

## Comparative study between Lugol's Iodine and Forsythia suspensa loading nanoparticles for endometritis therapy in repeat-breeder dairy cows

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### Abstract

The current study was conducted on 75 crossbred dairy cows, ages ranging from (4-8) years suffering from endometritis, including (75 cows) were divided equally into five groups ; 1st group (control group) kept untreated, 2nd group (Lugol's iodine solution group) that administered 1% (Lugol's iodine), 3rd group (T1) treated with 10% green synthesized nanocomposite (Forsythia suspensa-AgNPs), 4th group (T2) treated with 10% plant extract only, and 5th group (T3) treated with 10% Silver nanoparticles only.

The results of the current study showed that there was no significant difference between the groups of animals studied in the length of the estrous cycle. While the group T1 recorded the highest pregnancy rate (86.66%) and was statistically superior to the groups in the current study: the control group, Lugol's iodine solution group, T2, and T3 (0%, 53.33%, 66.66% and 26.66 %) respectively.

The results showed the percentage of cows not returning to estrus after artificial insemination and treatment in the T1 group (93.33% ) after 21 days compared with the other groups: control group, Lugol's iodine solution group, T2, and T3 (0%, 60%, 73.33%, and 26.66% ) respectively.

**Keywords:** *Lugol's Iodine, Forsythia Suspense, nanoparticles, endometritis, dairy cows.*

### INTRODUCTION

Endometritis is common in dairy cows and causes infertility by dysfunction of the uterus and ovarian functions (Sheldon, et al, 2009). It has significant negative consequences on the economy since it decreases dairy industry profitability and results in losses (Gurunathan et al., 2018).

Antibiotics, prostaglandins, and Lugol have all been used in various regimens for cows with endometritis (Drillich, 2006; MidoS et al., 2016; Szenci, 2016), in addition to Lugol (Ahmed et al., 2014; Ahmed and Elsheikh,

2014; Alyasiri et al., 2015). Cattle reproductive diseases such uterine infection can be treated with Lugol 1% (Ahmed and Elsheikh, 2013). When used on buffaloes undergoing anesthesia, Lugol 2% produces positive results (Ahlawat et al., 2016). It is still not common practice to employ Lugol as an alternate treatment for endometritis (Mido et al., 2016) in local cows, especially those with uterine sonography observations (Szenci, 2016).

Treatment of cows by repeated intrauterine infusion with antimicrobials, this led to the

growth of bacteria resistant to treatment, so it is required to provide alternative antimicrobial treatment for endometritis (Gurunathan et al., 2018, Genís et al., 2018). As a result, it's critical to look for alternatives that are very successful in getting rid of pathogens with high potency and little resistance, as well as having a short treatment time and few to no adverse effects (LeBlanc, 2008).

Forsythia Suspensa extract is widely used in traditional Chinese medicine for the treatment of a wide range of conditions, including but not limited to: fever, tonsillitis, oral lesions, pharyngeal-laryngitis, nephritis, erysipelas, ulcers, gonorrhoea, and acute respiratory syndrome (Jiao et al., 2013). Prior studies have identified lignan, phenethyl alcohol glycoside, volatile oil, and pentacyclic triterpenoids as the principal active components of Forsythia suspensa extract (FSE) (Piao et al., 2009). Antioxidant (Wang et al., 2008), antibacterial (Han et al., 2012), antiinflammatory (Zhao et al., 2017), and antiallergic (Hao et al., 2010) effects have been attributed to these molecules. Current research in our lab has employed a variety of stress scenarios and animal species to show that FSE has antioxidant properties and can strengthen the immune system (e.g., piglets, broilers, laying hens, and mice). In addition to improving performance, studies have shown that FSE can alter immune function, intestinal permeability, and antioxidant status.

The current study aims to combine the therapeutic properties of Forsythia Suspensa extract with the critical properties of Silver nanoparticles to create a green synthesis composite and assess their antimicrobial activity for the treatment of clinical endometritis in dairy cows and comparative with Lugol's iodine.

## Material and method

### Animals and study design

Animals of the study the current study included (75) crossbred dairy cows, their ages ranged between (4-8) years in separate places in Thiagar Governorate.

### Experimental study

Cows were distributed into five equal groups (15 cows for each group and artificial insemination after 20 hours from treated) as follow:

1st group (control group) kept untreated.

2nd group (Lugol's Iodine solution group) administered 1% Lugol's Iodine solution.

3rd group (T1) treated with 10% green synthesized nanocomposite (Forsythia suspensa-AgNPs).

4th group (T2) treated with 10% plant extract of Forsythia Suspensa only.

5th group (T3) treated with 10% Silver nanoparticles only.

### Selection of clinical cases

The cows were selected based on:

1. Breed of the cow: crossbred dairy cows.
2. Age: between 4-8 years old.
3. Condition: With three or more previous inseminations.
4. Health status:
  - a) It was confirmed that the ovaries were free of any pathological lesions through performing a rectal palpation.
  - b) Presence of a turbid vaginal secretions.

Lugol's iodine solution Ingredients and preparation:

Two grams of potassium iodide, one hundred milliliters of distilled water, and one gram of iodine crystals are used to make Lugol's iodine. To make Lugol's iodine, we dissolved two grams of potassium iodide in 100 ml of DW, while continuously shaking of the mixture. Next, we added 1 g of iodine crystals while continuing shaking, and last, we filtered the solution and stored it in a firmly sealed brown bottle at room temperature (Manimaran et al.,2022).

Preparation of ethanolic Forsythia Suspensa extract:

The alcoholic Forsythia suspensa extract was prepared in the laboratory of the Zoonotic Diseases Unit / College of the Veterinary Medicine / Qadisiyah University by using the Soxhlet apparatus (Redfern, et al., 2014). It was stored under sterile circumstances at 5°C until they are used in farther analysis (Gas-Chromatography-Mass Spectrometry).

Green synthesized nanocomposite (Forsythia suspensa-AgNPs):

The Forsythia suspensa-AgNPs were prepared according to (Du et al., 2019). Conducting tests such as UV-Vs spectroscopy (Thermo® Scientific, USA), SEM (JSM-7001F, JEOL, Japan), and FTIR spectroscopy (Vertex 70, Bruker, Germany) were used to identify the functional groups capped on the surface of the AgNPs.

Statistical analysis:

The results were analyzed by using computerized SPSS system version 24 ( $P \leq 0.01$ ) (Pallant, 2007).

## Result and discussion

The length of the estrous cycle in experimental cows:

The length of the estrous cycle in all study groups did not have a significant difference (control, Lugol's iodine, T1, T2, and T3) and

the rates were (20.4, 20.5, 20.1, 20.3 and 20.2 ) respectively, and this is agree with (Squires, 2003) showed the cycle lasts for approximately 21days for cattle, table 1.

**Table (1) The length of the estrous cycle in experimental cows**

Parameters Groups	Estrous cycle (days)
Control	20.4
Lugol's iodine	20.5
T1	20.1
T2	20.3
T3	20.2
Total	20.3

Pregnancy rates of treated experimental cows

A clinical evaluation of cows using rectal palpation and a pregnancy indication in table 2 were further results, the control group had the pregnancy rate (0%), whereas T1 had the greatest pregnancy rate (86.66%). The results for the other groups were consecutively 0%,53.33%,86.66%,66.66%,26.66%, for control group, Lugol's iodine, T1, T2, and T3 respectively. This results is more than with what (Resum &Singh, 2016) found that the pregnancy rate using Lugol's iodine solution was 30% for cross bred cows suffering from repeat breeder and the results are also more than (Singh, et al., 2018) who found 42.86% of endometritis in cows and agreement with (Ceti, et al., 2019) who fined 62% in dairy cows and (Bhardwaz, et al., 2018) who recorded conception rate 40% in infectious repeat breeder crossbred cows and it agrees with what (Lv, et. al., 2021) showed, as it was found that the herb Forsythia suspensa has a positive effect from a histological point of view in the uterus of cows suffering from endometritis due to it works as an antibacterial and antiviral.

The study's findings support those of (Liu et al. 2005) discovered that extracts of Forsythia

Suspensa are antibacterial and antifungal, and the findings agree with (Bai et al., 2019) that forsythiaside is an active anti-bacterial agent. This could be explained by loading the extract on the surfaces of the nanoparticles, thus increasing the treatment area of the endometrium and the results agree with what was shown (Zahoor et al., 2021) who indicate that AgNPs are widely used in a variety of fields because of their unique physio-chemical properties, morphological characteristics (size, shape, and high surface area), and dispersion.

Our results are in accordance with (Khatib, et al., 2022; Daud, et al., 2022 and Naganthran, et al., 2022) those confirmed that Toluene works as antibacterial, antiviral and antifungal, the results are compatible with (Isnaini, et al., 2022 ; Burlou-Nagy, et al., 2022 and Mlambo, et al., 2022) those found that Hexanal its properties as an antibacterial, antifungal, anti-inflammatory, and antiviral, as well as, compatible with (Kuttithodi, et al., 2022; Nabi, et al., 2022 and Mondal & Mistri 2022) those obtained that Cyclohexanol its function as an antioxidant, antimicrobial, antiviral, these results agree with results are obtained by (Gazwi, et al., 2022 ; Abdel-Motaal, et al., 2022 and Rodrigo, et al., 2022) those explained that Benzoic acid-2-bis-trimethylsilyloxy ester it works as antimicrobial, antioxidant, antifungal and antibacterial activity, our results agree with Zhan, et al., 2022 who found that 3-Cyclohexen-4-methyl-1-methylethyl-R- its act as an antimicrobial, our results consistent with (Fazal, et al., 2022) Who explained that 3-Tetradecene, (Z)- it's as antioxidants and antimicrobials.

The current study results are agreement with (Zazouli, et al., 2022) who noticed that Nonadecan-1-ol trimethylsilyl ether its

antimicrobial activity, the results are consistent with (Gajera, et al., 2020 and Lammers, et al., 2021) who confirmed that 1-Tridecene its antibacterial, antiviral, antifungal and antioxidant qualities, the results are concordant by (Bora & Devi 2023 and Nasser Alahmari, et al., 2022) those found that (S)-(+)-5-Methyl-1-heptanol it works as antioxidant, antiviral, and antiparasitic.

The present study finding were in accordance with (Tania, et al., 2022) who showed that 3-Trifluoroacetoxytridecane its anti-inflammatory properties, the results agree with (Yarazari, & Jayaraj 2022; Adeyinka, et al., 2022 and Amankwah, et al., 2022) those found that E-15-Heptadecenal its function as an antimicrobial, antioxidant, antifungal, anti-inflammation and anticancerous, the findings are in agreement with the observation of (Khaled, et al., 2021; Shivhare, et al., 2023 and Tyagi, et al., 2020) those who confirmed that 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione its properties as an antifungal, antibacterial, antiviral, anticancer, antioxidant, the results are concordant with the explanation of (Sousa Mourão, et al., 2022 ; Roselin, & Parameshwari, 2022 and Abdalla, 2022) those notice that 1-(+)-Ascorbic acid 2,6-dihexadecanoate its action as an antioxidant, anti-inflammatory, antiviral, and antimicrobial, the findings are consistent with those of (Rawat et al., 2022 and Das et al., 2022), who explored that trifluoroacetic acid, the pentadactyl ester is an antibacterial and antioxidant, the results agree with (Mohammad, et al., 2022 ; Alshamrani, 2023 and Joshi, 2022) those explained that 1,4,5,7-Tetramethyl-6H-pyrrolo[3,4-d]pyridazine its works as antifungal, antibacterial, antioxidant, antiglycation, analgesic antiviral, and anticancer activity.

**Table (2): Pregnancy rates of treated experimental cows**

Parameters Groups	Total No. of cows	Pregnant cows		Non pregnant cows	
		No.	%	No.	%
Control	15	0	0	15	100
Lugol's iodine	15	8	53.33	7	46.66
T1	15	13	86.66	2	13.33
T2	15	10	66.66	5	33.33
T3	15	7	26.66	11	73.33
X <sup>2</sup>		23.51			
P value		0*			

\* Significant difference at P<0.05

The cows returning to estrus after 21 days in experimental study

In table 3 the control group, which consists of fifteen (15) cows, the percentage cows non returning to estrus after 21 days was 0%. Clinical examination findings revealed that a group of Iodine lugols (9 cases out of 15) were healed with a percentage of (60%), whereas (6 cases out of 15) were not cured with a percentage of (40%).

InT1 group (treated with 10% green synthetic nanocomposite (Forsythia suspense- AgNPs), the outcome was (14 cases out of 15) completely healed from uterine inflammation(93,33%), whereas (1 case out of 15) did not react to therapy (6,66%). While T2 (treated with 10% plant extract only) was (11 out of 15) cases, with a percentage of (73,33%) healing was gained, and (4 out of 15) cases (26,66%) that did not gain healing.

The results of treatment T3 (treated with 10% Silver nanoparticles only.) indicated that (4

cases out of 15) were cured from uterine inflammation with a rate of (26,66%), while (11 cases out of 15) were not cured with a percentage of (73,33%).

The non-return of the exchanger may explain the loading of the extract on the nanomaterial and its effect on the endometrium and this is consistent with Kumari, et al, (2022) who showed that the use of nanomaterials to enhance therapeutic efficacy and reduce unwanted effects of bioactive materials and also, the results agree with Ying, et al, (2022) who concluded nanoparticles metals have larger surface area compared with their non-nano.

As for the cows returning to estrus, this may be explained by the inappropriateness of the uterine environment, and this is consistent with what was shown by Noakes, et. al., (2010), that most cases are due to a change in the uterine environment.

**Table (3): The cows returned to estrus after 21 days in experimental study**

Parameters Groups	Total No. of cows	non return cows		Return cows	
		No.	%	No.	%
Control	15	0	0	15	100
Lugol's iodine	15	9	60	6	40
T1	15	14	93.33	1	6.66
T2	15	11	73.33	4	26.66
T3	15	4	26.66	11	73.33
X <sup>2</sup>		19.9			
P value		0*			

\* Significant difference at  $P \leq 0.05$

### Conclusion:

Depending on the experiment's findings, utilizing *Forsythia suspensa*-AgNPs as a treatment after artificial insemination in dairy cows with endometritis was found to increase the pregnancy rate more effectively than using Lugol's iodine.

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Standard setting, methodology, organization, formal analysis, investigation, data organization, validation, visualization, writing - original draft done by Ahmed Hassan Al-Safi and Ali Habeeb Jaber; Writing, reviewing, editing, supervising, and managing the project was done by Ali Habeeb Jaber. All authors approved the final version of the manuscript.

Availability of data and materials: "The data used and/or analyzed during the current experimental available from the corresponding author on reasonable request".

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Conflict of Interest "The authors declare that there are no conflicts of interest regarding the publication of this manuscript"

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