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Assess the Efficiency of Silver Nanoparticles for Treatment of Endometritis in Iraqi Breed Cows

Elias Khudhur Hussein[1] and Khawla A. Hussein[2]

[1]*Department of Surgery and Obstetrics, College of Veterinary Medicine, Mosul University, Iraq.*

[2]*Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University, Iraq.*

THE AIM of the present study was to assess the efficacy of intrauterine infusion of silver nanoparticles for the treatment of endometritis in cows. The research was carried out in a dairy cow field with Iraqi breed cows. The study involved 20 cows with clinical endometritis (CE). cows were randomly allocated into two groups (G1and G2). Clinical endometritis were clinically diagnosed depending up on a case history, clinical signs, ultrasound and estimate polymorphoneuclear cells (PMNC) in uterine mucus. Before treatment, the involved cows in the study were subjected to the following examinations: Color and pH of uterine mucus, Measurement of the thickness of endometrium by Ultrasonography, percentage of PMNCs in the uterine mucus, The first group (G1) was given a 20 ml Intrauterine infusion containing 100 mg of AgNPs. The second group (G2) was left untreated. After 21 days of treatment, all of the animals were Re-examined for: Color and pH of uterine mucus, thickness of the uterine wall, and percentage of PMNCs in the uterine mucus and response rate. Results of present study revealed that the cure rate for endometritis in G1,G2 were 70% and 20%, respectively, based up on both color and pH of uterine mucus, thickness of endometrium, and percentage of PMNCs before and after treatment with a significant difference (P = <0.001). We conclude that Silver nanoparticles are very effective in treating endometritis in cows.

Keywords: AgNPs, Endometritis, Cows, Uterine Mucus.

Introduction

Endometritis is a common disease affects about 40% of dairy cows, causing a significant impact on cattle health and reproductive performance [1, 2] with significant economic consequences and decreased profitability for the dairy [3]. Clinical endometritis in cows is identified by the presence of purulent (>50% pus) uterine discharge visible in the vagina or vulva twenty one days or more after birth, or mucopurulent (approximately 50 percent pus, 50 percent mucus) discharge visible in the vagina after twenty six days after parturition [4]. Intrauterine antibiotic infusion is the most common treatment for clinical endometritis (5). However, antibiotic overuse and indiscriminate usage in the treatment of uterine infections have led to the increase of antibiotic-resistant strains, necessitating the development of alternative therapeutics for bovine metritis and endometritis [3].

In veterinary medicine, nanoparticles have just lately been utilized as reproductive aids and drug and nutrient delivery systems [6], but they have the potential to be used as antimicrobial alternatives [7]. Silver nanoparticles (AgNPs) have aroused the interest of researchers in biological applications, the most important of which is antimicrobial treatment, among the various metallic nanoparticles accessible [8]. Silver nanoparticles have been widely employed as effective antibacterial drugs against a variety of bacteria, fungi, and viruses [9,10]. AgNPs have been shown to be effective against a wide range of antibiotic-resistant bacteria [6,9].

Corresponding author: Elias Khudhur Hussein: eliaskhudhr@uomosul.edu.iq, 07735482370
Khawla A. Hussein: khawla.a.h@covm.uobaghdad.edu.iq, 07719341503

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Due to the resistance of some bacteria to the traditional treatment of endometritis, it has become necessary to find an alternative treatment for endometritis due to the disease's importance and the economic losses caused by the high percentage of infection in cows. For this reasons, the present study was proposed to assess the efficacy of Intrauterine infusion of silver nanoparticles for the treatment of endometritis in cows, and the use of AgNPs as an alternative to conventional antibiotics for the treatment of endometritis in cattle.

Materials and Methods

Animals of the Study

The current study was conducted in a dairy cow's field that contains Iraqi local breed cows. The field contains 600 cows distributed in the form of six stations. This field is located in Sheikh Amir area of AL-Hamdaniya district, east of Nineveh northern Iraq, and the study included ٢٠ cows with clinical endometritis, aged between 3 to 6 years old, while their weight ranges from 400 - 600 kg with an average of 500 kg and were Multiparous.

Preparation of Silver Nanoparticles

Green biosynthesis was used to synthesize silver nanoparticles from *Curcuma longa* tuber powder. The prepared Ag/C. longa was characterized by X-ray diffraction (XRD), Fourier-transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM)11]].

Diagnosis of the Cases

Cows with clinical endometritis were diagnosed through case history, clinical examination (vaginal discharge), in addition ultrasound was used to measure uterine wall thickness and estimation of polymorphoneuclear cells (PMNC) from the uterine mucus which was collected by massage from the uterus through rectal palpation. Vaginoscopy has also been used to confirm the source of mucus. The clinical endometritis animals were randomly divided into two groups (G1 and G2), each with ten cows.

The following examinations were performed before treatment on all cows that were included in the study.

Color of the uterine mucus

Color visual examination of the uterine mucus was examined after collection. The collection was done as follows: Wash and cleanse the perineum region and the external opening of the female reproductive system thoroughly with pull the tail aside by the assistant. Inserting the hand into the rectum after wearing a long plastic glove, palpating the uterus and doing a uterine massage to get the mucus out from the vaginal opening. Then the mucus was placed in sterile plastic test tubes and transferred to the laboratory at a temperature of 4 ^{o}C. The color and nature of the mucus were observed, and the different types of secretions were identified, which were divided into: clear mucus, Cloudy or turbid mucus, mucus containing white or white pus flecks and mucopurulent or yellow mucus [12].

Measuring the pH of the uterine mucus

After the uterine mucus was collected, the pH is measured by using a portable digital pH meter.

Measurement thickness of the uterine wall

A portable ultrasound was used, a type (STATEMED, STT3-VUS29, CHINA), with a 7.5-MHz linear probe, where the probe was inserted into the rectum after cleaning the rectum from feces and placed over the uterus. The thickness of the endometrium was measured sagittally in the lumen-bounded uterus wall and the presence of purulent and mucus using the ultrasound.

Calculation percentage of PMNC (Neutrophil) in the uterine mucus

In the laboratory, smears of this mucus were made on glass slides and left to dry. Then they were Immerse in fixative (A) five times for 5 seconds, then on Eosin solution (B) five times for 5 seconds and finally on thiazine staining solution (C) four times for 4 seconds and After that, the slides are washed with running water to remove the remnants of the dye and the slide is left to dry. After that, a drop of oil was placed and the slides were placed under the oily lens (1000x magnification) of the light microscope. If Percentage of PMNC is more than 18%, this means that there is clinical endometritis and from 12% – 18% is considered subclinical endometritis while less than 12% is normal.

Treatment

The first group (G1) was treated with 20 ml containing 100 mg of AgNPs by Intrauterine infusion (The dose was calculated by 0.2 mg / kg, Considering the average weight of the cows is 500 kg, Through that the dose required for each cow is 100 mg) (13–15). AgNPs concentration is calculated on the basis of the following equation: (total dose / uterine content ×100), uterine content in cows with endometritis is 2 L, so be-

comes the desired AgNPs concentration is 0.1g / 2000 ml ×100 = 0.5% (16). Intrauterine infusion was done by using 20 ml disposable syringes to infusion the drug and disposable transcervical catheters. The catheter inserted into the uterus using a vaginoscopy as a guide to the catheter to the opening of the cervix in addition to the use of trans rectal palpation of the cervix and its hold to facilitate the passage of the catheter into the uterine cavity

The second group (G2) was left untreated.

Follow-up of animals after treatment

The animals were followed up after 21 days of treatment, when all of them were measured: Color of the uterine mucus, pH of uterine mucus, thickness of the uterine wall, the percentage of PMNC in the uterine mucus, Response rate to treatment and the number of times of treatment.

Statistical Analysis

Data of the study were expressed as Mean ± SE by T-test paired, Normally distributed data were compared by ANOVA 1 (One-Way Analysis of Variance). Significant differences were determined by Duncan's Multiple Range Test. statistical analyses were performed by Sigma Plot 12.5 and ($P<0.001$) , ($P<0.05$) was consider as statistically significant.

Results

The results of first group (G1) showed that the color of the uterine mucus before treatment was distributed as follows: turbid mucus for two of the 10 cows (20%), mucus containing white flecks for 4 cows (40%) and mucopurulent in 4 cows (40%), while after treatment it became clear mucus For 8 cows (80%), and mucus containing white flecks in two cows (20%) with a significant difference (P= <0.001) before and after treatment, as shown in Table (1) and Fig. 1.

Also, the results this group before treatment showed that the average pH of uterine mucus, the percentage of PMNC, and the percentage of thickening of the uterine wall were (8.33), (62.3 %) and (0.33mm), respectively, while after the treatment it was (7.39), (14.8 %) and (0.16mm), respectively with a significant difference (P = <0.001), as shown in Table (2) and Fig. (2,3). The cure rate of cows with clinical endometritis in this group was 70%.

TABLE 1. Shows color of uterine mucus before and after treatment.

Color of uterine mucus	Before treatment	After treatment
Turbid mucus	2^a	0^b
Containing white flecks	4^a	2^b
Mucopurulent	4^a	0^b
Clear mucus	0^a	8^b

a,b means there is a significant difference (P= <0.001) within raw before and after treatment.

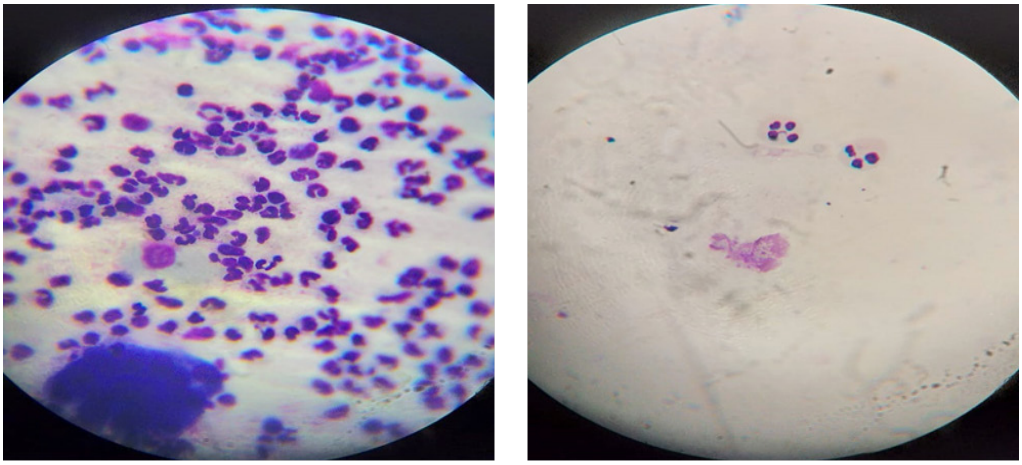
A **B**

Fig. 1. Shows color of uterine mucus before (A) and after (B) treatment.

TABLE 2. Shows the mean values of pH , PMNC, in the uterine mucus & thickening of endometrium before and after treatment.

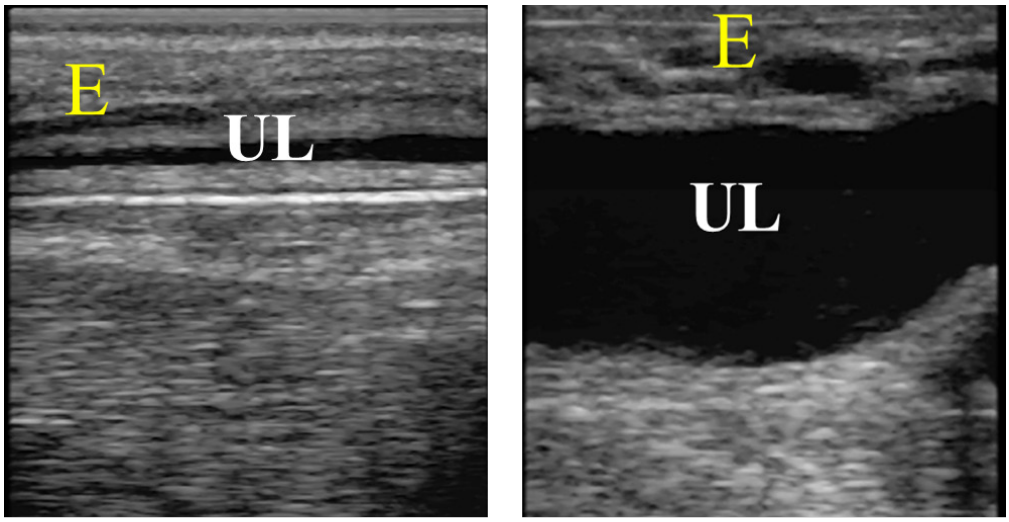
Test	Before the treatment Mean± SE	After the treatment Mean± SE
pH of uterine mucus	8.33 ± 0.04[a]	7.39 ± 0.10[b]
Polymorphoneuclear cells%	62.3% ± 0.04[a]	14.8% ± 0.02[b]
Thickening of the uterine wall (mm)	33.3 ± 0.01[a]	16.9 ± 0.01[b]

a,b means there is a significant difference (P= <0.001) within raw before and after treatment.



A B

Fig. 2. Shows the percentage of PMNC in the uterine mucus before (A) and after (B) treatment.



A B

Fig. 3. Shows the thickening of the uterine wall (E) & uterine lumen(UL) before (A) and after (B) treatment.

The results of the second group (G2) revealed that the color of the uterine mucus on day 0 was turbid mucus for three cows out of a total of ten cows (30%), mucus with white flecks for four cows (40%), and mucopurulent for three cows (30%). While After 21 days, it became clear mucus in two cows (20%), turbid mucus in two cows (20%), mucus with white flecks in three cows (30%), and mucopurulent in three cows (30 percent), with a significant difference, as shown in Table (3)

The results also revealed that on day 0, the rate of pH of uterine mucus, the percentage of PMNC, and the percentage of uterine wall thickening were (8.27), (63 percent), and (0.34mm), respectively, whereas on day 21, they were (7.99), (44.2 percent), and (0.26mm), Respectively, as shown in Table (4). It also showed that the cure rate in this group was (20%).

The current study found a significant and distinct difference between G1 and G2 in the color change and pH degree of uterine mucus, the percentage of PMNC, the percentage of uterine wall thickening, and the cure rate, as shown in Table (5).

TABLE 3. Shows the color of the uterine mucus on 0 and 21 days.

Color of uterine mucus	0 day	21 days
Turbid mucus	3^a	2^a
Containing white flecks	4^a	3^a
Mucopurulent	3^a	3^a
Clear mucus	0^a	2^a

a,b means there is a significant difference (P= <0.001) within raw

TABLE 4. Shows the mean values of pH , PMNC, in the uterine mucus and thickening of endometrium 0 and 21 days.

Test	0 day Mean± SE	21 day Mean± SE
pH of uterine mucus	8.27 ± 0.05^a	7.99 ± 0.16^a
Polymorphoneuclear cells%	$63\%\pm0.04^a$	$44{,}2\%\pm0.06^a$
Thickening of the uterine wall (mm)	0.34 ± 0.01^a	0.26 ± 0.02^a

a,b means there is a significant difference (P= <0.001) within raw.

TABLE 5. Shows the mean values of color, pH, PMNC, thickening of endometrium, the cure rate between two groups.

Test / Groups	G1: (100mg) AgNP	G2: Without treatment
Normal Color of uterine mucus	8^a	2^b
pH of uterine mucus	7.39 ± 0.10^a	7.99 ± 0.16^b
PMNC %	14.8 ± 0.02^a	44.2 ± 0.06^b
Thickening of the uterine wall (mm)	0.16 ± 0.010^a	0.26 ± 0.021^b
The cure rate	$70\%^a$	$20\ \%^b$

a,b means there is a significant difference (P<0.05) within raw.

Discussion

The results of the current study in first group showed, that the color of the uterine mucus before treatment was distributed between turbid, containing white flecks, mucopurulent and the difference is due to the severity of endometritis and the species of bacteria causing inflammation, as well as bacterial metabolism [11, 17]. While the color changed to clear mucus for eight cows after treatment because the AgNPs destroyed the bacteria causing inflammation and promoted healing, allowing the uterine secretions to return to normal (absence of bacterial metabolism) [3,18]. The AgNPs destroyed the bacteria by Penetration of the cell, causing damage to intracellular structures and biomolecules and generation of reactive oxygen species (ROS) and free radicals causing cell damage and oxidative stress [19]. As for the reason for the remaining abnormal color of two cows after treatment, this is mean that AgNPs not effective against some bacteria species or the presence of more than one cause of the disease and this agrees with some researchers [3].

The study's findings in this group also indicated a high pH value of uterine mucus (8.33 ± 0.04) before treatment, which may be related to the metabolism of bacteria and inflammatory exudates in uterine mucus [20]. While after treatment, the value decreased (7.39 ± 0.10) with a significant difference (P = <0.001) due to the destruction of bacteria causing inflammation by AgNPs [3,18].

The results of this group also revealed that the percentage of PMNC in the uterine mucus was significantly high (62.3% ± 0.04) in all of the cows before treatment, indicating that all of the cows in the study have clinical endometritis of varying degrees of severity. This study is in agreement with many investigators [17-22]. While after treatment with AgNPs, the percentage decreased significantly (14.8% ± 0.02) with a significant difference (P = <0.001), this indicates that the endometrium has healed from inflammation after the pathological microbe has been removed [17].

The results of the ultrasound examination in this group before treatment indicated the presence of thickening in the uterine wall with different degrees of severity, with an average of (33.3 ± 0.01) Which indicates the presence of chronic endometrial inflammation, and the cause of thickening is due to the formation of connective tissue in endometrium as response to the uterus's immune system [23]. After treatment, the degree of thickening of the uterine wall decreased (16.9 ± 0.01) with a significant difference (P = <0.001) , and this indicates that the endometrium has healed from inflammation [24].

The results of this group showed that the cure rate was 70%, and this indicates the high efficiency of silver nanoparticles in the treatment of endometritis and the destruction of most of the pathogens. In some cases, the lack of response is due to the type of causative agent or the presence of more than one cause of the condition and this is in agreement with Gurunathan et al. [3].

The results of the second group revealed that the color of the uterine mucus ranged from turbid to mucopurulent, the difference is due to the severity of endometritis and the species of bacteria causing inflammation [12,17]. After 21 days, the color of two cows' mucus turned to clear mucus, indicating that the animal's autoimmune was able to control the disease and repair itself [25,26]. The The study's findings also revealed that the pH value, the percentage of PMNC, and the percentage of uterine wall thickening were significantly higher (8.27±0.05) , (63%±0.04), and (0.34± 0.01) respectively, in all of the cows on 0 day due to the severity of endometritis (12,17), while after 21 days were (7.99±0.16), (44,2%±0.06), and (0.26±0.02) respectively, with no significant differences due to disease persistence (endometritis) [17,22].

As shown in Table 5, there is a significant and clear difference (P<0.05) between G1 and G2 in the cure rate, which was 70% and 20%, respectively, indicating the high efficiency of AgNPs in the treatment of endometritis and the destruction of most pathogens and promoted healing, allowing the endometrium to return to normal [3,18].

The study also found that in G1 and G2, the percentage of uterine mucus color change to normal was 80% and 20%, respectively, with a

significant difference (P<0.05) between the two groups. While the average pH of G1 and G2 uterine mucus was 7.39 and 7.99, respectively, there was a significant difference (P<0.05) between the two groups [3,18]. The percentage of PMNC in G1 and G2 uterine mucus was 14.8 percent and 44.2 percent, respectively, with a significant difference between the two groups [3,17]. The uterine wall thickness in G1 and G2 was 0.16mm and 0.26mm, respectively, with a significant difference (P<0.05) between the two groups [24]. The strong effect of silver nanoparticles in the therapy of endometritis is responsible for the high significant difference (P<0.05) in all parameters between G1 and G2 [3].

Conclusion

We conclude that Silver nanoparticles are very effective in treating endometritis in cows and the biologically prepared AgNPs are safe for use in treating endometritis by Intrauterine infusion.

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Conflicts of Interest

There are no conflicts of interest declared by the authors.

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تقييم كفاءة جزيئات الفضة النانوية لعلاج التهاب بطانة الرحم في سلالات الأبقار العراقية

الياس خضر حسين و خولة عباس حسين
قسم الجراحة والتوليد - كلية الطب البيطري - جامعة الموصل - العراق.
قسم الجراحة والتوليد - كلية الطب البيطري - جامعة بغداد - العراق.

الهدف من الدراسة الحالية هو تقييم فعالية جسيمات الفضة النانوية المحقونة داخل الرحم في علاج التهاب بطانة الرحم للأبقار. تم إجراء البحث في حقل أبقار حلوب ذو سلالة محلية. شملت الدراسة على ٢٠ بقرة مصابة بالتهاب بطانة الرحم السريري. تم تقسيمها إلى مجموعتين عشوائياً. الأبقار المصابة شخصت سريريا بالاعتماد على تاريخ الحالة والعلامات السريرية والموجات فوق الصوتية وحساب نسبة الخلايا متعددة النواة في مخاط الرحم. قبل العلاج ، خضعت جميع الأبقار في الدراسة للفحوصات التالية: اللون ودرجة الحموضة لمخاط الرحم، قياس سماكة بطانة الرحم بالموجات فوق الصوتية ، نسبة الخلايا متعددة النواة في مخاط الرحم ، تم إعطاء المجموعة الأولى ٢٠ مل تحتوي على ١٠٠ ملجم من جسيمات الفضة النانوية عن طريق التسريب داخل الرحم. المجموعة الثانية لم تتلق أي علاج. بعد ٢١ يومًا من العلاج ، تم فحص المعايير التالية لجميع الحيوانات: اللون ودرجة الحموضة لمخاط الرحم وسمك جدار الرحم والنسبة المئوية للخلايا متعددة النواة ونسبة الاستجابة للعلاج. أظهرت نتائج الدراسة الحالية أن معدل الشفاء من التهاب بطانة الرحم في المجموعة الاولى والمجموعة الثانية كانت ٧٠٪ و ٢٠٪ على التوالي ، على اساس نسبة اللون ودرجة الحموضة لمخاط الرحم وسماكة بطانة الرحم ونسبة الخلايا متعددة النواة قبل وبعد العلاج مع وجود فرق معنوي ($P = <0.001$). نستنتج أن جسيمات الفضة النانوية فعالة جدا في علاج التهاب بطانة الرحم في الأبقار.

الكلمات المفتاحية: جسيمات الفضة النانوية ، التهاب بطانة الرحم ، الأبقار ، مخاط الرحم.