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The Presumptive Effectiveness of Silver Nanoparticles in Treatment of Postpartum Endometritis in Dairy Cows

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Abstract

Clinical postpartum endometritis due to bacterial invasion is one of the most common diseases affecting dairy cows in postpartum period; antibiotics are widely utilized as a treatment to overcome this affection. Recently, bacterial resistance to antibiotics due to uncontrolled usage became one of the most serious problems facing the medical community, so, the aim of study was to detect the effectiveness of nano-silver particles as a treatment for postpartum endometritis in dairy Holstein cows. At 35-40 days postpartum, twenty one Holstein cows were diagnosed to be affected with postpartum endometritis, depending on clinical and ultrasonography examination. The treatment protocol included intrauterine infusion with 50 ml of silver nanoparticles solution (50 ppm/ml) for five consecutive days, and single dose of PGF-2α analogue as a treatment for animals with a corpus lutetum on their ovaries. At the 7th day post-treatment, serum level tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP) and serum amyloid-A (SAA) and was significantly decreased ($P<0.01$). Also, the level of ceruloplasmin and haptoglobin (Hp) in serum recorded a significant decrement($P<0.05$) upon treatment with silver nanoparticles.In addition, uterine secretions were found with a significant decrease or disappeared completely, and endometrial thickness decrement was detected upon ultrasonography examination. At re-examination (49-54 days postpartum), 14 animals out of 21 animals (66.6%) were recovered successfully from endometritis. The pregnancy rate on 40th day post-service was 71.4 % (10 out of 14 animals were found pregnant after insemination on their observed estrus). As a conclusion, this study proved the efficacy of silver nanoparticles as a treatment for postpartum endometritis in dairy Holstein cows. So, it could substitute antibiotic treatment in such cases to avoid the antibiotic resistance problems.

KEYWORDS
Postpartum endometritis, Nano-silver, Dairy cows.

INTRODUCTION

Endometrial inflammation which occurs beyond three weeks or more after parturition in dairy cows without any apparent systemic illness signs can be called postpartum endometritis (Sheldon *et al.*, 2006). Uterine bacterial contamination after parturition is likely to occur, but occurrence of clinical postpartum endometritis depends on challenge between host immunity, virulence of invading microbes and finally some environmental factors (Potter *et al.*, 2010). Clinical endometritis would disturb ovarian cycle, delay involution of uterus or damage its tissues (Bonnet *et al.*, 1993; Sheldon *et al.*, 2002) which could prolong calving interval, decrease subsequent conception rate or lead to animal culling due to repeated failure of conception (Gilbert *et al.*, 1998). The most common bacterial species which are involved in occurrence of clinical enodometritis are *Escherichia coli*, *Fusobacterium necrophorum, Arcanobacterium pyogenes* and *Prevotella* species (Huszenicza *et al.*, 1991, Sheldon *et al.*, 2002). Presence of abnormal vaginal discharge is one of the most characteristic signs of clinical endometritis (Holt *et al.*, 1989). Treatment of clinical endometritis depends mainly on intrauterine antibiotic infusion (Singh *et al.*, 2014), but overwhelming and uncontrolled usage of antibiotics unfortunately have led to multidrug resistance (Yah and Simate, 2015; Gurunathan, 2015), so development of novel antibacterial agents is an urgent need to overcome bacterial resistant to antibiotics (Panáč̌Ek *et al.*, 2006). Silver nanoparticles (AgNPs) are one of the most commercialized nanoparticles in health care field due to their antibacterial effect (Chen and Schluesener, 2008). Antibacterial effect of AgNPs may depends on release of the silver ions (Ag+) which could attach to nucleic acids forming a complex which would inhibit bacterial replication (Yakabe *et al.*, 1980; Chouhan and Guleria, 2020). Also, AgNPs have electrostatic affinity to sulfer proteins of cell membrane and cytoplasm, after adherence, they could increase permeability of cell wall and disturb cell casing (Khorrami *et al.*, 2018). In this consistence, this study hypothesized that intrauterine infusion of silver nanoparticles would aid in effective elimination of bacterial infection which cause postpartumendometritis in dairy cows owing to its proved wide spectrum antibacterial activity against different ranges of bacterial strains, such recovery could be indicated in early stage through estimation of peripheral level of some inflammatory cytokines and proteins and subsequently through detection of complete recovery and pregnancy rates. So, this study aimed to evaluate the effectiveness of silver nanoparti-

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cles as a treatment for postpartum endometritis in dairy Holstein cows.

MATERIALS AND METHODS

Animals

In an extensive private dairy farm on Alexandria-Cairo desert road, twenty one Holstein cows, weight about 450-500 Kg were diagnosed suffering from clinical postpartum endometritis at 35-40 days postpartum, on the basis of clinical examination, as the animals were suffering from abnormal vaginal discharge, increased thickening of uterine wall and presence of uterine fluids upon ultrasonography examination (Real time, B-mode, 7.5MHz, Sonoscope®, China).

This study was approved by The Institutional Animal Care and Use committee (IACUC), Alexandria University, Egypt.

Treatment protocol

The treatment protocol was in form of intrauterine administration of 50 ml of aqueous solution of silver nanoparticles (silver nitrates nano-particles solution, 50ppm/ml) (Fig.1) (Seif Nanotechnolgy®, Egypt) for five consecutive days using intrauterine metal catheter, and single dose (Estrumate®, 500µg cloprostenol) (I/M) for animals with a corpus lutetum on the ovaries. Blood samples were obtained just before start of the treatment and on the 7th day from application of the treatment protocol, and animals were re-examined clinically and by ultrasonography on both the 7th and 14th day after start point of the treatment. After second examination, the recovered animals were artificially inseminated with trusted source semen straws at their upcoming estrous.

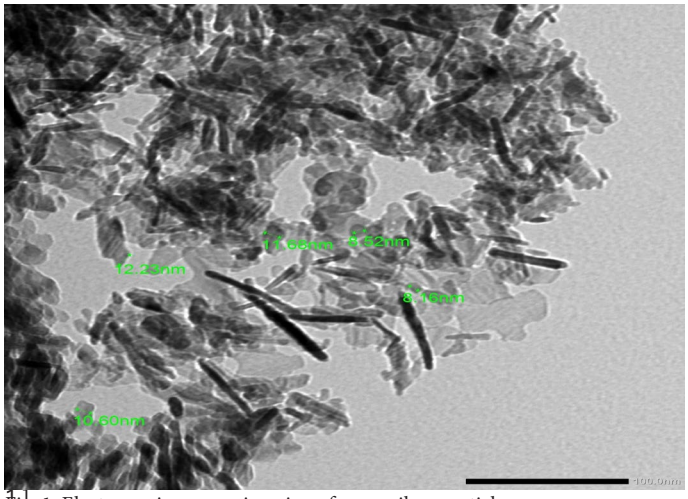

Fig. 1. Electron microscopy imaging of nano-silver particles.

Serum preparation and analysis

Blood aliquots were drawn from tail veinin plain vacutainers, left for 30 minutes to coagulate, and then centrifuged for 10 minutes at 3000 rpm to separate serum, which were kept at -20 °C for subsequent analysis. ELISA kits were used for determination of serum level of TNF-α, IL-6 (Abcam, USA) and SAA (Abbexa, UK). CRP level was detected using rapid latex slide test commercial kit (Spectrum, Egypt), serum concentration of haptoglobin and ceruloplasmin was evaluated by immuno-turbidimetry method using a commercially available kits (Randox, UK; Spectrum, Egypt).

Statistical analysis

The statistical differences in means of the serum concentration of the tested parameters before and after treatment were detected using independent samples t-test with aid of SPSS 16.0 for windows.

RESULTS

Changes in serum concentration of inflammatory cytokines and proteins

On the 7th day post-treatment, serum level of TNF-α, IL-6, SAA and CRP was decreased ($P<0.01$) significantly upon treatment with silver nanoparticles. Also, the level of haptoglobin and ceruloplasmin recorded a significant decrement ($P<0.05$) after the treatment.

Recovery and conception rate

Turbid vaginal secretions were decreased or disappeared, also decrement in endometrial thickness and uterine fluids were recorded upon ultrasonography examination on the 7th day post-treatment. Upon re-examination, 14 cows out of 21 (66.6%) were completely recovered from Endometritis (complete absence of vaginal secretion and uterine fluids with normal thickness of endometrium). Upon pregnancy diagnosis through rectal palpation, pregnancy rate was 71.4 % at 40th day after insemination (10 out of 14 animals were found pregnant).

DISCUSSION

Postpartum endometritis is one of the most common diseases affecting dairy cows, as its annual worldwide incidence may reach about 10-50% (Lewis, 1997; Noakes *et al*., 2001) and its incidence may reach 40% in some dairy herds (Lewis, 1997). Due to its potent antibacterial effect, nano-silver particles are attracting more interest nowadays; also, AgNPs have been proved as anti-inflammatory agent which would help in the healing process (Chaloupka *et al*., 2010). Several studies have talked over

Table 1. Mean values (mean± SD) of different evaluated parameters before and after treatments.

	Before treatment	After treatment	t-value
TNF-α (pg/ml)	209.95±8.84A	158.57±7.91B	4.33**
IL-6 (pg/ml)	219.05±9.95A	168.67±7.47B	4.05**
SAA (µg/ml)	37.90±2.64A	26.29±2.0B	3.47**
CRP (mg/L)	5.08±0.25A	3.42±0.16 B	5.56**
HP (µg/ml)	208.33±11.50A	205.67±11.14B	2.16 *
Ceruloplasmin (mg/dl)	62.71±3.13A	58.19±2.98B	2.55 *

Means within the same row of different litters are significantly different. * Significant at ($P < 0.05$; **: Significant at ($P < 0.01$)

about the increased level of inflammatory cytokines and proteins during course of endometritis in cattle (Li *et al*., 2010; Kim *et al*., 2014; Kaya *et al*., 2016a; Kaya *et al*., 2016b).The presence of bacterial lipopolysaccharide (LPS) as in case of endometritis induces inflammatory cytokines (as TNF-α) production, in order to attract inflammatory cells and increase their influx inside uterine lumen for elimination of the causative bacteria (Sheldon *et al*., 2009). Also, uterine endometrium can produce IL-6 in response to presence of pathogens (Turner *et al*., 2012) for leukocytes chemotaxis and enhancement of neutrophils and macrophages phagocytosis (Singh *et al*., 2008). In the same manner, acute phase proteins play a key role in inflammatory response and serve as an accurate marker of different cattle diseases (Maden *et al*., 2012; Tothova *et al*., 2012). After infection, SAA is produced from liver as one of first line of defense mechanism in response to stimulation of TNF- α and/or IL-1 (Petersen *et al*., 2004;Tothova *et al*., 2014).In the same consistence, CRP is one of acute phase proteins which play an important role in innate defense system against pathogens (Du Clos and Mold, 2001), and its level is proved to be increased in response to endometritis (Li *et al*., 2010; Kaya *et al*., 2016b). Second line response include Hp, which is secreted from liver in response to increased level of IL-6 in case of prolonged inflammatory process (Petersen *et al*.,2004; Tothova *et al*., 2014). In addition, ceruloplasmin is also one of acute phase protein which may enhance immune cells phagocytosis and antimicrobial effect (Ceron *et al*., 2000). Both of haptoglobin and ceruloplasmin are proved to be increased according to severity of endometritis (Kaya *et al*., 2016b). Concerning antimicrobial impact of silver nanoparticles, after cellular uptake of nano-silver ions (Ag+), respiratory system of the cell will be activated and produce reactive oxygen species (ROS) and inhibit ATP release, also ROS would disturb cell membrane integrity and damage DNA, which collectively may affect bacterial replication and propagation (Pareek *et al*., 2018; Das *et al*., 2020). In addition, antibacterial effect of silver ions may depend on its ability to frustrate cellular protein synthesis due to ribosomal component denaturation (Pareek *et al*., 2018). Side by side, anti-inflammatory effect of nano-silver ions was detected previously (Chaloupka *et al*., 2010; Morozova, 2021). Theprevious explanations may illustrate the cause of highly significant decrement in serum level of TNF-α, IL-6, SAA and CRP in response to treatment with nano-silver particles due to elimination of causative agents. On contrary, the minimal decrease in serum level of ceruloplasmin and hapotoglobin may be owed to their prolonged half-life time (Hellman and Gitlin, 2002; Eckersall and Conner, 1988). The recovery of the tested animals from endometritis upon treatment with nanosilver particles was further indicated by gradual decrease in vaginal discharge and thickness of endometrium, as abnormal vaginal secretions (Holt *et al*., 1989) and thickened endometrium (Barlund *et al*., 2008) are the most common signs for existence of postpartum endometritis.

CONCLUSION

In conclusion, this study indicated that silver nanoparticles solution seems to be an effective treatment for clinical postpartum endometritis in dairy cows. So, it could substitute the treatment of clinical endometritis with antibiotic to avoid the antibiotic resistance problems.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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