

Review on Subclinical Endometritis In Cows and Its Effect on Fertility in and around GondarAbebe Mequanent¹ and Nibret Moges²¹ University of Gondar College of Veterinary Medicine and Animal Science, Department of Veterinary Clinical Medicine, Gondar, Ethiopia, P.o. Box: 196² University of Gondar, college of Veterinary Medicine and Animal Science, department of Gynecology and Obstetrics.E-mail: abebequanent@gmail.com

Summary: A normal uterine environment is required for survival and transport of sperm cells and for maintenance of pregnancy. Subclinical endometritis is an inflammation of the uterus that results in significant reduction in fertility. Fertility is a successful reproduction, infertility is temporary loss of fertility, where as sterility is a permanent loss of fertility. Subclinical endometritis is the presence of inflammatory cells (PMNs) and inflammation within the uterine lumen, but without clinical signs. Polymorphonuclear cells (PMNs) are the first and most significant cell type recruited during uterine inflammation. Subclinical endometritis is diagnosed by endometrial cytology using uterine lavage. It is treated by removal of necrotic material, administrations of antimicrobials and the inductions of oestrus. Retained fetal membrane, bacterial loading, management factor and nutrition are predisposing factor for subclinical endometritis. The bacteria and fungi are the most common cause of subclinical endometritis. Subclinical endometritis is high in cows having poor calving hygiene, poor body condition. Subclinical endometritis is economically important disease which reduces profitability of dairy enterprise resulting in losses due to an extended calving interval, conception interval, increased culling rates, reduced milk yield and the cost of treatment. It is prevented by hygiene and good management system.

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1.Introduction

The key for an optimal fertility in dairy herds is a healthy uterine environment. A healthy uterus is the basis for high submission and conception rates (Ausder, 2010). Subclinical endometritis has been defined as the presence of inflammatory cells (PMNs), within the uterine lumen but without signs of clinical endometritis (Sheldon *et al.*, 2006). Polymorphonuclear cells are the first and most significant cell type recruited during uterine inflammation. Negative energy balance is known to influence the number as well as the functional properties of PMNs (Subandrio *et al.*, 1997).

The inflammation of the endometrium is characterized by the proportion of polymorphonuclear (PMN) cells in a cytological sample taken from clinically healthy cows. Cytological samples can be obtained by flushing the uterine lumen, taking an endometrial biopsy or by using the cytobrush technique. Studies on subclinical endometritis (SCE) found prevalence in the range between 12 and 94%. Subclinical endometritis result in increased days to first service as well as decreased conception and fertility rates followed by an increased risk of culling. There is evidence that periparturient depression of functional properties and number of PMNs is of great

importance for the pathogenesis of subclinical endometritis, as recently reviewed (Ausder, 2010).

Fertility is a successful reproduction, infertility is temporary loss of fertility, where as sterility is a permanent loss of fertility. Fecundity or prolificacy is a degree of reproduction for species (that is usually used for polytocous species to indicate relative litter size). Fertility, infertility and sterility are applied to both sexes, but fecundity and prolificacy usually are reserved for females (Mc Donald, 1980).

Finally, subclinical endometritis is one of the most common causes of reducing pregnancy in high producing dairy cattle, profitability and sustainability of farming (Gordon, 1996). The major constraints of dairy production in Ethiopia can be grouped in to two. These are technical and non-technical problems. Technical problems are those problems specific to animal production. These are animal diseases, animal genotype, lack of appropriate extension program and shortage of feed and water. Non technical problems are those mostly common to other agricultural sectors; including increased livestock and human populations. Other minor constraints are lack of infrastructure and market, poor education of farmers, seasonality of milk production and consumption pattern of people (Bath *et al.*, 1985). The term endometritis, usually referring to

clinical endometritis (CE) and subclinical endometritis are often used interchangeably (Sheldon *et al.*, 2004).

2. Subclinical Endometritis

Subclinical endometritis (SCE) can be defined as endometrial inflammation of the uterus usually determined by cytology, in the absence of purulent material in the vagina (Gilbert *et al.*, 1998). It is the presence of inflammatory cells (PMNs) within the uterine lumen, but without clinical signs. (Sheldon *et al.*, 2006). According to Sheldon *et al.*, (2006) these cows should also be greater than 26 days postpartum so that the condition is not confused with normal uterine involution. Subclinical endometritis is highly significant condition in postpartum dairy cows resulting in substantial economic losses due to decrease in both milk production and fertility. It can be speculated that the conditions are linked and are part of a continuum. Cows with subclinical endometritis do not show signs of systemic illness (Sheldon *et al.*, 2004).

Subclinical endometritis seems to be associated more with the postpartum recovery of the endometrium than with bacterial infections (Sheldon *et al.*, 2009). It is inflammation limited to the endometrium at least 21 days postpartum with no detectable discharge from the vagina (Sheldon *et al.*, 2006). Inflammation has been defined by the presence of greater than 18% polymorphonuclear neutrophils (PMNs) in uterine cytosmears prepared 21 to 33 days postpartum, or greater than 10% PMNs in cytosmears prepared 34 to 47 days postpartum (Kasimanickam *et al.*, 2005).

Subclinical endometritis is characterized by neutrophils in the uterine luminal fluid, but without visible purulent materials (Noakes *et al.*, 2009). It results in a significant reduction in fertility in the absence of clinical signs of endometritis. Although cows with subclinical endometritis do not have uterine discharge, impaired fertility has been documented in a number of studies (LeBlanc *et al.*, 2008 and Barlund *et al.*, 2008). The incidence of subclinical endometritis was 53% at 40–60 days postpartum, and was associated with delayed conception and increased culling (Santos *et al.*, 2004).

The current study revealed that the overall prevalence of subclinical endometritis (SCE) with 0.5 neutrophil on endometrial cytology was 68.3%. In conclusion SCE diagnosed by endometrial cytology cytobrush was associated with reduced rates of fertility. The diagnostic criteria for subclinical endometritis (SCE) should identify cows at risk of pregnancy failure with in an appropriate time. Subclinical endometritis is an emerging area of investigation and can be defined as an inflammations of the uterus that result in significant reduction in

fertility in the absence of overt clinical sign (Sheldon *et al.*, 2006).

2.1 Etiology

Most commonly organisms, particularly bacteria and fungi, contaminate the uterus during calving or the early post calving period. The bacterial agents commonly isolated from the uterus of postpartum cows are *Escherichia coli*, *Streptococci spp*, *Arcanobacterium pyogenes*, *Bacillus licheniformis*, *Prevotella spp* and *Fusobacterium necrophorum*. The reproductive tract is very susceptible at this time, especially if trauma or lesions in the vagina or vulva are present, and the animal's natural defenses are lowered. Any assistance or manipulations performed during parturition can easily introduce organisms into the uterus (Bulletin, 2007).

Infection in the uterus may result from an infection elsewhere in the body such as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) or leptospirosis which then spreads to the uterus (systemic infections). Infection also can enter the reproductive tract during natural breeding with a bull (venereal). The two most common venereal diseases are campylobacteriosis (vibriosis) and trichomoniasis. Infection can be transmitted either by an infected bull or carried by the bull to a susceptible female from an infected female (Drillich *et al.*, 2001).

2.2. Predisposing factors

2.2.1. Hereditary predisposition and sexes

In many cases the role of males as the possible cause for infertility in the herd is overlooked. Reproductive failure may be associated with inherited characteristics, defective semen quality, failure in mating performance, physiological and physiological causes, infectious disease, improper management or combination of these factors (Butler, 1998).

2.2.2. Season

Environmental temperature, radiant energy, relative humidity and wind speed are all components that influence the degree of heat stress. Therefore, heat stress gives rise to more conditions than those in the animal thermal neutral zone. The season of the year has been shown to have an effect on fertility but, the effect is mainly due to the changes in length of day light. Light appears to have an influence on fertility and it has been shown that in areas where there is a difference in the daylight length between seasons, the time when fertility is at highest in the spring when daylight hours are increased. Further more, humidity and improper nutrition management can also complicate fertility even if daylight length hours are sufficient (De Rensis and Scaramuzzi, 2003).

2.2.3 Nutrition

Feeding more dietary protein has been negatively associated with dairy cow fertility. Excess ammonia is conjugated to urea and then excreted. Thus, high urea

levels are consistent with excess protein intake, possibly with concomitant energy shortage, and are likely to be associated with high levels of ammonia circulation. This results in a high level of ammonia in the rumen, which is absorbed across the rumen wall into the blood stream, where it is carried to the liver and converted into urea. Excess urea in the blood or blood urea nitrogen (BUN) can be toxic to sperm, eggs and embryos (Elrod and Butler, 1993).

Nutrition is the one factor where most cow management system fails be it in over feeding or under feeding. It is often a difficult factor to manage and to master, but it is essential for the fertility in the cow. Severe under or over feeding and deficiency of specific nutrients, especially vit A are probably the most common cause of impaired fertility in females. Although over feeding has serious implications, under feeding is more common. It is likely that all vitamins needed for growth and maintenance are also needed for fertility. Inadequate intake of nutrients or inadequate body reserves, needed to meet production requirements, after calving, results in suppressed fertility in cattle (Harms *et al.*, 1994).

2.2.4 Age

The effect of age on fertility is difficult to measure due to the effect of environmental factor affecting fertility and selection pressure for both the ability to produce and reproduce. Cows' especially first calf heifers were more commonly affected by subclinical endometritis with high prevalence (Kadokawa and Martin, 2006).

2.2.5 Retained fetal membrane

In virtually every survey of the factor causing subclinical endometritis, retained fetal membrane is identified as being of a major importance. In one survey the incidence of subclinical endometritis was 28 times higher than in cows with retained fetal membrane than in normal cows. There is significant association between retentions of membrane and subclinical endometritis. Hence the conditions that leads to retained fetal membrane (RFM) are also associated with developments of SCE. These include multiple birth, abortion, induced calving the high incidence of retained fetal membrane (Gordon, 1996).

2.2.6 Management factor

Many management factors affect the incidence of subclinical endometritis. Thus high milk yield is associated to any increase in incidence of subclinical endometritis. Good management system is essential to reduce severity of subclinical endometritis (Noakes *et al.*, 2009).

2.2.7 Bacterial loading

The environment in which the parturient and post parturient cow has been kept affects the incidence of subclinical endometritis. In particular, a dirty unhygienic calving environment predisposes to the

disease. This bacterial loading is probably relate with the season of years. Since cows calving in winter or indoor in the spring are heavily contaminated environment (Noakes *et al.*, 2009).

2.3.Epidemiology

2.3.1. Prevalance

The overall prevalence of SCE is 12.4%. Prevalance of SCE in primiparous cows is 7.8% and in multiparous cows is 15.2% (Ausder, 2010). Studies focusing on subclinical endometritis found between 12 to 94%. The prevalence of subclinical endometritis depend on the occurrence of early postpartum disease, the time of examinations and the diagnostic technique (Bullein, 2007).

2.3.2. Transmission

Transmission is strictly venereal in the case of campylobacteriosis(vibriosis) and trichomoniasis. Although other means of transmission may be common for most of the other infection, many of them are semen borne. Semen borne infection are particularly dangerous if animals are not in oestrus when inseminated and if the resistance of uterus is lowered. *Coryne bacterium pyogenes*, as well as many of other miscellaneous bacteria gains their entrance during the puerperium, particularly in confined cattle under certain condition, *C.pyogenes* may develop in to a herd infection (Morrow, 1980).

2.4 Pathogenesis

The most common pathogenic species are *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella species*. Some bacteria, including *A. pyogenes*, *F. necrophorum* and *Prevotella spp.* acts synergistically to enhance the severity of uterine disease (Singh *et al.*, 2008). Each of these species produces substances to enhance bacterial growth. *Fusobacterium necrophorum* actively invades uterine tissues and produces a leucocidal toxin that inhibits phagocytosis (Singh *et al.*, 2008 and Sheldon *et al.*, 2004).

Arcanobacterium pyogenes, protected by the leucocidal toxin, in turn provides catalase and a growth factor which supports the proliferation of *F. necrophorum*. It has been reported that persistent infection with *A. pyogenes* after 21 days postpartum will reduce conception rates at the first postpartum service (Singh *et al.*, 2008). Studies to evaluate the appearance and odour of vaginal mucus have shown that *A.pyogenes*, *Proteus* species and *F. necrophorum* are associated with purulent or mucopurulent discharge evident in the vaginal mucus while *A.pyogenes*, *E.coli*, and non-hemolytic *Streptococci* are associated with foul smelling exudates. Polymorphonuclear cells recruited from the circulation to the infected uterus by chemotactic factors enhance phagocytosis of bacterial particles. During pregnancy the uterus is sterile but after parturition uterine lumen

is almost always contaminated with a wide range of bacteria (Williams *et al.*, 2005).

Fusobacterium produce a leukotoxin, *P.melaninogenicus* produce a substance that inhibits phagocytosis and *A.pyogens* produce a growth factor for *F.necrophorum*. However, infections of endometrium with *E.coli* preceds infection by *A.pyogens* is associated with the severity of subclinical endometritis and impacts of fertility. *Escherichia coli* produces on endotoxin ,lipopolysaccharides (LPS) responsible for many of the effect of bacteria. However, the most severe endometrial lesion are caused by *A.pyogens*, *Arcanobacterium pyogens* express several virulence genes. Pylosin is the most important encoding cholesterol dependent cytotoxin pylosin (PLO). Pylosin molecules are attracted to cholesterol rich domain in cell membrane where they aggregate to form a pore which leads to osmotic death of the cell (Bulletin, 2007).

2.5 Diagnosis

Subclinical endometritis is diagnosed by endometrial cytology using uterine lavage on postpartum dairy cows from 30-60 days. It is diagnosed by the presence of inflammatory cells (neutrophils or PMNs) after uterine lavage and cytology (Sheldon *et al.*, 2006). Subclinical endometritis is determined by endometrial cytology using uterine lavage and uterine sample centrifuged at 800 rpm for 5 minutes, if the amount of neutrophil or PMN is greater than or equal to 5% are categorized as SCE is present where as PMN or neutrophil less than or equal to 5% considered as normal or free from SCE. Endometrial cytology is used as diagnostic technique for identifications of subclinical endometris (Buckley *et al.*, 2003). The endometrial cytology findings were defined in the terms of impact on actual fertility of the all available informations including postpartum history per rectal palpations findings endometrial cytology only positive endometrial cytology is significantly associated with increased time to achievements of pregnancy (Ausder, 2010).

Since only a few portions of cows with endometritis display a visible discharge and because PMNs influx and visible discharge is associated with normal uterine involutions, there has been a concerted effort to develop reliable diagnostic tools and protocols that could be used to diagnose subclinical endometritis (Kasimanickam *et al.*, 2005). For example endometrial cytology cytobrush is the most precise method for diagnosing SCE in cows and that a threshold of greater than 8% PMNs between 28 to 41 days postpartum is significantly affecting fertility status at postpartum dairy cattle. Others defined SCE based on similar proportions of PMNs but with increasing the threshold of neutrophil as the postpartum periods (Dubuc *et al.*, 2010).

2.6 Treatment

Use of single administration of benzathine cephapirin improved fertility of cows with SCE (Diagnostic criteria greater than 18% PMNs using endometrial cytology cytobrush). Cephapirin a first generative cephalosporin is effective against most grame negative and positive bacteria in the uterus. The report shows that cows with SCE that reduced cephapirin between 20 and 33 DIM and had an 89% increase the risk of pregnancy compared to non treated cows (Kasimanickam *et al.*, 2005).

Success in treatment of uterine infections depends on: evacuation of the uterus, susceptibility of the infectious agent to the drug used, concentration and number of times the drug is used, exposure of entire endometrium, cervix, and vagina to the drug. Evacuation of the uterus contributes to the success of further antibiotic therapy. Evacuation can be done by repeated palpations of the uterus by a veterinarian and/or the use of drugs to expel the fluid or hasten the onset of estrus. Estrus is usually the best way of stimulating uterine contractions and expelling the fluids. Once fluids are expelled, the effectiveness of antibiotics in clearing the remaining infection is improved antibiotic therapy of the uterus is indicated only when uterine infection is present. Broad spectrum drugs are used most frequently (Barlund *et al.*, 2008).

The effect of subclinical endometritis treatment on the main fertility measure has been found to be modest (Sheldon *et al.*, 2006). Due to different types of infectious agents involved in uterine infections, a set recommendation of drugs is impossible. Ideally, identification of the infectious agent and drug sensitivity is done. Subsequent treatment with an approved drug is the most practical, economical and efficient approach. Withholding time for milk depends on the concentration and number of treatments that a drug has been used (LeBlanc *et al.*, 2008).

Many veterinarians routinely use hormones such as prostaglandins (PGF2 α) to induce estrus before treating the uterus with antibiotics (Galvo *et al.*, 2009). Antibiotic and antiseptics are not substitutes for sound sanitary and management practices, but are valuable additions when needed. The promiscuous use of antibiotics in uterine infections is not only expensive through loss of milk and cost of drugs but is usually not an effective or efficient practice (Drillich *et al.*, 2001). Treatment also done based on removals of necrotic material, administrations of antimicrobial and the inductions of oesterus (Bhat, 1985).

3. Consequence Of Subclinical Endometritis On Fertility

As uterine infections exerts detrimental effect at all levels of the reproductive system, it is not surprising that it causes considerable disruption of

fertility in cattle. Even when animals are treated the conception rate is about 20% lower for cows with subclinical endometritis the calving to conception interval 30 days longer and there are 3% more animals culled for failure to conceive, as well as the effect of uterine infection is associated with lower milk yield particularly it is associated with retained placenta. The financial loss associated with uterine infections are dependent on the cost of treatment, reduced milk yield and subfertility. In the UK, the direct costs of treatment and reduced milk yield are about £60 and the indirect cost of extra insemination a longer calving interval and increased culling rates are about £70 (Sheldon *et al.*, 2009).

Genetic selection for production traits in high producing dairy cows has inadvertently reduced reproductive and perhaps immune functions certainly. Conception rates for high producing dairy cows are persistently decreasing and uterine disease is an important component of this problem. The reproductive consequence of uterine disease is multiple. At simple level, cattle with sub clinical disease have reduced fertility components with that in animal with a healthy uterus. (Ausder, 2010).

Subclinical endometritis causes considerable subfertility and infertility in the presence of uterine bacterial contaminations that delicate hormonal milieu of the hypothalamic-pituitary ovarian axis and disrupt follicular growth and development of uterine infections have been reported to be associated with an increased incidence of cystic ovarian disease (Andrew *et al.*, 2006).

4. Economic Significance Of Subclinical Endometritis

The financial impacts of uterine disease is derived from infertility, increased culling for failure to conceive, reduced milk production and the cost of treatment. The cost of subclinical endometritis are a burden on the dairy industry and need to be quantified. Postpartum subclinical endometritis in cattle is a multifactorial disease with high economic impact (Drillich *et al.*, 2001).

Culling cows can be decided through the year and it is common discussion in the summer and winter months when grass and resources may be sparse. Many ranches use cows breeding history to decide or at least influence on ranch or needs to be culled (Santos *et al.*, 2009). Subclinical endometritis reduce profitability of dairy enterprise. The cost can be calculated by relating it to the increase in the calving-conception interval. Losses were mainly due to an extending calving, conception interval, increased culling rates, reduced milk yield and the cost of treatment (Noakes *et al.*, 2009).

5. Prevention And Control

A regular herd health program is beneficial in the prevention of many fertility problems including subclinical endometritis. If uterine infections are excessive (greater than 20%) in a herd, management of dry and recently calved cows should be thoroughly examined. Adequate housing, nutritionally balanced and palatable rations for the dry and fresh cows, satisfactory calving facilities with optimal ventilation and sanitary conditions, and avoidance of undue stress such as overcrowding and disease are absolute requirements for healthy fresh cows (Drillich *et al.*, 2001).

Little progress has been made in the terms of a structured approach to control or prevention of subclinical endometritis. The risk factors that have been associated with uterine infections include abortions, dystocia, twins, retained placenta and metabolic disease, on the other hand factors that intuitively might have been thought to influence the severity or incidence of disease such as the hygiene of the farm environment have proved difficult to confirm. As a consequence there are few control programmes for preventing uterine disease and approaches to investigate of high incidence of uterine disease is often to evaluate each of the known risk factor in turn. (Singh *et al.*, 2008).

Common thoughts on the control of subclinical endometritis focus on attentions to detail and optimizing animals husbandry. Thus paying attention to the hygiene of cattle accommodations and cullings facilities will pay dividends in terms of cleaner surfaces for animals to lie on and less contaminations of the skin and hairs of the animals with bacteria. Formulations of the diet is important for several reasons. There is some evidence that negative energy balance or deficiencies of micro nutrient such as selenium and vitamin E can suppress immunity. Perhaps of a greater importance is that the liquid feces common in many dairy herds likely contributes to bacterial infections of the genital tract as the vulva is often plastered with dung (Sheldon *et al.*, 2006).

6. Conclusions And Recommendations

Subclinical endometritis is an inflammation of the uterus that results in significant reduction in fertility in the absence of overt clinical signs. The diagnostic techniques used to confirm subclinical endometritis in cross breed dairy cows is uterine lavage. The present review indicated that the prevalence of subclinical endometritis was high and it has a great impact on pregnancy of dairy cows. Variation of the prevalence of subclinical endometritis had been observed on parity, hygienic condition, and method of services, body condition score and farm scales. Cows' especially first calf heifers were more

commonly affected by subclinical endometritis with high prevalence.

The prevalence of subclinical endometritis is also high in cows having poor calving hygiene and poor body condition. An improvement of the managerial systems such as increased calving and post calving hygiene, improve the hygienic condition of pregnant heifers and its housing at parturition and after calving are considered in to account to reduce prevalence of subclinical endometritis and increases the pregnancy rate of cows. The cows with high proportion of endometrial PMNs (greater than 18% PMNs) after artificial inseminations have decreased first service conception rates (FSCR), which might be indicative of subclinical endometritis.

Based on the above conclusion the following recommendations are forwarded:

- ❖ The owners should be aware to improve the managerial systems of their cows to minimize prevalence of subclinical endometritis.

- ❖ The owners should be responsible to keep sanitation of dairy cows through improved housing systems and nutrition to reduce bacterial exposure of cows at parturition.

- ❖ Calving ropes should be cleaned in order to reduce subclinical endometritis during pregnancy diagnosis and delivery problems.

- ❖ Semen evaluations should be taken in order to inseminated healthy bull's semen to cows during AI to increase fertility.

- ❖ Studies should be conducted on subclinical endometritis (SCE) to overcome problems of fertility.

- ❖ Herds should be managed to reduce calving problem and to decrease the incidence of postpartum uterine infections.

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