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Administration of ashwagandha and garlic extract in subclinical endometritis improves conception rate in cattle

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Abstract

Subclinical endometritis (SCE) is a major postpartum reproductive disorder that adversely affects fertility in dairy cattle. The present study evaluated the prevalence of SCE and the therapeutic efficacy of intrauterine Garlic (*Allium sativum*) and Ashwagandha (*Withania somnifera*) extracts in affected cows. A total of 92 postpartum cows were screened using the Whiteside test and endometrial cytology, revealing an SCE prevalence of 41.13%. Eighteen positive cows were allocated into three groups: control, Ashwagandha-treated, and Garlic-treated. Both herbal treatments significantly reduced uterine inflammation, as evidenced by lower polymorphonuclear neutrophil (PMN) counts and fewer positive Whiteside test results compared to the control group. The conception rate was significantly higher in the Ashwagandha and Garlic groups (83.33%) than in the control (50%). Herbal supplementation also improved serum total protein concentration, while serum NEFA levels showed no significant difference between healthy and SCE-affected cows. The findings indicate that intrauterine Garlic and Ashwagandha extracts effectively improve uterine health and reproductive performance, suggesting their potential as safe, economical, and natural alternatives for managing subclinical endometritis in cattle.

Keywords: Subclinical endometritis, garlic, ashwagandha, dairy cattle, endometrial cytology

1. Introduction

Infectious diseases of the female reproductive tract are among the major causes of impaired fertility in mammals, particularly in cattle, where they result in substantial reproductive and economic losses (Ross, 2002; Sheldon *et al.*, 2006; Sheldon *et al.*, 2020; Ribeiro *et al.*, 2022) [32, 34, 36, 31]. The detrimental effects of these infections extend beyond local tissue damage, as they interfere with the normal physiological functions of both the uterus and ovaries, ultimately compromising reproductive performance. Over the past several years, considerable progress has been made in understanding the innate immune mechanisms that enable the reproductive tract to recognize and respond to invading microorganisms (O'Neill, 2008; Akira *et al.*, 2006; Beutler, 2004) [26, 2, 4].

Research has demonstrated that innate immune activity within the female reproductive tract is not confined to circulating immune cells. Endometrial and ovarian cells also possess the ability to detect pathogenic organisms through specific receptors and actively participate in local defence responses (Herath *et al.*, 2006; Herath *et al.*, 2007; Hernandez-Gonzalez *et al.*, 2006) [13, 15, 16]. Although these responses are essential for controlling infection, microbial pathogens and their associated molecules may simultaneously disturb endocrine regulation by altering the synthesis of reproductive hormones and inflammatory mediators. Such alterations adversely influence ovarian function, uterine physiology and, consequently, fertility (Herath *et al.*, 2006; Herath *et al.*, 2007; Herath *et al.*, 2009; Sheldon *et al.*, 2002; Ribeiro *et al.*, 2022; Sheldon and Owens, 2023) [13, 15, 14, 37, 31, 35].

Among postpartum reproductive disorders, uterine infections remain one of the most frequently encountered conditions in dairy cattle. Metritis commonly develops during the first

week after calving, although its reported prevalence varies depending on the diagnostic criteria adopted. Several epidemiological studies have indicated that approximately one-third to one-half of dairy cows may be affected, while a smaller proportion develops systemic manifestations such as fever and depression (Markusfeld, 1987; Zwald *et al.*, 2004; Drillich *et al.*, 2001; Benzaquen *et al.*, 2007; LeBlanc, 2020; Sheldon and Owens, 2023) [24, 44, 7, 3, 21, 35].

Failure of complete uterine recovery following calving predisposes animals to persistent uterine inflammation. Consequently, nearly 15-20% of cows develop clinical endometritis after the third postpartum week, whereas a considerable proportion experiences subclinical endometritis without obvious external signs (Sheldon *et al.*, 2006; Gilbert *et al.*, 2005; Ribeiro *et al.*, 2022; LeBlanc, 2020) [34, 12, 31, 21]. These postpartum uterine disorders delay uterine involution, impair ovarian cyclicity and significantly reduce subsequent reproductive efficiency, thereby increasing the economic burden on dairy production systems.

Clinical endometritis is recognized by the presence of purulent uterine discharge after 21 days postpartum or mucopurulent discharge beyond 26 days postpartum, as described by Sheldon *et al.* (2006) [34]. In routine clinical practice, examination of vaginal mucus is widely used for diagnosis because its characteristics closely reflect the severity of uterine infection and inflammation (Williams *et al.*, 2005; Sheldon and Owens, 2023) [43, 35]. The nature of the discharge also provides valuable information regarding the likely bacterial involvement and the expected response to treatment.

Unlike clinical endometritis, subclinical endometritis progresses without visible abnormalities in vaginal discharge and therefore requires cytological evaluation for diagnosis. The condition is generally identified by an increased proportion of polymorphonuclear neutrophils (PMNs) in endometrial cytology samples collected during the postpartum period (Kasimanickam *et al.*, 2004; Santos *et al.*, 2009; Ribeiro *et al.*, 2022; LeBlanc, 2020) [17, 33, 31, 21]. Despite the absence of apparent clinical signs, persistent inflammation adversely affects uterine function, lowers conception rates and prolongs the interval required to achieve successful pregnancy. Early diagnosis and appropriate management of both clinical and subclinical endometritis are therefore essential for improving reproductive performance in dairy cattle.

The use of phytogetic feed additives has emerged as an important nutritional approach for improving animal health and reproductive performance in dairy cattle. Among the various medicinal plants, garlic (*Allium sativum*) has attracted considerable attention because of its antioxidant, antimicrobial, anti-inflammatory and immunomodulatory properties, which contribute to improved metabolic status, immune competence and reproductive efficiency in cattle (Puvača *et al.*, 2020; El-Tarabany *et al.*, 2021) [30, 10]. Similarly, ashwagandha (*Withania somnifera*) is recognized for its adaptogenic and antioxidant activities and has been reported to alleviate stress, improve physiological functions and support reproductive performance in cattle (Kumar *et al.*, 2022; Tewari *et al.*, 2023) [19, 42]. Although the individual biological effects of these phytogetic supplements have been documented, information regarding their use as nutritional interventions for improving reproductive performance under field conditions remains limited. Therefore, the present study was undertaken to evaluate the effect of dietary

supplementation with garlic and ashwagandha extracts to treat the subclinical endometritis in cattle and to increase reproductive performance in cattle.

Materials and Methods

The present investigation was carried out in the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science & Animal Husbandry, Mhow, in collaboration with the Central Semen Station, Bhopal.

Animals used for study

A total of 80 apparently healthy cows with no clinical signs of endometritis that calved between August 2017 and March 2018 were used for the study. Animals were maintained under an intensive housing system in cemented sheds with brick floor and stall-fed seasonally available green fodder, wheat straw, and concentrate. Supplements included mineral mixtures and *ad libitum* water. This management reflected standard private farming practices in the region.

The cows were screened for subclinical endometritis using endometrial cytology and the Whiteside test for prevalence study. Blood was collected via jugular puncture during the last week pre-partum, the day of calving, and the first week postpartum for estimation of NEFA concentration using the copper soap solvent extraction method modified by Shipe *et al.* (1980) [38], in healthy animals and animals affected with subclinical endometritis. From this population, 18 animals confirmed with subclinical endometritis (SCE) were selected and divided into three groups (n=6) to evaluate herbal therapeutic efficacy. Positive cows were randomly assigned to:

- Control group I (Group I, n=6): 20 ml intrauterine normal saline solution oid for 3 days.
- Treatment II (Group II, n=6): 20 ml intrauterine ashwagandha extract oid for 3 days.
- Treatment III (Group III, n=6): 20 ml intrauterine garlic extract oid for 3 days.

Treated animals were subjected to control breeding at 45 days using ovsynch protocol and conception rate were confirmed by per rectal examination at 60 days post artificial insemination.

Preparation of extract

1. Ashwagandha extract

Fresh Ashwagandha (*Withania somnifera*) leaves were dried in the dark, pulverized, and extracted with absolute alcohol using a Soxhlet apparatus until the solvent darkened. The extract was stored in sterile glass containers (Sunderam *et al.*, 2011) [40].

2. Garlic extract

Chopped Garlic (*Allium sativum*) bulbs were dried in the dark and subjected to successive Soxhlet extraction using different solvents such as Methanol. Extracts were concentrated and weighed according to Gangadhar *et al.* (2012) [11].

Statistical analysis

Prevalence and conception rates were expressed as percentages. The data of PMN per cent were analysed statistically by two-way analysis of variance (ANOVA) and means were compared using Duncan's new multiple range test (DNMRT) with significance at $p < 0.05$.

Results

a) Prevalence of sub-clinical endometritis in cattle

The prevalence of subclinical endometritis in cattle was determined by the formula mentioned below, when 41.13%

animals were found positive for sub-clinical endometritis:

$$\text{Prevalance of subclinical endometritis (\%)} = \frac{\text{No. of positive animals}}{\text{Total no of animals}} \times 100$$

Table 1: Prevalence of sub-clinical endometritis in cattle

No. of animals	Animals positive for sub-clinical endometritis	Sub-clinical endometritis (%)
92	38	41.13

b) Concentration of NEFA in healthy and diseased animals

Concentration of NEFA in healthy and sub clinical endometritic cattle is presented in Table 02. There was a highly significant difference ($p < 0.05$) between the means of

NEFA on all the three days, viz., a week prior to, on the day and a week after of calving in both healthy and diseased categories. However, no significant difference ($p > 0.05$) was found between healthy and SCE-positive animals.

Table 2: concentration of nefa in healthy and diseased animals

Metabolites	Time interval	Healthy animals (mean)	Animals positive for sub-clinical endometritis (mean)	Healthy animals (overall mean)	Animals positive for sub-clinical endometritis (overall mean)
NEFA (mmol/L)	-1	0.18±0.002	0.19±0.005	0.41±0.313	0.51±0.364
	0	0.49±0.009	0.63±0.012		
	+1	0.55±0.006	0.72±0.016		
	0	4.60±0.20	4.40±0.10		
	+1	5.00±0.18	4.70±0.06		

c) Treatment Efficacy

Post-treatment, the Ashwagandha group (33.33%) and Garlic group (50.00%) had fewer positive Whiteside tests than the control (83.33%) on next estrus.

Table 3: Whiteside test on CVM in sub-clinical endometritic cattle

Groups	Positive before treatment	Positive after treatment
Group I	06(100%)	05(83.33%)
Group II	06(100%)	02(33.33%)
Group III	06(100%)	03(50.00%)

Table 6: Total protein concentration (g/dl) in different groups of sub-clinical endometritic cattle

Groups	Protein concentration	
	Before treatment (day of estrous)	After treatment (day of next estrous)
Group I	6.31±0.39 ^a	6.98±0.04 ^a
Group II	6.47±0.03 ^a	7.16±0.81 ^b
Group III	6.79±0.04 ^a	7.71±0.05 ^b

Different superscripts within a row indicate significant difference ($p < 0.05$)

d) Conception & Cytology

Both herbal groups achieved an 83.33% conception rate vs. 50% in the control when inseminated on next estrous after treatment in previous estrous. PMN levels dropped significantly to 8.98% (Ashwagandha) and 9.26% (Garlic), compared to 15.34% in the control.

Table 4: Conception rate in sub-clinical endometritic cattle treated with different herbal extract

Groups	Animal	Pregnant after treatment
Group I	06	03(50.00%) ^a
Group II	06	05(83.33%) ^b
Group III	06	05(83.33%) ^b

Different superscripts within a column indicate significant difference ($p < 0.05$)

Table 5: Endometrial cytology in different groups of sub-clinical endometritic cattle

	Group I	Group II	Group III
Before treatment (day of estrous)	19.93±0.13 ^{aA}	21.08±0.07 ^{aA}	19.87±0.19 ^{aA}
After treatment (next estrous)	15.34±0.24 ^{aA}	8.98±0.11 ^{bB}	9.26±0.07 ^{bB}

Different superscripts in small letters within a column and in capital letter within a row indicate significant difference ($p < 0.05$)

e) Total Protein

Total protein concentration (g/dl) increased significantly ($p < 0.05$) in herbal groups (7.16–7.71 g/dl) post-treatment.

Discussion

In the present study, the relatively high prevalence observed indicates that subclinical uterine inflammation remains a common reproductive disorder during the postpartum period. Since affected animals did not exhibit obvious clinical signs, diagnosis through Whiteside test and endometrial cytology proved useful for identifying infected animals at an early stage. Similar observations were reported by Kasimanickam *et al.* (2004) [17], who demonstrated that a considerable number of postpartum cows without visible vaginal discharge were cytologically positive for endometritis, indicating that clinical examination alone is insufficient for diagnosis. Likewise, Gilbert *et al.* (2005) [12] reported an overall prevalence of approximately 53% in postpartum Holstein cows and observed that affected animals required a significantly longer period to conceive than healthy cows. Dubuc *et al.* (2010) [8] also reported that nearly one-fifth of postpartum dairy cows suffered from cytological endometritis, emphasizing that uterine inflammation frequently persists even in apparently healthy animals. Recent reviews by Parikh *et al.* (2022) [28] and Pascottini *et al.* (2023) [29] have further emphasized that postpartum uterine diseases remain one of the major causes of reduced fertility and economic losses in dairy cattle, particularly when diagnosis and treatment are delayed. Therefore, early diagnosis followed by timely therapeutic intervention is essential to restore uterine health and improve reproductive performance.

The concentration of serum non-esterified fatty acids (NEFA) increased significantly ($p < 0.05$) from one week before

calving to one week after calving in both healthy and subclinical endometritic cattle. In healthy animals, the mean NEFA concentration increased from 0.18 ± 0.002 mmol/L one week before calving to 0.49 ± 0.009 mmol/L on the day of calving and further to 0.55 ± 0.006 mmol/L one week after calving. A similar pattern was observed in subclinical endometritic cattle, where NEFA concentration increased from 0.19 ± 0.005 mmol/L before calving to 0.63 ± 0.012 mmol/L at calving and 0.72 ± 0.016 mmol/L one week postpartum. Although NEFA values were numerically higher in diseased animals throughout the transition period, the difference between healthy and diseased groups was not statistically significant ($p > 0.05$).

The increase in circulating NEFA during the transition period reflects the development of negative energy balance, which is a normal physiological response when energy demand for milk production exceeds dietary energy intake. Under these conditions, adipose tissue reserves are mobilized to meet energy requirements, resulting in increased release of NEFA into the bloodstream. Similar observations have been reported by Ospina *et al.* (2010)^[27] and Chapinal *et al.* (2011)^[5], who demonstrated that elevated NEFA concentrations during the transition period are associated with increased fat mobilization and a greater risk of postpartum metabolic and reproductive disorders. LeBlanc *et al.* (2011)^[22] further suggested that excessive lipid mobilization may impair immune function, thereby reduce uterine defence mechanisms and increase susceptibility to postpartum uterine infections.

Although serum NEFA concentration was numerically higher in the subclinical endometritis group in the present study, the absence of a significant difference between healthy and diseased animals indicates that NEFA alone cannot be considered a reliable diagnostic marker for subclinical endometritis. Development of uterine inflammation is influenced not only by metabolic status but also by bacterial contamination, immune competence and uterine defence mechanisms. Similar conclusions have been drawn by Pascottini *et al.* (2023)^[29], who reported that postpartum uterine diseases result from the combined effects of metabolic stress, microbial infection and host immune response rather than any single biochemical parameter.

Ashwagandha (*Withania somnifera*) contains withanolides, sitosterols and alkaloids that possess potent antioxidant, anti-inflammatory and immunomodulatory properties. These compounds reduce oxidative stress, regulate inflammatory cytokines and improve cellular immune responses, thereby promoting repair of the damaged endometrium and facilitating elimination of pathogenic microorganisms (Mirjalili *et al.*, 2009; Abd El-Hack *et al.*, 2022)^[25, 1]. Improved immune competence also enhances uterine defence mechanisms during the postpartum period, leading to faster resolution of subclinical infection.

Similarly, Garlic (*Allium sativum*) contains allicin and other organosulphur compounds that exhibit broad-spectrum antibacterial, antifungal and antioxidant activities. Allicin inhibits bacterial multiplication, suppresses inflammatory mediators and improves phagocytic activity of neutrophils and macrophages, thereby accelerating uterine involution and recovery (El-Saber Batiha *et al.*, 2020; Chen *et al.*, 2021)^[9, 6]. The reduction in Whiteside test positivity observed in the Garlic-treated group in the present study supports these pharmacological properties.

The present findings are in close agreement with Lawange *et al.* (2019)^[20], who evaluated Garlic extract with and without Ashwagandha in repeat breeding cattle associated with uterine

infection. They reported a significantly higher recovery rate in animals receiving the combined herbal treatment, accompanied by conversion of Whiteside test-positive animals to negative status and improvement in conception rate. Likewise, Swelum *et al.* (2021)^[41] suggested that phytochemical feed additives improve reproductive efficiency primarily through their antioxidant, antimicrobial and immunomodulatory effects. The superior response observed in the Ashwagandha-treated group may be attributed to its immunomodulatory activity, which improves both innate and adaptive immune responses while simultaneously reducing oxidative stress within uterine tissues. Reduced production of reactive oxygen species and inflammatory mediators promotes regeneration of the endometrial epithelium and restores normal uterine function (Abd El-Hack *et al.*, 2022)^[1]. Garlic supplementation also contributed to reduction in PMN percentage through its antimicrobial activity, which limits bacterial growth and minimizes tissue damage caused by persistent infection (El-Saber Batiha *et al.*, 2020)^[9].

The findings of the present investigation are consistent with those of Kasimanickam *et al.* (2004)^[17], who reported that reduction in PMN percentage following treatment was associated with improved uterine health and higher fertility. Similarly, Gilbert *et al.* (2005)^[12] demonstrated that persistence of elevated PMN levels was associated with reduced conception and prolonged days open. Pascottini *et al.* (2023)^[29] emphasized that successful treatment of subclinical endometritis should primarily be reflected by reduction in cytological inflammation rather than only disappearance of clinical signs.

Following herbal treatment, the reduction in uterine inflammation, as confirmed by the Whiteside test and PMN cytology, probably restored normal endometrial function, thereby increasing the probability of successful conception. Ashwagandha has been reported to improve antioxidant defence mechanisms and modulate immune responses, while Garlic enhances antimicrobial activity and reduces oxidative stress through sulphur-containing bioactive compounds such as allicin (El-Saber Batiha *et al.*, 2020; Abd El-Hack *et al.*, 2022)^[9, 1].

The present findings are comparable with those reported by Lawange *et al.* (2019)^[20], who evaluated Garlic extract alone and in combination with Ashwagandha in repeat breeding cattle suffering from uterine infection. They observed a significantly higher conception rate in animals receiving herbal therapy than in untreated animals and concluded that improvement in uterine health following herbal treatment contributed directly to enhanced fertility. Similarly, LeBlanc *et al.* (2011)^[22] reported that successful resolution of postpartum uterine inflammation substantially improves reproductive efficiency in dairy cattle. More recently, Swelum *et al.* (2021)^[41] suggested that phytochemical feed additives improve reproductive performance by reducing oxidative stress, enhancing immune competence and maintaining reproductive tract health.

The increase in serum total protein observed in the treated groups may indicate improvement in metabolic status, protein synthesis and immune function following herbal supplementation. During uterine infection, inflammatory stress and oxidative damage may adversely influence protein metabolism. Reduction of inflammation following Garlic and Ashwagandha treatment probably improved nutrient utilization and hepatic protein synthesis, resulting in increased serum protein concentration. Garlic contains several antioxidant compounds that protect tissues against oxidative

damage, whereas Ashwagandha enhances immune function and supports tissue repair through its adaptogenic and anti-inflammatory properties (El-Saber Batiha *et al.*, 2020; Abd El-Hack *et al.*, 2022) ^[9, 1]. The present findings are consistent with previous reports indicating that phytogetic feed additives improve metabolic health and immune response in livestock. Chen *et al.* (2021) ^[6] reported that Garlic supplementation enhances antioxidant status and improves nutrient utilization in farm animals. Likewise, Kholif *et al.* (2021) ^[18] emphasized that herbal feed additives improve metabolic efficiency and overall health by reducing oxidative stress and enhancing rumen and immune function.

Conclusion

The present study demonstrated that subclinical endometritis is an important postpartum reproductive disorder in cattle that adversely affects uterine health and fertility. The findings revealed that supplementation with Garlic (*Allium sativum*) and Ashwagandha (*Withania somnifera*) extracts effectively reduced uterine inflammation, improved endometrial health and enhanced reproductive performance. Both herbal treatments decreased uterine inflammatory changes, improved cytological findings and increased conception rate compared to the untreated control animals.

The beneficial effects of these herbal extracts may be attributed to their antimicrobial, antioxidant, anti-inflammatory and immunomodulatory properties, which promote uterine recovery and improve the uterine environment for successful conception. Overall, the study suggests that Garlic and Ashwagandha can be considered safe and promising herbal alternatives or supportive therapies for the management of subclinical endometritis and improvement of reproductive efficiency in cattle.

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Conflict of Interest

Not available

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Not available

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