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RISK FACTORS FOR ENDOMETRITIS AND ITS IMPACT ON FERTILITY OF POSTPARTUM DAIRY COWS IN WOLAITA SODO, SOUTHERN ETHIOPIA

MVSC THESIS

BY

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DEPARTMENT OF CLINICAL STUDIES

MVSC IN VETERINARY THERIOGENOLOGY

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BISHOFTU, ETHIOPIA

RISK FACTORS FOR ENDOMETRITIS AND ITS IMPACT ON FERTILITY OF POSTPARTUM DAIRY COWS IN WOLAITA SODO, SOUTHERN ETHIOPIA

A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Theriogenology

BY

Tsigereda Teshome Tadesse

June, 2024

Bishoftu, Ethiopia

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As MVSc research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance by Tsigereda Teshome Tadese, entitled " Risk Factors For endometritis and its Impact on fertility of postpartum dairy cows in Wolaita Sodo, Southern Ethiopia." We recommend that it be submitted as fulfilling the MVSc thesis requirement for the Degree of Master of Veterinary Science in Veterinary Theriogenology.

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DEDICATION

This thesis is dedicated to my beloved parents, Amarech Gamiyo and Teshome Tadesse, whose unwavering sacrifice, unconditional support, and boundless encouragement have been the foundation of my success and the guiding light in my life. Their selfless dedication, belief in my abilities, and constant presence as pillars of strength have shaped me into the person I am today. I am forever grateful for their love, guidance, and unwavering faith in me. This thesis is dedicated to them with profound gratitude and heartfelt appreciation for all they have done.

STATEMENT OF THE AUTHOR

First, I declare and affirm that this thesis is my own work. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I affirm that I have cited and referenced all sources used in this document. Every serious effort has been made to avoid any plagiarism in the preparation of this thesis. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
BCS	Body condition score
CCI	Calving to conception interval
CCS	Cows cleanliness score
CFSI	Calving to first service interval
CE	Clinical endometritis
CMT	California mastitis test
DIM	Day in milk
FSH	Follicle stimulating hormone
GDP	Gross domestic production
GnRH	Gonadotropin releasing hormone
LH	Luteinizing hormone
LPS	Lipopolysaccharide
NSPC	Number of service per conception
PGF2a	Prostaglandin F2
PMN	Polymorphnuclear cell
SCE	Subclinical endometritis
SCM	Subclinical Mastitis
WZFEDD	Wolaita zone financial and economic development department

ABSTRACT

Despite its significant impact on the dairy industry, there is limited research or documentation on the extent of endometritis, its specific risk factors and its impact on the reproductive performance in postpartum dairy cow in the study area. The present study was aimed to investigate incidence of postpartum endometritis, risk factors contributing to its development and reproductive consequences of endometritis in dairy farms in Wolaita Sodo, Southern Ethiopia, from October 2023 to May 2024. Longitudinal prospective study determined incidence, risk factors and reproductive outcomes prospectively. A study was carried out on 74 dairy cows with 69 clinically healthy and 5 cows diagnosed with clinical endometritis. Subclinical endometritis was diagnosed using endometrial cytology and subclinical mastitis was diagnosed using California Mastitis Test. The clinical, management and reproductive data were obtained from a weekly follow up visit of each cow and from record book of the farm. Incidence of endometritis was 44.59% (33/74) and subclinical endometritis was 40.57% (28/69). Retained fetal membranes (OR=9.23, P=0.007), assisted calving (OR= 5.06, P=0.026), dystocia (OR=7.79, P=0.014), hypocalcaemia (OR=6.49, P=0.027), mastitis (OR=5.06, P=0.026), male calf births (OR=3.06, P=0.04) and poor body condition scores (OR=2.78, P=0.003) were significantly related with subclinical endometritis. Clinical endometritis in this study were reported to be related to retain fetal membrane, vaginal prolapse, and abortion. Cows with endometritis had shown longer ($P < 0.001$) calving-to-conception interval, extended ($P < 0.001$) calving-to-first service interval and higher ($P < 0.001$) number of services per conception. Thus, it could be concluded that endometritis occur at higher incidence and causes a tremendous impact on the reproductive performance of dairy cows in the study area. Therefore, preventive measures should be done to reduce occurrence of endometritis through enhanced reproductive health and management practices and dairy farmers should take awareness creating trainings.

***Key Words**: Dairy Cows, Endometritis, Clinical endometritis, Subclinical endometritis, Reproductive Performance, Southern Ethiopia, Wolaita Sodo.*

1. INTRODUCTION

Livestock production accounts for approximately 40% of the total agricultural GDP and 20% of national foreign currency earnings (World Bank, 2017). Even though the huge number of cattle and their economic importance, the productivity is low due to the obstacles such as diseases of different origin, low quality and quantity nutrition, poor management and poor performance of indigenous breeds (Seid and Thomas, 2019). In addition, the successful operation of dairy farms is significantly impacted by the reproductive performance of lactating animals (Softic *et al.*, 2020).

Reproductive health stands as a cornerstone for the overall productivity of both individual animals and herds. Within female animals, uterine diseases like endometritis and metritis can significantly impair reproductive health, yielding substantial economic repercussions on the animal's overall productivity (Umer *et al.*, 2022). Uterine inflammation hampers the uterine involution process and prolongs the initiation of ovarian activity, leading to economic losses from systemic illness, reduced milk production and a substantial decline in fertility (Deori, and Phookan, 2015).

The postpartum period is a crucial period in the life of dairy cattle and management of the essential problems and diseases at an early stage represents the key to successful dairy herd management. Endometritis is an inflammation of the endometrial lining of the uterus and is one of the fundamental and striking diseases that hamper the reproductive performance of cows and lower the livestock profitability. Determining all the factors that boost endometritis risk of occurrence is more important than treating the disease because it is a multifactorial disease and is caused by nonspecific bacteria (Adnane *et al.*, 2017).

Endometritis is sub-categorized into clinical endometritis (CE) often characterized by purulent or mucopurulent uterine discharge and subclinical endometritis (SCE) characterized by the presence of ≥5% of polymorphonuclear (PMN) cells in endometrial cytology sample. Despite the lack of uterine discharge in cows with SCE, the condition's severity is still considered adequate to hinder reproductive performance (Sheldon *et al.,* 2006; Barlud *et al.,* 2008; Dubuc, *et al.,* 2010a). Endometritis is a common reproductive disorder in dairy cattle characterized by inflammation of the endometrium with or without obvious clinical signs. It can negatively impact reproductive performance, milk production, and overall herd health. Prolonged calving-conception intervals and low fertility in dairy cows have been linked to inflammation of the endometrium persisting after postpartum uterine involution. The subclinical nature of this condition makes it necessary in the use of endometrial cytology or biopsy for diagnosing it (Arias *et al.,* 2018).

In the Wolaita Sodo region, dairy farming plays a crucial role in the local economy, making it essential to understand the risk factors associated with endometritis in postpartum dairy cattle (Keshamo, 2020; Hirpassa *et al.,* 2023). Factors that increase the risk of endometritis include dystocia, stillbirths, twin deliveries, male offspring, assisted parturition, retained fetal membranes, hypocalcaemia, ketosis, metritis, displaced abomasum, induced calving, vaginal trauma during calving, and poor body condition score. In addition, unfavorable calving environment, nutrition, parity, calving season and lack of gloves during calving assist and poor management practice (Potter *et al.,* 2010; Cheong *et al.,* 2011; Adnane *et al.,* 2017). Reproductive performance is hampered by endometritis which reduces pregnancy to first insemination and increases median days open or calving to conception interval (Kasimanickam *et al.,* 2004; Gilbert *et al.,* 2005; Galvão *et al.,* 2009). Determining the risk factors for endometritis allow management interventions to aid in controlling this costly disease (Cheong *et al.,* 2011).

1.1. Statement of Problem

An important factor for a successful dairy operation is reproductive efficiency. Reproductive health disorders have been shown to be the main cause of dairy farms poor productive performance and results in considerable economic losses to the dairy industry. Consequently, upon closer examination of reproductive processes in the dairy cattle, the post-partum period is the miscellaneous and most susceptible to problems and that incidentally coincides with the peak of milk production, uterine involution, and resumption of ovarian activity, conception and greater risk to infection (Radostits *et al.,* 2007; Fesseha *et al.,* 2020).

Endometritis is a significant issue that directly affects the reproductive performance of dairy cows and is the most common clinical and economic problem in the industry. It leads to slower uterine involution, reduced reproductive rate, prolonged interconception period and calving interval, high medication costs, decreased milk production, lower calf crop, and early depreciation of potentially valuable cows. This results in considerable economic losses for the dairy industry (Gizaw *et al.,* 2007; Sheldon *et al.,* 2009). Identifying risk factors for endometritis is crucial for implementing dairy management interventions that aid in controlling this costly disease (Adnane *et al.,* 2017; Ahmadi, 2023).

Despite its significant impact on the dairy industry, there is limited research or documentation on the extent of endometritis, its specific risk factors and its impact on the reproductive performance in postpartum dairy cattle in the Wolaita Sodo region. So identifying and understanding these risk factors, assessing its prevalence and its impact on reproductive performance is crucial for developing effective solution and suggesting management strategies to improve the overall reproductive health and productivity of dairy cattle in study area.

In the present study, the hypothesis tested was that the risk factor to the postpartum period or during calving is high in the study area and contributes to the development of endometritis and this result in loss of fertility in dairy cows. Dairy cows exposed to these risk factors have higher exposure to endometritis and lower fertility than healthy dairy cows.

Therefore, the objectives of this study were:

- To determine the incidence of endometritis in postpartum dairy cow in Wolaita Sodo, Southern Ethiopia.
- To investigate the risk factors that contributes to the development of postpartum endometritis in dairy cows in the study area.
- To evaluate the impact of endometritis on the reproductive performance of dairy cows.
- To develop evidence based recommendations for improved preventive measures and management practices to reduce the identified risk factors and improve overall herd health.

2. LITERATURE REVIEW

2.1. Overview of Endometritis in dairy cattle

Uterine disease remains a significant concern on high-producing dairy cattle farms, influencing approximately half of all dairy cows and exerting substantial effects on reproductive performance and milk production (Sheldon *et al.,* 2004). Reproductive performance issues constitute the primary reasons for culling in large-scale dairy cattle operations (Bajcsy *et al.,* 2019), with reproductive-related culling accounting for an average of 30% of all premature disposals. The economic consequences of uterine disease encompass reduced milk yield, diminished pregnancy rates, heightened risk of premature culling, and increased replacement costs. Despite ongoing research on the disease itself, its economic implications, and treatment options in recent years (Gilbert *et al.,* 2005), uterine disease continues to pose significant challenges to dairy cattle management and productivity. These conditions also lower the levels of luteinizing hormone, affect the size and growth of the dominant follicle, and reduce the follicle's ability to produce estradiol, thus impacting ovulation. Additionally, cows that have experienced uterine disease may have prolonged luteal phases after resuming ovulation postpartum (Mateus *et al.,* 2002; Williams *et al.,* 2008; Galvao, 2018).

The postpartum period stands out as a pivotal period in a cow's reproductive life and is a key area for veterinarians to address in terms of prevention, diagnosis, and appropriate treatment of uterine diseases and their sequelae. This period marks the occurrence of the majority of reproductive problems in cattle, making it essential for the cow to calve normally and navigate the postpartum period with minimal complications as the ultimate goal (Palmer, 2014). Postpartum uterine disease has garnered considerable attention in the veterinary literature as a leading cause of reproductive inefficiency in dairy cattle (Barlud *et al.,* 2008). Essential postpartum uterine disease of economic importance in dairy cows is endometritis. The disease disrupts cows' fertility performance and reduces dairy herd productivity and profitability (Pascal *et al.,* 2021).

The endometrium is the innermost lining of the uterus and is comprised of two layers called stratumspongiosm and stratum compactum. The stratum spongiosum (or basalis) is the deeper layer (rich in endometrial glands) and an attaches to the underlying myometrium, and the stratum compactum (or functionalis) is the superficial layer that lines the uterine cavity. Endometritis is defined as inflammation limited to the endometrium that does not extend beyond the stratum spongiosum and and not associated, with systemic illness. It involves damage of the luminal epithelium, inflammatory cell infiltration and lymphocyte accumulations, vascular congestion, stromal edema, macrophages and polymorphonuclear nutrophils (Bondurant, 1999; Barlud *et al.,* 2008; LeBlanc, 2008; Várhidi *et al.,* 2024). This localized inflammation of the uterine lining is associated with chronic postpartum infection of the uterus with pathogenic bacteria (Dubuc, *et al.,* 2010a).

In contemporary classification, endometritis has been sub-categorized into clinical and subclinical endometritis. CE is characterized by purulent or mucopurulent uterine discharge present after 21 or 26 days postpartum, respectively. Cows with clinical endometritis can be easily assessed using a straightforward scoring system based on the characteristics and smell of their vaginal mucus. SCE is defined by the presence of >18% polymorphonuclear (PMN) cells in uterine cytology samples collected 21–33 days postpartum, or >10% PMNs in samples obtained at days 34–47. Despite the lack of uterine discharge in cows with subclinical endometritis, the condition's severity is still considered adequate to hinder reproductive performance (Kasimanickam *et al.,* 2004; Sheldon *et al.,* 2006). When no other uterine or ovarian conditions have been identified, subclinical endometritis was once thought to be the reason for infertility. Its incidence was imprecisely approximated for many years despite the fact that subclinical endometritis was thought to have a significant impact on cattle reproduction due to the lack of a feasible method for identifying this disorder (Barański *et al.,* 2012).

2.2. Etiology of Endometritis

Notably, the most prevalent microorganisms in the uterus during the postpartum period (10–14 days for the majority of cows) include *Escherichia coli, Streptococcus, Trueperella pyogenes, B. licheniformis, Prevotella spp., and Fusobacterium necrophorum*. This happens because the physical barriers that typically keep germs out of the uterus relax and because blood and leftover necrotic tissue serve as appropriate culture medium inside the uterus. When these microorganisms adhere to the mucosa, colonize, or penetrate the epithelium and release bacterial products (toxins, enzymes), it can be considered a uterine disease (Sheldon *et al.,* 2006; Yáñez *et al.*, 2022). In cases of severe endometritis, it is speculated that *Fusobacterium necrophorum* and *B. melaninogenicus* act synergistically with *A. pyogenes* in severe endometritis. Especially, *Arcanobacterium pyogenes, coliforms, gramme-negative anaerobes, Fusobacterium, and Bacteroides species* are commonly encountered. Neutrophils constitute the first line of defense against invading pathogenic organisms postpartum, resulting in an increase in the PMN population within the uterine lumen (Kasimanickam *et al.,* 2004; Azawi *et al.,* 2008; Negasee, 2020).

Table 1: Bacterial isolates from the uterine lumen based on their possible pathogenicity

Uterine pathogens	Potential pathogens	Opportunist contaminants
		Clostridium perfringens
Trueperella pyogenes	*Bacillus licheniformis*	*Klebsiella pneumonia*
Bacteroides sp.	*Enterococcus faecalis*	*Micrococcus sp.*
Prevotella melaninogenicus	*Mannheimia haemolytica*	*Proteus sp.*
Escherichia coli	*Pasteurella multocida*	*Streptococcus acidominimus*
Fusobacterium necrophorum	*Peptostreptococcus sp.*	*Staphylococcus sp., coagulase-negative*
	Staphylococcus aureus	*α-Hemolytic streptococci*
	Streptococci, nonhemolytic	*Aspergillus sp.*

Source (Williams *et al.*, 2005).

SCE is characterized by inflammation of the endometrium, leading to a significant reduction in reproductive performance without overt clinical signs. The condition may arise from resolving bacterial infections, immune-pathological processes in the absence of pathogenic bacteria, or abnormalities in postpartum tissue regeneration and repair. The precise etiology of SCE remains unclear (Sheldon *et al.,* 2019); however, Arias *et al.* (2018) suggested that alterations in the postpartum inflammatory response could be implicated in the development of this condition. Cows affected by endometritis experience disruptions in ovulation, attributed to bacterial products or associated inflammatory responses that can inhibit pituitary luteinizing hormone (LH) secretion and disrupt ovarian follicular growth and function (Opsomer *et al.,* 2000).

2.3. Pathogenesis

Endometritis is usually a cause for bovine infertility. The causal organisms usually reach the uterus at coitus, insemination, parturition and post-partum. The retention of foetal membrane, abortion, dystocia, mounting by infected bull, unhygienic practices at insemination, hypocalcaemia, season and poor nutrition are the main factors associated with the development of endometritis (Colazo. and Kastelic, 2012). Endometritis, a significant postpartum disease in dairy cattle, hampers productivity and causes substantial economic losses (Turk *et al.,* 2011). Following calving, the warm, fluid-filled uterus with necrotic debris creates an environment conducive to pathogenic microorganism proliferation (Deori and Phookan, 2015). Evidence indicates that pathogens may access the uterus hematogenously (Jeon *et al.,* 2017). Multiple bacterial infections contribute to endometritis. The uterus mobilizes neutrophils around 21 days postpartum to combat these pathogens (Sheldon *et al.,* 2008). This immune response leads to inflammation, exudation, and pus formation. Unfortunately, postpartum uterine infections disrupt normal physiological processes in dairy cattle, leading to the development of uterine diseases. The severity of infection is influenced by factors such as bacterial load, pathogenicity, and the cow's immune status (Shortall, *et al.,* 2017).

2.4. Risk factors associated with endometritis

The risk factors for endometritis classified in to cow level risk factors (intrinsic risk factors) and herd level risk factor (extrinsic risk factor). Intrinsic risk factors are specific variable characteristics of the individual cow while extrinsic risk factor concern all conditions and environmental characteristics shared by animals in the same herd, the characteristics of the dairy herd can directly or indirectly affect the prevalence of endometritis. Cow level risk factors are acute metritis, ketosis, milk yield, age, parity, body condition score (BCS), twins, stillbirths, retained placenta, milk fever, displaced abomasum, negative energy balance, mastitis and cyclicity. Herd-level risk factors identified were housing, bedding material, herd size, number of moves during the dry period, calving season and nutrition (Cheong *et al.,* 2011; Fesseha, 2020; Negasee, 2020). According to Ribeiro *et al.* (2013), described CE is also a risk factor for SCE.

2.4.1. Extrinsic Factors

Management factors

Many management factors and complications during parturition can contribute to an increased risk of uterine disease in cattle (Healy *et al.,* 2014; Prunner *et al.,* 2014; Wagener *et al.,* 2014). Some study found that the type of housing system could also influence the incidence of endometritis in beef cows. Specifically, cows that were housed and inseminated in tie stalls showed a higher incidence of endometritis compared to cows housed in free stalls. Interestingly, the parity of the cows did not appear to be a significant factor associated with the development of endometritis (Ricci *et al.,* 2015). Some researchers evaluated the effects of stocking density at different percentages of headlocks on the occurrence of retained placenta, metritis, and purulent vaginal discharge. Surprisingly, no significant effects were observed for these uterine conditions across different stocking densities. Thus, considering management practices, such as housing systems and stocking densities, in relation to the incidence of uterine diseases in cattle is important for herd management and welfare (Silva *et al.,* 2014).

Nutrition

Nutrition and its management are critical factors influencing the uterine environment of cows. The amount and quality of proteins included in the food ration are crucial for the effectiveness of the immune system. Protein quantity in food rations affects cellular immunity and cellular phagocytosis dramatically reduced due to protein deficiency. Conversely, excess protein intake can induce high serum ammonia, which in turn reduces lymphocyte production and increase the risk of endometritis. It is important to carefully balance protein intake to maintain optimal health and reproductive outcomes in cows (Bencharif and Tainturier, 2005; Fesseha, 2020).

Moreover, numerous vitamins are involved in the functioning of the immune system, including vitamins A, B and C. Vitamin B have a role in antibody synthesis, and vitamin C ensures the integrity of immune cell membranes and protects them from free radicals. Vitamin A is important for epithelial tissue development and cell differentiation, which are very important in the early postpartum stage. It also has immunological functions by increasing the early inflammatory phase through enhancing the number of macrophages at the site of a wound, which improves localization and stimulation of the immune response in the case of uterine infection (MacKay and Miller, 2003; Ingvartsen and Moyes, 2013).

Different mineral plays roles in uterine defense mechanism. Some of those minerals were selenium, magnesium, calcium, copper, zinc, and iron. Selenium is associated with neutrophil function and its inadequacy disrupts the reproductive performance of the cow and increases the risk of endometritis. Magnesium is involved in opsonization mechanisms. Calcium deficiency delays uterine involution and prolongs uterine infection and it acts as a complement activator. Calcium deficiency can be induced by excessive phosphorus consumption. In addition, copper, zinc, and iron intervene in lysosome production and their deficiency dramatically reduces phagocytosis and favors the growth of bacteria and development of endometritis (Rutigliano *et al.*, 2008; Doumtsop *et al.*, 2021).

Calving season

Research findings indicate that calving between November and April significantly increases the likelihood of uterine infections within the first month postpartum. This heightened risk during winter months may be attributed to the overall decreased health status of cows, rendering them more susceptible to such infections (Bruun *et al.*, 2002). A study involving 57,301 dairy cows revealed a clear link between winter calving and clinical metritis, a key risk factor for endometritis (Ghavi Hossein-Zadeh and Ardalan, 2011).

2.4.2. Intrinsic risk factors

Bod Condition Score (BCS) and Parity

In a study by Zobel (2013), it was observed that cows with a body condition score (BCS) of 2 and 5 had the highest rates of CE and SCE. Conversely, cows with a BCS of 3 to 4 had the lowest rates of CE and SCE. It is suggested that BCS may have a significant impact on the development of SCE, potentially due to varying metabolic disorders in both underweight and overweight cows. However, in contrast, Gautam *et al.* (2010) found no significant association between body condition score and the incidence of postpartum endometritis.

There are conflicting evidence concerning the relation between the presence of endometritis and parity. Interestingly, some studies found no significant association between development of endometritis and parity, except for primiparous cows. Given that primiparous cows frequently experience dystocia, endometrial lesions, and endometritis, it is expected to observe a correlation between these factors (Potter *et al.*, 2010; Ricci *et al.*, 2015). Uterine involution was more rapid in the primiparous group due to this persistent uterine infection and clinical endometritis were less frequent in primiparous than multiparous cows (Kim and Kang, 2003). However, some authors excluded an effect of the cow's parity number on the prevalence of endometritis (Adnane *et al.*, 2017).

Retained fetal membranes (RFM)

Retained fetal membrane (RFM) is the inability of fetal membrane to be expelled from 8 to 48 hours average 8 hours after parturition. In cattle, it can lead to adverse health effects that ultimately affect reproductive performance (Beagley *et al.*, 2010). During parturition, the normal physiological stages of birth include the dilatation of the parturient canal, delivery of the fetus, and expulsion of the fetal membranes. Typically, the fetal membranes are expelled within two to eight hours after parturition. If the fetal membranes are retained beyond 12 hours, it may be considered a pathological condition (Tucho, and Ahmed, 2017).

Residual placental tissue in cases of retained placenta provides a favorable environment for bacterial growth within the uterus, while necrotic tissue delays uterine involution and impairs endometrial repair. Additionally, the presence of residual tissue may lead to cervical dilation and increased bacterial contamination of the endometrium (Potter, 2010; Robert and Walter, 2007). Cows affected by endometritis exhibit elevated levels of bacterial lipopolysaccharide (LPS) and immunosuppressive products, which hinder the recruitment of leukocytes into the uterus for bacterial clearance (Sheldon and Owens, 2018; Adnane *et al.,* 2017). The positive association between retained placenta and endometritis is likely attributed to impaired neutrophil function (Salasel *et al.*, 2010).

Dystocia and assisted calving

Dystocia in cattle can arise from various factors, including fetal-maternal disproportion, abnormal fetal presentation (such as breech, head, or limb back), or disruptions in the normal parturition process due to factors like maternal weakness, stress, or hormonal imbalances (Gaafar *et al.,* 2011) which can lead to endometrial disruption and increase the risk of endometritis (Finan and McKinnon, 2015; Jackson, 2004). In cows, dystocia is commonly linked to postpartum complications such as retained fetal membranes and delayed uterine involution, both of which can predispose to the development of endometritis (Ghavi Hossein-Zadeh and Ardalan, 2011).

Assisted calving refers to a birthing process that requires intervention, such as when only one foot of the calf is visible outside the vulva, although this may not always lead to dystocia. Following the correction of malpositions, the cow may proceed with delivery normally or with minimal assistance (Schuenemann, 2012). However, the act of calving assistance can introduce bacteria into the uterus, potentially increasing the risk of endometritis development (Bruun *et al.*, 2002). In cases of dystocia, calving assistance significantly raises the likelihood of endometritis incidence (Prunner *et al.*, 2014). The parturition of a male calf, typically characterized by a larger size compared to a female, can elevate the likelihood of dystocia or lead to assisted calving and subsequently increase the risk of endometritis (Fesseha, 2020).

Hypocalcaemia

Hypocalcaemia is characterized by a decrease in serum calcium levels below 2.0 mmol/l and is observed in various livestock species (Quader *et al.*, 2017). Inadequate calcium mobilization during the periparturient period can lead to hypocalcaemia, as calcium plays a crucial role in uterine involution, uterine contractions related to reduction of myometrial contractility, and parturition. Deficiencies in calcium can delay uterine involution, increase the risk of dystocia, prolong gestation, and predispose animals to retained fetal membranes, metritis, and endometritis (Negasee, 2020).

Subclinical hypocalcaemia lead to reduced feed intake by causing decreased rumen motility, which increases the risk of ketosis as a result which contributes to incidence of endometritis, and since hypocalcaemia affects rumen motility it indirectly increases the risk of developing displaced abomasum which maybe bestow to the occurrence of endometritis in return (Kim and Kang, 2003; Guterbock, 2004). Some researchers found positive association between hypocalcaemia and endometritis such as Ghavi Hossein-Zadeh and Ardalan (2011) (OR=12.36), Kim and Kang (2003) (OR=3.5).

Cyclicity

Endometritis is a common cause of delayed resumption of ovarian activity after calving. The timing of ovarian activity resumption plays a crucial role in uterine involution and the potential risk of developing endometritis. Dairy cows that resume cyclicity early are less likely to develop endometritis at 30 days postpartum compared to those with a later resumption (Sheldon and Owens 2018). In cases where endometritis is induced by gram-negative bacteria such as *Escherichia coli*, the LPS released inhibits the secretion of GnRH and LH without affecting FSH secretion, but it also suppresses the sensitivity of the pituitary gland to GnRH. This leads to the development of follicular waves without ovulation of the dominant follicle. In cases where ovulation does occur, the resulting corpus luteum persists due to inadequate synthesis of uterine prostaglandin F2α (PGF2α). Persistent progesterone secretion from the corpus luteum delays the recruitment of phagocytic cells, reducing the efficiency of the immune system. This compromised immune response can further exacerbate the development and persistence of endometritis in dairy cows (Sheldon, and Dobson, 2004; Salasel *et al.,* 2010).

Mastitis

Mastitis, an inflammatory condition of the mammary gland caused by physical trauma or microbial infections, is a prevalent issue in dairy industries leading to significant economic losses due to decreased milk yield and quality (Gomes and Henriques, 2016). This condition serves as a potential source of bacterial contamination in the environment, which can contribute to the development of endometritis. The bacteria isolated in cases of uterine infections are often nonspecific and typically originate from environmental sources that contaminate the uterus during parturition (Madoz *et al.,* 2014). Risk factors associated with mastitis, such as retained placenta, milk fever, and calving during winter, are also considered potential risk factors for endometritis (Ghavi Hossein-Zadeh and Ardalan, 2011). Subclinical mastitis can directly affect the incidence of endometritis at both 30 and 60 days postpartum (Robert and Walter, 2007).

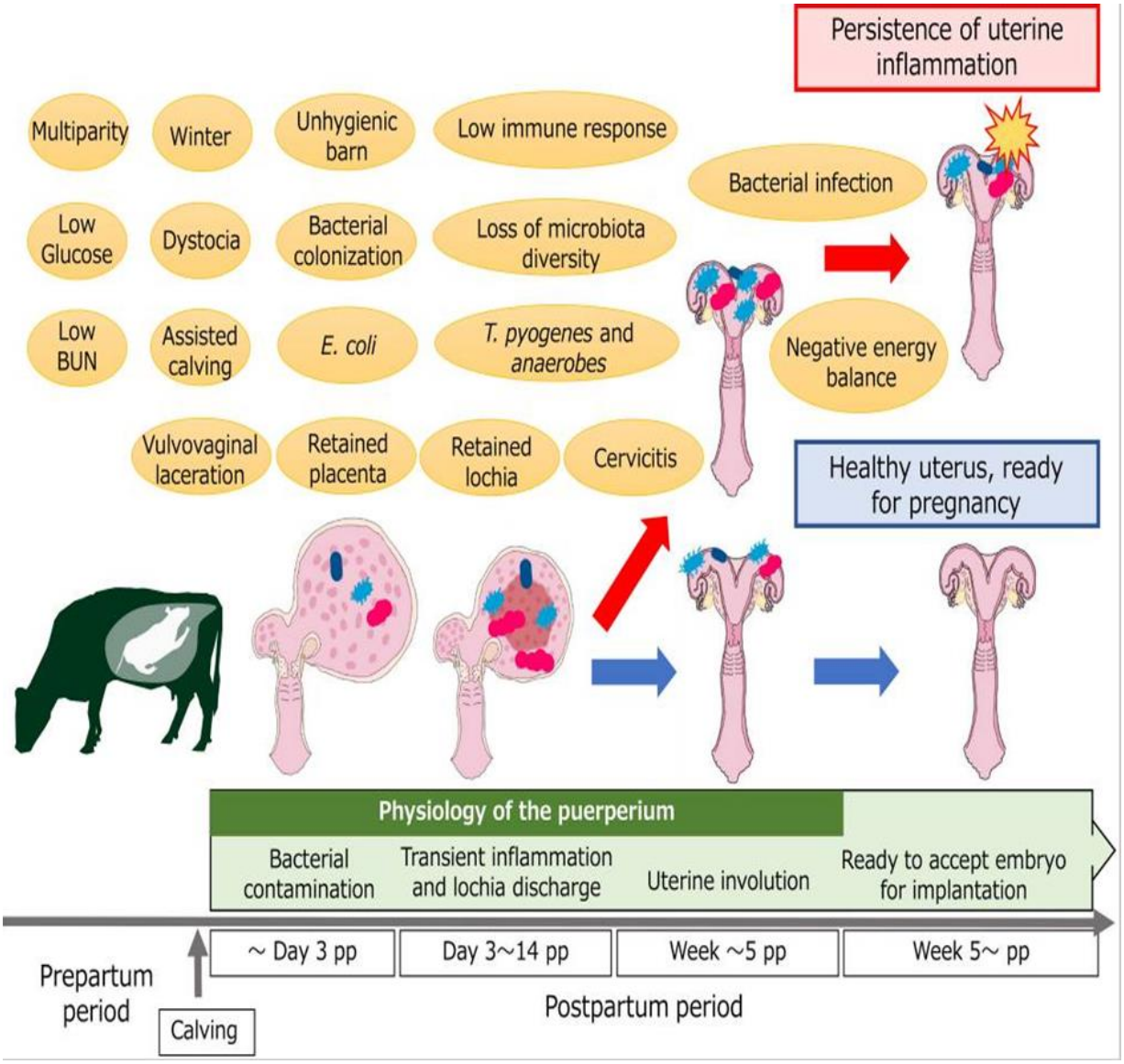

Figure 1: Predisposing factors for endometritis

Figure 1 shows predisposing factors for endometritis along with prepartum and postpartum periods in the cow including factors like improper metabolism, abnormal calving, and unhygienic barn conditions. Uterine infections can result from significant bacterial colonization following dystocia, inadequate hygiene practices, and compromised uterine defense mechanisms. Pathogenic microorganisms induce endometrial inflammation, which can impede the process of uterine involution (Osawa, 2021).

2.5. Clinical signs

Endometritis, a condition that typically occurs from 21 days post-calving, can be categorized as CE or SCE and affects approximately 20% of dairy cows. CE is characterized by the presence of visible mucopurulent (50% mucus; 50% pus) or purulent discharge at the vulva or in the vagina. The severity of CE is commonly assessed by grading the vaginal discharge. Grade 0, representing a normal state without endometritis, is indicated by clear or translucent discharge. In Grade 1, the mucoid discharge contains flecks of white or off-white pus. In Grade 2, the discharge consists of less than 50% white or off-white mucopurulent material. Grade 3, purulent discharge, often white or yellow in color, and may also contain blood, along with a large number of uterine exudates containing a foul smelling red and brown watery fluid. SCE, on the other hand, is characterized by infection and inflammation of the endometrium without resulting in visible discharge (Parmar, 2021). CE is observed in animals from 21 days post-parturition and is abnormal mucopurulent or purulent vaginal discharge, clear mucus with flakes of pus, cloudy mucus, or mucopurulent discharge, as well as a uterus that has not fully involute (Dobson *et al.*, 2007).

2.6. Diagnosis

The classical method of diagnosing endometritis relies on clinical signs and rectal examination, which is commonly used in practice. However, ultrasonography and new diagnostic tools like Metricheck are also effective for diagnosing this condition. Rectal palpation is the most frequently used diagnostic technique for endometritis, while vaginoscopy is less commonly utilized in clinical practice (Parmar, 2021). Subclinical endometritis in animals may not show any clinical signs, making it challenging to diagnose. To accurately identify this condition, different diagnostic tools such as ultrasonography, cytology, and uterine biopsy can be used (Williams *et al.*, 2005, McDougall *et al.*, 2011).

2.6.1. Cytology

Microscopic examination of endometrial smears is considered one of the most reliable and reproducible techniques for diagnosing endometritis in animals. This method allows for the accurate determination of the proportion of neutrophils present in the endometrial tissues. Cytology stands out as the most dependable technique for diagnosing endometritis, surpassing the accuracy of ultrasonography and vaginoscopy in this regard (Wagener *et al.*, 2017). Over the last decade, cytology has developed and been used to diagnose this condition. For uterine cytology, samples are obtained by lavage technique or cytobrush method. The first step is to take endometrial surface scrapings, preferably with a cytobrush, as this method is thought to be more reliable than uterine flushing due to its higher reproducibility (Sheldon, 2004).

To calculate the PMN to epithelial cell ratio, the endometrial surface scrapings are examined under a microscope. It is possible to classify cows as healthy or having SCE using this ratio, which is given as a percentage. The threshold ratio varies between 4% and 18% depending on the author and the sampling time following parturition (Barański *et al.,* 2012). In the uterus, PMN are the main immunologic defense system. A mild local inflammation is indicated by an increased PMN count (Plöntzke *et al.,* 2010). One drawback of cytobrush is that just a small portion of the endometrium is sampled, whereas uterine lavage yields cells from the entire endometrial surface (Arias *et al.,* 2018).

The uterine lavage procedure involves injecting sterile saline solution into the uterus with a catheter, gently massaging the uterus to spread the fluid throughout the lumen, then aspirating some of the fluid with the same catheter to retrieve some of it. The fluid sample is centrifuged, with the supernatant being discarded and the sediment being spread out onto a microscope slide. Using common stains (such Diff-Quick), cytological smears are fixed and stained regardless of the collection method (Kasimanickam *et al.,* 2005).

2.6.2. Ultrasonography

Based on the detection of intrauterine fluid and the measurement of uterine diameter, ultrasonography has been utilised to make the diagnosis of SCE. Endometrial inflammation can be detected by the presence of a modest amount of fluid in the uterine lumen and/or thicker uterine walls. But in several trials, it was discovered that endometrial cytology was more accurate at diagnosing SCE than ultrasound (Meira *et al.,* 2012). While ultrasonography alone is not specific enough to correctly identify unhealthy animals and biopsies are time-consuming and money-consuming. This is because intrauterine fluid, the primary ultrasound indicator of cytologically determined SCE, is not always present in cows with this condition (Williams *et al.*, 2005, McDougall *et al.,* 2011). In physiological circumstances like estrus or the early postpartum period, the presence of intrauterine fluid and a thick uterine mucosa may be normal observations. Perhaps the study of fluid properties could increase the sensitivity of ultrasound diagnosis (López-Helguera *et al.,* 2012, Quintela *et al.*, 2016). Doppler ultrasonography is still being researched; however, it may be helpful in the diagnosis of endometritis in cattle. It was discovered that cows with acute endometritis that had been experimentally produced had considerably increased blood flow in their uterine arteries. It has to be explored whether the vascular flow patterns between SE-affected and healthy uteri are different (Debertolis *et al.,* 2016).

2.7. Treatment, Control and prevention of endometritis

A single intramuscular injection of oxytetracycline at a dosage of 20 mg/kg is an effective treatment for uterine bacterial diseases. Penicillin is widely utilized for endometritis due to its ability to penetrate all layers of the uterus and eradicate a majority of bacteria in the endometrium (Kocabagli, 2012). Various intrauterine therapies, such as antiseptics, antibiotics, and immune modulators, are administered to eliminate bacterial infections, stimulate normal uterine defense mechanisms, and enhance blood flow to the uterus. The infusion of iodine solution in water or saline is a commonly employed intrauterine therapy (Deori and Phookan, 2015).

Hormones act on the corpus luteum to induce luteolysis, resulting in the formation of a corpus albicans and cessation of progesterone production. Prostaglandin F2 alpha (PGF2α) is particularly beneficial when the corpus luteum is not present and is essential for treating clinical endometritis when corpus luteum is detected during diagnosis (Abdullah *et al.,* 2015; Smith and Risco, 2002). Implementing sound management practices in the prepartum period can minimize or prevent uterine infections in cows and reduce the prevalence of endometritis (Azawi *et al.,* 2008). Conversely, suboptimal management of transition cows can expose susceptible cows to uterine diseases, including endometritis (Niraj *et al.,* 2014).

Preventing endometritis hinges on boostering the innate immune system through strategic management practices. Key strategies include optimizing bunk space to facilitate feed consumption, providing clean rest areas to minimize contamination of the reproductive tract in freshly calved cows, and maintaining ambient temperature within a comfortable range. Implementing rigorous monitoring protocols and establishing clear targets enable timely identification and intervention for potential issues. Early intervention in risk factors plays a pivotal role in reducing the incidence of endometritis. Ensuring prompt resolution of these underlying factors is crucial. Maintaining cleanliness in calving pens through regular bedding changes and hygienic calving assistance is essential to mitigate post-partum infections (Wagener *et al.,* 2014; Noakes *et al.,* 2018)

Targeted control measures focus on addressing primary causes of endometritis. Enhanced hygiene practices in calving areas and meticulous calving assistance protocols are paramount in reducing endometritis prevalence, particularly in herds with historical hygiene challenges (Semivolos *et al.,* 2018). Optimal nutritional management pre- and post-calving is critical, emphasizing the avoidance of excessive body condition at calving and preventing drastic weight fluctuations during the transition period (Blowey and Weaver, 2011)

2.8. Impact of Endometritis on Reproductive Performance

SCE is recognized as a significant contributor to poor reproductive performance in dairy cows. This condition, characterized by endometrial inflammation without overt clinical signs or detectable infection, is often rooted in postpartum alterations in the inflammatory response (Pereira *et al.,* 2023). Uterine pathogens have the capacity to directly impair fertility by damaging the endometrium and indirectly through the production of harmful toxins. Bacterial endotoxins, for instance, can disrupt reproductive processes by inhibiting LH production, impeding ovulation, affecting follicular growth and corpus luteum development, prolonging the lifespan of the corpus luteum, and inducing embryo mortality (Arias *et al.,* 2018).

Molecular changes in the endometrium and surrounding tissues are also linked to SCE. Postpartum SCE at 45-56 days post-delivery has been associated with elevated levels of blood mononuclear cells and increased expression of genes encoding inflammatory mediators. This genes encoding inflammatory mediators are tumor necrosis factor (TNF), interleukin-12 (IL12), and chemokine ligand 8 (CXCL8), as well as complement proteins (C2, C3), Toll-like receptors (TLR2, TLR4), antimicrobial peptides (DEFB, S100A8), and acute phase proteins (Duvel *et al.,* 2014; Foley *et al.,* 2015).

Studies by Salasel *et al.* (2010) have suggested a potential association between subclinical endometritis and the repeat breeder cow syndrome, although findings remain inconclusive. Both clinical and subclinical endometritis have been linked to reductions in milk yield by 0.6-1.03 kg per cow per day drop in milk output, decreases in milk fat and protein content, and increases in somatic cell counts in milk (McDougall *et al.,* 2011). Endometritis negatively affect the performance of the global dairy industry. Delayed resumption of ovarian activity, heightened number of services per conception (NSPC), diminished milk yield, escalated treatment costs, and the risk of septicemia-related mortality are key factors contributing to these economic loses (Kasimanickam *et al.,* 2014).

Furthermore, endometritis prolongs the calving to first service interval (CFSI) approximately by 11 days and delays calving to conception interval (CCI) by 32 days compared to unaffected animals. Cows afflicted with postpartum endometritis exhibit a 1.7-fold higher likelihood of being culled due to reproductive failure, underscoring the significant repercussions of this condition on herd productivity and sustainability (Youngquist and Shore, 1997; LeBlanc *et al.*, 2002; Barnes *et al.*, 2023).

2.9. Prevalence of Endometritis in Ethiopia

Endometritis is a common reproductive disease in dairy cows that can have significant impacts on their reproductive performance and overall herd productivity (Nyabinwa *et al.,* 2020). However, there is limited data available on the current prevalence and impact of endometritis in dairy cows in Ethiopia. More research is needed to assess the prevalence, risk factors, and management strategies for endometritis in Ethiopian dairy cows in order to develop effective control and prevention measures to improve the reproductive health and productivity of the dairy industry in Ethiopia (Abdeta and Hailu, 2020)

Table 2: The status of endometritis in Ethiopia

Study area	Prevalence (%)	Source
Horro Guduru, Wollega Zone	7	(Shiferaw *et al.*, 2016)
In and around Fitich towns	5.4	(Abera *et al.,* 2017)
Gondar town	46	(Moges, 2019)
Jimma town	2.5	(Seid and Thomas, 2019)
Assela	2.6	(Yohannes *et al.,* 2019)
Alage dairy farm	6.36	(Gebremeskel *et al.,* 2019)
Wolaita sodo	1.6	(Hirpassa, *et al.*, 2023)
Hawassa, wolaita sodo and Arsi Negelle towns	7.6	(Mekibib *et al.*, 2024)
Wolaita sodo	14.33	(Mekonnen, 2024)

3. MATERIALS AND METHODS

3.1. Description of the Study area

The study was conducted in Wolaita Sodo from October 2023 to May 2024. Wolaita Sodo is located about 390 km south of Addis Ababa at latitude of 8°50' N and longitude of 37°45' E. The altitude varied from 1100-2950 meters above sea level. The area experienced a mean annual temperature of about 20°C and a maximum of 26.2°C. The rainfall regimes over much of the area were typically bimodal, with the big rainy season extending from June to September and a small rainy season occurring from February to April. The mean annual rainfall of the area ranged from 801-1600 mm. The district is surrounded by the Damot Gale district to the north, Humbo district to the south, Damote Woyde district to the east, and Damote Sore district to the west. The site is classified under a midaltitude ("Woyina dega" in the local Amharic language) agro ecological environment. The dry season lasts from September to February, and the rainy season remains from March to August (WZFEDD, 2022).

Common feed resources utilized by dairy producers in the study area include grazing, natural grass, hay, purchased green grass, and cereal crop residues. Additionally, other animal feed resources consist of pseudo stems of enset (false banana) and banana plants along with their leaves, maize and sorghum stover, improved forages like elephant grass (*Pennisetum purpureum*) and pigeon pea (*Cajanus cajan*) are also commonly employed. Fruits and fruit seeds like avocado, root crops such as sweet potato and sugar cane (*Saccharum officinarum*), including its tops especially during dry seasons, are frequently fed to animals. Furthermore, plant weeds and non-conventional feeds such as Attalla (a local beverage by-product), kitchen wastes, and edible leaves from plants like 'Korch' and grabble are included in the animal diet (Asrat *et al.,* 2016).

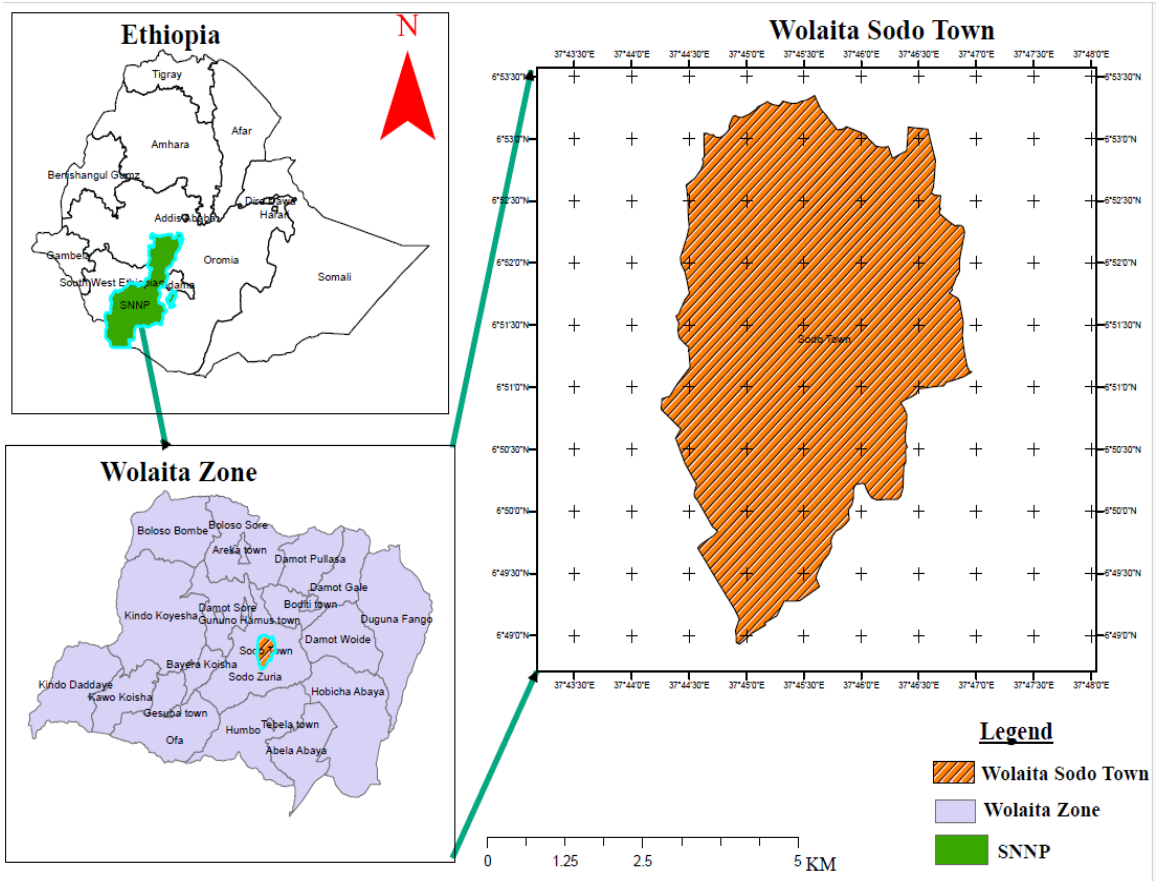

Figure 2: Map of the study area

3.2. Study animals and sample size of dairy farms

A total of 74 dairy cows with 69 clinically healthy and 5 diagnosed with clinical endometritis from one large farm, three medium farm and 23 smallholder farms were taken for this study. Both the farm and dairy cows were selected purposively according to inclusion criteria of the study. This inclusion criterion includes good recording system using record-keeping books on factors such as age, parity, calving information, reproductive performance, and conditions like mastitis and reproductive health disorders. In addition, the farms had an established arrangement of monthly visits to the farm by a veterinarian and willingness of the farm owner. The dairy cows that included in this study were the cow calved during a seven-month period or recently gave birth between October 2023 and May 2024.

The age of cows was categorized as young (3–6 years), adult (7–9), and old (>9) (Wakeman and Pace, 1983) based on birth records. The parity was recorded and grouped into few (one-to-three calves), moderate (four to six calves), and high (seven and above calves) (Fesseha *et al.,* 2021). The herd size was classified as smallholder (<5 heads of cattle), medium (6–50 heads) and large farm (>50 heads) (Bacha, and Regassa, 2010). Body condition was assessed by examining eight areas of the cow's body, using established criteria. Briefly, animals were assigned a body condition score along a 1 to 5 scale with 0.25 unit increments; score 1 indicated an emaciated condition and score 5 an obese condition (Edmonson *et al.,* 1989) and later classified as poor (score >2.5) and good (score <2.5) (Bacha and Regassa, 2010). The measurement was conducted by palpating and visually inspecting the transverse processes for the lumbar vertebrae (loin) and the spinous processes for the tail head.

3.3. Study Design

A prospective longitudinal study was employed to study the risk factors for endometritis in postpartum dairy cow and the impact of endometritis on the reproductive performance of dairy cows in Wolaita sodo, Southern Ethiopia, from October 2023 to May 2024. The study also aimed to assess the incidence of endometritis. The regular follow up during study period was employed in order to quantify days of reproductive performances after the cow calved and to see if any potential risk factors occur. In the beginning of the study dairy farms information were collected from Ministry of Agriculture and Rural Development in Wolaita Sodo, which consist a list of registered farms.

3.4. Data Collection

3.4.1. Regular Follow-up and Use of farm record books

A weekly follow-up program was implemented for each cow enrolled in the study, commencing from the time of parturition. During each scheduled visit, thorough clinical examinations of the reproductive tracts were conducted to assess potential risk factors, and the udder of each animal was examined for signs of mastitis.

Reproductive tracts checked for any signs of abnormal discharge, pain, or swelling and abnormalities in vaginal canal and uterine cytology were conducted 8th week to assess the health of uterine lining. Sign of clinical mastitis such as udder abnormalities (swelling, heat, redness and pain), milk abnormalities (watery appearance, flakes or clots in milk and milk contain pus) and behavior abnormalities like being reluctant to be milked were observed and for subclinical mastitis CMT were conducted.

Additionally, reproductive traits, including calving to conception interval, calving to first service interval, and number of services per conception, were meticulously monitored. DIM were recorded for both cows testing positive and negative for endometritis to evaluate its impact on reproductive performance. Pregnancy status was ascertained by observing non-return to estrus after a 24-day period of insemination and confirm by rectal palpation after 60 days of insemination. The farm record books were monitored every time during regular visit to the farm to collect any risk factors like RFM, dystocia, abortion, assisted calving, hypocalcaemia, calving with or without glove, mastitis (also it were confirmed by CMT test) that were happened. In addition age, parity, calve sex and herd size were collected from farms record book.

3.4.2. Cytology Sample Collection

Subclinical endometritis was diagnosed using endometrial cytology. The endometrial cytology sample was collected at 8th week after calving. A modified cytobrush, made by cutting a human cervibrush to a shorter length to fit onto an artificial insemination (AI) gun without contamination, was used to collect the sample. Both the AI gun and the cytobrush were protected by sterile plastic tube to maintain cleanliness. The AI gun, with the cytobrush covered in sterile plastic tube, was inserted through the vagina and into the uterus. Once inside the uterus, the cytobrush was exposed and rotated against the uterine wall to collect the sample (Appendix 7).

After collection, the cytobrush was replaced inside plastic tube to prevent cellular contamination from the cervix or vagina. After removal, the sample was spread on a glass slide, fixed with methanol, and stained with Giemsa stain (Appendix 1). Each slide was then examined under an optical microscope at 400x magnification (Appendix 2). If the PMN count was ≥10% it was cindered the cows positive for subclinical endometritis and if <10% the cow was cindered negative for Subclinical endometritis (Appendix 3) (Gilbert *et al.*, 2005).

3.4.3. Milk Sample collection and determination of subclinical mastitis

At sampling for SCE and subclinical mastitis (SCM) were concomitantly evaluated using the California Mastitis Test (CMT) methodology. To conduct CMT test udder was thoroughly washed and dried by towels and after milking down some drops to the ground 5ml of milk from each quarter of the udder was collected into the central ring on the CMT paddle. Subsequently, an equivalent volume of CMT reagent was added to each ring, and the outcomes were systematically documented for analytical purposes. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. The result of the test was indicated based on gel formation (Appendix 4). The CMT results were scored as 0 (negative), trace, 1(weak positive), 2 (distinct positive) and 3 (strong positive) based on gel formation. All CMT scores of 0 and trace were considered as negative while CMT scores of 1, 2, and 3 were considered indicators of subclinical mastitis. Positive cows were defined as having at least one quarter with CMT score of 1+ (Ibrahim *et al.*, 2023).

3.4.4. Clinical examination

The animal was examined for body conditions score (BCS), cow cleanliness score (CCS) and fecal consistency score. Each animal was checked for cleanliness and given a score based on established criteria from previous research: (1) completely free of dirt or has very little dirt; (2) slightly dirty; (3) mostly covered in dirt; or (4) completely covered, caked in dirt. To assign CCS the flanks, hindlegs, udder and tail of each cow were checked because dirty legs sign to faecal splashing from the passageways, a dirty tail hints loose faeces or overhanging in the passageway, and dirty flanks may reflect the state of the beds (Hughes, 2001) (Appendix 5).

And also each animal was checked for fecal consistency and given a score based on established criteria from previous research: (1) very dry, lumpy; (2) dry, stiff, semi formed pats; (3) circular, moist raised pat with petal like symmetrical rings surrounding a dipped center; (4) flat, loose, thinly spread; or (5) liquid pools of feces (Schreiner, and Ruegg, 2002). Hypocalcaemia in targeted dairy cow were examined by observing clinical signs such as recumbency, reduced appetite, stiff gait and muscle tremors specially 24-74 hours after calving.

Clinical endometritis cases were checked after 21 day of calving. Then identified by visual examination for the presence of pus in the vaginal lumen or by manual extraction of vaginal contents while wearing gloves to maintain hygiene and prevent infection. The process of opening the vagina for manual extraction of vaginal contents typically involves the use of a lubricated glove-covered hand to carefully and gently insert fingers into the vaginal lumen (Appendix 8). This allows for the manual extraction of any accumulated vaginal contents, such as pus. The vaginal mucus was assessed using an endometritis scoring system. Where each sample was assigned a character score based on the following criteria: unaffected animals exhibited clear or translucent mucus; a score of 1 indicated mucus with flecks of white or off-white pus; a score of 2 denoted mucopurulent exudate containing at least 50% white or off-white material; and a score of 3 represented exudate containing over 50% purulent material, typically white or yellow, occasionally sanguinous (Williams *et al.*, 2005).

3.5. Data Management and Statistical Analysis

Data generated from field and laboratory investigations were recorded, screened and coded in the format developed for this study, entered in to Microsoft Excel spreadsheet, and analyzed using STATA 14.0 statistical software. The incidence of endometritis was determined by the rate of affected animals out of the total animal population determined by descriptive statistics. The logistic regression, reporting the odds ratio (OR), was used to evaluate the association of the incidence of endometritis with associated risk factors. A pie chart used to compare the incident of endometritis in different herd size and to compare the occurrence of SCE and CE in the study area. The reproductive performances, such as calving to CCI, CFSI and NSPC were compared between normal and diseased dairy cows by using a t-test.

3.6. Ethical consideration

Cytobrush samples were collected during regular follow up in accordance with a high standard of veterinary care. Before sample collection, the permission of the farm owners (animal owners) and informed consent were obtained from the owners for animal use. Ethical clearance was sought and granted by the Animal Health Ethical Committee at the College of Veterinary Medicine and Agriculture, Addis Ababa University (Appendix 9). Upon receiving the official letter of ethical clearance, all relevant parties, including the farm manager and data recorder, were informed in a transparent and legal manner. The objectives of the study were clearly communicated and understood by all concerned bodies. Furthermore, any data utilized was handled with utmost confidentiality.

4. RESULT

4.1. Overall Incidence of Endometritis in the study area

In total, 74 dairy cows that calved during study period and meet inclusion criteria were sampled from 27 herds among them 69 were clinically healthy and 5 were showed sign of clinical endometritis. Cytologically, PMNs identified by their characteristic multi-lobed nucleus and granular cytoplasm (Fig. 4). Out of the 69 dairy cows evaluated, 40.57% (28/69) cows were found to have subclinical endometritis (PMN ≥10%). During regular follow-ups, an additional 5 dairy cows displayed signs of clinical endometritis, characterized by purulent discharge. In total, 33 cows or 44.59% (33/74) cows were diagnosed with endometritis both SCE and CE together, comprising 37.84% (28/74) with subclinical and 6.76% (5/74) with clinical endometritis (Fig. 3).

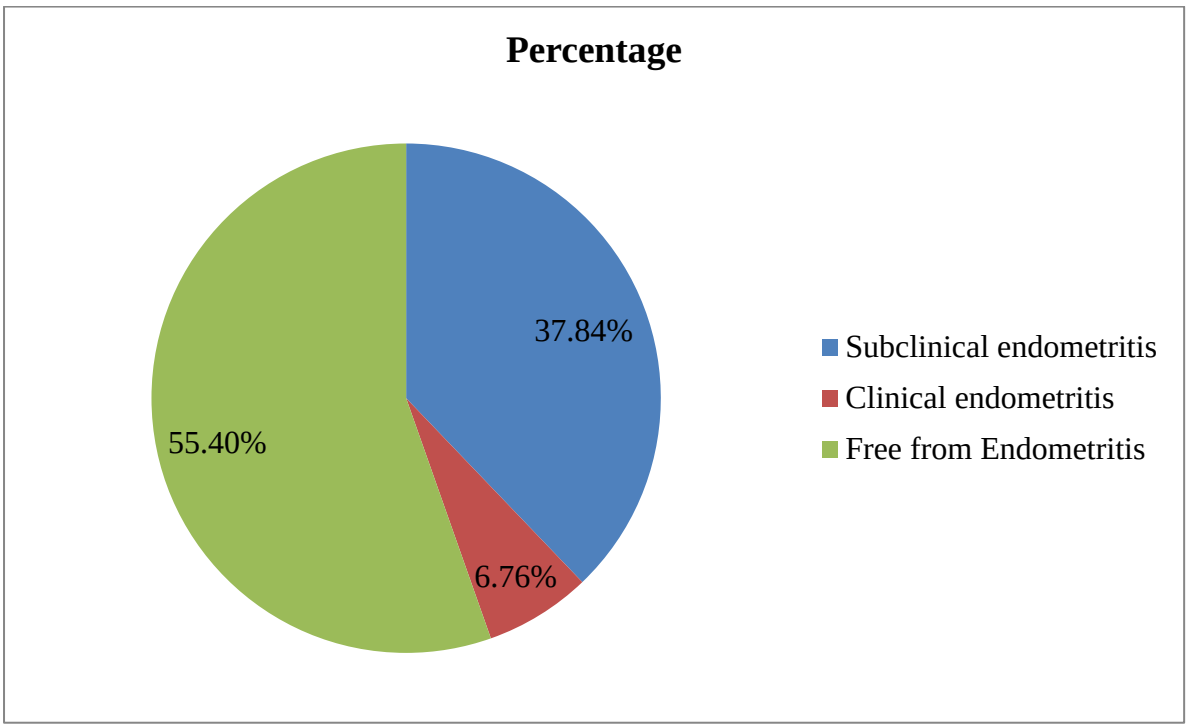

Figure 3: Percentage of endometritis based on its category and incidence

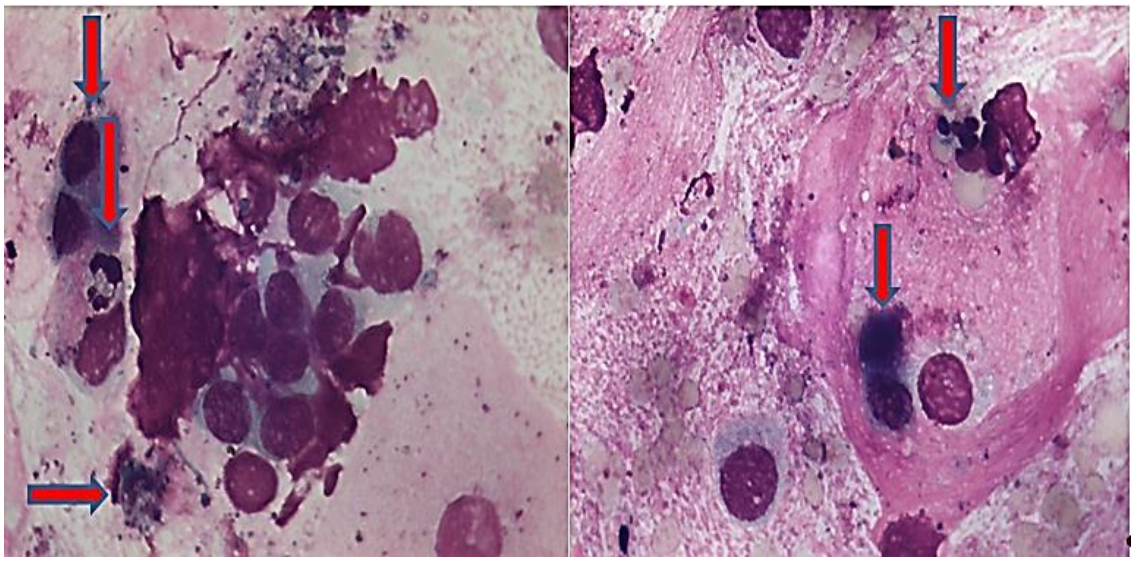

Figure 4: Cytological Characterization of PMNS

4.2. Association of Risk Factors with Subclinical endometritis

4.2.1. Association of health related risk factor with SCE

According to logistic regression analysis, the dairy cows with RFM were 9.23 times more likely to develop SCE compared to those without RFM (P<0.05), indicating a significant impact of RFM on SCE occurrence. Cows with dystocia was 7.79 more prone to have SCE (P<0.05), than dairy cows with normal calving. Cows with hypocalcaemia were 6.49 times significantly exposed to SCE (P<0.05) than dairy cows without hypocalcaemia. In contrast, no statistically significant association was observed between abortion and SCE (P>0.05), although dairy cows that experienced abortion had a 2.010 times higher prevalence of SCE compared to healthy cows (Table 3).

Table 3: Logistic regression analysis on association of health related risk factors with SCE

Serial No.	Risk factors	Odd ratio	P value	95% confidence interval
1	RFM	9.236841	0.007	1.814-47.018
2	Dystocia	7.799994	0.014	1.512-40.233
3	Hypocalcaemia	6.499991	0.027	1.237-34.137
4	Abortion	2.01087	0.333	0.488-8.269

4.2.2. Association of cow related risk factor with SCE

The analysis of age did not reveal any statistically significant association between age and SCE incidence. There was also no significant difference in SCE incidence between adult and young dairy cows. However, the incidence of SCE was found to be 1.5 times higher in older animals compared to younger ones, although this difference was not statistically significant ($P>0.05$). Furthermore, the incidence of SCE was 1.23 times higher in dairy cows with more than 7 calves compared to those with 1-3 calves, but this difference was not statistically significant ($P>0.05$). Additionally, the incidence of SCE was 58.1% lower in dairy cows that had calved 4-6 times compared to those that had calved 1-3 times, with no statistically significant difference ($P>0.05$). Overall, the findings indicated that parity did not have a statistically significant impact on the incident of SCE in dairy cows.

The incidence of SCE was found to be 2.78 times significantly higher (Table 4) in dairy cows with a body condition score below 2.5 compared to those above 2.5 ($P<0.05$). Statistically significant relationship was observed between calf sex and SCE ($P<0.05$), in which incidence of SCE is 3.06 times higher in dairy cows giving birth to male calves compared to female calves. The logistic regression analysis revealed a statistically significant association between the incidence of SCE and mastitis ($P<0.05$). SCE occurrence was 5.06 times higher in dairy cows with mastitis compared to those without mastitis.

Table 4: Logistic regression analysis on association of cow related risk factors with SCE

Serial No.	Risk factors		Odd ratio	P value	95% confidence interval
1	Age	Young	Referent		
		Adult	1.0227	0.964	0.085-26.361
		Old	1.5	0.782	0.046-5.392
2	Parity	1-3 Calves	Referent		
		4-6 calves	0.581	0.314	0.202-1.671
		7 and above	1.235	0.810	0.220-6.921
3	BCS	>2.5	Referent		
		<2.5	2.788	0.045	1.023-7.595
4	Calf sex	Female	Referent		
		Male	3.0666	0.040	1.0526-8.934
5	Mastitis		5.066	0.026	1.208-21.238

BCS = Body condition Score

4.2.3. Association of hygiene related risk factor with SCE

There was a statistically significant relationship between assisted calving and the incidence of SCE. The incidence of SCE was found to be 5.06 times higher in dairy cows that required assistance during calving compared to those that calved normally ($P<0.05$). Incidence of SCE was 44% lower in dairy cows when an arm-length glove was used during calving compared to bare hand assistance however, not statistically significant ($P>0.05$). When comparing different cleanliness levels, cows that were mostly covered in dirt had a 2.181 times higher likelihood of developing SCE ($P>0.05$), while cows completely covered in dirt had a 5.909 times higher likelihood of SCE occurrence ($P > 0.05$, CI = 0.991-35.213). In terms of fecal consistency, cows with flat, loose, thinly spread feces and cows with circular, moist raised pats with petal-like symmetrical rings surrounding a dipped center faces were found to have a 3.851 and 1.33 times higher chance of developing SCE compared to cows with dry, stiff, semi-formed feces ($P>0.05$). Details of factors with SCE are indicated in table 5.

Table 5: Logistic regression analysis on calving management and cow cleanliness related risk factors with SCE

Serial No.	Risk factors		Odd ratio	P value	95% confidence interval
1	Assisted calving		5.066	0.026	1.208-21.238
2	Calving Equipment		0.448	0.350	0.083-2.404
3	CCS	Score 2	Referent		
		Score 3	2.181	0.147	0.759-6.264
		Score 4	5.909	0.051	0.991-35.213
4	Fecal	Score 2	Referent		
	consistency	Score 3	1.333	0.706	0.298-5.957
	score	Score 4	3.851	0.093	0.796-18.621

CCS = Cow Cleanliness Score

4.2.4. Association of herd level risk factor with SCE

The distribution of SCE cases across different farm sizes is indicated in figure 5.

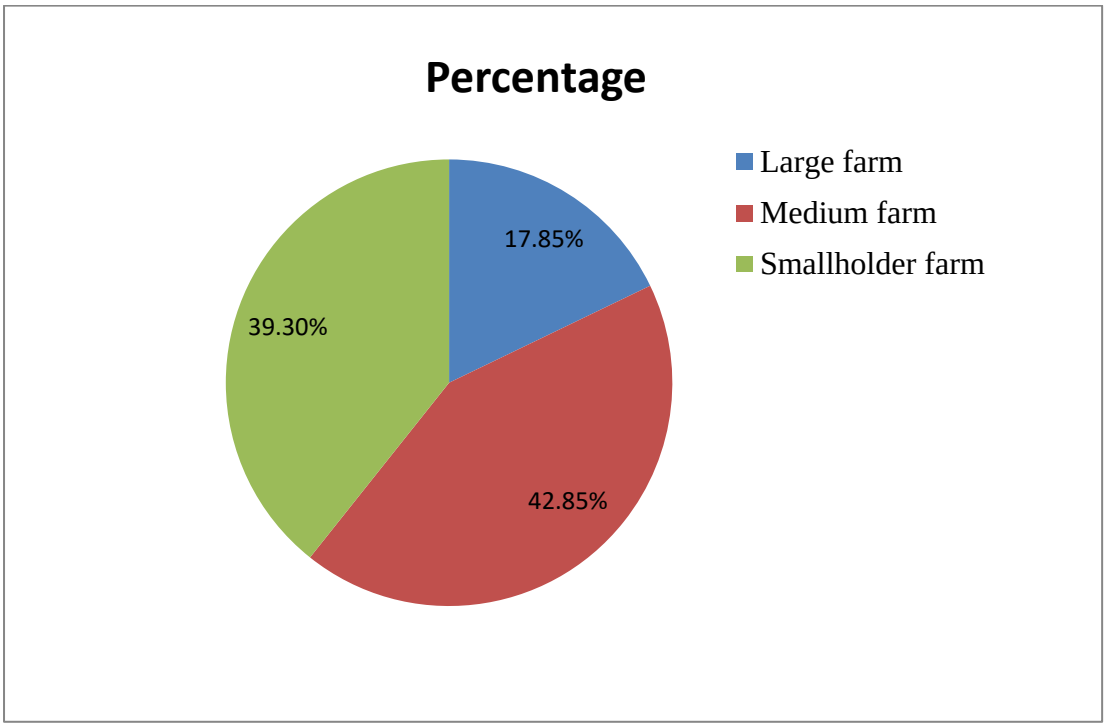

Figure 5: Percentage of subclinical endometritis in different scale of farms

Herd size did not have a significant (P>0.05) impact on the incidence of SCE. However, SCE occurrence was 1.44 times higher in medium farms and 1.16 times higher in smallholder farms compared to large farms. Regarding calving pen bedding materials, there was no statistically significant (P>0.05) relationship with SCE incidence, but dairy cows using concrete bedding had 1.33 times higher odds of experiencing SCE compared to cows using straw bedding. In terms of calving season, the prevalence of SCE was 3.31 times higher in December to February compared to September to November.

Table 6: Logistic regression analysis on association of herd level risk factors with SCE

Serial No.	Risk factors		Odd ratio	P value	95% confidence interval
1	Herd Size	Large	Referent		
		Medium	1.44	0.591	0.380-5.449
		Smallholder	1.1647	0.822	0.307-4.406
2	Type of bedding	Straw	Referent		
		Concrete	1.3360	0.556	0.509-3.501
3	Calving Season	Sep-Nov	Referent		
		Dec-Feb	3.3157	0.026	1.152-9.539

4.3. Impact of Subclinical Endometritis in Reproductive performance

The presence of endometritis had significantly (P< 0.05) prolonged CCI in dairy cows (Table 7). Similarly cows with SCE showed significantly (P<0.05) extended CFSI compared with cows without SCE. Endometritis was significantly correlated with NSPC (P<0.05), with affected cows requiring more services per conception than healthy cows as supported by statistical analysis. Details of presence of SCE on fertility of dairy cows are indicated in table 7 below.

Table 7: Mean ±SD for reproductive traits with or without subclinical endometritis

Trait	SCE status	Mean ±SD	95% confidence interval	T	P value
CFSI	Negative	77.34±15.877	72.33-82.35	-8.1816	0.0000
	Positive	110.25±17.160	103.59-116.90		
NSPC	Negative	2±0.836	1.735-2.264	-3.4624	0.0009
	Positive	2.64± 0.621	2.401-2.883		
CCI	Negative	99.24+19.871	92.97-105.51	-8.9467	0.0000
	Positive	147+24.317	137.57-156.42		

SCE = subclinical endometritis CFSI = Calving to first service interval

CCI = Calving to conception interval NSPC = Number of service per conception

4.4. Clinical Endometritis

During the study period, five cows after 21 days giving birth presented with clinical endometritis accompanied by various reproductive health issues, including vaginal prolapse, retained fetal membranes, abortion, abscess around the gluteal muscle, and hind leg. One case exhibited vaginal prolapse three days postpartum, which was promptly addressed through adjustment. Another cow had a history of RFM, which was manually removed one day after parturition. A third case experienced an abortion. The fourth cow displayed pus around the gluteal muscle and hind leg, while the fifth cow, despite no prior reproductive health issues, suffered from poor management practices. During the study period, the dairy cow with clinical endometritis identified by their purulent or mucoprulent vaginal discharge. Notably, all cases had received intramuscular antibiotic treatment prior to the onset of clinical endometritis. After onset of clinical endometritis one of the case were treated by hormone called prostaglandin and penstripe combination. The other cases were treated by only penstrip for five days (Appendix 8).

5. DISCUSSION

5.1. Overall Incidence of Endometritis in the study area

An essential factor for optimal fertility in dairy herds is a healthy uterine environment. In cattle, the regeneration of damaged endometrial tissue typically occurs between the 2nd and 4th week postpartum, ensuring minimal evidence of the previous pregnancy around 6 weeks after calving. However, approximately half of high-producing dairy cows do not exhibit these normal processes, leading to an absence of a "clean uterus" during the postpartum examination at the 4th week (Hoedemaker *et al.,* 2009). These cows are classified as having CE (Kasimanickam *et al.,* 2004). According to Sheldon *et al.* (2006), CE cases were identified by the presence of purulent vaginal discharge (PVD) after 21 DIM.

Some of the apparently healthy cows exhibit varying degrees of endometrial inflammation, as indicated by the presence of polymorphonuclear (PMN) cells in a cytological analysis of the endometrium (Kasimanickam *et al.,* 2004). According to Asfar *et al.* (2021), the most effective minimally invasive diagnostic technique for identifying SCE is endometrial cytology using a cytobrush. The study found that cows with SCE had a percentage of PMN $\geq$10% at 8^{th} week. This finding is consistent with previous reports by Gilbert, (2005), Carneiro *et al.,* (2014), and Moscuzza *et al.,* (2015). Additionally, Singh *et al.* (2016) and Sharma and Singh (2019) supporting the use of 10% PMN as a diagnostic cutoff for subclinical endometritis. In the present study, the incidence of endometritis including both clinical and subclinical endometritis was 44.59% (33/74) which is much higher than incidence 14.33% reported by Mekonnen (2024) in Wolaita Sodo. The difference between in this study was maybe due to study population and study methodology.

In the present study, 40.57% (28/69) of dairy cows found to have SCE in the study area. This finding is in agreement with the reports of Plontzke *et al.* (2010), who found 38% incidence in Argentina, Moges (2019), who found 46% in dairy cows with a PMN level of ≥3 neutrophils in the uterine sample in and around Gondar and Bacha and Regassa (2010), who found 47.5% in Debrezeit. In other studies, subclinical endometritis has been reported as 43% for cows between 20 and 33 days in milk (DIM) 45% for cows between 34 and 47 DIM (Sheldon *et al.*, 2006).

However some authors found higher incidence of SCE such as Gilbert (2005), who found 53% at 40 to 60 days postpartum and Moges and Jebar (2012), who found 68.3% in and around Gondar town with >5 neutrophil on endometrial cytology and some found lowere incidence like Madoz *et al.* (2013) found 17% in Argentina and Madoz *et al.* (2014) found 14% incidence. The difference of SCE in the current study as compared to the other cited studies could be due to the difference in the management system of dairy cows. Regarding CE only five cases were found 6.76% (5/74). This finding is comparable with Pillai (2012) who found eight in Coimbatore and Abera *et al.* (2017) who found 5.4% incidence of vulval discharge or clinical endometritis in and around Fitche town.

5.2. Association of Risk Factors with Endometritis in dairy cows

The findings of the present study suggest that there was no statistically significant association between age and the incidence of endometritis. However, it was observed that older dairy cows, specifically those above 9 years of age, exhibited a higher incidence of endometritis. This aligns with previous reports by Moges (2019), which indicated that cows aged above 6 years were more susceptible to endometritis. The discrepancy between the current study and the findings reported by Moges (2019) regarding the age at which cows are most affected by endometritis could be attributed to be geographical location and study populations. Older cows are more prone to persistent uterine infections due to reduced uterine elasticity and slower uterine involution, but they may have developed some immunity from prior infections. This can lead to quicker elimination of bacteria compared to younger cows, which may experience prolonged infections due to delayed immune responses (Chaffaux *et al.*, 1991; Adnane *et al.*, 2017).

The current study found that parity did not have a statistically significant impact on the incidence of SCE, which is consistent with the findings reported by Al-Absy *et al.* (2023). However, it was observed that the incidence of SCE was 1.23 times higher in dairy cows with more than 7 calves compared to those with 1-3 calves. This finding aligns with the report by Pascal *et al.* (2021), who also noted a higher incidence of SCE in cows with higher parity. This association may be explained by the cumulative exposure of multiparous cows to uterine bacterial contamination over time due to unsanitary calving aid interventions.

Furthermore, the study revealed that the incidence of SCE was 58.1% lower in dairy cows that had calved 4-6 times compared to those that had calved 1-3 times. This finding is in contradicts with a previous report by Bruun *et al.* (2002), who suggested that heifers are more vulnerable to endometritis than second parity cows due to the higher incidence of uterine injury in heifers. Third parity cows were found to be more sensitive to endometritis than second parity cows, attributed to the longer involution period of the uterus and a higher risk of infection. Interestingly, this study finding indicates all 1-3 parity cows were sensitive to endometritis. The discrepancy in the findings of this study compared to previous reports, such as Bruun *et al.* (2002), may be attributed to the grouping of parity levels differently. In our study, parity was categorized into few (1-3), moderate (4-6), and high (>7) based on Fesseha *et al.* (2021). The focus of the study was on identifying which parity groups were more sensitive to endometritis, rather than pinpointing specific parities that were sensitive. The cows included in this study were predominantly in their 4th to 6th parity due to their availability.

The relationship between body condition and the incidence of endometritis in dairy cows has been a focal point of recent research endeavors. A notable study by Bacha and Regassa (2010) illuminated a compelling correlation between BCS and the incidence of endometritis in dairy cows, which is similar to the current study. Their insightful findings revealed that cows with a BCS below 2.5 exhibited a staggering 4.5-fold higher risk of developing SCE compared to cows with a BCS exceeding 2.5.
The present study's revelation underscores the heightened risk of endometritis occurrence in cows with a BCS above 2.5, increasing incidence of endometritis by only 2.7 in contrast to the research work by Bacha and Regassa (2010). The

divergence in SCE incidence between the current study and prior literature could potentially be attributed to variations in dairy cow management practices. Insufficient nutrition during the transition period can lead to cows having a lower body condition score (BCS), which increases the risk of metabolic disorders and imbalances in energy and protein. This can weaken the immune system, making cows more susceptible to endometritis. To prevent this, it's important to ensure that cows are well-fed with high-quality food during the later stages of lactation in the previous cycle, so they enter the next lactation period with a good BCS. This can help reduce the incidence of endometritis and other related health issues (Walsh *et al.*, 2011).

According to present studies finding the incidence of endometritis is associated with delivery of male calf more than female calf which agreed with the reports described by Potter *et al.* (2010), Pascal *et al.* (2021) and Al-Absy *et al.* (2023) that endometritis is associated with delivery of male calf more than female calf. This is because the male calf is often larger than a female calf so it is related to their size. Cows that give birth to male calves are at a higher risk of developing SCE, which is linked to an increased risk of stillbirth. This is likely due to physical trauma, human interventions during the birthing process, and contamination of the cow's reproductive tract (Sheldon *et al.*, 2009).

The current study's findings align with previous research by Ibrahim *et al.* (2023) and Bacha and Regassa (2010) (odds = 4.5), demonstrating a significant association between the incidence of endometritis and mastitis. Specifically, subclinical mastitis (SCM) was found to be significantly and directly linked to subclinical endometritis (SCE). However, there is a slight discrepancy in the magnitude of the association, with the current study showing that the likelihood of endometritis occurrence in dairy cows with mastitis was 5.06 times higher, compared to 2.97 times higher as reported by Ibrahim *et al.* (2023). This variation may be attributed to differences in management practices and geographical locations between the studies. Mastitis occurs due to bacterial contamination in the environment, while a weakened immune system increases susceptibility to both mastitis and SCE. These findings indicate that implementing regular practices for preventing and controlling mastitis and endometritis together may lower the incidence of SCLE in smallholder dairy herds (Bacha and Regassa, 2010; Nyabinwa *et al.*, 2020).

Endometritis demonstrated a significant association with retained placenta, hypocalcemia, and dystocia in the study. The increased risk of endometritis (odds ratio = 9.23) in cows with retained placenta aligns with the findings of Kim and Kang (2003) (odds = 5.67), Ghavi Hossein-Zadeh and Ardalan (2011) (odds = 9.45) and Potter *et al.* (2010) (odds = 34.29). RFM may serve as a perfect medium for bacterial growth so that any treatment that reduces the severity of the ensuing endometritis would be beneficial in cows with retain placenta (Kim and Kang, 2003; Negasee, 2020). However, Cheong *et al.* (2011) reported no significant association between endometritis and retained placenta.

Furthermore, cows experiencing dystocia demonstrated a heightened risk of endometritis (odds ratio = 7.79), in line with Pascal (2021) and Dubuc *et al.* (2010b). This may be because dystocia induce endometrial trauma and subsequent postpartum complications, which contribute to, delayed uterine involution, fostering the occurrence of endometritis. Injuries sustained during dystocia can trigger the release of heparin at the site of damage, which may inhibit collagenases and delay uterine involution, ultimately contributing to the retention of fetal membranes, contributing to the occurrence of endometritis (Beagley *et al.*, 2010).

Hypocalcemia was also identified as a significant risk factor for endometritis (odds ratio = 6.49), which is lower than the findings of Ghavi Hossein-Zadeh and Ardalan (2011) (odds = 12.36). The difference in odds between two study may be due to study population, study size and geographical area. The potential mechanism underlying the association involves hypocalcaemia impairing uterine contractions and increasing the likelihood of RFM and endometritis.

Interestingly, while some studies such as Galvão (2012) and Faradillah and Agustina (2023) have highlighted abortion as a risk factor for endometritis, our study did not find a statistically significant association between abortion and SCE. However, it is noteworthy that cows with a history of abortion exhibited a 2.01 times increased risk of developing endometritis. When an abortion occurs, there is an increased risk of retained fetal membranes and uterine contamination, which can lead to inflammation and infection of the endometrium.

The study identified several risk factors for endometritis in dairy cows, including hygiene related risk factors like assisted calving (P<0.05, OR=5.06), calving equipment (P>0.05, OR=0.44), cow cleanliness scores, specifically cows mostly covered in dirt (P>0.05, OR=2.18) and completely covered in dirt (P=0.051, OR=5.909). As well as fecal consistency scores, particularly cows with flat, loose, thinly spread feces (OR=3.85), except assisted calving all of which showed no statistical significance. These findings are consistent with a study by Potter (2010) which reported odds ratios of 1.7 for assisted calving, 0.83 for cow cleanliness score, and 1.27 for fecal consistency score in relation to endometritis. However, while Potter (2010) did not find a significant association between hygiene or fecal contamination and endometritis, our study not revealed any association however only a little difference in odds. This difference could potentially be attributed to variations in management practices that may introduce bacteria to the uterus.

In unsanitary conditions, bacteria that contaminate the reproductive tract can enter through the vagina from both the environment and the cow's skin and feces. This is often a result of the cow being heavily soiled with dirt and feces, allowing for fecal matter to enter the vagina and potentially lead to ascending bacterial infections in the genital reproductive tract (Potter *et al.*, 2010; Piersanti and Bromfield, 2019). When cows experience diarrhea, they may inadvertently spread feces around their pelvis area, leading to dirt accumulation on their vulva, udders, and legs that may enter to reproductive tract (Hulsen, 2016).

Current research indicates that herd-level risk factors such as herd size and calving pen bedding material do not show a significant association with endometritis. However, dairy cows using straw bedding material exhibit lower odds (25.16%) of experiencing endometritis compared to other bedding materials in calving pens, aligning with a study by Cheong *et al.* (2011) which found a 10.7% decrease in the incidence of endometritis (SCE) with straw bedding. Mogese (2015) reported that large farms (18%), medium farms (44%), and smallholder farms (38%) are exposed to endometritis, consistent with the current findings of 17.85%, 42.85%, and 39.3% exposure, respectively.

The incidence of endometritis appears to be linked to the calving season, as supported by Al-Absy *et al.* (2023), who associated endometritis with cold weather. However, there are slight variations due to country-specific differences and the data collection period of this study being limited to the months of September to November, with data only available for November. The correlation between cold weather and endometritis may be due to cows' decreased health during wet seasons, making them more vulnerable to uterine infections as suggested by Bruun *et al.* (2002). While some researchers like Kim and Kang (2003) and Carneiro *et al.* (2014) found no effect of calving season on endometritis, discrepancies in findings could stem from variations in diagnostic criteria and climatic conditions across studies, particularly differences in average environmental temperatures among research locations as noted by Adnane *et al.* (2017).

5.3. Impact of Subclinical Endometritis in Reproductive performance

Endometritis in dairy cows is a significant global issue that has been linked to a decline in reproductive performance. The current study revealed that endometritis had a statistically significant adverse impact on the reproductive performance of dairy cows. During the study period, it was observed that dairy cows suffering from endometritis had a significantly longer CCI than those without endometritis. Additionally, affected cows had significantly high NSPC compared to normal cows, and had a potential impact on the CFSI of dairy cows in the study area. These findings are consistent with those reported by Mekonnen (2024), who found that cows with endometritis had longer CCI, higher NSPC, and significantly different CFSI compared to normal cows in the Wolaita Sodo region.

The current findings on CCI is supported by previous studies such as Tayebwa *et al.* (2015) in Central Uganda and Mohammed *et al.* (2019) in the UK, which also reported that cows affected by endometritis had prolonged calving-to-conception intervals and delays in resumption of ovarian activity after calving. Similarly, the impact of endometritis on CFSI aligns with the findings of Belachew (2007) in Debrezeyit and Ghanem *et al.* (2002) in Egypt, who observed significantly higher differences in CFSI between cows with endometritis and normal cows.

Furthermore, the significantly higher NSPC in affected cows compared to normal cows is consistent with the results of studies by Leblanc *et al.* (2000) in Canada and Plontzke *et al.* (2010) in Argentina, which also found an increased number of inseminations per conception in cows affected by endometritis. Additionally, the present investigation is consistent with the findings of Pascal (2021) in Rwanda, who observed longer CFSI and more services required per conception in endometritis-positive cows. It has been widely reported that endometritis prolongs the CFSI and CCI, increases NSPC, and has a detrimental impact on reproductive performance.

Comparably, it have also found that endometritis leads to prolonged CFSI and CCI, as well as an increase in the NSPC, all of which have a significantly negative impact on reproductive performance (Raab *et al.,* 2003; Gilbert *et al.,* 2005). The decline in reproductive parameters associated with endometritis is likely due to the toxic effects of the disease on uterine tissues, which in turn affect reproductive function. This may include delayed uterine involution, disruption of endometrial function, reduced steroid concentrations in ovarian follicles, and ultimately, ovarian dysfunction. These factors collectively contribute to the observed decline in reproductive performance in cows affected by endometritis (Shrestha *et al.,* 2004; Herath *et al.,* 2007; Green *et al.,* 2011; Dawod *et al.,* 2015; Chaudhari *et al.,* 2017;).

5.4. Clinical Endometritis

The present study found five cows with endometritis. Abortion and retain placenta was a risk factor for one of the case that occur which agrees with Faradillah and Agustina (2023), who report the case of holstein Friesian cross heifer pregnant and suffered from Foot and Mouth Disease that caused abortion and have retain placenta the next day after abortion and after some day showed clinical endometritis. Vaginal prolapse also one of risk factor for endometritis in current study. While there is no direct evidence linking vaginal prolapse to clinical endometritis, some factors may contribute to uterine trauma, potentially leading to inflammation. Postpartum vaginal prolapse has been associated with an increased risk of CE. The compromised vaginal and cervical anatomical integrity resulting from prolapse may facilitate the ascension of pathogens into the uterus, triggering an inflammatory response and contamination (Rees, 2016).

An abscess in the gluteal muscle or leg of a dairy cow can lead to clinical endometritis or be a risk factor due to the spread of infection. Bacteria from the abscess can enter the bloodstream and travel to the uterus, causing inflammation and infection (endometritis). This can impair reproductive health and increase the risk of infertility in dairy cows (Dubuc *et al.*, 2010b).

6. CONCLUSION AND RECOMMENDATION

The postpartum period is a critical period in the life of dairy cattle and management of the most important problems and diseases at an early stage represents the key to successful dairy herd management. In the present study, a notable number of postpartum dairy cows in the study area were diagnosed with endometritis. The significant risk factors included retained fetal membranes, assisted calving, dystocia, hypocalcaemia, mastitis, poor body condition scores with <2.5 and birth of male calves. The findings also suggest that cow cleanliness and fecal consistency had a tendency to play a role in the development of SCE, highlighting the importance of maintaining good hygiene practices. Cows with subclinical endometritis had longer calving-to-conception interval, extended calving-to-first service interval and higher number of services per conception. This study had shown that endometritis had a tremendous impact on the reproductive performance of dairy cows in the study area

Based on above conclusion, the following recommendations are forwarded:

- The dairy farmers and veterinarians prioritize preventive measures to reduce the incidence of endometritis in dairy herds. This may include implementing strategies to improve overall cow health and management practices, such as ensuring proper nutrition, minimizing stress during calving, and promptly addressing any reproductive health issues.
- Additionally, regular monitoring and early detection of endometritis through appropriate diagnostic methods can help in timely intervention and treatment, ultimately improving the reproductive efficiency and overall productivity.
- Good feeding system should be applied around the transition period, particularly in the last trimester of pregnancy as poor body condition can lead to the occurrence of endometritis.
- Further, the farmers should be aware that keeping clean cow environment could improve reproductive performance.

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8. APPENDIX

Appendix 1: Geimsa staining procedures

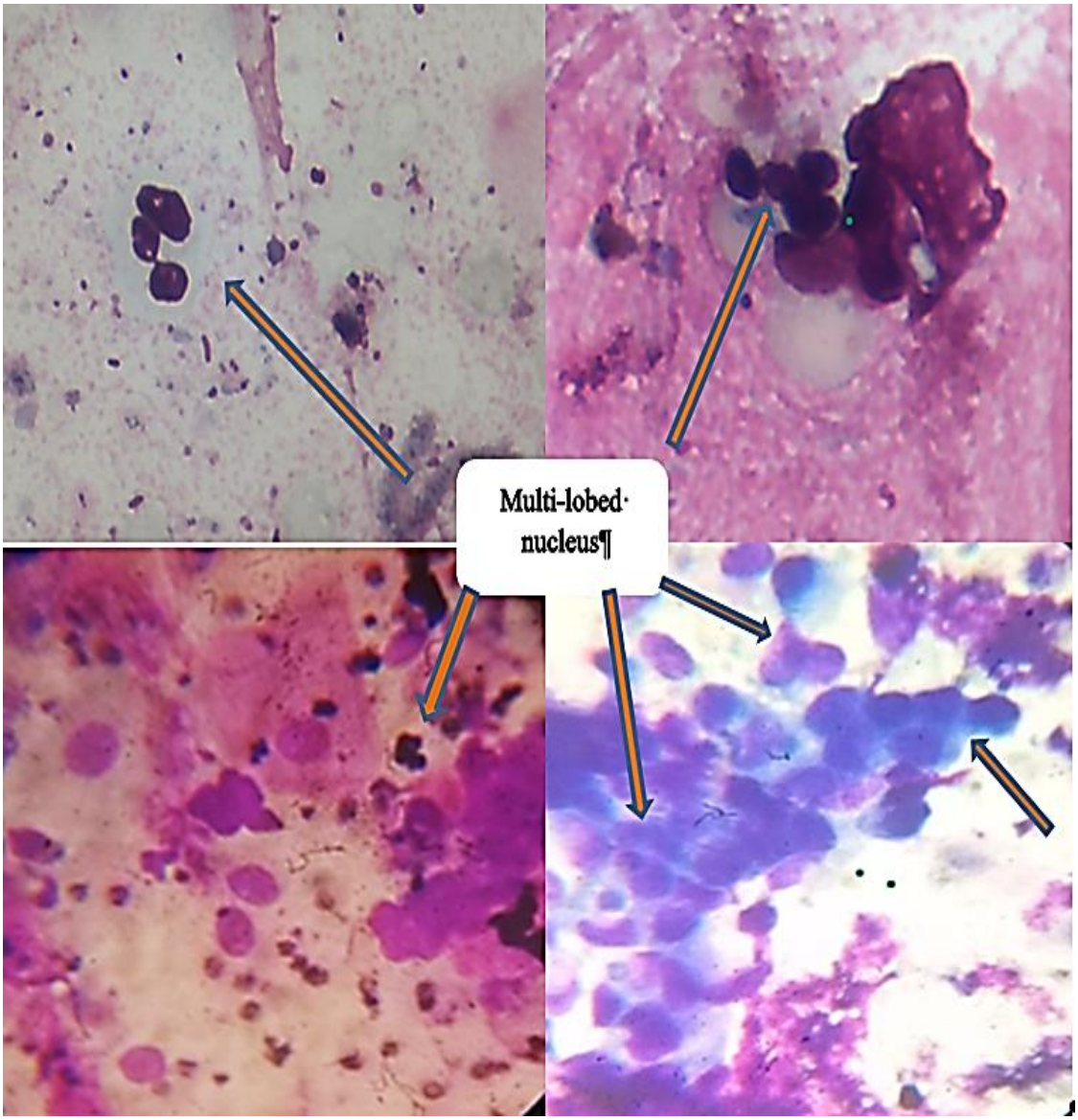
Place the dried smear on a test tube rack and immerse it in methyl alcohol for approximately 3 minutes to fix the smear. Subsequently, remove the alcohol and air-dry the slide. Transfer the slide to diluted Giemsa stain for 30-40 minutes. Rinse the stain with tap water, dry the slide, and examine it under an oil immersion microscope.

Appendix 2: Microscopic evaluation

After the smear has been prepared, stained and dried, samples were scanned at 400X magnification using a light microscope to evaluate cell morphology in detail. This process aimed to differentiate inflammatory cells and count polymorphonuclear leukocytes (PMNs). When viewed under microscope, PMNs typically appear as round or oval cells with granules in the cytoplasm. The nucleus of a PMN may have two to five lobes connected by thin strands of chromatin. PMNs are white blood cells with a multi-lobed nucleus, giving them a characteristic segmented or "polymorphonuclear" appearance.

Appendix 3: PMNs count

After the smears has been prepared, fixed and stained, the sample were placed on the microscope stage and set the microscope to 400x magnification to bring the cells into focus and then start counting PMNs. When counting, use a systematic approach to ensure thorough coverage of the sample by using a grid pattern. Starting at one edge of the slide, scanning fields of view in a consistent manner and then identifying PMNs by their characteristic multi-lobed nucleus and granular cytoplasm, which is frequently segmented with pink/purple staining. Exclude other cell types such as lymphocytes, erythrocytes, or debris from the count. Keep track of the total number of PMNs counted in each field. Calculate the percentage of PMNs by dividing the number of PMNs counted by the total number of cells and then multiplying by 100.



In the figure above the arrow points to multi lobed nucleus that is connected with thin thread, called chromatin and granular cytoplasm.

Appendix 4: California mastitis test

Subclinical mastitis was identified based on gel formation. The CMT results were scored as 0 (negative), trace, 1(weak positive), 2 (distinct positive) and 3 (strong positive) based on gel formation. All CMT scores of 0 and trace were considered as negative while CMT scores of 1, 2, and 3 were considered indicators of subclinical mastitis.

In this figure the green arrow points for gel formation that shows being positive for subclinical mastitis.

Appendix 5: Cow Cleanliness Score

Score 1 is free from dirt and Score 2 is slightly dirt then Score 3 is covered by dirt however, Score 4 is completely caked by dirt

Appendix 6: Data Record Format

Record sheet for investigation on the risk factors for endometritis and its impact on fertility of postpartum dairy cows in Wolaita Sodo, Southern Ethiopia.

RISK FACTORS FOR ENDOMETRITIS IN POSTPARTUM DAIRY CATTLE IN AND AROUND WOLAITA SODO

S.No	Risk Factors															Treatment outcome		Traits	
Cow No.	Endom + or –	RFM	Dystocia	Mastitis	Abortion	hypocalcaemia	CCS	Age	Fecal consistency	BCS	Calving equip	calving indoors	Calving outdors	Parity	Assisted calving	Others……	Type of Treatment	Response	CFSI	NSPC
1																				
2																				
3																				
4																				
6																				
7																				
8																				
9																				
10																				
11																				
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Appendix 7: Preparation of Cytobrush and Cytology sample collection

The human cervibrushes were meticulously modified through precise incisions to facilitate secure attachment to an AI gun using durable shoemaking thread (Fig B and C). Subsequently, these tailored cervibrushes were carefully placed within sterile plastic tubes for preservation and handling (Fig. D, E and F). Following this, the bovine pelvic region was meticulously cleansed, and the rectal area meticulously cleared of fecal matter (G). Finally, with precision and care, the cytobrushes were gently introduced through the vaginal canal to collect uterine sample (H).

A) Human Cervibrush B) Cervibrush prepared to be cutted

C) Cutted cervibrush D) Cervibrush being tied to AI gun by shoemaking thread

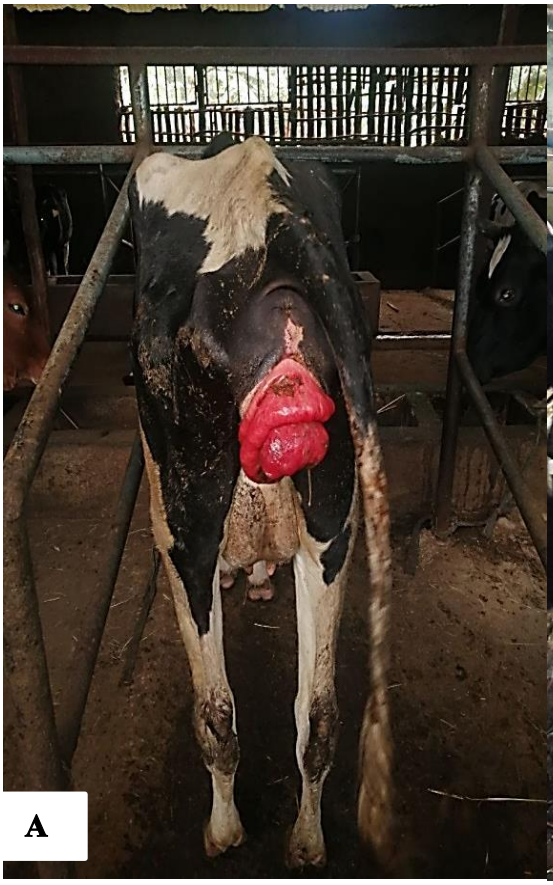
E) Cervibrush tied and ready for use

F) Placed within sterile plastic tube

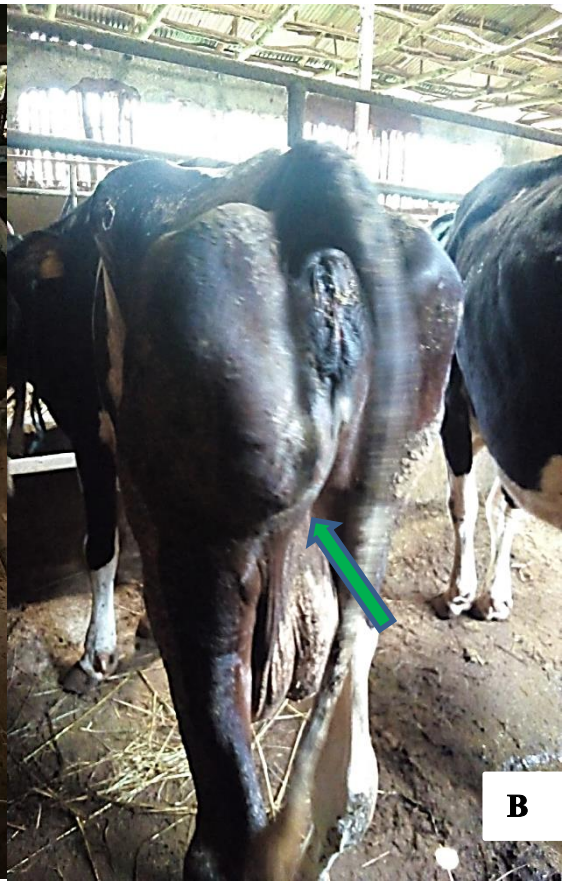
G) Removing faces from rectum H) Uterine sample collection by Cytobrush

Appendix 8: Clinical endometritis clinical signs and its associated risk factor

Clinical endometritis is characterized by notable clinical sign such as pale vaginal mucosa membrane (F), the presence of purulent vaginal discharge and mucoprulent discharge (E) sometimes that hangs around dairy cows vagina.



A) Postpartum vaginal prolapse



B) Abscess around gluteal muscle

C) Abscess in hind leg

D) Extracting vaginal discharge from vagina

E) Purulent Vaginal discharge F) Pale mucosa membrane

Appendix 9: Ethical Clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/03/16/16/2024

Name of Applicant: **Tsigereda teshome (DVM, MSc student)**

Address: Department of Clinical studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Risk factors for endometritis in postpartum dairy cattle in and around Wolaita Sodo*

Date of application:	**December, 2023**
Nature of the project:	**Farm investigation**
Target animal species:	**cattle**
Number of animals involved:	**69**
Study area:	**Wolaita Sodo- Ethiopia**

Minutes No. and date of review: **VM/ERC/02/16/024, 26/03/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Tsigereda teshome.**

The Federal Democratic Republic of Ethiopia

Professor Getachew Tereffe (DVM, PhD)
Chairman

Signature

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