Original Research

Advanced Clinical Trials in Treatment of Postpartum Endometritis in Holstein Dairy Cows

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ABSTRACT

In the current study, we aimed to evaluate the efficacy of the treatment of the dairy cows suffering from endometritis using nano-oxytetracycline (OTC-NPs), platelet-rich plasma (PRP), and conventional oxytetracycline (OTC) based protocols. To accomplish this study a group of 30 cycling Holstein lactating dairy cows suffering from post-partum endometritis were divided into three equal groups. Group I was given 10 ml of nano-oxytetracycline intrauterine infusion, two doses with one week interval, group II was treated with three doses of 20 ml of oxytetracycline 5% intrauterine infusion at one week interval and group III was given two doses of 10 ml of their own platelets rich plasma (PRP) one week apart and third one in day four post estrus. Results showed that serum level of TNF-α and IL6 recorded a significant decrease in all treated groups and the higher average decrease was recorded in group-I followed by group-III and finally group-II. In addition, all the treatment protocols significantly and successfully decreased the uterine thickness as compared to uterine thickness before treatment. In conclusion, and based on the changes in serum pro-inflammatory cytokines level, treatment with nano-oxytetracycline was more efficient in treatment of endometritis, followed by PRP and finally the conventional oxytetracycline.

Keywords: Endometritis, Nano-oxytetracycline, Conventional oxytetracycline, Cytokines, PRP.

INTRODUCTION

Reproductive performance is one of the most important key components of dairy production management. Diagnosis and treatment of postpartum uterine disease, and its negative impact on reproductive performance, have traditionally attracted more attention from veterinarians and producers**(LeBlanc et al., 2002).**On dairy farms, post-partum uterine diseases result in significant financial losses. These losses are primarily caused by treatment costs, milk production decrease, milk disposal, infertility, repeated breeding, and a high annual culling rate of dairy cows, which represents immediate losses in the event of treatment failure**(Paiano et al., 2021;Ghallab et al., 2023)**.Postpartum uterine illnesses affect dairy herds at a median rate of 10%, as it can range from 3% to 40%. Bovine uterine inflammation reveals as one of four clinical or subclinical forms: pyometra, metritis, clinical endometritis (CE), and subclinical endometritis (SE)**(Barański et al., 2022)**.About 40% of dairy cows suffer from endometritis, a frequent postpartum illness that has a substantial negative influence on the health and reproductive abilities of cattle. This has serious financial consequences and lowers dairy farmer profitability**(Hussein and Hussein, 2022).**Many treatment strategies are commonly used to deal with postpartum uterine problems in cattle, including the intrauterine administration of antibiotics, disinfectants, and antiseptics. However, these strategies may have an adverse effect on the uterine cellular immune response. Consequently, in order to mitigate the detrimental effects of uterine illnesses on fertility, it is essential that they must be correctly identified and treated**(Ahmadi and Dehghan, 2007).** In recent years, various substances such as hormones and antibiotics have been investigated for the treatment of uterine infections. However, in addition to being costly, these traditional therapies could harm animals even more if they could keep remnants in animal products or if they cause bacteria to get resistant to them **(Kumar et al., 2004).** Due to the high concentration of growth factors that give platelet-rich plasma (PRP) its mitogenic and anti-inflammatory qualities, PRP has been used therapeutically in tissue regeneration. **(Farghali et al., 2022)**. Animal studies demonstrated that treatment with PRP could a decrease the expression of inflammatory markers and fibrosis, and increase endometrial proliferation rate, increase expression of proliferative genes, and increase pregnancy rates **(Mouanness et al., 2021).** Moreover, nano-antibiotics (nAbts) have more delivery and membrane penetration to reach target sites. Consequently, nAbts are viewed as a possible alternative strategy to get around antibacterial resistance and increase therapy efficacy. Since most antibiotics are no longer effective against resistant bacteria in their molecular forms, research on and usage of nAbts have been increased dramatically. **(Mamun et al., 2021).** Oxytetracycline is a broad-spectrum antibiotic that is recommended for the management and treatment of illnesses brought on by or related to bacteria that are sensitive to oxytetracycline. It has a well-established antibacterial efficacy against a wide range of infections brought on by both gram-positive and gram-negative bacteria. When treating postpartum endometritis in dairy cows, the most popular method of giving this antibiotic

is through intrauterine infusion **(Gohar et al., 2018).** In this context, this study aimed to evaluate the efficacy of some recent advanced treatment protocols as platelets rich plasma (PRP), and oxytetracycline nano- particles based protocols in treatment of post-partum endometritis in dairy cows, in comparison with treatment efficiency of the treatment with conventional oxytetracycline.

MATERIALS AND METHODS

Animals:

This study was conducted during April to December 2022. A group of 30 cycling Holstein lactating dairy cows, belonging to milk production project (farm- key) Faculty of Agriculture, Alexandria University with age ranging from 4 to 5years, body weight 400-450 kg and body score 3.5 – 4 were used to accomplish this study. They were suffering from repeat breeding condition due to affection with endometritis. The ration composition was in form of yellow corn 30%, bran 30%, cotton seed meal 25%, soybean 5%, molasses 0.3%, sodium bicarbonate 1%, common salt 1%, and lime stone 2% (formulated to contain 16% crude protein). Milking was done twice daily in automatic milk parlor (Average daily milk production was about 25 kg). According to farm records, proper vaccination programs against common disease were applied. Also, regular De- worming program was applied.

Methods of endometritis diagnosis in cows:

1) History: repeat breeder cows which did not conceive when bred for three or more consecutive times using artificial insemination with excellent semen quality.
2) Clinical signs: which ranged from presence of turbid cervical mucus during estrus and inter-estrus period to purulent cervical discharge at 21th day post-partum or more **(Sheldon et al., 2006).**
3) Rectal palpation: enlarged uterus and cervix with an increase in uterine wall thickness.
4) Ultrasonography: uterine examination through ultrasonography was performed by using real time B-mode scanners (M 12 Sonoscape – China) with 5-7.5 MHz frequency linear-array trans-rectal to confirm the increase in uterine wall thickness and accumulation of uterine fluid.
5) Cytological examination: This was used for endometritis presence confirmation in suspected cases via cytobrush which was inserted within its sheath into uterus through vagina and rolled clockwise to obtain uterine content. Immediately after retraction, cytobrush was rolled on to glass slide, air dried, fixed and stained using Leishman's stain for 10 min. After washing and dryness, the slides were examined microscopically at X1000 at which approximately 100 cells were counted to detect the percent of neutrophils (PMNs %) for quantitative assessment of endometrial inflammation. Endometrial threshold value of 10% PMNs was used for diagnosis of endometritis (**El-Amrawi and El-Karim, 2019)**.

Preparation of oxytetracycline nanoparticles (OTC-NPs):

Oxytetracycline 20% **(Oxytetra® 20% L.A. kela N.V., Belgium)** was transferred to oxytetracycline 20% nanoparticles (reduced to about 50% of their original size) by high pressure micro-fluidic homogenizer according to referenced principles (**Lammari et al., 2020)**.

Preparation of PRP:

Blood aliquots were collected from tail vein (80ml) using the 16- gauge needle into tubes containing anticoagulant (citrated tubes), then the samples were transferred into ice box to the laboratory within 2 hours of collection for immediate processing and preparation of PRP using double centrifugation method as described before **(Bos-Mikich et al., 2019)**

Treatment protocol:

The animals were divided equally into 3 groups (N= 10) as follow:

Group I: Cows suffering from endometritis which were treated with 10 ml of nano- oxytetracycline intrauterine infusion into two doses with one week interval by using catheter.

Group II: Cows suffering from endometritis which were treated with 20 ml of oxytetracycline 5% **(Oxyvet-Pharma® 5%),** 3 times with one week interval via intrauterine infusion using catheter.

Group III: Cows were given two doses of 10 ml of their own platelets rich plasma (PRP) one week apart via intrauterine catheter **(Farghali et al., 2022)**. Another 3rd dose of PRP was given to each animal, at day 4 post estrus. **(Marini et al., 2016)**

Assessment of the Pro-inflammatory cytokines:

Blood samples were collected weekly starting from first week after treatment protocols application till third week after application (7^{th} day, 14^{th} day and 21^{st} day after treatment start). Blood samples were drawn from tail vein in plain vacutainers (5 ml) and left to clot at room temperature. Blood aliquots were centrifuged at 3000 rpm. for 10 min for serum separation and serum was stored at -20° C. Serum level of inflammatory biomarker as tumor necrosis factor-alpha (TNF-α,), and interleukin-6 (IL-6) were detected using species specific ELISA kits **(abcam, USA).**

Evaluation of the recovery:

Examination using ultrasonography was performed at day 21 from start of treatment by using real time B-mode scanners (M 12 sonoscape – China) to measure the decrease in uterine wall thickness and fluid accumulation

Statistical analysis:

Statistical analysis was carried out using two ways analysis of variance (ANOVA) according to **(SAS, 2004).**

RESULTS

1.Change in serum TNF-α

As present in Table (1), serum level of TNF-α recorded a significant decrease in starting from first week post-treatment in both of group I and III, and this decrement continued significantly till third week post treatment. In contrary, the decrement begins in group-II in second week post treatment and continued till third week post treatment. Generally, the higher average decrease was recorded in group-I followed by group-III and finally group-II.

Table (1): Serum TNF-α (pg/ml) among different periods in different groups.

Groups	Pre-treatment	First week	Second week	Third week	Average change
Group-I (Nano)	211.20±12.02a	169.30±13.34b	103.40±11.14c	61.60±5.56d	136.38±10.62C
Group-II Oxy (5%)	269.40±11.69a	254.60±12.31a	203.41±7.23b	190.80±8.28c	229.55±4.96A
Group-III PRP	239.33±13.71a	214.60±12.85b	172.60±14.3c	137.40±15.95d	191.00±9.12B

Means within the same raw of different small litters are significantly different at (P < 0.01).
Means within the last column of different capital litters are significantly different at (P < 0.01).

1. Change in serum IL-6

Table (2) showed that, serum level of IL-6 decrement was detected in both of groups I and II starting from first week post treatment and its level continued to decrease in a significant manner till third week post-treatment. On the other hand, its level begins to decrease in serum of group-III at first week post treatment and remain constant without any significant change at second week post- treatment, but its level decreased significantly again at third week post-treatment. The concluded decrease was the higher ingroup-I followed by group-III and finally group-II.

Table (2): Serum IL-6 (pg/ml) among different periods in different groups.

Groups	Pre-treatment	First week	Second week	Third week	Average change
Group-I (Nano)	301.30±12.41a	241.80±11.9b	198.20±8.79c	170.50±5.78d	230.20±9.6C
Group-II Oxy (5%)	338.10±5.58a	326.70±15.31b	310.30±10.32c	293.80±12.52d	317.23±6.14A
Group-III PRP	326.70±15.31a	288.60±18.67b	280.20±16.01b	251.20±14.48c	286.68±8.9B

Means within the same raw of different small litters are significantly different at (P < 0.01).
Means within the last column of different capital litters are significantly different at (P < 0.01).

B- Change in uterine thickness

At the end of the treatment period (3 weeks), all the treatment protocols significantly and successfully decreased the uterine thickness as compared to uterine thickness before treatment, as present in table (3)

Table (3): Change in uterine thickness in different groups before treatment and at the end of treatment protocols.

Groups	Period	Mean Std. Error	t-value
Group-I (Nano)	Before	13.90±0.80 a	9.007
	After	5.89±0.30 b	
Group-II Oxy (5%)	Before	14.78±0.63 a	0.98
	After	7.40±0.18 b	
Group-III PRP	Before	12.98±0.55 a	9.32
	After	6.50±0.25 b	

For each group: Means within the same column of different litters are significantly different at p < 0.001

DISCUSSION

Appropriate diagnosis and treatment of endometritis is imperative because uterine diseases cause significant financial losses for dairy farms, primarily because of the expenses related to treating affected animals, disposing of milk, and worsening postpartum fertility in cows. These factors can also lead to a decrease in pregnancy rates, which may lead to the culling of affected animals during early lactation. **(Bos-Mikich et al., 2018).** In this study, we would discuss a new insight into the treatment of cows suffering from endometritis by using nano oxytetracycline (OTC-NPs) and platelet-rich plasma (PRP) in comparison with conventional oxytetracycline (OTC), to evaluate the efficacy of these treatment protocols. Concerning serum pro-inflammatory cytokines level, the presence of the pathogens inside uterus causes recruitment of a large number of uterine neutrophils, as well as an increase in pro-inflammatory cytokines production such as IL-6 and TNF-α (**Loyi, et al., 2013).** So, our results showed higher level of serum TNF- α level, and IL-6 in cows suffering from endometritis before treatment in agreement with **(Brodzki et al., 2015);** as innate immune cells use Toll-like receptors to identify bacterial components, subsequently, pro-inflammatory cytokines like interleukins and tumor necrosis factor-α are produced, then neutrophil movement occurs which is followed by the phagocytosis of pathogenic organisms that invade the uterine lumen, and these intricate signaling events are related to the development of endometritis in cows. Additionally, **Kim et al. (2014)** confirmed this mechanism and reported that cows with endometritis have higher inflammatory cytokine concentrations in uterine flush than healthy cows, but there are no abnormalities in their serum level. Since oxytetracycline is a broad-spectrum antibiotic, it is used to treat and manage diseases brought on by or associated with bacteria that are sensitive to oxytetracycline and proliferate rapidly. **(Gohar et al., 2018).** Because of its potent anaerobic postpartum activity and its ability to impact a broad range of bacteria, oxytetracycline is the recommended treatment for

clinical endometritis when administered intrauterine. **(Kaczmarowski et al., 2004).** On the other hand, according to **Cohen et al. (1995)**, oxytetracycline failed to successfully cross endometrium level and eradicate the uterine infection. As a result, oxytetracycline is not the best therapeutic option. In addition, the potential of bacteria to acquire resistance to antimicrobial drugs makes the treatment of bacterial infections more challenging. The more antibiotics used improperly, the more chances for bacterial mutation. Therefore, we must find alternative treatment to overcome the drawbacks of using traditional antibiotics. **(Tenover, 2006).** Nano-scale antibiotic has more cell membrane penetration to target area, with more efficacies in bacterial protein synthesis disruption **(Mamun et al., 2021).** As a consequence, the results concerning biochemical and reproductive parameters obtained through treatment with nano-oxytetracycline recorded more mitigation than the results obtained from animals treated with conventional oxytetracycline. PRP contain several growth factors for endothelium and fibroblasts, besides its ability to boost macrophages and neutrophils functions, which collectively would enhance angiogenesis, re-epithelization and eradication of bacteria **(Anitua et al., 2004).** Also, anti-inflammatory effect of PRP is well documented **(Reghini et al., 2016). Marini et al. (2016)** discovered that PRP could be used as a treatment for endometritis in vivo after testing it as a therapy for endometritis in cows in vitro and observing a potential anti-inflammatory response in the examined endometrial cells. As a result, for the previously listed effects of different treatment protocols on eradication of uterine infection, the uterine wall thickness, elimination of intrauterine discharge, and improvement in overall uterine status after the administration of the treatments was recorded in our study.

CONCLUSION

It could be concluded that, all treatment protocols treated endometritis and nano-oxytetracycline was the best one in the treatment of endometritis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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