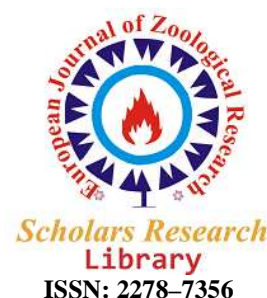




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The effect of intrauterine injection of super-oxidized water on the improvement of postpartum endometritis in dairy cows

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ABSTRACT

Endometritis is a kind of uterine infection that causes decreased fertility in cows. This study aimed to determine the effect of intra-uterine injection of super-oxidized water on the improvement of the clinical symptoms in the cows with Endometritis. The study examined 60 dairy cows 25 days or more in the postpartum period. Thus, 80 cows with different degrees of Endometritis were selected applying rectal examination and vaginoscopy and their endometrial thickness was measured and recorded applying ultrasonography. Finally, about 20 cows were withdrawn from the study due to reasons such as withdrawal from the herd. 60 remaining cows that received treatment were divided into 6 groups based on their degree of Endometritis; 3 experimental groups and 3 control groups. Control groups received the common treatment by antibiotics and Metricure while the experimental groups were treated by intra-uterine injection of 50 ml of super-oxidized water. The results indicated that after the treatment, the cows in the experimental groups were significantly different from the cows in the control groups considering the endometrial thickness, presence of infectious liquids in the uterus, and higher rate of successful conception compared to the number of inseminations. According to the significant results of the study, it can be concluded that applying super-oxidized water in treating cows with Endometritis will be more effective.

Key words: dairy cow, Endometritis, super-oxidized water, Metricure

INTRODUCTION

Following some standards like calving, the period between the two parturitions, parturition until estrus, and the number of inseminations for each conception is one of the basics of success and profitability in successful dairy farms[8]. That is, if any of the issues above are not followed based on the standards, the number of the calves every year and following that the rate of milking and the profit will also reduce. One thing that affects the above mentioned issues is the female reproductive organs diseases [2]. One of the important reasons for the increase in the time period between calving, parturition until the first estrus, delivery until conception, and number of inseminations compared to the number of conceptions is the postpartum infections, especially Endometritis. Endometritis is defined as the inflammation of the uterine endometrium[3]. There are some disorders in the function of uterus in dairy cows postpartum due to the bacteria in the uterine cavity. Infectious bacteria cause uterine diseases which are the main reasons of infertility in dairy cows [17]. Although most cows are able to fight and get rid of those bacteria five weeks postpartum, in 10 to 15% of them the continuing infections resultant from those bacteria cause uterine diseases which are diagnosed by physical examinations[10]. The most important factor in diagnosing Endometritis is the presence of purulent on day 21 or mucopurulent on day 26 postpartum. There are several methods to treat

Endometritis all of which are time-consuming and depend on the consumption of different antibiotics[8]. These methods are not advised because they not only result in resistance to antibiotics, which in turn make it difficult to treat the disease at subsequent times, and remain in the milk or the meat, but also impose a huge financial burden on the farmer[4, 7]. This research study was an attempt to examine the impact of intra-uterine injection of super-oxidized water on the treatment of the Endometritis without the application of antibiotics or a very little use of them in a short time and with the least financial cost.

MATERIALS AND METHODS

This study was conducted in a farm in Moghan Agro Industry and Animal Husbandry with 1200 Holstein cows. The cows were studied under identical conditions and had the same feeding and breeding management. Cows were milked three times daily and the average herd milk yield was 25 ± 1 .

In this study, 80 cows that were diagnosed with Endometritis and yielded almost the same amount of milk were treated. The cows were at clinical state of 2-3 and had no clinical problems other than uterine problems. The cows that had been selected were 25 or more days postpartum and the Endometritis symptoms were observed through uterine discharge and vaginoscopy. Moreover, the thickness of endometrium was measured by ultrasonography and was recorded[4, 7].

Finally, 20 cows were withdrawn from the study because of some reasons such as withdrawal from the herd. The remaining 60 cows were divided into 3 groups based on the grade (1, 2, and 3) of their Endometritis. Cows in each group were then randomly assigned into two groups; control and experimental.

The cows in the control group received an intrauterine injection of 50 ml of Metricure. After 9 days, the cows further received one dose of PGF_{2α} injection and were observed and examined by vaginoscopy after 48 hours. The thickness of endometrium was recorded for these cows. In the experimental group, also, the above procedure was repeated, but the super-oxidized water was applied instead of Metricure. At the end of the procedure, the cows in both groups that showed estrus symptoms were inseminated based on the farm policies and were examined for pregnancy after 30-35 days by uterus palpation and rectal examination.

The substance used in this study was super-oxidized water with FAC (the amount of the total free chlorine in the solution) of 600-PH7.2-7.1 and ORP (potential of decreased oxidization in millivolts) of 860. The trade mark of this substance is Envirolite and had been produced by the machinery of Envirolite factory. The substance was purchased from the Khosro Medisa Teb Company which imports Envirolite machinery.

Anolyte is super-oxidized water with effective substance of HClO. The other existent compounds include OH, H₂O, H₂O₂, and O₂. This substance is a very strong disinfectant and can be effective on bacteria, viruses and fungi. This substance definitely has fewer compounds compared to Chlorine, halogens, and trihalomethanes (carcinogenic) and when it is diluted by water, it has no chronic or acute effects. It is used in some countries like Germany to disinfect drinking water. Other advantages include its ease of production and cheap price.

RESULTS

The thickness of the endometrium before and after treatment, the amount of purulent in uterine discharge, and the number of successful conception compared to the number of inseminations were recorded and analyzed statistically. Data was statistically analyzed using the Microsoft Excel software and employing paired samples T-test. Results showed that in both experimental and control groups and in all three grades of 1, 2, and 3, the employed treatment had a significant effect on reducing the thickness of endometrium ($P < 0.05$). However, since there is a natural difference in the thickness of endometrium before treatment (every animal has a different endometrium thickness according to the severity of the disease), the resulting numbers from subtracting the endometrium thickness before and after treatment were converted into percentages and were then compared, so that they were statistically acceptable. After that, more statistical analyses were done using the SPSS software. The ANOVA test was employed for the analysis of variances (see table 1). Because the results of the ANOVA test were significant ($P < 0.05$), the Welch and Brown Forsythe tests were employed for further analysis (see table 2). The results of both tests indicated a difference between the experimental and the control groups ($P < 0.05$).

		Sum of Squares	df	Mean Square	F	Sig.
BAD	Between Groups	1283.148	5	256.630	75.242	.000
	Within Groups	184.179	54	3.411		
	Total	1467.327	59			
AAD	Between Groups	968.987	5	193.797	133.344	.000
	Within Groups	78.482	54	1.453		
	Total	1047.469	59			

		Statistic ^a	df1	df2	Sig.
BAD	Welch	100.092	5	22.632	.000
	Brown-Forsythe	75.242	5	28.786	.000
AAD	Welch	108.749	5	23.123	.000
	Brown-Forsythe	133.344	5	31.535	.000

The following results indicated that the cows in all three experimental groups were in a worse condition than the cows in the control groups considering the severity of their disease. Therefore, the significant statistical results showing the difference between the experimental and the control groups shows that the treatment procedure employing the super-oxidized water was very effective. To compare the experimental and the control groups from the same grade of the disease, the independent samples T-test was employed. The results of this test are as follows: According to table3, the comparison between the two groups of the Endometritis grade 1 (experimental grade 1 and control grade 1), showed that the mean of the difference between the thickness of the endometrium before and after the treatment equals 10.94 in the experimental group and 2.41 in the control group. The difference between the two groups was statistically significant at 95% confidence rate according to the results of the independent samples T-test ($P=0.000<0.05$). This means that the improvement in the experimental group was more than the control group.

	Group	N	Mean	Std. Deviation	Sig.
Grade I	Experimental	10	10.9430	1.85782	.000
	Control	10	2.4140	.53054	

According to table A9, the comparison between the two groups of the Endometritis grade 2 (experimental grade 2 and control grade 2) showed that the mean of the difference between the thickness of the endometrium before and after the treatment equals 11.10 in the experimental group and 3.04 in the control group. The difference between the two groups was statistically significant at 95% confidence rate according to the results of the independent samples T-test ($P=0.000<0.05$). This means that the improvement in the experimental group was more than the control group.

According to table A10, the comparison between the two groups of the Endometritis grade 3 (experimental grade 3 and control grade 3), showed that the mean of the difference between the thickness of the endometrium before and after the treatment equals 25.11 in the experimental group and 6.43 in the control group. The difference between the two groups was statistically significant at 95% confidence rate according to the results of the independent samples T-test ($P=0.000<0.05$). This means that the improvement in the experimental group was more than the control group.

Finally, a precise study of the above results showed that the application of the super-oxidized water in treating Endometritis is not only more effective than the application of Metricure, but also in the cows with a high grade of Endometritis, the effect of super-oxidized water was stronger compared to the cows with lower grades of Endometritis. In other words, the worse the disease is the better the super-oxidized water affects. For a better understanding, the rate of improvement (endometrium thickness) in each group was calculated in percentages and was compared to the pairing group in the opposing group.

To analyze the qualitative data (Endometritis grading based on presence of purulent in the uterin discharge), both McNemar and Kruskal-Wallis tests were employed (see table 4). The results of both tests showed that the experimental group was statistically different from the control group. To apply the Kruskal-Wallis test, we had to assign a number to each observed clinical symptom.

C (clean)-no infection =5.
 P.d (post discharge) =4.

1 (grade 1) which is indicative of 50% infection, =3.
 2 (grade 2) which is indicative of 50 to 75% infection, =2.
 And finally,3 (grade 3) which is indicative of more than 75% infection, =1.

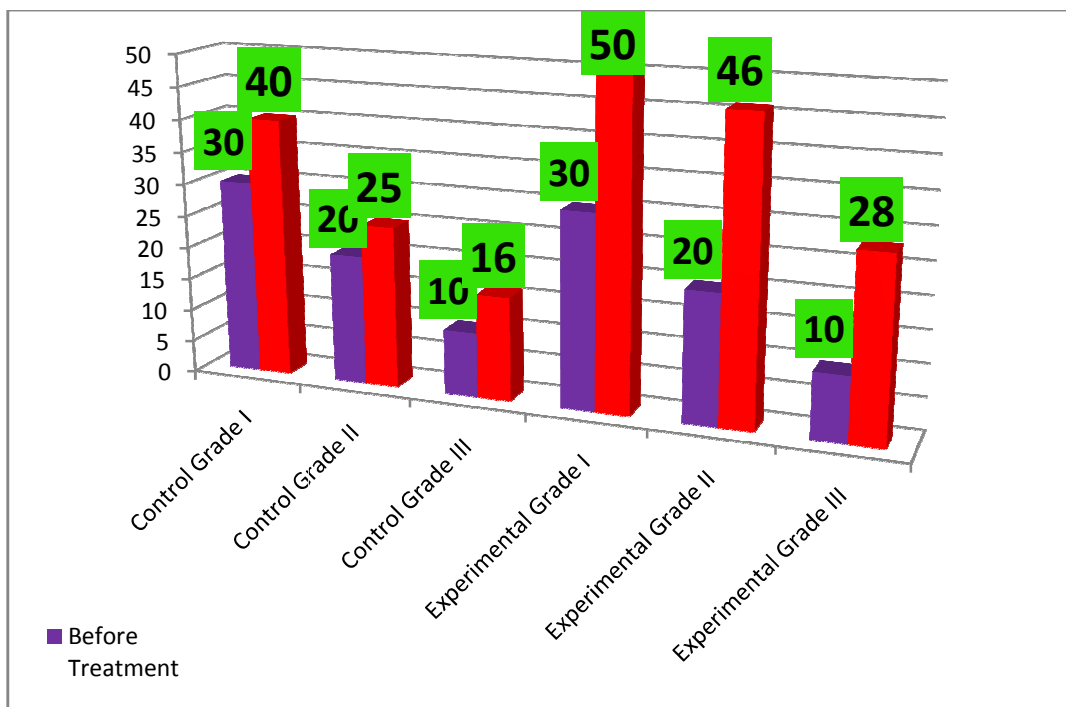
The above assigned numbers show that the bigger the number assigned, the less severe the disease in that group is and vice versa (see Figure1).

To employ McNemar statistical tests, only the improvement of the disease was analyzed without considering how much it was improved.

Table 4: Kruskal-Wallis-Test

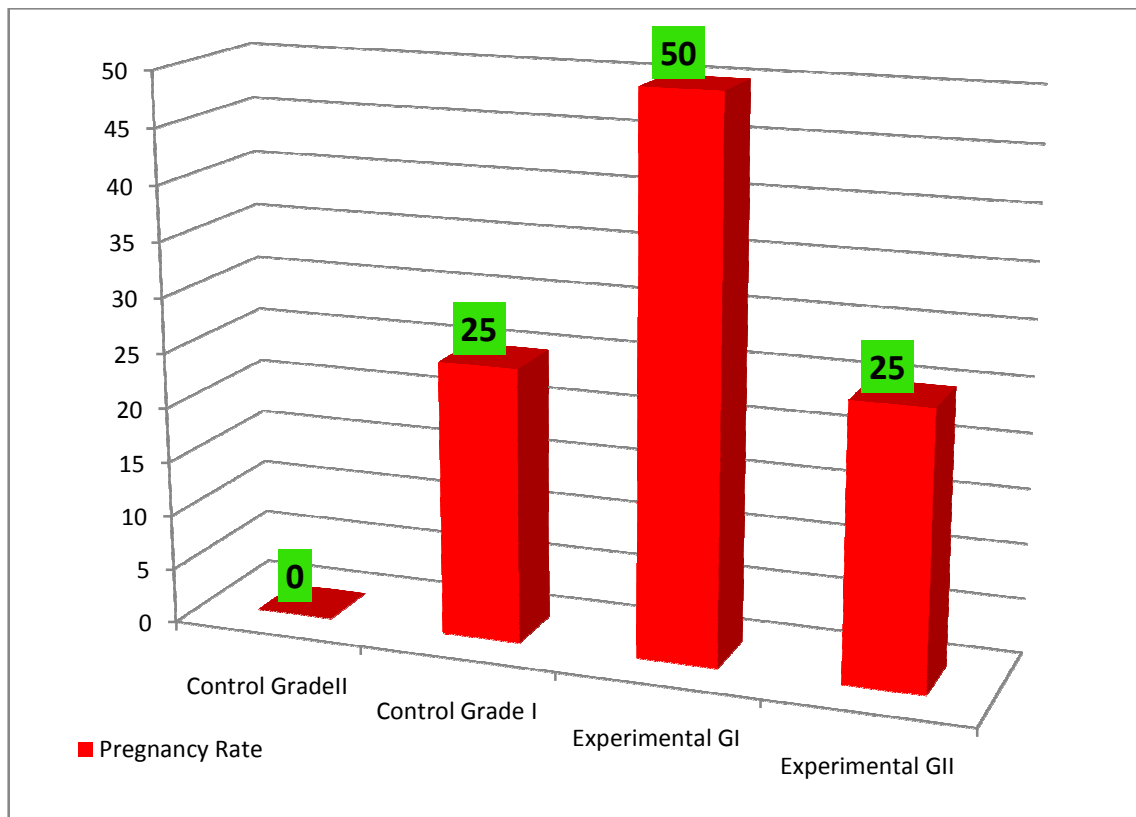
Test Statistics ^{a,b}	
	rank
Chi-square	48.008
df	5
Asymp. Sig.	.000

Figure 1: Comparison of clinical symptoms before and after treatment based on hypothetical values



The comparison between the results of the pregnancy of the both groups is depicted in figure2. This comparison shows a better performance of the experimental group compared to the control group.

Figure 2. The percentage of conception rate per insemination in each group



DISCUSSION

Since a healthy uterine area is the most important factor for the optimal conception in dairy herds, treatment of Endometritis [12, 14], which is one of the most common uterine disorders in dairy cows and which reduces the conception rate and in turn results in great financial and economic loss, has always been significant for the researchers. The present study proposed the intrauterine injection of super-oxidized water for treating Endometritis and showed that this method resulted in better and faster improvements of the clinical symptoms of Endometritis in dairy cows compared to the common treatments. Therefore, this method can improve reproductive index in dairy cows.

For a long time, veterinarians have been employing different kinds of antibiotics for treating clinical and subclinical Endometritis. The most important intrauterine antibiotics that are used for Endometritis treatment are divided into three groups; tetracycline, cephalosporin, and penicillin. It is believed that overall, the profitability rate of the antibiotics in treating diseases is needed to be examined periodically because the new generation of bacteria are not only resistant to them, but also have new characteristics [16]. Counting these cells can be an appropriate index in characterizing Endometritis in dairy cows [5]. It has also been reported that frequent use of oxytetracycline causes necrosis of endometrium and formation of fibrosis and subsequently, significantly decreases the conception potential of the area being treated [8]. In addition to the burning characteristics, as mentioned, oxytetracycline is absorbed to the blood circulation of the uterine area in 12 hours and remains in milk and meat between 48 to 144 hours [8]. The first generation cephalosporins under the business name of Metricure, is used intrauterine to treat acute and sub-acute Endometritis. These antibiotics are also effective on specific kinds of microorganisms in anaerobic environments and function by creating the appropriate thickness in endometrium and are resistant to penicillinase bacteria [8]. Findings of the present research study indicated that employing super-oxidized water as a non-toxic and non-antibiotic substance can be used as an alternative for the common antibiotics by reducing the bacteria in the uterine area. Moreover, since it is not present in the milk and meat after the treatment, it is highly proposed and is very appropriate to be employed. By and large, there have not been many laboratory experiments regarding the

employment of antibiotics for the treatment of Endometritis in cows. Moreover, the resultant clinical improvements and the conception rate after the employment of antibiotics are not consistent from one study to the other. However, the intrauterine injection of antibiotics is known as one of the desired methods in treating Endometritis and is used widely. It is reported that the clinical symptoms improved and the time between the parturition and the subsequent conception reduced as a result of intrauterine injection of antibiotics [10, 11]. However, it is not argued whether or not antibiotics remain in the milk or meat after they are employed. There are several reports which prove that in European Union, different dilutions of super-oxidized water are used to disinfect the drinking water. This is accepted by the World Health Organization (WHO). In Iran, license has been granted for this substance to be used for similar purposes. It is concluded that the remaining dilutions of this substance in the milk and the meat does not put the consumers' life at risk and is totally safe. 0.5 grams of Cephapirin was injected intrauterine on days 27-33 postpartum to the cows that had been selected by rectal examinations and vaginoscopy on days 20 and 30 postpartum and had unusual uterine discharge [10, 11]. Results indicated that compared to the control group, the experimental cows showed to have higher conception rate. For the intrauterine injection to be more effective, the employed antibiotic not only should be effective on the microorganism, but also should not cause the destruction or the dysfunction of the uterine defensive mechanisms. The antibiotic should also stay active in the infected area and should not remain in the milk or meat. It should additionally, preserve its dilution and be economically appropriate [8]. Recently, employment of non-antibiotic substances has been of attention as an alternative treatment for Endometritis such as PGF₂ α or Estrogens. In a recent study, it is reported that PGF₂ α had a significant effect on the treatment of Endometritis grade I and the improvement of conception rate compared to estrogens [1, 9]. However, according to the findings of the present study and the shortcomings mentioned with the application of other medication, it seems that super-oxidized water as a non-toxic substance, which is also easily available, cheap, and with better clinical results compared to Metricure, can be an appropriate alternative for antibiotics in treating Endometritis of any grade [13]. In a study, super-oxidized water was used to disinfect the medical instruments and the surgery area (rib cage and Sternum bone) in an open-heart surgery [15]. That is, the surgery area was scrubbed by the solution for 5 minutes. The study was conducted on 25 patients who were going through an open-heart surgery and the researchers reported that the results were positive. In another study, the solution was used to disinfect the teeth root canals after the nerves and their derivations were extracted [6]. The researchers then reported that the super-oxidized water as the main disinfectant resulted in significantly more positive effects compared to the control group. Analyzing the similar research studies in the literature, we found out that there had been no use of this solution in veterinary to date and the present study is the first study to be conducted in the area of midwifery and reproduction diseases in veterinary.

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