three, six, two and two, respectively, in the groups at the beginning of the dry period. These numbers were determined as zero, two, five, three and seven, respectively, in the groups at the time of delivery. The number of cows in which isolation was not detected at the time of delivery was observed to be the most (seven) in Group 5.

While Groups 3, 4 and 5 were found to be similar with regard to the number of cows determined not to have microbiological isolation at the postpartum 6th week (four, three, and four, respectively), Groups 1 and 2 were found to be similar with regard to the microbiological analysis results (positive in all).

When the udder health statuses at the beginning of the dry period and at the time of delivery were compared, the frequency of cows with the best status was the highest in Group 5. Similarly, the frequency of cows with the best status was the highest in Groups 5 and 3 when udder health statuses were compared between the beginning of the dry period and the postpartum 6th week.

Discussion

Some procedures such as dry period antibiotic administration have an important place among management procedures aimed at preventing and controlling mastitis in dairy herds. However, alternative methods may be required as these antibiotics may be ineffective, leading to residue problems and resistance to antibiotics. The present study investigates the influence of intra-mammary ozone administration on udder health in herds with contagious mastitis as an alternative to the dry period procedures used to prevent and control mastitis in cows.

Contagious micro-organisms enter the udder via the hands or milking equipment and easily spread from one cow to another. Prevention against subclinical mastitis includes an effective dry period treatment, post-milking teat dipping procedures and correct milking hygiene (Pinedo et al. 2012, Vural et al. 2016).

Bradley et al. (2011) administered cefquinome, a wide-spectrum antibiotic in one group, cloxacillin, a narrow-spectrum antibiotic in another, and cloxacillin+ internal teat seal in the third group to investigate three dry period treatment regimens. The authors reported no difference between the groups with regard to the recovery rate of intra-mammary infections related to major agents. The clinical mastitis rates

were determined to be lower in Groups 1 and 3 than in Group 2.

Krömker et al. (2014) reported the novel intra-mammary infection rate as 3.4% in cows on which teat seal was applied and 10% in cows that did not undergo treatment; teat seal may thus be effective in reducing intra-mammary infection rates. With regard to udder health, the best results in the present study were determined in the teat seal group.

Ozone is used to treat and prevent mastitis, washing the udder before and after milking with ozone water is reported to be useful for udder health (Shumway 2007). Ozone fumigation in barns is reported as an effective method for prevention of fungal mastitis in water buffalo (Atef et al. 2016).

To the best of our knowledge, no studies in the literature investigate the role of ozone or ozone-containing preparations for prevention of mastitis in the dry period. In the present study, the postpartum SCC, microbiological isolation and clinical mastitis rates were found to be higher in the groups to which ozone was administered at the beginning of the dry period or at the time of delivery.

In the inter-group comparison of the ozone-administered groups, the minimum SCC was determined in Group 1 (203.00 ± 11.00 cell/mL×1000) at the postpartum 6th week (microbiological isolation positive and negative cases).

When the microbiological analysis results at the postpartum 6th week were analysed, all cows in Groups 1 and 2 were detected to have subclinical and clinical mastitis, and the number of cows with mastitis was found to be the lowest in Group 3 (subclinical mastitis in three and clinical mastitis in three) and Group 5 (subclinical mastitis in four and clinical mastitis in two). While Groups 3, 4 and 5 were found to be similar with regard to the number of cows that did not have microbiological isolation in milk at the postpartum 6th week, Groups 1 and 2 were found to be similar with regard to the microbiological analysis results (positive in all). This is suggested to have resulted from the contagious micro-organisms common in the management and intra-mammary ozone administration, accelerating the isolation of this type of micro-organism (micro-aerophilic organism).

In conclusion, intra-mammary ozone administration in the dry period and at the time of delivery does not help the control and prevention of contagious mastitis in cows; moreover, it results in increased clinical mastitis rates. However, considering the SCC values at the time of delivery, a regular decrease was determined during the period including the postpartum 5th week. When the udder health status at the beginning of the dry period and at the time of delivery (after administration) were compared, the number of cows with a good status was the highest in Group 5; when the status at the beginning of the dry period and the postpartum 6th week were compared, the number of cows with the best status was the highest in Groups 3 and 5. It was concluded that intra-mammary ozone administration should be investigated, particularly in pregnant heifers and healthy cows, at the beginning of the dry period to reveal its effectiveness for the prevention and control of mastitis in herds with contagious mastitis.

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