

**ADDIS ABABA UNIVERSITY  
FACULTY OF VETERINARY MEDICINE**

**SUBCLINICAL ENDOMETRITIS AND ITS EFFECT ON REPRODUCTIVE  
PERFORMANCE IN CROSSBRED DAIRY COWS IN DEBRE ZEIT**

**BY  
BELACHEW BACHA**

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Veterinary Obstetrics and Gynecology

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## LIST OF ABBREVIATIONS

AAU	Addis Ababa University
AI	Artificial insemination
BHBA	Beta-hydroxybutyrate
CFSI	Calving to first service interval
DO	Days open
CI	Calving interval
CL	Corpus luteum
CSA	Central statistical authority
DMI	Dry matter intake
DIM	Days in milk
DPP	Days of postpartum
EC	Endometrial Cytology
ESAP	Ethiopian society of animal production
FSCR	First service conception rate
FVM	Faculty of Veterinary Medicine
GnRH	Gonadotropin releasing hormone
Ig	Immunoglobulin
NEFA	Nonesterified fatty acids
NMSA	National meteorology service agency
NSPC	Number of services per conception
PGF <sub>2α</sub>	Prostaglandin F 2 alpha
PP period	Postpartum period
RFM	Retained fetal membrane
SCE	Sub clinical endometritis

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## ABSTRACT

In this study 59 postpartum crossbred dairy cows were selected from three different farming scales (smallholders, medium sized and large scale dairy farms). Each cow had a normal parturition and had no abnormal uterine discharge by vaginoscopy or no abnormality in rectal palpation. Then the cows were examined for subclinical endometritis by examination of endometrial lavage sample for the presence of neutrophils at the 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum. The prevalence of cytologically diagnosed subclinical endometritis was 47.5% at the 4<sup>th</sup> and 30.5% at the 8<sup>th</sup> weeks of postpartum. In the three farming scales the prevalence of subclinical endometritis was 64.7% in small holders, 40.7% in medium sized and 40.0% in large-scale farms at the 4<sup>th</sup> week of postpartum ( $P = 0.240$ ). While prevalence of 41.2%, 26.7% and 25.9% was recorded at the 8<sup>th</sup> week of postpartum ( $P = 0.526$ ) in small, medium and large scale dairy farms respectively. Parity did not influence the prevalence of subclinical endometritis at the 4<sup>th</sup> week of postpartum ( $P = 0.492$ ). Cytologically diagnosed subclinical endometritis was greatly associated with impaired reproductive performance. Cows that had subclinical endometritis at the 4<sup>th</sup> week postpartum required a mean of 2.4 services per conception compared to 1.7 services per conception for normal cows ( $P = 0.000$ ) where as at the 8<sup>th</sup> week postpartum 2.7 services per conception were required for cows with subclinical endometritis, compared to 1.7 for normal cows ( $P = 0.001$ ). For cows with subclinical endometritis at the 4<sup>th</sup> weeks postpartum the median calving to first service interval were 96 days and 80 days for normal cows ( $P = 0.000$ ) while at the 8<sup>th</sup> week postpartum it was 100 days for cows with subclinical endometritis and 82 days for normal cows. For cows with subclinical endometritis at the 4<sup>th</sup> weeks postpartum the median days open were 174 and 152 for normal cows ( $P = 0.001$ ) while at the 8<sup>th</sup> week of postpartum it was 178 for cows with subclinical endometritis and 155 days for cows without subclinical endometritis ( $P = 0.000$ ). Also, there was a significant difference in first service conception rate between cows with and without subclinical endometritis at the 8<sup>th</sup> week of postpartum (3.4 versus 27.1%;  $P < 0.05$ ). From those cows diagnosed negative for subclinical endometritis at the 8<sup>th</sup> week postpartum Proportionally 51% of cows confirmed to be pregnant with in 180 days, whereas from those positive cows for subclinical endometritis only 2% confirmed to be pregnant. The proportion of cows confirmed to be pregnant with in 180 days were 41 and 12% for normal cows and cows with subclinical endometritis at the 4<sup>th</sup> week of postpartum respectively. These results also indicated that there was an association between subclinical mastitis and

subclinical endometritis at the 4<sup>th</sup> (P = 0.012) and 8<sup>th</sup> (P = 0.031) week of postpartum. From the follow up findings, it was observed that risk factors like poor body condition scores at the 4<sup>th</sup> week of postpartum had an association with subclinical endometritis (P = 0.017). Dairy cows with no regular exercise had a higher prevalence of subclinical endometritis than those exercising regularly (53.6 versus 46.4%; P = 0.026). In conclusion subclinical endometritis diagnosed by endometrial cytology in the postpartum period had a detrimental effect on current reproductive performance.

**Keywords:** Cows, Dairy, Endometritis, Inflammation, Reproduction, Subclinical, Uterus

## 1. INTRODUCTION

Four major dairy production systems have been recorded in Ethiopia. These include: the low land pastoral dairy production system, rural highland smallholder dairy production system, urban and peri-urban small and medium scale dairy production system and large-scale (intensive) dairy production system (Ketema and Tsehay, 1995; Kelay, 2002).

Urban and peri-urban dairy production system developed in and around major cities and towns, which have a high demand for milk. The main feeds sources are agro-industrial by products (oil Seed cakes, brans, brewers residue, etc) and purchased roughage. The system comprises small and medium size dairy farms based on exotic dairy cattle and their crosses located mainly in the highlands of Ethiopia. Generally, the primary of the production system is to sale milk as a means of additional cash income (Kelay, 2002).

Urban and peri-urban large-scale dairy production system is a more specialized market oriented dairy operation practiced by state sector and very few individuals on commercial basis. Most of the large-scale dairy farms are concentrated in and around Addis Ababa and are basically based on high-grade exotic stock. The urban and peri-urban and intensive dairy farmers produce 2% of the total milk production of the country (Azage *et al.*, 2000; Kelay, 2002).

This recently growing urban /periurban dairy production system has been contributing to almost all of the fluid milk supply to the major urban centers of the country (Azage and Alemu, 1997). However, among other constraints fertility and reproductive problems have become important setbacks to improve the profitability of this emerging production system (Yoseph *et al.*, 1999).

The dairy industry's major goal is to provide milk for the consumer market. Over the past several decades, milk yield of cows has increased markedly. However, one negative impact of this improvement is reduced fertility (e.g., cows open for longer, more services per conception) and higher incidence of reproductive problems, which in turn, contribute to reduced fertility (Dhaliwal *et al.*, 1996).

Reproductive performance is one key component of dairy production and the goal of reproductive management in dairy cattle is to have cows become pregnant in an efficient manner and at a profitable interval after calving (Plazier *et al.*, 2002). So, high reproductive efficiency is necessary for a successful dairy operation and requires a calving interval that maximizes milk production within the herd (Ferguson and Galligan, 1999). Good estrus detection, good insemination technique, quality semen, and a healthy uterine environment are critical components of high reproductive efficiency (Nebel, 1999).

Under normal circumstances many cows have some degree of postpartum uterine contamination (Leslie, 1983). However, the natural resistance mechanisms of the uterus usually eliminate uterine infection and inflammation, but infection persists in 10–15% of the animals and causes sub fertility even after resolution of clinical signs. When inflammation occurs in the uterus of the dairy cow, the clinical consequences include reduction in fertility as measured by calving-to conception intervals, first service conception rates, and other performance indices (Bondurant, 1999). Hence, a persistent postpartum uterine infection will result in poor reproductive performance in dairy cows (Kasimanickam *et al.*, 2004).

Reproductive performance of dairy cows after the voluntary waiting period is highly related to the health status of the uterus after calving (Dijkhuizen and Stelwagen, 1985; Ferguson and Galligan, 2000). Particularly abnormalities such as delayed uterine involution, bacterial elimination, restoration of endometrium and return to cyclical activity and endometritis can result in prolonged luteal phases leading to increased calving to conception interval in dairy cows (Nakao *et al.*, 1997; Noakes *et al.*, 2001).

Endometritis is defined as inflammation of the endometrium without systemic signs and is associated with delayed uterine involution. Postpartum endometritis has a negative effect on reproductive performance as it increases services per conception, calving to first service interval and calving to conception interval (Butt *et al.*, 1999; Heuwieser *et al.*, 2000), it reduces the risk of pregnancy (LeBlanc *et al.*, 2002), and decreases the conception rate (Ferguson and Galligan, 2000). It has also been reported that Subclinical endometritis (SCE) is common at the end of voluntary waiting period, and severely impedes reproductive performance (Gilbert *et al.*, 2004).

The diagnosis of endometritis by palpation per rectum or by fortuitous observation of a genital discharge is insensitive and non-specific (Miller *et al.*, 1980). To gain clarity of some of the questions surrounding bovine endometritis, a simple, rapid, inexpensive means of diagnosing the condition is essential. Endometrial biopsy has been studied in cows. It is neither simple nor inexpensive for use in large numbers of animals. In addition, it has been suggested to impair subsequent reproduction in biopsied cows. Endometrial cytology has proved exceptionally valuable in equine theriogenology (Slusher *et al.*, 1984; Ball *et al.*, 1988; Roszel and Freeman, 1988), and was investigated in endometrial cytology at endometritis diagnosis in cows (Kasimanickam *et al.*, 2004).

In the absence of clinical signs of endometritis, alterations in the uterine lumen or uterine wall can be defined as subclinical endometritis. It has been demonstrated that cows with subclinical endometritis have a depressed reproductive performance in the current lactation (Raab *et al.*, 2003; Kasimanickam *et al.*, 2004).

From recent discussions held with different dairy cow owners and /or dairy farmers at Debrezeit during ILRI training on health and welfare of dairy cows it has been clearly indicated that the need of repeated service (repeat breeding) for the cows to conceive is one of the important problems of dairy farms. The cause for this problem could be multifactorial and need to be explored. Also, the cause is yet to be described though, the fertility of lactating dairy cows has been declining worldwide and this could be associated with factors influencing uterine health in the postpartum cow (Nebel, 1999).

In Ethiopia, the number of services perconception increased from an average of 1.6 in 1980s to over 3.5 in early 2000s (Wubet, 2005). However, the reasons for this progressive declines in the fertility of dairy cows in Ethiopia is not vary well understood and there is an urgent need for the investigation of the problems, as well as providing educational programme for those who farm the animals. Therefore the objectives of the present study are:

- To determine the prevalence of subclinical endometritis and its effect on calving to first service interval (CTFSI), days open (DO), number of service per conception (NSPC), first service conception rate and other reproductive traits
- To determine association between mastitis and subclinical endometritis

## **2. LITERATURE REVIEW**

### **2.1. Immunity of the uterus and the postpartum period**

The normal uterus is a sterile environment in contrast to the vagina, which hosts numerous microorganisms. Opportunistic pathogens from the normal vaginal flora or from the environment may invade the uterus from time to time. Such opportunities exist primarily, but not exclusively at mating, parturition and /or postpartum (Jones *et al.*, 1997; Aiello and Mays, 1998). Postpartum uterine infections occur commonly in the cow as a sequel to retention of the fetal membranes and dystocia. This predisposes cows to endometritis, the least severe form of uterine infection, which is characterized by inflammation of the endometrial lining (Hafez, 1993).

#### 2.1.1. Immunity of the uterus

Most inflammatory lesions of the non gravid uterus are infectious in origin and result either from ascending infection by organisms that normally inhabit the lower genital tract or infectious agents introduced into the uterine cavity during mating, artificial insemination (AI) or postpartum. In most cattle the uterus seems to be able to prevent bacteria that typically reside in the postpartum uterus from proliferating and creating infections. Effective defense against reproductive tract invasion by environmental organisms is mediated by anatomical and functional barriers as well as nonspecific and specific immune responses (Smith, 1996; Jones *et al.*, 1997; Lewis, 1997).

#### *Anatomical and Physical Protective Mechanisms*

The major anatomical barriers between the contaminated world and the relatively sterile environment of the uterus include the vulva, the vestibule (guarded by a muscular sphincter), and the cervix. In the cow the cervix is a formidable barrier composed of a series of mucosa lined collagenous rings. In addition, the cervico vaginal mucus (especially the scant, tenacious mucus of the luteal phase) can function as a physical barrier for organisms that would otherwise ascend the reproductive tract. The circular and longitudinal muscular layers of the uterus provide physical propulsion of particulate material, including microbes. As with nearly every other aspect of the reproductive tract, the properties of these structures are dynamic and change with the hormonal environment (Bondurant, 1999).

### *Cellular and Molecular Events in Uterine Inflammation*

Once the physical defenses are breached, the next line of defense in the endometrium is the innate immune system, which includes neutrophils, macrophages, and serum complement. The presence of all serum complement proteins in bovine uterine secretions is not thoroughly documented, but mechanisms for its delivery into the uterine lumen probably exist. For example, in the cycling cow, there is frequently a small amount of physiological hemorrhage from the caruncular endometrium shortly after ovulation (Ke`uther *et al.*, 1998). This hemorrhage would bring cellular and serum components, including complement, to the uterine lumen. In addition, vasoactive substances released from mucosal mast cells (Tizard, 1996) would be expected to increase permeability of small vessels such that serum may leak into the superficial endometrial tissue space and, perhaps, into the lumen. Activation of mast cells and damage to basement membranes can be accomplished by the action of neutrophil lysosomes (Cheville, 1988).

In cycling heifers at dioestrus, mast cells were noted immediately beneath the uterine epithelium (Galeotti *et al.*, 1997). In cows, endometrial mast cell numbers were highest around estrus and lowest at midcycle (Tibbits *et al.*, 1989).

Neutrophilic influx into the superficial endometrium and then into the lumen characterizes the early response of the uterus to surface infection. Neutrophils infiltrate tissue spaces and cavities and phagocytize and kill microbes by several mechanisms. They can be directly attracted to microbial products (e.g., *N*-formulated peptides of low molecular weight) (Tizard, 1996; Trowbridge and Emling, 1997). In addition, complement component C5a is a powerful chemotactic agent for neutrophils (Tizard, 1996). Once neutrophils are in the lumen, phagocytosis is enhanced by opsonization of microbes or other particulate material (Watson, 1985). Opsonins include the complement component C3b and specific antibody; the neutrophil surface has receptors for C3b and receptors for the Fc component of the antibody, such that binding and engulfing of opsonized microbes is greatly enhanced (Watson, E. D. 1989; Tizard, 1996).

Phagocytized organisms can be killed by oxygen dependent (respiratory burst) and oxygen independent (lysozymes and proteolytic enzymes) mechanisms (Klucinski *et al.*, 1995; Trowbridge and Emling, 1997). The ability of circulating neutrophils to phagocytize and kill

bacteria by generation of superoxide anions [reactive oxygen species (ROS)] is apparently diminished as parity of the cow increases (Gilbert *et al.*, 1993). In addition, cyclic fluctuations in opsonin-mediated phagocytosis and killing also apparently occur. Watson (1985) showed that uterine flushings from follicular-phase cows had enhanced phagocytosis and killing by neutrophils over that produced by flushings from luteal phase uteri. Several researchers have shown that cows with naturally occurring persistent endometritis have altered neutrophil function. In addition, both phagocytic activity and bactericidal activity were apparently reduced in cows with experimental endometritis (Klucinski *et al.*, 1995 and Zerbe *et al.*, 1996). Neutrophils flushed from normal uteri on postestrus day 2 to 6 had a greater degree of expression of Fc receptors (as detected by rosette formation) for IgG1 and IgG2 antibodies than did peripheral blood neutrophils. Neutrophils from cows with experimental endometritis showed a decrease in the expression of Fc receptors for IgG antibodies and a lower index of Fc-mediated phagocytosis (34, 35). However, the neutrophils were apparently able to at least partially compensate by increasing phagocytic activity mediated by non-immunological receptors (Klucinski *et al.*, 1990), the nature of which was not described. In the LTB<sub>4</sub>-induced endometritis model, Zerbe *et al.*, (1996) reported a similar decrease in phagocytic activity of uterine versus blood neutrophils with or without complement opsonization, but uterine neutrophils retained their ability to generate ROS after stimulation. They (Zerbe *et al.*, 1996) also noted that other surface molecules on uterine neutrophils, including class I major histocompatibility complex (MHC I) and the integrin LFA-1, showed diminished expression relative to circulating neutrophils, but also that CD11b and another uncharacterized surface antigen were upregulated in endometritic cows. It is interesting to note that the upregulation of CD11b, an important component of the C3b-binding integrin, CR3, did not appear to increase opsonin-aided phagocytosis.

Immune protection of the reproductive tract requires most of the same cellular and chemical strategies, as do other mucosal surfaces. However, some unique influences of the endocrine environment that mediate reproductive events also significantly affect this mucosal immune system (Bondurant, 1999).

Increased uterine leukocytes activity during estrous plays a role in clearing infectious agents entering the uterus during this time. Local antibody production to the invading organism may also be important in limiting the course of infections (Jones *et al.*, 1997).

The predominant immunoglobulin within the uterus is IgG. If bacteria such as *Campylobacter fetus* infect the genital tract vaginal IgA antibodies will immobilize and agglutinate the organisms. If the mucus membrane becomes inflamed, IgG antibodies derived by transudation from serum will also assist in protection (Tizard, 1998).

At calving and immediately postpartum, the vulva is relaxed and the cervix is dilated thus allowing bacteria to gain entry into the vagina and thereafter the uterus. In the PP period blood, cell debris and sloughed caruncular tissue provide an ideal medium for bacterial growth; however, in most cases the bacteria do not colonize the uterus to produce endometritis. The main mechanism involved in the elimination of the bacteria is phagocytosis by migrating leucocytes. Also, persistence of uterine contractions, sloughing of caruncular tissue and uterine secretions all assist in the physical expulsion of the bacteria (Arthur *et al.*, 1989).

Early return to cyclical activity is probably important since the estrogen-dominated uterus is more resistant to infection. However, there is evidence that in some cases early return to estrus may be disadvantageous (Arthur *et al.*, 1989; Hafez, 1993).

#### 2.1.2. The Postpartum period and postpartum uterine involution

Postpartum period (PP period) is the time required for the completion of uterine involution that is it is the interval from parturition to complete uterine involution (Olson *et al.*, 1986). It takes three weeks under normal conditions, from size point of view, and functional resumption needs six weeks. It has been reported that uterine involution in normal cows is largely completed as indicated by the size of the uterus and the condition of the endometrium at about 30 days postpartum (Lewis, 1997; Jones *et al.*, 1997). The complete uterine involution range is 26 to 52 days after calving, but the changes after 20 to 25 days post calving are generally almost imperceptible. Complete re-epithelialization of the caruncle is complete from 25 days onwards (Noakes *et al.*, 2001).

The PP period of the dairy cow determines productive and reproductive responses during lactation and is, therefore, a pivotal time in the production cycle of the cow. During this period, dairy cows are at risk of developing calving related diseases, such as hypocalcaemia,

endometritis, metritis (uterine infection), ketosis and displacement of the abomasums (Curtis *et al.*, 1985).

The PP period is a crucial event in the life of the dairy cow, during which the animal should re-establish normal uterine and ovarian activities. Understanding of the physiology of these organs is thus imperative to the reproductive management, as failure of either one or both to return to normal function may delay the first postpartum ovulation (Lamming *et al.*, 1981; Kindahl *et al.*, 1982).

The PP period is divided into three sub periods: puerperal, intermediate and postovulatory periods. The puerperal period was defined as the interval from calving until the pituitary becomes responsive to GnRH (i.e., at approximately 7-14 days postpartum). The intermediate period is the interval from the time that the pituitary becomes responsive to GnRH to the first postpartum ovulation. The postovulatory period is the interval from the first ovulation to complete uterine involution, which was defined loosely as the time when the uterine horns and the cervix were approximately 40 mm in diameter and the caruncles were, reepithelialized (Olson *et al.*, 1986).

The physiologic changes that occur in the uterus during the PP period when it is returning to its normal non-gravid functional and anatomic state are referred to as postpartum uterine involution. Postpartum involution in cows is generally complete by 45 to 50 days postpartum (Jones *et al.*, 1997).

PGF<sub>2α</sub> is likely the most important contributor to uterine size reduction in the immediate PP period for all domestic species. This can be inferred from the episodes of discomfort that parturient animals undergo during the hours immediately after delivery (Cunningham, 2002). The CL of pregnancy begins to regress prior to parturition and is in an advanced stage of degeneration within few days after calving. During the first few days after delivery, the uterus contracts gradually so that by the fourth day it is approximately one-half that during pregnancy and by day 7-8 it is only one-third the size of gravid uterus. By day 14, the uterus has reached its approximate non-pregnant size. Cows may continue to expel uterine fluid and lochia for up to 18 days postpartum; those that continue to discharge beyond this time should be investigated for abnormality. As long as the discharge does not have a foul odor, it is probably normal (Jones *et al.*, 1997).

### 2.1.3. Postpartum ovarian activity and utero-ovarian relationship

Ovarian activity during the early PP period exerts an important influence on the ability of the uterus to resist or eliminate bacterial infections. The uterus of the cow can resist uterine infection during the estrogenic phase or estrus but are very susceptible during the progesterone or luteal phase of the cycle, since cellular defense mechanisms are potentiated during estrus it has been generally assumed that a delay in return to cyclical activity would predispose cows to endometritis (Arthur *et al.*, 1989; Hafaze, 1993).

Studies showed that in those cows that ovulated early, the bacterial contamination was such that it was probably not eliminated at the estrus, so that when there followed a luteal phase, the bacteria were able to proliferate and colonize the uterus (Olson, 1986; Arthur *et al.*, 1989). It was also shown that a short exposure to luteal or exogenous progesterone will down regulate immune functions and in some animals transform the uterus from an organ that is resistant to one that is susceptible to infection (Lewis, 1997)

If cows with uterine infection resume cyclicity, relatively early in the PP period, pyometra is likely to occur when elevated levels of progesterone coincide with the presence of high number of pathogenic bacteria. Therefore the practice of injecting cows with GnRH to induce cyclicity early in the postpartum period should be avoided, as it could lead to pyometra (Hafez, 1993).

### 2.1.4. Postpartum uterine infections

Uterine infections are generally classified according to clinical signs and degree of severity (Youngquist and Little, 1988). The term uterine infection is used to indicate that the uterus is contaminated with pathogenic organisms. Endometritis indicates that the endometrium is inflamed, and, when inflammation occurs after the puerperal period, endometritis is considered to be the least severe classification of uterine infection (Lewis, 1997). Endometritis, metritis, and pyometra are related (i.e., they may develop sequentially) and are after collectively called the metritis-pyometra complex (Bartlet *et al.*, 1986).

Different forms and degrees of uterine infections affect the dairy cow, and of several bacteria that have been implicated, *Actinomyces pyogenes* is the most frequently encountered

organism in the cow.  $\text{PGF}_{2\alpha}$  is released in postpartum cows with either normal puerperium or with uterine infection; but higher levels persist for a longer period in cows with uterine infections. Apparently, bacterial infections and toxins stimulate the uterus to secrete abnormally higher levels of prostaglandin (Fredrikson *et al.*, 1988), which delay the onset of cyclicity until the infection is cleared and the prostaglandin levels are low. Another possibility is that uterine infection may delay the initiation of folliculogenesis and suppress the rate of follicular growth in dairy cows during the early puerperium by inhibiting LH release. The inhibition is believed to be due to endotoxins produced by gram-negative bacteria in the postpartum uterus of the cow (Hafez, 1993). Severe endometritis results from coinfection of the uterus with gram-negative anaerobes and *A. pyogenes* due to pathogenic synergy (Arthur *et al.*, 1989; Bekana *et al.*, 1994).

## **2.2. Endometritis and factors predisposing to endometritis**

Endometritis implies inflammation of the endometrium, which is a common condition in the cow and although it has a profound effect on the fertility of the animal it does not affect its general health (Arthur *et al.*, 1989). During the puerperal period, bacteria invade the uterus of most cows, and the composition of the uterine flora fluctuates constantly during the first 7 weeks after calving, which is the entire PP period. The uterus seems to become contaminated repeatedly, to clear the organisms and to become recontaminated until uterine involution is complete. The majority of the cows resolve endometritis spontaneously. However, when a uterus becomes infected (i.e. unable to readily clear the organisms and the organisms proliferate) endometritis progress (Del Vecchio *et al.*, 1994)

### **2.2.1. Endometritis**

As a pathological entity, endometritis is an inflammation of the endometrial lining of the uterus with out systemic signs (Lewis, 1997). DeBois and Manspeaker, (1986) also describe endometritis as a superficial inflammation of the endometrium only, extending no deeper than the stratum spongiosum. Histologically, endometritis is characterized by some disruption of surface epithelium, infiltration with inflammatory cells, vascular congestion, and stromal edema and by varying degrees of lymphocyte and plasma cell accumulation in the superficial layers. Several specific diseases are associated with endometritis or metritis. These include

brucellosis, leptospirosis, camphylobacteriosis and trichomoniasis. However, often endometritis is the result of nonspecific infections. The non-specific opportunist pathogens are the most important cause of endometritis and have a significant effect upon fertility (Aiello and Mays, 1998).

#### *Acute endometritis*

Acute endometritis usually follows parturition, copulation, AI, and invasion of the uterine lumen with infusion solutions and instruments. In most cases acute endometritis is transient and offending microorganisms are removed with in several oestrus cycles by the uterine defense mechanism (Smith, 1996).

#### *Acute metritis*

Acute metritis is also referred to as postpartum metritis, toxic puerperal metritis or septic metritis and occurs within the first 10 days after parturition. It is characterised by fetid, watery, and reddish- brown to purulent vulvar discharge and an elevated body temperature  $\geq 39.5^{\circ}\text{C}$ . Referees on which body temperature can be regarded as fever range from  $39.2^{\circ}\text{C}$  to  $39.7^{\circ}\text{C}$  (Drillich, 2006). The palpation of the uterus per rectum reveals an enlarged and flaccid uterus. Acute metritis is often associated with a depressed general attitude, reduced feed intake and decreased milk yield. Risk factors for acute metritis were categorized by Sheldon and Dobson into uterine damages (stillbirth, dystocia, twins, cesarean section, retained placenta, delayed uterine involution), metabolic conditions (milk fever, ketosis, left displaced abomasum) and the balance between pathogenicity and immunity (disruption of neutrophil function, type of bacterial flora, progesterone and glucocorticoide administration, early formation of a corpus luteum, level of hygiene).

#### *Chronic endometritis*

Chronic endometritis is also referred to as clinical endometritis and is characterised by the presence of mucopurulent or purulent exudate in the vagina three weeks or more after parturition (Drillich, 2006). In contrast to acute metritis, chronic endometritis is not associated with elevated temperature and depressed general attitude. Several methods for diagnosing endometritis have been established, including adspection of the vulva, perineum and the tail, combined with manual palpation of uterus and cervix per rectum, and/or vaginal inspection.

Clinical findings by *rectal palpation* of the uterus are asymmetric uterine horns, thickened uterine wall, and palpable presence of fluid (Lewis, 1997), and a cervical diameter of more than 7.5 cm (LeBlanc *et al.*, 2002).

*Vaginal inspection* via speculum, however, is more accurate than rectal palpation (Lewis, 1997; LeBlanc *et al.*, 2002). Rectal palpation as well as vaginoscopy are indirect diagnostic methods and cannot verify the inflammation of the uterus itself. A uterine swab for microbiology as a routine diagnostic tool for chronic endometritis is not practical and associated with high costs (Lewis, 1997).

### 2.2.2. Factors predisposing to endometritis

When the greater majority of cows suffer from bacterial contamination after calving, the uterine flora is frequently changing because of elimination and recontamination. In cows that develop endometritis the bacterial flora colonize the uterus and causes the endometrium to respond. A number of factors have been shown to predispose cows to endometritis (Arthur *et al.*, 1989), and some of these predisposing factors stated as follows:

#### *Dystocia and Retained fetal membrane (RFM)*

Endometritis, frequently following an acute puerperal metritis, is more prevalent in cows and heifers that have had severe dystocia. Since RFM is a problem in case of twin pregnancy and induction of calving, it is not surprising to have more prevalence of endometritis and /or metritis (Arthur *et al.*, 1989)

#### *State of ovarian activity*

It has been known for sometime that the uterus of the cow is more resistant to infection at estrus than during the luteal phase of the cycle. Since cellular defense mechanisms are potentiated during estrus it has been generally assumed that a delay in return to cyclical activity would predispose cows to endometritis. It is also showed that in those cows that ovulated early, and the bacterial contamination not fully eliminated at the estrus, when the luteal phase followed the bacteria will proliferate and colonize the uterus to cause

endometritis. So the state of cyclical ovarian activity could act as a predisposing factor to endometritis (Arthur *et al.*, 1989; Hafez, 1993).

### *Cleanliness and hygiene*

The cleanliness of a farm especially the calving area and hygiene during assisted calvings are generally thought to affect the incidence and outcome of endometritis. A dirty, unhygienic calving environment in which the parturient and post parturient cow occupies can have a negative effect and predispose to endometritis since the bacterial load is high in such environment (Olson *et al.*, 1986; Youngquist and Little, 1988).

### *Metabolic diseases*

Metabolic diseases like ketosis, hypocalcaemia and overfeeding during the dry period can predispose to endometritis. This is probably associated with the fatty liver syndrome (Arthur *et al.*, 1989).

## **2. 3. Subclinical endometritis and its diagnosis**

On the basis of relative number of neutrophils from endometrial cytology, we can diagnose and define Subclinical endometritis (SCE) (If greater than 5 neutrophils per 400-x-microscope power field or greater than 2 % of the cells are neutrophils it can be categorized as SCE). SCE is inflammation of the endometrium without external indicators such as vaginal discharge or other apparent clinical manifestations (Smith, 1996; Drillich, 2006). The percentage of PMN in the cytological preparation provides information on the presence of subclinical endometritis. The threshold value for PMN varies between authors from 5 to 18% (Raab *et al.*, 2003; Kasimanickam *et al.*, 2004; Gilbert *et al.*, 2005). Inflammation in the uterus due to SCE has an effect on reproductive performance (Gilbert *et al.*, 2004).

### **2. 3.1. Diagnosis of subclinical endometritis**

Some recent studies described the diagnosis of subclinical endometritis. Ultrasonography as a non-invasive method visualizes small amounts of fluid in the uterine lumen. False positive findings might result from clear mucus in the uterus appearing during estrus. Therefore, the ovaries should be scanned as well to define the stage of the estrus cycle (Lenz, 2003;

Kasimanickam *et al.*, 2004). Subclinical forms of endometritis, not detected by per-rectal palpation and vaginal discharge, can cause depressed fertility, therefore the diagnosis of SCE need assessing of endometrial cytology (Drillich *et al.*, 2004). Endometrial cytology can be performed by flushing the uterus to obtain endometrial cells or by taking samples with a cytobrush from the endometrium (Kasimanickam *et al.*, 2004; Gilbert *et al.*, 2005).

Cytological examination of the reproductive tract is often used to evaluate possible reproductive lesions in humans and domestic animals. Endometrial cytologic (EC) examination in mares (Wingfield-Digby, 1987) and cows (Gilbert *et al.*, 1998) are accepted diagnostic techniques. Endometrial and inflammatory cells may be collected by a guarded cotton swab, uterine biopsy and uterine lavage or cytobrush techniques to evaluate endometrial cytology, especially as an aid in the diagnosis of acute endometritis (Kasimanickam *et al.*, 2004).

Endometritis in dairy cows is one of the most controversial topics discussed among practitioners due to the lack of a diagnostic gold standard. However, improved diagnostic procedures help to enhance diagnostic accuracy. A technique that yields well-preserved cells representative of a large uterine surface area without causing harm to the reproductive tract is required for consistent and reliable cytological results. The uterine lavage technique harvests cells from a larger uterine surface area and provides a more representative sample of luminal contents than does either a swab or a uterine biopsy (Miller *et al.*, 1980; Bourke *et al.*, 1997), but it may cause irritation to the endometrium (Ball, 1988). Cotton swabs are routinely used to evaluate the vagina cytologically in the bitch. In both humans and the equine species, the cytobrush technique has been determined to be superior to other methods for the collection of cervical and endometrial cells (Kasimanickam *et al.*, 2004).

Cytologic examination by uterine lavage with low volumes of saline to recover neutrophils has recently been studied in dairy cattle as a method to define subclinical endometritis (Gilbert *et al.*, 1998) and endometritis (Hammon *et al.*, 2001).

## 2. 4. Measures of reproductive performance

Various methods are used for calculating reproductive indices and assessing reproductive efficiency in dairy cows. Proposed standards for measuring dairy herd reproductive efficiency include calving to conception interval, number of services perconception, non-return rate, calving interval and reproductive culling rate for infertility (Fertrow *et al.*, 1990).

As far as management factors go, it is generally assumed that a calving interval of 12 to 13 months is economically optimal (French and Nebel, 2003). One report (Louca and Legates, 1968) suggests that a 12-month calving interval is desirable for mature cows, while an interval of 13 months for first calf heifers maximizes their production (due to their greater persistency of milk production). Several studies support this idea, but some studies have found that a longer calving interval could be economically advantageous (van Amburgh *et al*, 1997). For example, there is experimental data from 19 dairy farms in Israel that suggest intentional delayed breeding may be economically feasible in using modern Holsteins in year-round calving herds. Washburn and coworkers (2002) suggested that some of the increases in days to first service and average days open in Southeastern herds may be due to the increasing popular idea that delayed breeding can be economically advantageous. However, they also found that the number of days open after the first breeding still increased in the last 20 years, suggesting that it takes longer to get cows pregnant after the start of breeding.

The length of the calving interval is effectively determined by the number of days from calving to conception, which is referred to as the open interval and which must average 85 days if a herd is to have an average calving interval of 12 months. The open interval consists of two components: the calving to first service interval and the interval from first service to conception. The first of these is a function of the time required for a cow to start cycling following calving, the efficacy of heat detection on the farm and the management decision on when to start breeding. The second component, the interval from first service to conception, is a function of the number of services per conception and the efficacy of the heat detection program. It has been shown that a herd's average number of services to conception has relatively little effect on the open interval compared to the efficacy of the heat detection program and the management decision on how soon after calving to start breeding (Oltenucu *et al.*, 1981).

### **3. MATERIALS AND METHODS**

#### **3.1. Description of the study area and study population**

##### **3.1.1. Study area**

The study was carried out at Debrezeit, from October 2006 to May 2007. The town is located at 9°N and 40°E 47km south east of Addis Ababa with a human population of about 95,000 people. The altitude is about 1850m above sea level. It is an important small town where most governmental institutions, national and international research centers are located. The single major soil type of the area is Vertisol, which covers about 60.8% of the district. Other soil types are Cambisols and Luvisols (23.5%) and Rendzinas and Phaeozems (14.7%). It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800mm. The mean annual minimum and maximum temperatures are 12.3°C and 27.7°C respectively with an overall average of 18.7°C (NMSA, 20003; CSA, 2001). Highest temperatures are reached in May and the mean relative humidity is 61.3%. Debre-zeit is the center of Ada'a Liben woreda (district), and, the Woreda has a total land area of about 1610.56 Km<sup>2</sup>. and is divided in to three agro-ecological zones namely mid land (94%) high land (3%) and low land (3%) (Ada'a Woreda agricultural and rural development office)

##### **3.1.2. Study population and study animals**

This study was conducted on postpartum dairy cows owned by dairy farmers in Debre Zeit area. The majority of dairy producers in the town are market oriented smallholder dairy farmers with average herd sizes of three cows which are organized under, one dairy cooperative called, Ada'a milk and milk products marketing cooperative share company. There are also few government and private owned large (commercial) scale dairy farms with milking herd size of over 50 in the town. The Adaa Milk and Milk products Marketing Cooperative (formerly Adaa Liben milk and milk products marketing cooperative) was founded in September 1999 with initial founding members of 34 (Mohamed *et al.*, 2004). According to the administrative office currently the cooperative is reported to have some 850 member farms including few large dairy farms with milking herds size of >30.

Thirty-four dairy farms (30 from the Adaa Milk and Milk products Marketing Cooperative and 4 private commercial large scale farms) were originally selected at random for inclusion in the study; however, 2 private farms were left out because of lack of cooperation and accessibility problems. A total of 66 crossbred postpartum dairy cows suited for the prospective longitudinal study were originally obtained from the 32 selected dairy farms however 7 were excluded from the study 5 because of failure to recover any uterine lavage fluid and 2 because of uterine discharge beyond the 4<sup>th</sup> week postpartum.

The study Population was all crossbred dairy cows (Holstein x zebu) found in Debrezeit that calved during October 2006-January 2007. And from these postpartum dairy cows those with abnormal uterine discharge were excluded from the study and clinically normal dairy cows were selected and followed up for five to six months until they were served and the results were carefully recorded. Therefore the study animals consisted of 66-crossbred dairy cows selected from smallholder, medium sized and large-scale dairy farms. During the follow up study, after calving each cows were visited weekly and clinically normal cows and with no abnormal vaginal discharge were selected, based on visual inspection, vaginoscopy and rectal palpation.

### **3.2. The study design**

A prospective follow up study was conducted on dairy cows (crossbred) found in Debre Zeit that calved from October, 2006 – January, 2007 and weekly based follow up was done until the end of May, 2007.

### **3.3. Sampling method and determination of sample size**

Selection of study animal's i.e. crossbred postpartum dairy cows was conducted purposively after an original random selection of the farms.

A sampling frame i.e. the list of current members of Adaa Milk and Milk products Marketing Cooperative was acquired from the administrative office, at the beginning of study. Dairy farms / herds were selected from this list using a stratified random sampling procedure to ensure the selection of proportional and representative sampling of herds. Stratification was done based on herd size in similar manner to previous works in the urban peri-urban

production systems (Lemma *et al.*, 1999), as small holder (< 5 heads of dairy cattle), medium scale (6-50 heads of dairy cattle), and large/commercial scale (>50 heads of dairy cattle). A total of 30 dairy farms (17 small holders, 12 medium scales, and 1 large commercial scale) were in this manner selected from the cooperative. Two more large commercial scale farms were additionally selected purposively based on cooperation, to improve representation of the large-scale farms.

### **3.4. Study methodology**

#### 3.4.1. Endometrial cytology (EC) using the uterine lavage technique

To determine the presences of subclinical endometritis endometrial samples were taken using the uterine lavage technique as described by Gilbert *et al.* (2005) and Kasimanickam *et al.* (2004). The uterine body was lavaged by infusing sterile 50 ml of 0.9% sodium chloride solution into the uterine body with a 50 ml syringe attached to a 52 cm disposable plastic infusion rod (We also used a sterile AI sheath). The uterus was massaged and then retracted to recover the fluid. In some of the cases we used sterile saline water more than 50 ml to increase the amount of recovered fluid. As much fluid as possible, was recovered by negative pressure aspiration into the syringe and transferred to test tube without preservatives. Uterine lavage samples were brought to the laboratory within 2 h and centrifuged at 800-rpm for 5 min. A drop of sediment was streaked on to a clean microscopic slide and air-dried. All slides were fixed with methanol and stained with giemsa stain (Kasimanickam *et al.*, 2004), and examined under a microscope at 400x magnification by counting 80 – 100 cells to determine the percentage of neutrophil. Initially the whole slide was assessed and a representative area was selected to determine the percent neutrophil. If greater than 5% of the cells are neutrophils per 400-x-microscope power field, it was categorized as subclinical endometritis (Smith, 1996; Gilbert *et al.*, 2005).

For the determination of effects of SCE on reproductive performance, reproductive data of cows with and with out SCE were recorded (emphasis on calving to first service interval, NSPC, days open, first service conception rates) for six months after enrolment until confirmed pregnant or not and the findings on these parameters were compared (Annex 1 and 8).

#### 3.4.2. Determination of subclinical mastitis

Subclinical mastitis was determined by taking milk samples at the 4<sup>th</sup> and 8<sup>th</sup> weeks of the postpartum period at the same time with determination of subclinical endometritis was done. A squirt of milk from each quarter of the udder was placed in each of the four shallow caps in the CMT paddle. An equal amount of commercial CMT reagent was added to each cap. The interpretation of the result was done as described by Quinn *et al* (1994) (Annex 3). Confirmation of the presence of subclinical mastitis was done by culturing for those milk samples with CMT score of trace and above and categorized as positive or negative. The prevalence of subclinical mastitis in SCE positive and negative cows recorded and its association with endometritis was determined. Cows with clinical mastitis were not included in the study.

#### 3.4.3. Follow-up observation and questionnaire survey

Dairy cows from the selected farms that calved from October 2006 to the first week of January 2007 were selected and followed for a minimum of five months. A weekly-based follow up program was assigned for each farm and the reproductive events and reproductive performance including hygienic conditions and management aspects were recorded. Associated risk factors were determined using data from structured questionnaire survey and follow up findings recording format (Focusing on current reproductive events and overall management practice) (Annex 2 and 8). Body condition changes postpartum were monitored and determined on a 1- 5 scale (1 = emaciated, 5 = extremely fat) (Matthew man, 1993). The scoring period were at calving, 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum (Annex 1 and 4).

#### 3.4.4. Clinical examination of the reproductive tracts

During the routine follow-up reproductive tracts of cows were evaluated by vaginoscopy and rectal examination from 28 – 30 and from 49 – 56 days postpartum and checked for the presence of uterine abnormalities (discharge, endometritis, etc). At examination, cows were first inspected for the presence of fresh discharge on the vulva, perineum, or tail. If discharge was not visible externally, cows were examined vaginoscopically. The vaginal speculum lined with sterile aluminum foil (or disinfected with Savelon between uses). The vaginoscope was inserted into the vagina up to the level of the external os of the cervix. Inspection of the

vagina and the cervix was performed with illumination from a penlight (LeBlanc *et al.* 2002). Those cows with apparent clinical endometritis were excluded from data analysis.

### **3.5. Data management and analysis**

The raw data were collected and entered in to MS excel program and the statistical analysis was performed by using SPSS® program for windows (version 11.5, 2000) and Intercooled Stata® (version 7.0). Outcomes measured to assess reproductive performance were calving to first service interval (days to first service), interval from calving to pregnancy (days open, DO), first service conception rate (FSCR, %), and cows pregnant with in 180 days postpartum (CPWI 180 DPP). Pregnancy was diagnosed by transrectal palpation at around 60 days post-insemination. Pregnancy status at 60 days after successful insemination was used for the analysis. Categorical variables like subclinical endometritis (positive and negative) at the two points in the postpartum period and their interaction with different parameters (like farm scale, parity body condition score, with subclinical endometritis) were compared using chi-square analysis. Continuous variables like percent neutrophil count, number of services perconception, days to first service and days open were compared using *Independent- samples T- test or Students T- test*. The level of significance was set at  $\alpha = 0.05$ .

#### 4. RESULTS

A total of 66 crossbred dairy cows of different parity, which calved between October 2006 and January 2007, were included in the study. Of the total 66 uterine lavage attempts, 7.6% (5/66; at the 4<sup>th</sup> week PP) failed to recover any fluid and 2/66 manifest uterine discharge beyond the 4<sup>th</sup> week postpartum and these were excluded from the study. The majority of endometrial cytology slides were readable and assessment of endometrial cytology was done on samples preparation taken from 59 crossbred dairy cows and findings were correlated with fertility parameters.

Table 1 shows the prevalence of subclinical endometritis at the 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum to be 47.5% and 30.5% respectively. The number of cows, which were positive for subclinical endometritis decreased between the 4<sup>th</sup> and 8<sup>th</sup> week (Fig. 1). The prevalence of SCE significantly ( $P < 0.05$ ) dropped at the 8<sup>th</sup> week compared to that at the 4<sup>th</sup> week postpartum

Table 1. Mean ( $\pm$  SEM) of neutrophil count and prevalence of subclinical endometritis in 59 cows at different postpartum period

Postpartum Period (weeks)	SCE	Prevalence N (%)	% Neutrophil count ( $\pm$ SEM)	95% CI for the mean % neutrophil count
4 <sup>th</sup> week	SCE P+	28 (47.5)	8.23 $\pm$ 0.362	7.48, 8.97
	SCE N-	31 (52.5)	2.55 $\pm$ 0.281	1.97, 3.12
8 <sup>th</sup> week	SCE P+	18 (30.5)	7.34 $\pm$ 0.478	6.33, 7.34
	SCE N-	41 (69.5)	1.45 $\pm$ 0.215	1.06, 1.49

SCE P+= Subclinical Endometritis Positive cows; SCE N- = Subclinical Endometritis Negative cows;

Table 2 shows the proportion of subclinical endometritis positive and negative cows in the three dairy farming scales. From these farming scales 64.7, 40.0 and 40.7% incidence of subclinical endometritis were registered in smallholders, medium sized and large-scale dairy farms at the 4<sup>th</sup> week PP respectively. At the 8<sup>th</sup> week PP, 41.2% of the smallholder dairy cows, 26.7% of the medium sized and 25.9% of the large-scale dairy cows were positive for subclinical endometritis demonstrating no significant difference ( $P > 0.05$ ) between farm scales.

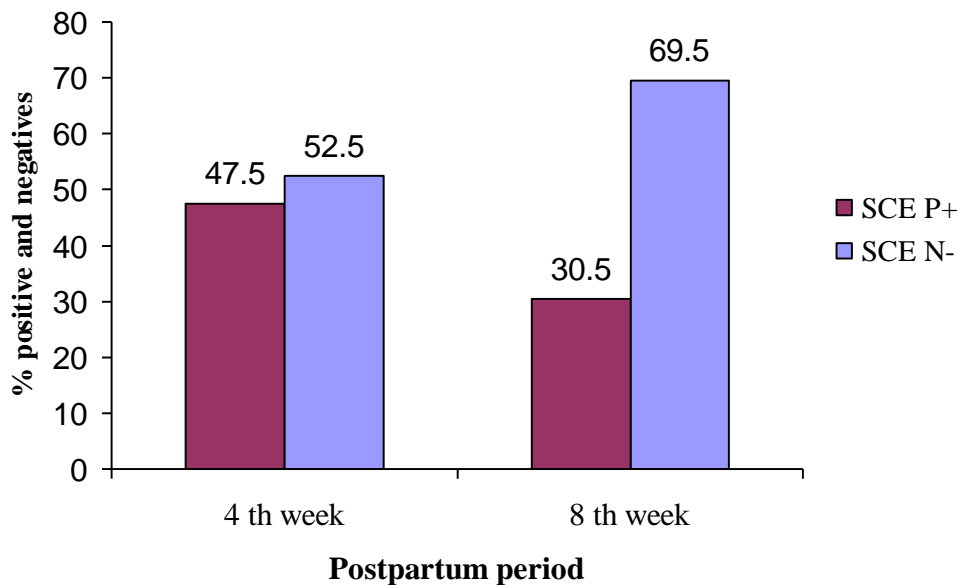


Fig 1. Subclinical endometritis positive and negative cows at the 4<sup>th</sup> and 8<sup>th</sup> week postpartum

Table 2. The association of Subclinical endometritis with the three dairy farming scales

Postpartum period	SCE findings	Smallholders (N= 17) n (%)	Medium sized (N= 12) n (%)	Large scale (N= 3) n (%)	P- value
4 <sup>th</sup> week	SCE P+	11 (64.7)	6 (40.0)	11 (40.7)	0.240
	SCE N-	6 (35.3)	9 (60.0)	16 (59.3)	
8 <sup>th</sup> week	SCE P+	7 (41.2)	4 (26.7)	7 (25.9)	0.526
	SCE N	10 (58.8)	11 (73.3)	20 (74.1)	

N= number of farms, n= number of dairy cows in each farm type, SCE P+= Subclinical Endometritis Positive cows; SCE N- = Subclinical Endometritis Negative cows.

Table 3 describes the proportion of subclinical endometritis in cows with different parity. Out of the 59 cows examined by the uterine lavage technique, 56.2, 36.8 and 50.0% of the dairy cows with first, second and third or more parity number were positive for SCE at the 4<sup>th</sup> week postpartum respectively. At the 8<sup>th</sup> week postpartum still 50.0% of the dairy cows with parity one were positive for subclinical endometritis whereas there were a proportional reduction in the case of dairy cows with parity of two, three and above. Thus, it appeared that first parity had some effect ( $P = 0.09$ ) on the incidence of subclinical endometritis.

Table 3. The association of parity with subclinical endometritis

Weeks postpartum	Parity No	SCE P+ (%)	SCE N- (%)	<i>P-value</i>
4 <sup>th</sup> week	1 (n = 16)	9 (56.2)	7 (43.8)	0.492
	2 (n = 19)	7 (36.8)	12 (63.2)	
	3 <sup>+</sup> (n = 24)	12 (50.0)	12 (50.0)	
8 <sup>th</sup> week	1 (n= 16)	8 (50.0)	8 (50.0)	0.089
	2 (n= 19)	3 (15.8)	16 (84.2)	
	3 <sup>+</sup> (n= 24)	7 (29.2)	17 (70.8)	

SCE P+= Subclinical Endometritis Positive cows; SCE N- = Subclinical Endometritis Negative cows;

Table 4 shows the number of services required for subclinical endometritis positive and negative cows to conceive. At the 4<sup>th</sup> week postpartum, out of the 45 cows that needed only one or two services per conception, 30 of them were negative whereas only 15 cows positive for subclinical endometritis conceived by one or two services. From 59 cows examined and followed up only 8 cows, which were positive for SCE at the 8<sup>th</sup> week postpartum conceived by one or two services while 37 dairy cows that were negative for endometritis need one or two services per conception.

Table 4. Percent subclinical endometritis and different number of services required for SCE positive and normal cows.

Weeks postpartum	SCE	Number of cows (%)	Number of services per conception (NSPC)			
			1(n= 17)	2(n=28)	3(n=10)	4(n=4)
4 <sup>th</sup> week	SCE P+	28(47.5)	5(29.4)	10(35.7)	9(90.0)	4(100.0)
	SCE N-	31(52.5)	12(70.6)	18(64.3)	1(10.0)	0(0.0)
8 <sup>th</sup> week	SCE P+	18(30.5)	1(5.9)	7(25.0)	7(70.0)	3(75.0)
	SCE N-	41(69.5)	16(94.1)	21(75.0)	3(30.0)	1(25.0)

SCE P+= Subclinical Endometritis Positive cows; SCE N- = Subclinical Endometritis Negative cows;  
n = Number of cows that require different number of services per conception

Table 5 presents the effect of subclinical endometritis (SCE) on the number of services per conception. A mean of 2.4 services per conception was required for cows that were positive for SCE at the 4<sup>th</sup> week postpartum where as 1.7 services per conception was required for cows negative for SCE. For those cows that were positive for subclinical endometritis at the 8<sup>th</sup> week postpartum a mean of 2.7 services per conception was required, compared to 1.7 NSPC for normal cows. At both 4<sup>th</sup> and around 8<sup>th</sup> weeks postpartum, the presence of subclinical endometritis had a significant effect (P = 0.001) on the number of services per conception indicating that cows with subclinical endometritis required more services per conception.

Table 5. The effect of subclinical endometritis on number of services per conception

Weeks postpartum	Findings	N (%)	Number of services per conception (Mean ± SEM)	95% CI for mean	P- value
4 <sup>th</sup> week	SCE P+	28(47.5)	2.4 ± 0.2	2.1, 2.8	0.001
	SCE N-	31(52.5)	1.7 ± 0.1	1.4, 1.9	
8 <sup>th</sup> week	SCE P+	18(30.5)	2.7 ± 0.2	2.6, 3.1	0.001
	SCE N-	41(69.5)	1.7 ± 0.1	1.5, 2.0	

SCE P+= Subclinical Endometritis Positive cows

SCE N- = Subclinical Endometritis Negative cows

SEM = Standard error of the mean

Table 6 and 7 shows the effect of subclinical endometritis on calving to first service interval and days open. The presence of subclinical endometritis at both the 4<sup>th</sup> and 8<sup>th</sup> weeks of postpartum had a significant effect (P = 0.001) on calving to first service interval and days open. The median calving to first service interval for cows with subclinical endometritis at the 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum were 96 and 100 days, compared to 80 and 82 days for cows without subclinical endometritis respectively. The overall days open for subclinical endometritis positive cows were 174 and 178 days (which are positive at the 4<sup>th</sup> and 8<sup>th</sup> weeks of postpartum), compared to 152 and 155 days for cows without subclinical endometritis.

Table 6. Effect of subclinical endometritis on calving to first service interval

Weeks postpartum	SCE	N (%)	Days to first service or CFSI (Mean $\pm$ SEM)	95 % CI	P- value
4 <sup>th</sup> week	SCE P+	28(47.5)	96 $\pm$ 3.2	89,102	0.000
	SCE N-	31(52.5)	80 $\pm$ 2.4	75, 84	
8 <sup>th</sup> week	SCE P+	18(30.5)	100 $\pm$ 4.1	91,108.	0.001
	SCE N-	41(69.5)	82. $\pm$ 2.7	78,86.	

DPP = days of postpartum; SCE P+= Subclinical Endometritis Positive cows;

SCE N- = Subclinical Endometritis Negative cows; CFSI = calving to first service interval

Table 7. Effect of subclinical endometritis on Days open

Weeks postpartum	SCE	N (%)	Days open (Mean $\pm$ SEM)	95 % CI	P- value
4 <sup>th</sup> week	SCE P+	28(47.5)	174 $\pm$ 4.0	166, 182	0.001
	SCE N-	31(52.5)	152 $\pm$ 3.2	146, 160	
8 <sup>th</sup> week	SCE P+	18(30.5)	178 $\pm$ 4.9	168, 189	0.000
	SCE N-	41(69.5)	155 $\pm$ 2.9	150, 161	

SCE P+= Subclinical Endometritis Positive cows; SCE N- = Subclinical Endometritis Negative cows.

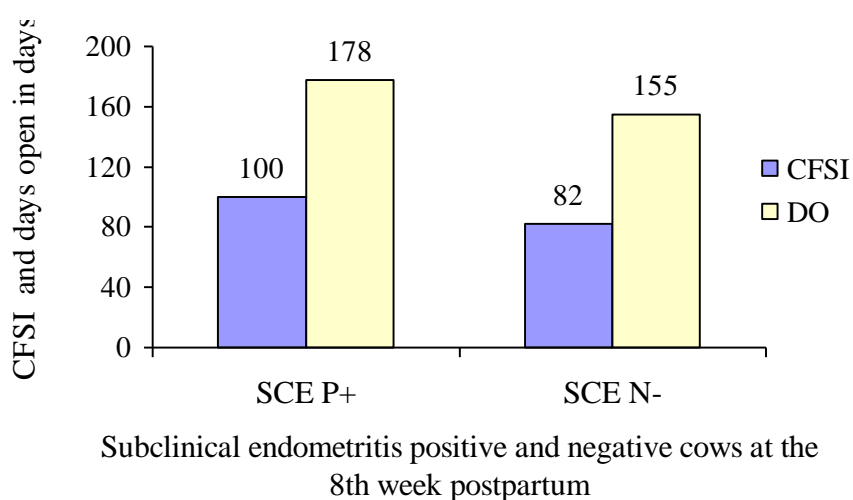


Fig 2. CFSI and days open for subclinical endometritis positive and negative cows at 8<sup>th</sup> week postpartum

Table 8 describes the reproductive performance of cows with subclinical endometritis and normal cows at the 4<sup>th</sup> and 8<sup>th</sup> weeks of postpartum. The FSCR was significantly different between cows with subclinical endometritis and normal cows at the 8<sup>th</sup> week, but not at the 4<sup>th</sup> week postpartum. The CFSI and DO were significantly different between cows with subclinical endometritis and normal cows at the 4<sup>th</sup> and 8<sup>th</sup> week postpartum. The proportion of cows confirmed pregnant within 180 days postpartum was 40.7 % for subclinical endometritis negative cows and 11.9% for endometritis positive cows.

Table 8. Reproductive performance of cows with subclinical endometritis and normal cows at the postpartum period

Parameters	4 <sup>th</sup> week postpartum		8 <sup>th</sup> week postpartum	
	Subclinical endometritis	Normal cows	Subclinical endometritis	Normal cows
N <sub>o</sub>	28 (47.5)	31 (52.5)	18 (30.5)	41 (69.5)
FSCR (%)	6 (10.1)	12 (20.3)	2 (3.4)	16 (27.1)*
DO (95% CI) *	173.7 (165.5,181.9)	152.4 (145.9,158.9)	178.7 (168.4,188.9)	155.4 (149.5,161.4)
CFSI (95% CI)*	95.6 (89.1, 102.1)	79.8 (74.9, 84.7)	99.5 (90.9,108.1)	81.9 (77.6,86.3)
CPWI180 DPP	7 (11.9)	24 (40.7)	1 (1.7)	30 (50.8)

\*P < 0.05). CFSI = Calving to first service interval DO = Days open

Table 9. The status of subclinical mastitis in SCE positive and negative cows

Weeks PP	Findings	N (%)	Subclinical mastitis		P- value
			Positives (%)	Negatives (%)	
4 <sup>th</sup> week	SCE P+	28(47.5)	13(46.4)	15(53.6)	0.012
	SCE N-	31(52.5)	5(16.1)	26(83.9)	
8 <sup>th</sup> week	SCE P+	18(30.5)	9(50.0)	9(50.0)	0.031
	SCE N-	41(69.5)	9(22.0)	32(78.0)	

PP = Postpartum; SCE P+=subclinical Endometritis Positive cows

SCE N-= Subclinical Endometritis Negative cows.

Table 9 shows the status of subclinical mastitis in the dairy cows that were diagnosed for subclinical endometritis. From 28 (47.5%) dairy cows that were positive for subclinical

endometritis at the 4<sup>th</sup> week PP, 13 (46.4%) of them were also positive for subclinical mastitis. However, in those cows negative for subclinical endometritis only 5(16.1%) had subclinical mastitis. From 41 (69.5%) cows that were negative for SCE at the 8<sup>th</sup> week postpartum, 32 (78.0%) of them were also negative for subclinical mastitis. This shows that the presence of subclinical endometritis at both points in the postpartum period had an association ( $P < 0.05$ ) with subclinical mastitis.

### Follow up observation and questionnaire result

Table 10 shows the association of subclinical endometritis with BCS at calving, 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum. At the 4<sup>th</sup> week postpartum, from cows that had a body condition score range of 2.5 – 3.5, only 28.6% were positive for compared to 63.3% for those cows that had a BCS < 2.5. This shows that BCS had a significant effect ( $P = 0.02$ ) on the incidence of SCE at the 4<sup>th</sup> week of postpartum but not ( $P > 0.05$ ) at calving and at the 8<sup>th</sup> week of postpartum.

Table 10. Effect of body condition on subclinical endometritis at the 4<sup>th</sup> week postpartum

Scoring period and BCS Category	No of observation N= 59	4 <sup>th</sup> week postpartum		P- value
		Subclinical endometritis	Normal cows	
BCS at calving at calving		28	31	
2.5 –3.5	35	18(51.4)	17(28.8)	0.474
< 2.5	7	4(57.1)	3(42.9)	
> 3.5	17	6(35.3)	11(64.7)	
BCS at the 4 <sup>th</sup> week PP		28	31	
2.5 –3.5	28	8(28.6)	20(71.4)	
< 2.5	30	19(63.3)	11(36.7)	0.017
> 3.5	1	1(100)	0(0)	
BCS at the 8 <sup>th</sup> week PP		18	41	
2.5 –3.5	23	4	19	
< 2.5	34	13	21	0.204
> 3.5	2	1	1	

BCS = Body condition score; PP = postpartum period

Table 11 describes the association between some management practice and SCE at the 4<sup>th</sup> week postpartum. It is observed that there was an association between SCE and the presence and absence of regular exercise. Out of the 28 cows that were positive for subclinical endometritis, 15 (53.6%) have no regular exercise and confined in the tie stall barn throughout the year.

Table 11. The association between management practice and subclinical endometritis at the 4th week postpartum

Management practices	Category	SCE positive	SCE negatives	P - value
Post calving hygiene	Poor	16(57.1)	13(42.0)	0.055
	Good	12(42.9)	18(58.1)	
Washing frequency	Twice a week	11(39.3)	20(64.5)	0.142
	Once a week	12(42.9)	7(22.6)	
	Every two weeks	5(17.8)	4(12.9)	
Regular exercise	Yes	13 (46.4)	25(80.6)	0.026
	No	15(53.6)	6(19.4)	

## 5. DISCUSSION

Results of this study showed that in 59 dairy cows examined by the uterine lavage technique, the prevalence of subclinical endometritis at the 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum were 47.5% and 30.5% respectively. Gilbert *et al.* (2005) reported that at 40 – 60 days postpartum in five United States commercial dairy herds the overall prevalence of subclinical endometritis to be 53%. In the study by Kasimanickam *et al.*, (2004) the prevalence of subclinical endometritis was 45.1% at visit 1 (20 – 33 days of postpartum) and 41.4% at visit 2 (34 – 47 days of postpartum). Kasimanickam *et al.*, (2004) used a cytobrush instead of low-volume lavage for obtaining cytological samples for the diagnosis of subclinical endometritis. Examining cows at 20–33 days postpartum, they found 80 of 215 cows (37%) to have endometritis by their definition (over 18% neutrophils in the sample). The overall prevalence of endometrial inflammation of these two studies was comparable to this study. In all 59 cows selected for this study, there was no clinical evidence of uterine exudate. LeBlanc *et al.* (2002) indicated that the absence of uterine discharge by vaginoscopic examination was not truly indicative of absence of uterine inflammation. The vaginoscopic identification of discharge may be influenced by the severity of the infection, myometrial contraction, uterine clearance mechanisms, perineal conformation, body condition score, postural changes and exercise. Alternatively, uterine discharge may not be detected externally or by vaginoscopy in cows in which the cervix is closed. Cows may have abnormal uterine fluid but fail to show clinical signs, leading to false negative results. Similarly, cows with subclinical endometritis may not be identified by routine diagnostic procedures (rectal palpation and vaginoscopy). Le Blanc (2002) concluded that clinically normal, treated cows performed better than untreated controls, indicating many cows regarded as normal had an undiagnosed uterine condition. This suggests that cows identified, as clinically normal by routine diagnostic procedures during the postpartum period should be screened for the presence of subclinical endometritis to identify cows at risk of poor reproductive performance. In this study, the number of cows, which were positive for subclinical endometritis decreased between the 4<sup>th</sup> and 8<sup>th</sup> week of postpartum. The presence of high proportion of uterine inflammation at the 4<sup>th</sup> week postpartum may be due to the occurrence of uterine inflammation perhaps as a result of bacterial contamination during uterine involution. Because, it has been reported that uterine involution in normal cows is largely completed as indicated by the size of the uterus and the condition of the endometrium at about 30 days postpartum (Lewis, 1997; Bondurant, 1999).

The pathogenesis of subclinical endometritis remains unknown and an experiment by Gilbert *et al.*, (1998) suggested that, most or all cows have uterine inflammation early in the postpartum period, but some resolve this inflammation more expeditiously than others. However, those failing to eliminate endometrial inflammation suffer detrimental reproductive consequences.

In this study, the percentage neutrophil counts threshold was 8.23% and 7.34% for subclinical endometritis positive cows at the 4<sup>th</sup> and 8<sup>th</sup> weeks of postpartum respectively. Similarly Gilbert *et al.* (2005) who examined cows at 40–60 days postpartum used > 5% neutrophil count for determination of subclinical endometritis. On the other hand Kasimanickam *et al.* (2004) definition (>18% neutrophils at 20–33 days postpartum or >10% neutrophils at 34–47 days in milk) seems somewhat compatible with the present study for samples taken at the 4<sup>th</sup> and 8<sup>th</sup> weeks postpartum. The validity of endometrial cytology for diagnosis of endometritis is confirmed by the substantial impact of the condition so diagnosed on reproductive parameters. It is possible that certain factors cause false negative or false-positive diagnoses. In particular, the effect of stage of cycle on endometrial neutrophilia needs to be examined. Nevertheless, the overall impact of cytologically diagnosed endometritis validates the use of this methodology to identify cows with significantly impaired reproductive performance. Even with the decrease in percentage neutrophil threshold, suggesting that endometrial cytology can be successfully employed at different times postpartum. However, the percentage neutrophil threshold to define subclinical endometritis will differ at different times postpartum. The decline in neutrophil count at the late stage postpartum may reflect gradually improved uterine immunity and completion of uterine involution.

In this particular study it was found that there was no significant difference on the occurrence of subclinical endometritis between the three farm scales. From these farming scales 64.7, 40.0 and 40.7% incidence of subclinical endometritis were registered in smallholders, medium sized and large-scale dairy farms at 4<sup>th</sup> week postpartum respectively. At the 8<sup>th</sup> week postpartum, still 41.2% of the smallholder dairy cows, 26.7% of the medium sized and 25.9% of the large-scale dairy cows were positive for subclinical endometritis. Gilbert *et al.* (2005) did investigation on five different herds (on five commercial dairy farms) and found great variation in prevalence of endometritis. This may be due to the variation in management practice. The high non-significant prevalence of SCE in smallholder farms can be attributed various reasons. For example, from the follow up result, we found that the absence of regular

exercise has an association with subclinical endometritis and confinement in the tie stall for a longtime throughout the production period, poor hygienic condition of the barns and cleanliness of the cows, absence of separate maternity pen in most of these farms may be responsible for the high incidence of SCE, particularly in smallholder dairy producers.

This study found no effect of parity on prevalence of subclinical endometritis. This agrees with the result of Gilbert *et al.* (2005). In contrast, LeBlanc *et al.* (2002) found that clinically relevant endometritis was more prevalent in mature cows. Cows in the third or higher lactation had a prevalence of 21%, compared to 12 and 13% for first and second-lactation cows respectively. Although not significant, in this study, cows with first-parity had a prevalence of 56.2% compared to 36.8 % in cows with second parity and 50.0% in those with their third or higher parity at 4<sup>th</sup> week postpartum. This could be due to higher stress of pregnancy and parturition in heifers. Several other diseases and conditions of postpartum cows have been found to increase in prevalence with advancing parity (Gilbert *et al.*, 1993) and an effect of parity on periparturient immune function has been established (Hammon *et al.*, 2001). However, the higher incidence rate of SCE in first calf heifer may be due to first time exposure of these cows for parturition hence their uterine environment could be new for contamination by microorganisms in the postpartum period. At the 8<sup>th</sup> week PP still 50.0% of the dairy cows with first parity were positive for subclinical endometritis whereas in the case of dairy cows with parity of two (15.8%) and three and above (29.2%) there was a higher reduction in the prevalence.

Substantial impairment of reproductive performance of SCE affected cows was reflected by delayed calving to first service interval, extension of days open, reduction in overall pregnancy rate, increased calving to conception interval and higher number of services per conception. In addition, in this particular study it was observed that the presence of subclinical endometritis prolong the median calving to first service interval and days open. Similarly, it had also been reported that SCE prolongs the interval from calving to first service, calving to conception interval, decreases first service conception rate and increases NSPC (Raab *et al.*, 2003; Gilbert *et al.*, 2004).

In this study it was observed that there was a significant difference in first service conception rate between subclinical endometritis positive and negative cows. The first service conception rate in dairy cows that were positive for subclinical endometritis at 8<sup>th</sup> week postpartum was

only 3.4% whereas those cows, which were negative for subclinical endometritis, had a higher (27.1) first service conception rate. But being positive or negative for subclinical endometritis at around the 4<sup>th</sup> week postpartum had no effect on FSCR. Gilbert *et al.*, (2005) also reported that the major contributor to impaired reproduction in cows with subclinical endometritis to be a severe reduction in first service conception rate (FSCR).

From dairy cows that were positive for subclinical endometritis at the 4<sup>th</sup> week postpartum, 46.4% of them were also positive for subclinical mastitis. However, in those cows negative for subclinical endometritis only 16.1% had subclinical mastitis. Dairy cows that were negative for SCE at the 8<sup>th</sup> week postpartum the majority of them (78.0%) were also negative for subclinical mastitis. Thus, these results indicate the direct strong association between subclinical endometritis and subclinical mastitis.

In this study, relevant risk factors such as body condition score and management were included and it was found that body condition status at the 4<sup>th</sup> week of postpartum had a considerable effect on the incidence of SCE. At this stage of postpartum period, from cows that had a body condition score of < 2.5, 63.3% of them were positive for SCE compared to 28.6% in those cows with a body condition score of 2.5 – 3.5. According to this observation the body condition score at calving or at the 8<sup>th</sup> week of postpartum has no effect on the occurrence of subclinical endometritis. The high incidence of subclinical endometritis at around the 4<sup>th</sup> week of postpartum may be attributed to the negative energy balance or establishment of a luteal phase, which down regulates uterine immunity (Lewis, 2003). Negative energy balance in early lactation requires cows to mobilize body tissue in support of lactation (de Vries and Veerkamp, 2000). A study by Hammon *et al.* (2002) describes, cows with subclinical endometritis had significantly lower dry matter intake (DMI) from 1 week before calving to week 5, higher nonesterified fatty acids (NEFA) from 2 week - to wk 4, and beta-hydroxybutyrate (BHBA) from wk 1 to wk 4, compared to cows without subclinical endometritis. Negative energy balance and excessive body tissue mobilization are associated with increased incidence of metabolic disorders and poor fertility (Loeffler *et al.*, 1999)

In this study we considered the different management factors that could be a risk for the occurrence of subclinical endometritis. However, because of lack of proportionality in the number of the different farms observed, the risk factors considered on the cow level. Management factors like post-calving hygiene, washing frequency and access to regular

exercise were considered. Although post calving hygiene had no significant association with the prevalence of subclinical endometritis, 58.1% of the cows with good post calving hygiene were negative for subclinical endometritis. Out of the 28 cows that were positive for subclinical endometritis at the 4<sup>th</sup> week of postpartum, 46.4% have no regular exercise and confinement in the tie stall barn throughout the production period may be act as a stress.

## 6. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, subclinical endometritis may occur in many postpartum cows previously presumed to be normal. Subclinical endometritis diagnosed by endometrial cytology in the postpartum period was highly prevalent and resulted in poor reproductive performance. The results of this study indicate that assessment of endometrial cytology will assist in the identification of animals with subclinical endometritis that will benefit from early prevention and treatment. Subclinical endometritis is also strongly associated with subclinical mastitis and mastitis control program can reduce the risk of subclinical endometritis or vice versa.

Parity had no effect on the prevalence of SCE and it was observed that there was no strong association between the occurrence of subclinical endometritis in the different farming scales (herds); this could be attributed to the minimum difference in management practices. However, management risk factors like poor hygiene, absence of regular exercise and covariates like poor body condition score had an association with the occurrence of subclinical endometritis particularly at around the 4<sup>th</sup> week of postpartum.

In line with the above conclusion the following recommendations are forwarded:

- Postpartum reproductive events and reproductive performance of dairy cows should be thoroughly followed since subclinical endometritis have a detrimental effect on reproductive performance of dairy cows.
- Management problems like absence of regular exercise and poor calving hygiene (post calving hygiene) must be considered very well since general hygienic conditions could be the root of the problem.
- Further study should be done on relatively large sample size and different breeds by combining the endometrial cytology with ultrasonography to ameliorate out put of the result.

## 7. REFERENCES

- Aiello, S. E. and Mays, A. (1998): Metritis and Endometritis in Large Animals. The Merck Veterinary Manual. 8<sup>th</sup> ed., Merck and co. Inc., USA, Pp 1017.
- Arthur, G. H., Noakes, D. E. and Pearson, H. (1989): None specific causes of infertility in dairy cows. In: Veterinary reproduction and obstetrics. 6<sup>th</sup> ed., Lea and Febiger, Philadelphia, Pp 386-389.
- Azage, T. and Alemu, G. W. (1997): Prospects for peri-urban dairy development in Ethiopia. In: Proceedings of the Fifth National Conference of Ethiopian Society of Animal Production, May 15-17, 1997, Addis Ababa, Ethiopia, Pp 55 - 70.
- Azage, T., Million, T., Yoseph, M. and Alemu, Y. (2000): Market oriented urban and peri – urban dairy production system. Urban Agricultural Magazine (The Netherlands) Pp 23-24.
- Ball, B. A. (1988): Use of a low-volume uterine flush for microbiologic and cytologic examination of the mare's endometrium. *Theriogenology*, **29**:1269 - 1283.
- Bartlett, P. C., Kirk, J. H., Wilkes, M. A., Kaneene, J. B. and Mather, E. C. (1986): Metritis complex in Michigan Holstein Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. *Prev. Vet. Med.*, **4**: 235.
- Bekana, M., Jonsson, P., Ekman, T. and Kin Dahl, H. (1994): Intrauterine bacterial findings in postpartum cows with retained fetal membranes. *J. Vet. Med.*, **41**: 663 - 670.
- Bondurant, R. H. (1999): Inflammation in the bovine female reproductive tract. *J. Anim. Sci.*, **77**:101 - 110.
- Bourke, M., Mills, N., Barnes, L. (1997): Collection of endometrial cells in the mare. *Aust. Vet. J.*, **75**: 755 - 758.
- Butt, B. M., Senger, P. L. and Widders, P. R. (1991): Neutrophil migration into the bovine uterine lumen following intrauterine inoculation with killed *Haemophilus somnus*. *J. Repro. Fert.*, **93**: 341 - 345.
- Cheville, N. (1988): Pathogenesis of acute inflammation. In: Introduction to Veterinary Pathology. Iowa State Univ. Press, USA, Pp 309 - 330.
- CSA (2001): Central statistical authority, Federal democratic Republic of Ethiopia, central statistical investigatory, statistical abstract, 2001.
- Cunningham, J. (2002): Physiology of reproductive system. In: Textbook of veterinary physiology. 3<sup>rd</sup> ed., W. B. Saunders Co., Philadelphia, Pp 402.

- Curtis, C. R., Erb, H. N., Sniffen, C. J., Smith, R. D. and Kronfeld, D. S. (1985): Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and Mastitis in Holstein cows. *J. Dairy Sci.* **68**: 2347 - 2360.
- de Vries, J., and Veerkamp, F. (2000): Energy balance of dairy cattle in relation to milk production variables and fertility. *J. Dairy Sci.*, **83**:62 - 69.
- DeBois, C.W., and Manspeaker, J. E. (1986): Endometrial biopsy of the bovine. In: Current therapy in Theriogenology. 2<sup>nd</sup> ed., W. B. Saunders Co., Philadelphia, PA Pp 424 - 426
- Del Vecchio, R. P., Matsas, D. J., Fortin, S. D., Sponenberg, P. and Lewis, G. S. (1994): Spontaneous uterine infections are associated with elevated prostaglandin F<sub>2α</sub> metabolite concentrations in postpartum dairy cows. *Theriogenology* **41**: 413
- Dhaliwal, G. S., Murray, R. D., Dobson, H., (1996): Effects of milk yield, and calving to first service interval, in determining herd fertility in dairy cows. *Anim. Reprod. Sci.*, **41**(2): 109 - 117.
- Dijkhuizen, A. A. and Stelwagen J. R. (1985): Economic aspects of reproductive failure in dairy cattle. I. Financial loss at the farm level. *Preventive Vet. Med.*, **3**: 251 - 263.
- Drillich, M. (2006): Review paper: An update on uterine infections in dairy cattle. *Slov. Vet. Res.*, **43** (1): 11 - 15
- Drillich, M., Kaufmann, T., Raab, D., Lenz, M. and Heuwieser, W. (2004): Comparison of new techniques for the diagnosis of chronic endometritis in dairy cattle. *Abstract 23<sup>rd</sup> World Buiatrics Congress*, Quebec city, Canada, 11-16<sup>th</sup> July 2004. Amsterdam Elsevier.
- Ferguson, J. D, and Galligan, D. T. (2000): Assessment of reproductive efficiency in dairy herds. *Compend Contin Ed. Prac. Vet.*, **22**: 150 - 158.
- Ferguson, J. D., and Galligan, D. T. (1999): Veterinary Reproductive Programs. In: Proceedings of 32<sup>nd</sup> Annual Conference of American Association of Bovine Practitioner. Pp 131 - 137
- Fertrow, J., McClary, D., Butcher, K., and Williamson, N. (1990): Calculating reproductive indices: Recommendations of the American association of bovine practitioners. *J. Dairy Sci.*, **73**(1): 78 - 90.
- Fredriksson, G., Kindahl, H., Alenius, S., Carlsson, U., Cort, N., and Uggal, A. (1988): Hormonal and physiological factors, which influence the establishment of infection in the uterus. In: Proceedings of the 11<sup>th</sup> International Congress on Animal Reproduction and Artificial Insemination, Dublin, Ireland, 26 - 30<sup>th</sup> June 1988.

- French, P. D. and Nebel, R. L. (2003): The simulated economic cost of extended calving intervals in dairy herds and comparison of reproductive management programs. *Abstract J. Dairy Sci.*, **86**: 54.
- Galeotti, M., Belluzzi, S., Volpatti, D., Bergonzoni, L., D'Agaro, E., and Volpelli, A. (1997): Evaluation of mast cells in calf and heifer uteri. *Theriogenology*, **48**: 1301 - 1311.
- Gilbert, R. O., Grohn, Y. T., Miller, P. M. and Hoffman, D. J. (1993): Effect of parity on periparturient neutrophil function in dairy cows. *Vet. Immuno. Immunopatho.*, **36**: 75 - 82.
- Gilbert, R., Frajbiat, M., Guard, C.L., Erb, H. N. and Roman, H. (2004): The effect of sub clinical endometritis and routine treatment with prostaglandin F<sub>2α</sub> on reproductive performance of dairy cows. In: 23<sup>rd</sup> World Buiatrics Congress, July 11 - 16<sup>th</sup> 2004 Quebec city, Canada, [Abstract] Pp 11.
- Gilbert, R., Shin, S. T. and Guard, C. L. (2005): Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*, **64**: 1879 - 88.
- Gilbert, R., Shin, S. T., Guard, C. L. and Erb, H. N. (1998): Incidence of endometritis and effects on reproductive performance of dairy cows [Abstract] *Theriogenology*, **49**: 251
- Hafez, E.S. (1993): Reproduction in farm animals. 6<sup>th</sup> ed., Le and Febiger, Philadelphia, Pp 284 - 285
- Hammon, D. S., Dhiman, R. and Goff, J. P. (2002): Negative energy balance during the periparturient period is associated with uterine health disorders and fever in Holstein cows. [Abstract] *J. Anim. Sci.*, **82**: 279 - 282.
- Hammon, D., Holyoak, G., Jenson, J. and Bingham, H. (2001): Effects of endometritis at the beginning of the breeding period on reproductive performance in dairy cows. In: Proceedings of the 34<sup>th</sup> Annual Conference, Vancouver. [Abstract] *Am. Assoc. Bov. Pract.* Pp 142 - 143.
- Heuwieser, W., Tenhagen, B. A., Tischer, M., Luhr, J. and Blum, H. (2000): Effect of three programs for the treatment of endometritis on the reproductive performance of a dairy herd. *Vet. Rec.*, **146**: 338 - 341.
- Jones, T. C., Hunt, R. D. and King, N. W. (1997): Pathology of Genital system. In: Veterinary pathology. 6<sup>th</sup> ed., Williams and Wilkins, Philadelphia, Pp 1173 - 1177
- Kasimanickam, R., Duffield, T. and Foster, R. (2004): Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, **62**: 9 - 23.

- Kasimanickam, R., Duffield, T., Foster, R., Gartley, C., Leslie, K. Walton, J. and Johnson, W. (2004): A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. *Can. Vet J.*, **46**(3): 255 - 259.
- Ke`uther, K., Audiga, L., Kube, P., and Well, M. (1998): Bovine mast cells: distribution, density, heterogeneity, and influences of fixation techniques. *Cell and Tiss. Res.* **293**: 111 - 119.
- Kelay, B. (2002): Analysis of dairy cattle breeding practiced in selected areas of Ethiopia. PhD thesis, Department of animal breeding in the tropics and sub tropics, Humboldt University of Berlin, Berlin.
- Ketema, H. and Tsehay, R. (1995): Dairy production System in Ethiopia. In: proceedings of a workshop entitled: Strategies for market orientation small-scale milk producers and their organizations, March 20 - 24, 1995, Morogono, Tanzania.
- Kindahl, H., Edqvist, L. Larsson, K. and Malqvist, A. (1982): Influence of prostaglandin on ovarian function postpartum. In: *Current topics in veterinary medicine and animal science*, **20**: 173 - 196.
- Klucinski, W., Dembele, K., Kleczkowski, M., Sitarska, E., Winnicka, A., and Sikora, J. (1995): Evaluation of the effect of experimental cow endometritis on bactericidal capability of phagocytizing cells isolated from the blood and uterine lumen. *J. Vet. Med.* **42**: 461 - 466.
- Klucinski, W., Targowski, S., E., Dego`rska, M. and Winnicka, A. (1990): The phagocytic activity of polymorphonuclear leucocytes isolated from normal uterus and that with experimentally induced inflammation in cows. *J. Vet. Med.* **37**: 506 - 512.
- Lamming, G. E., Wathes, D. C. and Peters, A. R. (1981): Endocrine patterns of the postpartum cow. *J. Repro. and Fert.*, **30**: 155 - 170.
- LeBlanc, S. J., Duffield, T. F., Leslie, K. E., Bateman, K. G., Keefe, G. P., Walton, J. S. and Johnson, W. H. (2002): Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci.*, **85**: 2223 - 2236.
- Lemma, M., Kassa, T. Azage, T. (1999): Major health problems of dairy cattle in market oriented urban and peri-urban production systems in the central highlands of Ethiopia. In: Proceedings of the seventh annual conference of Ethiopian Society of Animal Production, May 26-27, 1999, Addis Ababa, Ethiopia, Pp 353 - 363.

- Lenz, M., Drillich, M., Heuwieser, W., (2003): Ultrasound examination in postpartum dairy cows. *Wien Tierärztl Monatsschr*; 90(Suppl.1), 18 In: Drillich, M. (2006): An update on uterine infections in dairy cattle, *a review Paper Slov. Vet. Res.*, **43** (1): 11 - 15
- Leslie, K. E. (1983): The events of normal and abnormal postpartum reproductive endocrinology and uterine involution in dairy cows. *A review. Can Vet. J.* **24**: 67 - 71.
- Lewis, G. (1997): Uterine Health and Disorders. In: Health problems of the postpartum cow. *J. Dairy Sci.* **80**: 984 - 994.
- Lewis, G. S. (2003): Steroid regulation of uterine resistance to bacterial infection in livestock. *Reprod. Biol. Endocrinol.* **1**: 47
- Loeffler, H., de Vries, J. and Schukken, H. (1999): The effects of time of disease occurrence, milk yield, and body condition on fertility of dairy cows. *J. Dairy Sci.* **82**: 2589-2604.
- Louca, A., Legates, E. (1968): Production losses in cattle due to days open. *J. Dairy Sci*, **51**: 573-583.
- Matthew man, R., W (1993): Dairying. Center for tropical veterinary medicine. University of Edinburgh, McMillan press Ltd, Scotland, UK. Pp 67 - 68.
- Miller, V., Kimsey, B., Kendrick, W., (1980): Endometritis of dairy cattle: Diagnosis, treatment and fertility. *The Bov. Pract.*, **5**: 13 - 23.
- Mohamed, A.M.A., Simeon, E. and Yemesrach, A. (2004): Dairy Development in Ethiopia. International Food Policy Research Institute, Environment and Production Division. EPTD Discussion paper No123.
- Nakao, T., Gamal, A., Osawa, T., Nakada, K., Moriyoshu, M. and Kewata, K. (1997): Postpartum plasma PGF<sub>2α</sub> metabolite profile in cows with dystocia and/or retained Placenta and effect of fenoprostalene on uterine involution and reproductive Performance. *J. Vet. Med. Sci.*, **59**: 791 - 794.
- Nebel, R. L. (1999): Optimizing fertility in the dairy herd. In: Proceedings of the annual. Conference of Society of Theriogenology, Nashville, TN, USA, Pp 443 - 452.
- NMSA (2003): National meteorology service agency. Addis Ababa, Ethiopia
- Noakes, D. E., Parkinson, T. J. and England, G. C. (2001): Arthur's Veterinary Obstetrics. 8<sup>th</sup> ed., W. B. Saunders, Philadelphia, Pp 189 -202.
- Olson, J. D., Bretzlaff, K. N., Mortimer, R. G. and Ball, L. 1986. The Metritis-Pyometra Complex Diagnosis and Treatment. *Theriogenology*, **2**: 21.

- Oltenucu, A., Rounsaville, R., Milligan, A., Foote, H. (1981): Systems analysis for designing reproductive management programs to increase production and profit in dairy herds. *J. Dairy Sci*, **64**: 2096 - 2104.
- Plazier, J. C., King, G. J., Dekkers, C. M. and Lissemore, K. (1997): Estimation of economic values of indices for reproductive performance in dairy herds using computer simulation. *J. Dairy Sci*. **80**, 2775 - 2783.
- Quinn, P.T., Carter, M.E., Markey, B. and Carter, G. R. (1994): Clinical veterinary microbiology. Wolfe Pub. Spain, Pp. 127 - 156
- Raab, D., Drillich, M., and Heuwieser, W. (2003): Diagnosis of subclinical endometritis and its effect on reproductive performance. In: 36th Annual conference of the American Association of Bovine Practitioners, Columbus, Ohio/USA, Pp 166.
- Sheldon, I. M. and Dobson, H. (2004): Postpartum uterine health in cattle. *J. Anim. Repro. Sci*. **83**: 295 - 306
- Smith, B. P. (1996): Uterine infections and Mechanisms resulting in Irregular cyclicity In: Large animal internal medicine 2<sup>nd</sup> ed., Mosby-year book Inc., USA California, Pp 1535 - 1541.
- Tibbits, F. D., Formigli, L., Foote, W. D., Randel, R. D. and R. P. Del Vecchio. (1989): A suggested role for mast cell heparin in prostaglandin synthesis in bovine endometrium. *Proc. Western Section, Am. Soc. Anim. Sci*. **40**,337 - 338.
- Tizard, I. R. (1996): Hypersensitivity. In: Veterinary Immunology: An Introduction. 3<sup>rd</sup> ed., W. B. Saunders Co., Philadelphia, Pp 343 - 401
- Tizard, I. R. (1998): Immunity of the urogenital tract and Immunity at body surfaces. In: Veterinary Immunology. 5<sup>th</sup> ed., W. B. Saunders Co., Philadelphia, Pp 262.
- Trowbridge, H. O. and Emling, F. E. (1997): Hypersensitivity reactions. In: Inflammation: A Review of the Process. 5<sup>th</sup> ed. Quintessence Publishing Co., Inc., Chicago, IL, Pp111 - 127
- van Amburgh, E., Galton, D., Bauman, D. and Everett, R. (1997): Management and economics of extended calving intervals with use of bovine somatotropin. *Livestock Production Science*, **50**: 15 - 28.
- Velez, J. S., Randel, R. D. and Neuendorff, D. A. (1992): Effect of uterine manipulation on postpartum fertility and plasma 13, 14-dihydro-15-keto prostaglandinF<sub>2α</sub> in Brahman cows and first calf heifers. In: 12<sup>th</sup> international congress on animal reproduction, August 23 - 27, 1992. The Hague Netherlands.

- Washburn, P., Silvia, C., Brown, H., McDaniel, B. and McAllister, A. (2002): Trends in reproductive performance in Southeastern Holstein and Jersey DHI herds. *J. Dairy Sci.*, **85**: 244 - 251.
- Watson, E. D. (1985): Oponising ability of bovine uterine secretions during the oestrous cycle. *Vet. Rec.*, **117**: 274 - 275.
- Watson, E. D. (1989): In vitro function of bovine neutrophils against *Actinomyces pyogenes*. *Am. J. Vet. Res.*, **50**: 455 - 458.
- Wingfield-Digby, N. (1987): The technique and clinical application of endometrial cytology in mares. *Equine Vet. J.*, **10**: 176 - 180.
- Wubet, S. (2005): Study on semen quality and field efficiency of AI bulls kept at National Artificial Insemination center. MSc thesis, Addis Ababa University Faculty of Veterinary Medicine Debre Zeit, Ethiopia,
- Yoseph, M., Azage, T., Alemu, Y. and Ummuna, N. (2000): Reproductive management and reproductive performance of dairy herds in urban periurban dairy production systems in Addis Ababa milk shed. In: Proceedings of the 18<sup>th</sup> annual conference of ESAP, August 24 - 26, 2006, Addis Ababa, Ethiopia.
- Youngquist, R. S. and Little, T. A. (1988): Anoestrus and Infertility in the Cow. In: Fertility and Infertility in Veterinary Practice, 4<sup>th</sup> ed., Laing, J.A., Brinley, W.J. and W.C., Wanger, Baillier Tindall, Philadelphia, Pp 210 - 215.
- Zerbe, H., Schuberth, H. J., Hoedemaker, M., Grunert, E. and Leibold, W. (1996): A new model system for endometritis: Basic concepts and characterization of phenotypic and functional properties of bovine uterine neutrophils. *Theriogenology*, **46**: 1339 - 1356

## 8. ANNEXES

### Annex 1. Individual dairy cow follow up and collected sample findings recording format

Date \_\_\_\_\_  
Sample code \_\_\_\_\_

Owners/ Farm/ name \_\_\_\_\_  
Address \_\_\_\_\_  
Animal identification: ID. No. \_\_\_\_\_ Age \_\_\_\_\_ Calving date \_\_\_\_\_  
Breed \_\_\_\_\_ Parity No. \_\_\_\_\_  
Nickname of the cow \_\_\_\_\_

#### Endometrial cytology

4<sup>th</sup> week \_\_\_\_\_

8<sup>th</sup> week \_\_\_\_\_

#### Laboratory result

\_\_\_\_\_

\_\_\_\_\_

#### Information about recent reproductive performance

Calving to first service interval \_\_\_\_\_

Days open (Calving to conception interval) \_\_\_\_\_

Number of services per conception (NSPC) \_\_\_\_\_

First service conception rate \_\_\_\_\_

Pregnancy rate with in 180 days in milk (150-180 days) \_\_\_\_\_

Cow ID	Previous calving date	*First service date	Confirmation of recent pre
		__ __/__ __/__ __	*→
		__ __/__ __/__ __	
		__ __/__ __/__ __*	

#### Body condition score (BCS)

BCS at calving \_\_\_\_\_

BCS 4<sup>th</sup> week \_\_\_\_\_

BCS 8<sup>th</sup> week \_\_\_\_\_

BCS at first service \_\_\_\_\_

Remarks (Notes) \_\_\_\_\_

#### Mastitis: Physical examination of the udder

Conformation even \_\_\_\_\_ uneven \_\_\_\_\_

Teat lesion present \_\_\_\_\_ absent \_\_\_\_\_

Tick infestation present \_\_\_\_\_ absent \_\_\_\_\_

#### Gross milk quality

Watery \_\_\_\_\_ blood tinged \_\_\_\_\_ Clots/ flakes \_\_\_\_\_

Normal \_\_\_\_\_

#### CMT score (0, T, 1, 2, and 3):

RF \_\_\_\_\_ RR \_\_\_\_\_ LF \_\_\_\_\_ LR \_\_\_\_\_

Forms of mastitis: Clinical \_\_\_\_\_ Sub clinical \_\_\_\_\_

Remarks \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## **Routine follow up activity**

### Reproductive tract

- Diagnosis of endometritis and avoiding clinically positive cows from the study population
- Vaginoscopy (using vaginal speculum)
- Presence and nature of uterine discharge (on the tail, perineum and vulva)

**Annex 2.** Questionnaire survey format and observed management practice recording

Date \_\_\_\_\_

Questionnaire code \_\_\_\_\_

**I. Owners and animal identification**

Owners / Farm name \_\_\_\_\_

Address \_\_\_\_\_

Occupation \_\_\_\_\_

Animal identification: ID. No. \_\_\_\_\_ Age \_\_\_\_\_

Breed \_\_\_\_\_ Parity No. \_\_\_\_\_

Nickname of the cow \_\_\_\_\_

**II. Composition of dairy holdings holding:**  Calves \_\_\_\_\_  Bull \_\_\_\_\_

Heifers \_\_\_\_\_

Primiparous cows \_\_\_\_\_

Pluriparous cows \_\_\_\_\_

➤ Number of dairy cows (Herd size) \_\_\_\_\_

Large scale (> 50)  Medium scale (5-50)  small scale (<5)

**III. The management decision on when to start breeding**

1. Interval from calving to first service? (Mean interval from calving to first service)

Two months  three months  four months

2. Interval from calving to first conception (DO) \_\_\_\_\_

\*3. Conception at first service (first service conception)  Yes  No

\*4. What was the number of services perconception? \_\_\_\_\_

\*5. Parity No. \_\_\_\_\_

**IV. Dairy cow management**

*a. Housing management*

6. What type of house do you provide to your dairy cattle?

Open barn  Closed lose housing  Tie stalls  Others \_\_\_\_\_

7. What is the building material used? \_\_\_\_\_

\*8. What is the floor type?  Concrete  Stone layer  others

\*9. Do houses provide sufficient space and maternity pen?

9.1. Degree of crowding (stocking density)

Very good (above average)  good (average)  Poor (below average)

9.2. Maternity pen  yes  No

10. Manure removal frequency? \_\_\_\_\_

11. Hygiene of the pen  Good  Bad

12. Do you provide bedding material to your dairy cows?  Yes  No

Type of bedding material? \_\_\_\_\_

13. Do you practice washing of dairy cows?  Yes  No How often? \_\_\_\_\_

13.1. Cleanliness of the animals

Very good (above average)  Good (average)  Poor (below average)

14. Hygiene maintenance at calving  V. good  good (average)  Poor  
Post-calving hygiene  V. good  good (average)  Poor

*b. Other management aspects*

15. Do the animals receive regular exercise?  Yes  No

16. Do you practice regular deworming and vaccination? To which parasites and diseases

---

---

17. How do you perform heat detection, how often and what are the major indicator signs of heat? \_\_\_\_\_

18. What is the method of service to your dairy cows?  AI  Bull  Both

How do you decide the timing of service with regards to detected estrus? \_\_\_\_\_

---

Good heat detection and  Poor heat detection

Annex 3. Interpretation of the California mastitis test result and the somatic cell count and formulas for some reproductive performance measures

CMT score	Interpretation	Visible reaction	Total cell count
0	Negative	Milk fluid and normal	0-200,000 0-25% neutrophils
T	Trace	Slight precipitation	150000-500000 30-40% neutrophils
1	Weak Positive	Distinct precipitation but not gel formation	400000-1500000 40-60% neutrophils
2	Distinct positive	Mixture thickens with gel formation	800000-5000000 60-70% neutrophils
3	Strong positive	Viscosity greatly increased. Strong gel that is cohesive with a convex surface	$\geq 5000000$ 70-80% neutrophils

**Reproductive performance measures**

Parameter	Definition
Service rate	$\frac{\text{Cows inseminated 72 - 92 DIM}}{\text{Number of cows} > 72\text{DIM}} \times 100$
Days to first service	Date of first service – Date of calving
Days open	Date of successful AI – Date of calving
First service conception rate	$\frac{\text{Cows pregnant to 1st AI}}{\text{No of cows inseminated}} \times 100$
Conception rate to further services	$\frac{\text{Cows pregnant to 2nd or greater AI}}{\text{Total No of AI} - \text{No of 1st AI}} \times 100$
Conception rate to all services	$\frac{\text{No: of cows pregnant}}{\text{Total No of AI}} \times 100$
Cows pregnant within Y DPP	$\frac{\text{No of cows pregnant within Y DPP}}{\text{Total No of cows enrolled}}$

#### Annex 4. Body Condition Scoring System

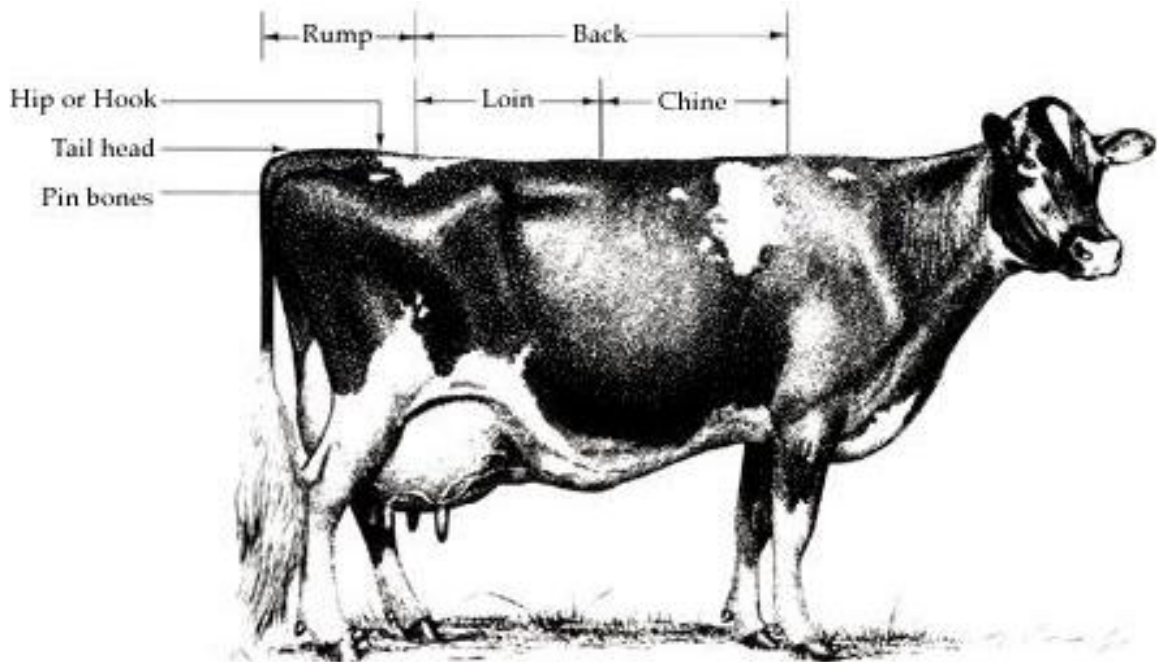


Fig 3. Anatomical regions used for determining BCS

##### ***Score of 1 (very poor body condition)***

- Individual short ribs have a thin covering of flesh.
- Bones of the chine, loin, and rump regions are prominent.
- Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones.
- Deep cavity under tail and around tail head (between pin bones)
- Bony structure protrudes sharply, and ligaments and vulva are prominent.

##### ***Body condition score 2 (poor body condition)***

- Individual short ribs can be felt but are not prominent.
- Ends of ribs are sharp to the touch but have a thicker covering of flesh.
- Short ribs do not have as distinct an "overhanging shelf" effect.
- Individual bones in the chine, loin, and rump regions are not visually distinct but are easily distinguished by touch.
- Hook and pin bones are prominent, but the depression between them is less severe.
- Area below tail head and between pin bones is somewhat depressed, but the bony structure has some covering of flesh.

***Body condition score 3 (good body condition)***

- Ends of short ribs can be felt by applying slight pressure.
- Short ribs appear smooth and the overhanging shelf effect is not so noticeable.
- The backbone appears as a rounded ridge; firm pressure is necessary to feel individual bones.
- Hook and pin bones are rounded and smooth.
- Area between pin bones and around tail head appears smooth, without signs of fat deposit.

**Body condition score 4 (fat)**

- Individual short ribs are distinguishable only by firm palpation.
- Short ribs appear flat or rounded, with no overhanging shelf effect.
- Ridge formed by backbone in chine region is rounded and smooth.
- Loin and rump regions appear flat.
- Hooks are rounded and the span between them is flat.
- Area of tail head and pin bones is rounded, with evidence of fat deposit.

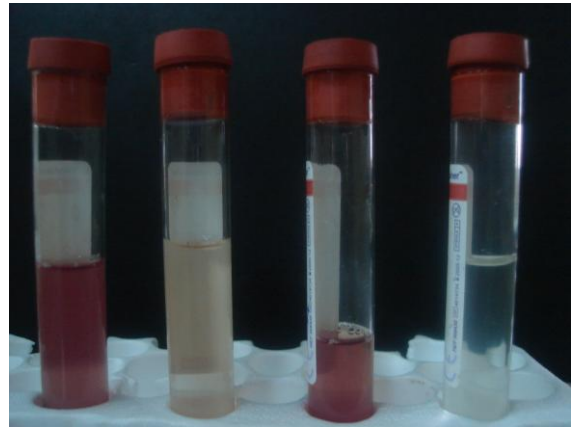
***Score of 5 (very fat)***

- Bony structures of backbone, short ribs, and hook and pin bones are not apparent; subcutaneous fat deposit very evident.
- Tail head appears to be buried in fatty tissue.

**Annex 5.** Different plates taken during the study period



**Plate 1.** Uterine lavage technique for collecting endometrial sample



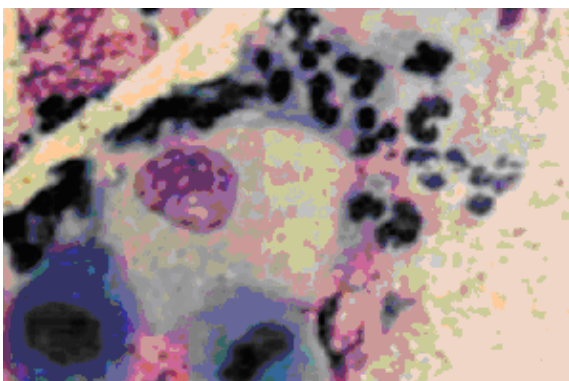
**Plate 3.** Uterine lavage samples taken at the 4<sup>th</sup> and around 8<sup>th</sup> weeks of postpartum



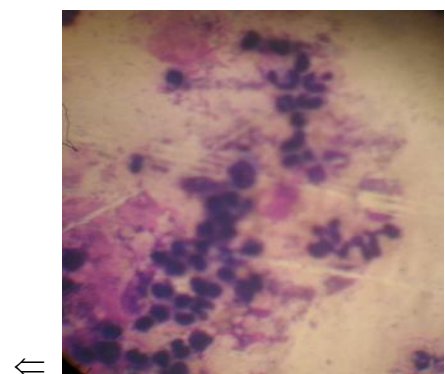
**Plate 2.** Uterine lavage samples taken at the 4<sup>th</sup> week postpartum



**Plate 4.** Sample after centrifugation (look at the sediment)



**Plate 5.** Neutrophils in smear from uterine lavage sample, Giemsa stain X 1000 magnification





**Plate 3.** Large-scale dairy farm tie stall housing with cubicle



**Plate 6.** Small scale (Small holder) Dairy farms



**Plate 4.** Medium scale dairy farm with poor barn hygiene



**Plate 7.** Medium scale dairy farm with poor barn hygiene



**Plate 5.** Medium scale dairy farm with poor barn hygiene



**Plate 8.** Medium scale dairy farms with poor barn hygiene



**Plate 9.** Medium scale dairy farm with good barn hygiene



**Plate 10.** Dairy cows having a sort of exercise at shading.



**Plate 12.** Dairy cows at shading



**Plate 11.** Tie stall housing with cubicle (Medium scale)



**Plate 13.** Tie stall housing without cubicle (Medium scale)

Annex 6. Map of the study area (Map of Debre Zeit)

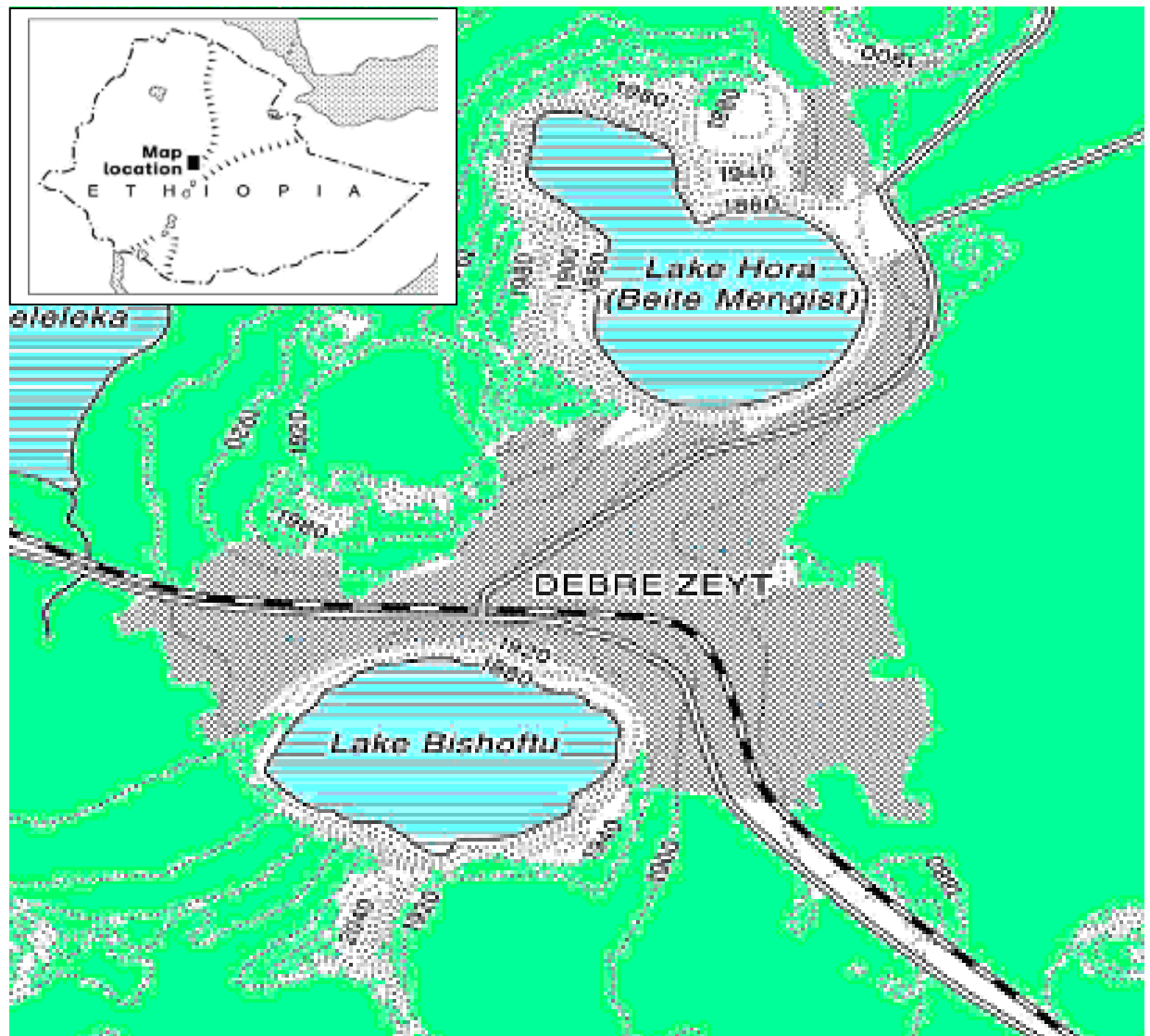


Table 12. Effect of body condition on subclinical endometritis at 49-52 days postpartum

Scoring period	8 <sup>th</sup> week postpartum		P- value
	Subclinical endometritis	Normal cows	
BCS at calving at calving			
2.5 –3.5	12	23	0.356
< 2.5	3	4	
> 3.5	3	14	
BCS at 4 <sup>th</sup> week PP			
2.5 –3.5	5	23	0.059
< 2.5	12	18	
> 3.5	1	1	
BCS at 8 <sup>th</sup> week PP			
2.5 –3.5	4	19	0.204
< 2.5	13	21	
> 3.5	1	1	

**Annex 7.** Some of the follow up and Questionnaire results

Table 13. Some of the follow up observation and questionnaire results

Management practice		Small holders N= 17 (%)	Medium scale N= 12 (%)	Large scale N= 3 (%)
Farm program to start breeding after				
calving:	45 – 60 days	–	3 (25)	2 (66.7)
	60 – 90 days	17 (100)	9 (75)	1 (33.3)
	> 90 days	–	–	–
Type of house:	Tie stall with cubicle	3 (17.6)	3 (25)	3 (100)
	Tie stall without cubicle	14 (82.3)	9 (75)	–
Floor type:	Stone layer	2 (11.8)	–	–
	Concrete	15 (88.2)	12 (100)	3 (100)
Sufficient space:	yes	1 (5.9)	4 (33.3)	–
	No	16 (94.1)	8 (66.7)	3 (100)
Maternity pen:	Yes	3 (17.6)	2 (16.7)	3 (100)
	No	14 (82.3)	10 (83.3)	–
Hygiene of the pen:	Good	2 (11.8)	8	2
	Poor	15 (88.2)	4	1
Method of services	AI only	–	1 (8.3)	2
	Bull only	–	–	–
	Combined (AI+ Bull)	17 (100)*	11 (91.7)	1 (33.3)

\* Smallholders use communal bull from bull service providers found in the surroundings

**Annex 8.** Subclinical endometritis survey, follow up and uterine lavage test result recording sheet

## **9. CURRICULUM VITAE**

### **I. Personal data**

Name	Belachew Bacha
Date of Birth	June 20, 1981 G.C.
Place of birth	Addis Ababa
Sex	Male
Nationality	Ethiopian
Marital status	Single
Religion	Orthodox Tewahido
Language Proficiency	Amharic, Oromifa and English
Address:	Mobil. 251911 391700 Home: 251113486905, e-mail bell_wsb@yahoo.com

### **II. Educational background**

1980 - 1987 E.C.	Repi elementary and junior secondary school, Addis Ababa
1987-.1991 E.C.	Ayertena senior secondary school, Addis Ababa
1992 E.C.	Addis Ababa University, Faculty of Science, Addis Ababa (Fresh man courses).
1993-1997 E.C.	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Courses of general Veterinary medicine).
1998-1999 E.C.	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit (Master of Veterinary Science in Veterinary obstetrics and gynecology)

### **III. Research paper**

Cattle fattening practice in Nazareth and Its surroundings

**Other papers:**

- Principal Anti Fungal Drugs and Their Applications In Veterinary Medicine. (Seminar paper 2004)
- Effects of Sub Clinical Endometritis on Reproductive Performance of Dairy Cows. (2006)

**IV. Additional trainings and certificates**

Computer literacy: Basic computer application software courses, June/2003-December 15/2004 (Certificate).

**V. Work experience**

As externship student from September, 2004-June, 2005 in Adama (Nazareth), abattoir work experience in the municipality abattoir; laboratory and clinical work activities at the Adama district veterinary clinic.

## **SIGNED DECLARATION SHEET**

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Belachew Bacha

Signature \_\_\_\_\_

Date of submission \_\_\_\_\_

This thesis has been submitted for the examination with my approval as advisor.

Dr. Fekadu Regassa (DVM, PhD, Asso. Proff.)

Signature \_\_\_\_\_