ENDOMETRIAL ECHOTEXTURE, CYTOLOGY AND BIOPSY IN POSTPARTUM SUBCLINICAL ENDOMETRITIS OF CROSSBRED DAIRY COWS

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Endometrial echotexture, cytology and biopsy in postpartum subclinical endometritis of crossbred dairy cows"**is a bonafide record of research done by me during the course of research and that this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"**entitled **"Endometrial echotexture, cytology and biopsy in postpartum subclinical endometritis of crossbred dairy cows"** is a record of research work done independently by **Gayathri Prathap (17-MVM-35)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Gayathri Prathap (17-MVM-35)**, a candidate for the degree of Master of Veterinary Science in Animal Reproduction, Gynaecology and Obstetrics, agree that this thesis entitled **"Endometrial echotexture, cytology and biopsy in postpartum subclinical endometritis of crossbred dairy cows"** may be submitted by **Gayathri Prathap (17-MVM-35)** in partial fulfillment of the requirement for the degree.

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EXTERNAL EXAMINER

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Gayathri Prathap

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1.	Area under curve	AUC
2.	Body condition score	BCS
3.	Calving to conception interval	CCI
4.	Clinical endometritis	CE
5.	Cervical diameter	CD
6.	Corpus luteum	CL
7.	Cytobrush	CB
8.	Days postpartum	DPP
9.	Dominant follicle	DF
10.	Endometrial cytology	EC
11.	Fluid in uterus	FIU
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Introduction

1. INTRODUCTION

Reproduction is the key pillar of dairy farming. Reproductive performance must be at its optimum so that the production efficiency in the herd is unaffected, it has a large influence on the calving to conception interval, duration of lactation and the culling rate. When dealing with reproduction in dairy bovines, long term purpose is to have cows become pregnant without delay after previous calving so that there is maximum utilization of the time. Selection of cows with the sole purpose of high milk production has compromised the reproductive efficiency of cows in recent decades.

Grohn and Rajala-Schultz (2000) expressed that poor reproductive performance subsequent to parturition affects herd profitability by reducing the milk yield, the number of calves produced, increasing the number of days open, enhancing the culling rate and thus is a production limiting factor.

Lucy (2001) stated that fertility was influenced by a multitude of interwoven factors and its decline had arisen due to a complex system of genetic, environmental, housing, nutritional and other supervisory factors which may include poor reproductive management, insufficient welfare conditions or reproductive diseases and not entirely associated to an increase in milk production. These complex interactions between them make it arduous to pinpoint the reason for its deterioration.

Subsequent to parturition the uterus tolerates the proliferation of aerobic and anaerobic microbes. In most scenarios the bacterial load reaches its zenith between seven and 14 days postpartum (DPP). An infection is established when they adhere to the mucosa, colonize or penetrate the epithelium, produce bacterial toxins causing inflammation, damaging the different layers of endometrium, delay uterine involution, suppress luteinizing hormone production from pituitary gland, disrupts postpartum ovarian follicular dynamics and disturb ovulation in cattle. The origin of corpus luteum (CL) after calving and secretion of progesterone can become a predisposing factor for the onset of uterine infections as progesterone encourages the establishment of uterine infection.

But, by the second week postpartum the lochia and placental discharges are evacuated and the bacteria are eliminated during the first 5 weeks after parturition. Elimination of the contaminants depends on the time taken for involution of uterus, rejuvenation of the endometrium, the inherent defence mechanisms of the uterus and the inpour of polymorphonuclear neutrophils (PMNs), attracted by chemokines. At times if the animal's immune system is severely affected or the bacterial load is great enough to overwhelm the immune system, the infection could persist, leading to metritis (within 21 days) or clinical endometritis.

Metritis affects all layers of the uterus showing evidence of inflammation such as oedema, infiltration by PMNs and damage to the muscle tissues. Endometritis is defined as a peripheral inflammation of the endometrium, which doesnot progress beyond the stratum spongiosum and is evident by histological examination of inflammation. During recovery period, there would be fibrosis and leukocytosis accompanied by atrophy of endometrial glands. The presence of corpus luteum results in endometritis which could later culminate in pyometra.

Subclinical endometritis (SCE) is inflammation of the uterus typically determined by the use of cytology with the absence of supporting clinical signs. Endometritis (clinical and subclinical) was analogous with poor conception and increased culling rates. SCE is most commonly diagnosed by measuring the PMNs in uterine samples gathered by uterine lavage/ cytobrush/ cytotape technique or uterine biopsy. Cytobrush technique is considered a safe and reliable technique with little repercussions. Ultrasound examination is an objective, noninvasive, practical and precise method for the diagnosis of endometritis by evaluating uterine horn diameter, cervical diameter and presence of fluid in uterus.

The use of uterine biopsy in bovine practice is debatable. Though it provides rich details about the endometrial inflammation it is believed to be difficult and dangerous, performed with skill and expertise to obtain and analyse biopsies. But if biopsy is performed with due care, valuable data can be obtained without long lasting effects on health, oestrous cyclicity or fertility.

In Kerala the present status of research in this area is scanty and hence the present study was undertaken with the following objective.

- Comparison of the efficacy of endometrial ultrasonography, cytology and biopsy in postpartum dairy cows for the diagnosis of subclinical endometritis.

Review of literature

2. REVIEW OF LITERATURE

2.1 INVOLUTION AND OVARIAN REBOUND

Hussain and Daniel (1991) stated that the complications during the puerperial period led to failure of defence mechanisms of uterus and retarded the time for complete uterine involution.

2.1.1 Involution

Okano and Tomizuka (1996) noted that to shorten the calving interval, it was necessary to diagnose the progression of uterine involution postpartum. The author had collected samples at day 18, 23, 29, 46 and 54 and reported that the surface epithelium did not reappear by day 18 and that there were large masses of aggregated erythrocytes and oedematous caruncles. The blood cells that infiltrated the endometrium were phagocytized by macrophages aiding in involution.

LeBlanc *et al.* (2002) showed that animals with a delayed involution of cervix often demonstrated poorer reproductive performance when compared to animals diagnosed as normal.

Sheldon *et al.* (2006) stated that complete involution of the uterus and cervix took about 40–50 days postpartum (DPP). Breed, age, BCS, plane of nutrition, parity and other factors could affect involution of the reproductive tract.

Chapwanya *et al.* (2010) defined involution as the process where the postpartum uterus returned to its normal size and location and was able to support the next pregnancy within six to eight weeks, post calving. During this time uterus underwent a cascade of events including contraction, atrophy of muscle and glands and their regeneration, necrosis, sloughing and development of fresh layer of endometrium. In cows with normal puerperium, greatest reduction in size of uterus occurred as early as 10 – 14 days post calving.

Leblanc *et al.* (2011) observed that after parturition the uterine endometrium underwent massive tissue revamping which included shrinkage of the uterus, especially the muscular layers, sloughing of the caruncles and a new endometrium was built.

Salah and Yimer (2017) observed that in bovines, involution of the genital tract took almost four to five weeks postpartum in healthy cows but was delayed in infected cows.

2.1.2 Effect of body condition score (BCS) on fertility

Patton *et al*. (2007) reported that a lower conception rate was observed in cows with poor BCS (≤2.25) at first service.

Anitha *et al.* (2011) reported that energy requirement in dairy animals had to be duly taken care for efficient production and reproduction.

Senosy *et al.* (2012) stated that loss of BCS during the early postpartum period could cause subclinical endometritis (SCE) at four and five week postpartum. His studies were based on description of BCS by Ferguson *et al*. (1994). Moreover, animals with lower BCS (less than 2.75) were susceptible to SCE and subsequently had reduced conception rates and fertility.

2.1.3 Ovarian rebound

Savio *et al.* (1990) and Beam and Butler (1999) studied the resumption of ovarian cyclicity and noted that following the regression of corpus luteum (CL) of gestation, there was a period of anovulation and quiescence, this was succeeded by first ovulation and was characterized by an absence of oestrus behaviour and lack of progesterone secretion by the ovary and a return to basal concentrations of oestradiol during the first week postpartum. After this period there was a leap in the FSH concentration and consequently a rise in the FSH concentrations every seven to 10 days. The first postpartum dominant follicle (diameter ~ eight mm or more), was observed about 10 days after parturition and it could have three fates

viz., to ovulate and become the first postpartum CL, to undergo atresia leading to a second follicular wave and to develop into an ovarian cyst.

Duffy *et al.*(2000) and Cheong *et al.* (2016) had observed that the future of the first postpartum dominant follicle was affected by the LH pulse frequency and if there was an inadequate frequency or insufficient levels of follicular oestradiol ovulation failure could occur.

2.2 UTERINE INFECTION

Sheldon *et al.* (2006) observed that uterine contamination at periparturient period was unavoidable and in about 80 to 100 per cent of animals bacteria had invaded the uterus in the first two weeks after calving. But its continued existence was problematic, causing inflammation, development of uterine disease and impaired fertility.

LeBlanc (2008**)** reported that the there was increased threat of infection in cows with assisted calving, dystocia, twinning, stillbirth, or retained fetal membranes due to the possible entry of bacteria into the uterus and genital tract.

Sheldon *et al.* (2009) in another study suggested that the tissue debris and fluid of the previous gestation and calving harboured a favourable environment for the bacterial growth during the postpartum period. No less than 20 per cent of cows were unable to fight and eliminate the infection which resulted in metritis within 21 DPP and in approximately 20 per cent of the herd; these persistence could extend beyond three weeks and cause uterine infections.

Szenci (2016) observed that up to 40 per cent of dairy bovines could develop clinical metritis within first fortnight after parturition and infection could continue to thrive in 10 to 15 per cent of the herd longer than three weeks after calving which could cause endometritis (clinical or subclinical).

2.2.1 Causative agent

Sheldon *et al*. (2009) stated that the most common uterine pathogens in infected animals were, *E. coli*, *T. pyogenes, F. necrophorum* and *Proteus spp.* These organisms had an important role in causing endometrial inflammation and purulent uterine and vaginal discharge.

Yilmaz *et al.* (2012) conducted a detailed study in buffaloes after slaughter and found that *S. aureus, E. coli* and *Bacillus spp.* were associated with mild type of endometritis. In moderate and severe uterine inflammatory changes the major pathogen involved was *T. pyogenes*. In buffaloes without any vaginal discharge, histopathological studies showed that almost all the pathogens were present and no abnormal quantity could be identified.

Sicsic *et al.* (2018) reported that the genus *Fusobacterium*, was found in abundance in cows with metritis. However, the genera which were previously thought to be causative agents for causing metritis identified using culture studies, *Escherichia* and *Trueperella*, were found to be of low concentrations in the metritic group in the same study.

Cunha *et al.* (2018) also made a similar observation that *Escherichia coli* and *T. pyogenes* were found in almost equal quantity in both healthy and metritic groups, while there was a significant variation in the quantity of *F. necrophorum* and *Porphyromonas levii* quantification between the groups.

2.2.2 Patho-physiology

Soto *et al.* (2003) had observed that the presence of bacterial endotoxins could induce embryo mortality.

Herath *et al.* (2007) and Williams *et al.* (2007) observed that uterine microbial invaders could negatively affect reproduction either by direct damage to the endometrium or by production of toxins.

Lavon *et al.* (2008, 2009) noted that bacterial endotoxins could interfere with the LH production and secretion thereby causing ovulation failure.

Herath *et al.* (2009) also found out that endotoxins could increase PGE_2 secretion and extend the life span of the CL.

Shimizu *et al.* (2012) reported that bacterial endotoxins could have numerous effects on reproduction such as effects on oestradiol and progesterone secretion, altering the progression of follicular development and the normal development of the CL.

2.2.3 Postpartum uterine diseases

Hussain and Daniel (1991) observed that the abnormal puerperium deleteriously affected the defence mechanisms of uterus and resulted in tardy involution uterus.

Sheldon *et al*. (2006) stated that among the reproductive diseases, postpartum uterine diseases (clinical endometritis, metritis and SCE) were the main reason for poor reproductive efficacy and resultant financial loss due to infertility, higher culling rates, reduced milk production and increased treatment cost.

Sheldon *et al.* (2006) systematically organized postpartum uterine diseases as puerperal metritis, clinical metritis, endometritis (clinical or subclinical) and pyometra and opined that these diseases resulted in lower conception rates, increased calving interval and failure to conceive.

2.2.4 Metritis complex

An important factor affecting fertility in cattle includes retention of fetal membrane (RFM), metritis, endometritis and pyometra and is named thus due to common aetiological factors and the fact that one disease predisposed to another. Depending up on the type of pathogens invading the animals immediately after

calving, the severity of colonization and the ability of animals to mount an immune response, the infection could vary from severe life threatening metritis to mild chronic endometritis (Parkinson, 2009).

2.2.4.1 *Clinical metritis*

Sheldon *et al.* (2006) described metritis as an infection that extends deeper into the uterine layers, through the serosa (perimetritis) or into the broad ligament (parametritis). Also these authors defined three grades of clinical metritis. Grade one clinical metritis (CM1) had an unusually engorged uterus with purulent discharge seen outside in the vagina, within 21 days of calving. Grade two or clinical metrits (CM2) or alternatively termed as puerperal metritis, had a distinctive foul reddish-brown watery discharge, enlarged thin walled and atonic uterus usually along with pyrexia (>39.5°C). In severe cases, systemic signs were also noticed such as inappetence or anorexia, reduced milk yield, lethargy, elevated heart rate and apparent dehydration. Toxaemic metritis/grade three clinical metritis (CM3) was characterized by toxaemic signs (extremities became cold, extreme dullness or even collapsed at times).

2.2.4.2 *Clinical endometritis*

Opsomer *et al.* (2000) had inferred that in the animals with clinical endometritis the resumption of ovarian cylcicity was likely to be delayed by 4.5 times and luteal phase was likely to be extended by 4.4 times compared to healthy herdmates.

Ahmadi (2005a) defined endometritis as inflammation of the endometrium without any visible signs and the one of the major determinant for SCE was delayed uterine involution.

According to a meta-analysis of 23 studies (Fourichon *et al.,* 2005) it was reported that mean days open was increased by 15 days as a result of endometritis, and the pregnancy rate reduced to an extend of 16 per cent.

Gilbert *et al.* (2005) described endometritis as an inflammation, which was limited only to endometrium, one that did not progress beyond stratum spongiosum and that occurred not less than three weeks post calving without exhibiting any general or typical systemic signs.

Sheldon *et al.* (2006) defined endometritis as an infection that was confined to the endometrium and stratum spongiosum of the submucosa without any systemic signs of illness.

Sheldon *et al.* (2009) had observed that even in cows that completed treatment for clinical endometritis, the rate of conception was about 20 per cent lower than that observed in unaffected animals and in which an extra three per cent did not conceive at all and was later culled.

Toni *et al.* (2015) reported an incidence range of 14 per cent to greater than 40 per cent for the clinical manifestations of metritis and endometritis in cows. Out of all the cows assessed within a week of parturition 40 per cent were positive for metritis, at 35 DPP (DPP), 43 per cent had SCE, with the calving to conception interval (CCI) increased by 27 days. They also defined RFM as the failure of detachment of fetal membranes for a minimum period of one day after calving.

Salah and Yimer (2017) reported that endometritis was highly likely to occur in dairy cows with high productivity and observed that this condition was associated with decreased conception rates, extended pregnancy intervals and higher culling rates.

2.2.4.3. *Pyometra*

Parkinson (2009) defined pyometra as the cumulation of purulent or muco-purulent material within a distended uterus and a functional CL and a closed cervix though some pus could escape through the cervix into the vagina. The authors further recorded that the condition was atypical and had an incidence rate

of less than two per cent. The proliferation of pathogenic bacteria in the uterus along with the development of the first CL was deemed to be the cause of this condition.

2.3 SUBCLINICAL ENDOMETRITIS (SCE)

Kasimanickam *et al.* (2004) defined SCE as the endometrial inflammation identified by cytology where >18 per cent of polymorphoneuclear neutrophils (PMNs) between 21-33 DPP and > 10 per cent of PMNs between 34-47 DPP were considered positive. They also observed that SCE was the cause for reduced first service conception rate which led to increased days open.

Sheldon *et al*. (2006) defined SCE as an inflammation of the endometrium without any clinical signs of endometritis.

Foldi *et al*. (2006) characterized SCE by PMNs infiltration without or with minimal intrauterine exudate.

Dubuc *et al.* (2012) described cytological endometritis as a hike in ratio of PMNs in endometrial cytology (EC) samples procured through cytobrush (CB) or uterine lavage (UL).

Salah and Yimer (2017) explained SCE (also known as cytological endometritis) as the inflammation of the endometrium of the uterus without any mucopurulent content accumulation in the vagina and an absence of systemic symptoms.

2.3.1 Occurrence

Kasimanickam *et al.* (2004) observed that approximately 45.1 per cent and 41.4 per cent of Holstein dairy cows had SCE at 20 to 33 DPP and at 34 to 47 DPP, respectively.

Kasimanickam *et al*. (2004), and Barlund *et al.* (2008) reported that SCE was the most prevalent uterine infection affecting nearly 30 per cent of lactating

dairy cows, with the prevalence ranging from 11 per cent to greater than 70 per cent.

Gilbert *et al*. (2005) observed that overall prevalence of SCE in Holstein dairy herds at 40-60 DPP varied from 37 to 74 per cent with an average of 53 per cent.

Toni *et al.* (2015) stated that at 35 DPP, 43 per cent of the cows had SCE and this led to an increase in CCI by 27 days.

Based on uterine cytobrush cytology and characteristics of cervico-vaginal mucous, Singh *et al.* (2015) reported an incidence of 29.4 per cent SCE in repeat breeding crossbred cattle.

Robert (2016) reported that the prevalence of cytological endometritis in Kerala Veterinary and Animal Sciences University farms (KVASU) was found to be 41.67 per cent.

2.3.2 Predisposing factors

Salasel *et al.* (2010) listed abnormal calving, RFM and postpartum uterine infections as the risk factors associated with SCE that increased prevalence of this condition in dairy cows.

2.4 DIAGNOSIS OF SCE

Kasimanickam *et al.* (2004) and Sheldon *et al.* (2006) reported that there were no distinguishable clinical signs and a complete lack of cervical discharge of pathognomonic significance.

2.4.1 Anamnesis

Markusfeld (1987) and McDougall (2001) individually suggested that the clinical records regarding any periparturient complications was helpful in identifying those animals that are at risk of uterine disease.

2.4.2 Rectal examination

Purohit (2008) reported that the perrectal palpation of animals with SCE was fruitless and no abnormalities could be detected.

2.4.3 Transrectal ultrasonography (TRUS)

Rajamahendran *et al*. (1994) reported that TRUS was the most befitting diagnostic aid for use in bovine practice, specifically for the examining the reproductive organs. The technique was considered noninvasive, relatively simple, effective, safe, portable and ultrarapid, since the ultrasonic imaging allowed the interpretation and diagnosis of the conditions at the time of observation in most circumstances.

A two-dimensional ultrasonographic image could be described as a matrix of pixels, which varied from zero (solid black) to 255 (solid white) through the different shades of greyscale (Singh *et al.,*1997).

Ginther (2014) identified TRUS as an important diagnostic tool for use in large animal practice to assess physiological and pathological changes especially of the reproductive system. This mainly allowed for the diagnosis of pregnancy at early stages of gestation, likelihood of embryonic deaths, normal and variations in the follicular dynamics and prediction of ovulation time and infections.

2.4.3.1 *Echotexture of uterus*

Mateus *et al*. (2002) stated that ultrasonographic measurement of uterus was easy and allowed genuine comparison of results. Ultrasonographic intrauterine fluid determination three weeks after postpartum was a reliable diagnostic method for endometritis, with good sensitivity and specificity. The authors also suggested that the increase in uterine diameter (UD) was noticeable only with severe endometritis and rather hazy in mild endometritis.

Lenz *et al.* (2003) and Kasimanickam *et al.* (2004) described the use of TRUS for the diagnosis of SCE by visualizing small amounts of FIU.

Kasimanickam *et al.* (2004) specified that transrectal ultrasonographic examination to examine intrauterine fluid accumulation must be measured at the base of the horn and scored as less than or equal to three, three to five, greater than or equal to five.

Drillich *et al.* (2004) stated that an increased fluid accumulation within the uterine lumen which resulted in an increased uterine horn and cervical diameter (CD) and echo-textural changes after calving were suggestive of bacterial colonising and delay in uterine involution.

Barlund *et al.* (2008) observed that the alterations in endometrial thickness could be suggestive of detection of SCE.

Oral *et al.* (2009) stated that uterine horn diameters greater than three centimetres could be considered as a case of endometritis further they also opined that the uterine size and its content could be detected by TRUS only in clinical endometritis. The study was conducted in cows 45-180 DPP and seven out of the thirteen cows diagnosed as being non endometritic by scanning were found to be positive for SCE when the cytobrush technique was used.

Polat *et al.* (2015) evaluated echotextural changes of endometrium using TRUS digital images during SCE using a computer-assisted image analysis program. The authors concluded that the pathological changes in SCE were so minute and could not be easily distinguishable by real time TRUS. This limitation in use of TRUS could have been due to the inability of the human eye to discern these diminutive differences with the human eye.

2.4.3.2 ***Echobiometry and echotexture of cervix***

Gier and Marion (1968) stated that when measuring cervix and uterine horn size, a five centimetre cut-off point was used to diagnose endometritis.

LeBlanc *et al.* (2002) noted that cows exhibited reduced fertility later in their life if the cervix was greater than five centimetres even five weeks after parturition.

Meira *et al*. (2012) compared TRUS, histopathology and its combinations for the diagnosis of endometritis by keeping the cytobrush as the gold standard in cows at 21and 47 DPP. These authors observed that, though cervical measurement was an interesting tool for the diagnosis of endometritis, its efficiency could be increased by combining this tool with other observations such as the presence of intraluminal fluid which was a subjective assessment. The authors further classified cows under endometritis group on the basis of CD and if greater than five centimetre coupled with the presence of FIU, regardless of the nature of echogenicity (hyper or hypo) upon TRUS**.** A cow could be categorized as healthy based on ultrasonographic findings, if its CD was less than five centimetre and if no abnormal discharge was observed externally or in the uterus. The authors thus concluded that a combination of intrauterine fluid assessment and CD gave the most accurate diagnostic results of all the ultrasonographic parameters.

Salah and Yimer (2017) noticed that cows with CDs greater than five centimetre after week four of postpartum had developed uterine diseases.

2.4.3.3 ***Ovarian status and follicular dynamics during the postpartum period***

Savio *et al.* (1990) and Rajamahendran *et al.* (1994) could identify that in more than 80 per cent of cows, the first ovulation was unaccompanied by overt signs of oestrus, the length of the second cycle (luteal phase following first ovulation) was about 23 days and the length of the first cycle was either short, 17 ± seven days, or of normal 22 ± nine days, duration.

Savio *et al*. (1990) also noted that if a dominant follicle was detected after 20 DPP, the cycle duration was consistently short.

2.4.3.4 *Agreement between ultrasonographical detection of uterine fluid and other diagnostic aids*

The presence of uterine fluid detected by ultrasound was associated with negative effects on fertility (Kasimanickam *et al*., 2004; Barlund *et al*., 2008).

Barlund *et al.* (2008) and Meira *et al.* (2012) in their separate studies noted that weak levels of concurrence was obtained between ultrasound measurements of uterine fluids and the cytobrush methods in diagnosing cytological endometritis among dairy cows**.**

Feuntes *et al.* (2017) also stated that the presence of intrauterine fluid, either clean or abnormal, had no relationship with cytology outputs just like previous studies.

Salah and Yimer (2017) showed that there was a weak agreement between transrectal ultrasonographic evaluation and the cytological examination, specifically at week four and a moderate agreement at week five, PP. There were mainly two forms of endometritis, one that was associated with the influxion of PMN identifiable using cytology and the second, associated with the fluid build up within the uterine lumen diagnosed using ultrasound evaluation. Furthermore, a slower uterine clearness was analogous with a high PMN percentage.These authors further opined that both fluid in uterus (FIU) and CD were useful for the diagnosis of endometritis affected cows. A higher sensitivity, specificity and kappa agreement was obtained by their combination with cytological method, the standard procedure for diagnosing cytological endometritis.

2.4.4 Endometrial cytology

Neutrophils spearhead the first line of defence in the war against the invading pathogens with amassing large populations within the uterine lumen (Watson, 1989 and Hussain and Daniel 1991).

Chacin *et al.* (1990) and Hussain and Daniel (1991) had reported that the primary defence mechanism against the impending bacterial invasion was phagocytosis. For effective phagocytosis to take place within the uterine lumen, there had to be rigorous mobilization and migration of an ample amount of neutrophils from the peripheral circulation which responsed to a chemotactic stimulus dispatched either directly or indirectly by the attacking bacteria.

Kasimanickam *et al*. (2004) had identified uterine cytology as the most useful device for the diagnosis of SCE.

Ahmadi *et al.* (2005b) and Kasimanickam *et al*. (2005a) had noted a routine practise of cytological inspection of the reproductive tract to assess the status of the organs in terms of inflammation or lesions and it could be performed at different stages of postpartum period.

Sheldon *et al*. (2006) noted that PMN were the primary inflammatory cells that were drafted from the peripheral circulation into the endometrium to be the first in line for the defending the uterus from the postpartum invasion of harmful microbes. This was the reason behind a higher percentage of PMN in cows suffering from uterine inflammatory diseases when compared to hale cows. The authors further observed that the impairment in this mobilization and phagocytic ability of the neutrophils could render the animal vulnerable to the infections.

Among the many methods of collecting samples, the one with cytobrush was considered the best technique for endometrial sample collection as it was easy and quick to perform (Barlund *et al*., 2008).

Oral *et al*. (2009) deemed that the use of cytobrush for cytology studies as being safe and effective.

Evaluation of endometrial cytology had been done either by counting the mean number of PMN observed in 10 microscopic fields (Prieto *et al*., 2012) or number of PMN per 100 nucleated cells (Melcher *et al*., 2014).

Brodzki *et al.* (2015) suggested that cytological examination could be done in cows to rule out or confirm SCE in instances where there were no clinical signs and no significant changes were visible in TRUS. The author also opined that this technique could be used to monitor the postpartum period in cows.

2.4.4.1 *Diagnostic threshold for PMN per cent*

Kasmanickam *et al.*(2004) proposed that in cows with SCE a PMN threshold value of >18 per cent could be observed from 20 to 33 DPP and >10 per cent PMNs from 34 to 47 DPP.

Gilbert *et al.* (2005) supplemented the use of a cut-off mark of greater than five per cent PMNs as a significant cutoff point for diagnosis of cytological endometritis in bovines using low volume of uterine lavage 40-60 DPP. Other studies were based on these levels of PMN thresholds and its effects on reproductive performance.

Oral *et al.* (2009) diagnosed postpartum SCE based on the concentration of inflammatory cells (greater than five per cent) on the cytological analysis of endometrial cells.

Madoz *et al*. (2013) used endometrial threshold values less than or equal to eight per cent to rule out the existence of endometritis in the herds 20-35 DPP.

Polat *et al*., (2015) collected samples from cows which were apparently healthy and cytopathological classification was done based on extensiveness of PMN cells and lymphocytes (LYM) to estimate the inflammatory status. The samples with greater than or equal to five per cent PMN, five per cent PMN + LYM and five per cent LYM were considered acute, sub-acute and chronic, respectively.

Salah and Yimer (2017) restricted the use of cytology for calculating the percentage level of inflammatory cells infiltration in various ways. They used leukocytes mainly segmented neutrophils and lymphocytes. The results attained

using the CB method showed that 11.3 per cent and 9.4 per cent at four and five weeks postpartum showed PMN greater than or equal to eight per cent, which implied CE.

Sicsic *et al.* (2018) observed that cows exhibiting metritis had a remarkably higher PMN percentage at five to ten DPP as compared to healthy cows. The results were similar to other studies where healthy and endometritic cows were compared by diagnosis cytologically much later in lactation. The authors also stated that the presence of PMNs in normal cows could be the outcome of the early steps of the uterine involution process, which involved massive tissue reconstruction and remodelling, supervised by an inflammatory process or as part of the innate immune reaction to bacterial contamination.

2.4.4.2 *Association between cytology and other forms of diagnosis*

Other diagnostic methods such as TRUS, vaginoscopic examination were found to have low association when compared with cytology for identifying animals with SCE (Kasamanickam *et al*., 2004; Meira *et al*., 2012 and Madoz *et al*., 2013).

Feuntes *et al.* (2017) compared after slaughter endometrial cytology, biopsy and bacteriology in culled dairy cows using Receiver Operating Characteristic (ROC) curve analysis, Cohen's Kappa method and Chi-square test and reported that the agreement between histology and cytology was moderately high for presence of infiltrate (kappa = 0.55) and low for infiltrate distribution (kappa = 0.33). Failure of cytology to identify low degree of inflammation could be due to the fact that cytology had access only to the superficial layer of the endometrium and a negative result by cytology could be obtained from uterine samples with inflammatory infiltrate in deeper layers. Other histological parameters (status of endometrial epithelium, endometrial glands or fibrosis), intrauterine content or bacteriology were not correlated with cytology. The authors concluded that endometrial cytology, although less sensitive than biopsy, is a useful tool for diagnosing subclinical endometrial inflammation.

Feuntes *et al.* (2017) also noted that the phase of the oestrous cycle had no significant influence on endometrial cytology, microbiological results or the presence of intrauterine content but had an influence on the presence of inflammatory infiltrate diagnosed by histology.

Salah and Yimer (2017) opined that their work evinced a weak agreement between TRUS evaluation and the cytological technique, especially at week four, and a moderate agreement at week five PP.

Sicsic *et al.* (2018) observed that the analysis using cytology was similar to the findings in a histopathological analysis of healthy and metritis animals but it was restricted to the superficial layers of the endometrium and luminal fluid only. Hence results using these techniques could not be taken to infer results for the condition of the deeper layers of uterus.

2.4.5 Uterine biopsy

Studer and Morrow (1978) used modified biopsy devices in cattle. These devises were intended originally for use in mares and could be introduced transvaginally with a cutting tip that could collect 300–500 mg of uterine tissue. These devices could be easily guided by per rectal manipulation.

DeBois and Manspeaker (1986) and Chapwanya *et al.* (2010) found that the most dependable and efficient method for diagnosis of SCE especially to evaluate further reproductive efficiency was endometrial biopsy and histopathology. The technique was revealed to be harmless and fail-safe technique if done properly. They had used Yeoman biopsy forceps which was relatively smaller and took only a smaller fraction (8 mm x 5 mm).

Bonnett *et al.* (1991) had also mentioned that biopsy was rarely performed in heifers because of the difficulty in traversing the cervix using the traditional large devices.

Bonnet *et al.* (1993) had expressed concern regarding the effect of taking biopsy samples on reproductive performance. Their concern could be rooted by the fact that they used a punch biopsy which had wide diameter that took a larger fragment in postpartum (PP) cows.

Meira *et al.* (2012) used uterine biopsy as a histopathological diagnostic tool for endometritis in PP cows.

Ramirez-Garzone (2017) took endometrial biopsies on day four or day seven post-oestrus in pre-synchronized, unmated beef heifers to assess the efficacy of a human bronchoscopy biopsy device with a circular cup (10366L Karl Storz®, Germany) in *Bos indicus* cross-beef heifers. This instrument was slimmer, rigid and had a smaller diameter 0.35centimetre which allowed the extraction of a smaller sampler size and minimizing tissue damage with increasing likelihood of rapid healing.

2.4.5.1 *Diagnostic evaluation*

Bonnett *et al.* (1991) laid out the evaluation criteria of histopathological characteristics of endometritis which had a summation score developed by summing 4 scores that reflected the condition of the surface epithelium, the lamina propria, the endometrial glands and the vascular inflammation.

Ahmadi *et al.* (2005b) noted that on comparison, inflammatory cell densities in cervical and uterine fluid of healthy cows were remarkably low than in cows having SCE. In acute endometritis, infiltration of PMNs into the mucosa, submucosa and intraluminal glands along with degeneration and necrosis of surface epithelium, congestion of the vessels and stromal oedema was observed. In subacute endometritis, lymphocytes either focal or dispersed, haemosiderin-laden macrophages (indicative that they have completed their function), neutrophils and regenerative activity of glands in the absence of fibrosis were seen. In endometritis lasting over a period of time, infiltration of mononuclear

inflammatory cells accompanied by cystic dilatation of endometrial glands and periglandular fibrosis were observed.

Meira *et al.* (2012) deemed that for positive diagnosis a score above 15 was taken as positive defined using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The best combination was the score that represented the condition of surface epithelium, lamina propria and vascular inflammation status. The glandular inflammation was disregarded as its involvement in SCE was minimal and the animals had no uterine discharge or other clinical signs.

Yilmaz *et al.* (2012) examined buffaloes that had calved around 6 months back, aged five to seven years, weighing 450-550 kg that were having trouble conceiving even after three or more insemination with no history of abnormal vaginal discharge. The authors classified the endometrial condition histologically as being negative (no proof of inflammation), mild (light infiltration of PMN cells, lymphocytes and plasma cells with little involvement of glandular or vascular degeneration changes), moderate (some lymphocytes infiltration, medium fibrosis around the vessels and glands and some cystic glandular degeneration) and severe (with intense infiltration of PMNs and mononuclear (MNs) cells, drastic atrophy of glands, fibrosis, haemorrhage and congestion).

Suleymanaov *et al.* (2018) reported that on histopathological examination of cows with endometritis the mucosal epithelia was necrosed, the functional layer was infiltrated by leukocytes, and glandular epithelial layer was dilated with occurrence of microbial cells on the surface of the mucosa. The stroma of stratum functionalis, endometrial mucosal epithelium and the blood cells were also oedematous.

Sicsic *et al.*, (2018) on histopathological analysis of uterine biopsies observed that in normal and slightly infected animals the luminal side in between the caruncular region was covered by a whole and undamaged single layer of columnar epithelial cells with distinct vacuoles that displaced the nucleus in some cells. The author also opined that in metritic and septic metritic animals this

epithelial covering was absent or damaged in the entire endometrial luminal surface which allowed easy invasion of the bacteria and this might have occurred due to the inflammatory process or an inherent defect in the host defence mechanism. The lamina propria of healthy animals had a continuous line of haemorrhage accompanied by mild, diffuse PMN infiltration, meanwhile in the septic metritic animal, discontinuous layer of mostly thrombosis and vessel congestion and heavy PMN infiltration were noticed. Epithelial tubular cells of the uterine glands could be either columnar of cuboidal or atrophied in animals which varies according to the severity of infection.

2.5 DIFFERENTIAL DIAGNOSIS

The other important uterine infections such as puerperal metritis, clinical metritis and clinical endometritis (CE) could be differentiated in terms of the nature of vaginal discharge and other systemic signs.

2.5.1 Metritis – signs and symptoms

Toni *et al.* (2015) stated that the term metritis could be used in cows having a fetid, brownish, abnormal vaginal discharge with or without pyrexia three to ten DPP. According to this definition of metritis, puerperal metritis and clinical metritis have been essentially combined into one clinical condition.

2.5.2 Clinical endometritis – signs and symptoms

Ahmadi *et al.* (2005b) reported that endometritis could be distinguished by purulent or mucopurulent uterine discharge either observed directly or by using vaginoscopy, 21 days or more after calving. The disease was mostly unaccompanied by systemic signs.

Sheldon *et al*. (2006) and Barlund *et al*. (2008) stated that CE could be diagnosed based on the features such as abnormal vaginal discharge (foul smelling watery red-brown or mucoprulent), enlargement of uterus and cervix,

whereas the detection of SCE needed specific diagnostic examinations applying TRUS, cytology or biopsy.

Sheldon *et al.* (2006) laid out the basis for classifying vaginal mucous based on nature and odour of the vaginal mucous. Character wise the mucous was scored depending on the colour and volume of mucous. Score of zero was assigned when mucous was clear or translucent. A score of one was assigned in the cases where there was presence of flecks of white or off white pus. Score two was assigned if the discharge contained ≤ 50 per cent mucopurulent material and finally, a score three was given, if the discharge contained ≥ 50 per cent purulent material, usually white or yellow, but occasionally sanguineous. It was specified that scores greater than or equal to one was considered positive for endometritis

Oral *et al*. (2009) compared the techniques of vaginoscopy, cytobrush and TRUS for the diagnosis of postpartum endometritis in 31 infertile cows, from 45 to 180 days after parturition and concluded that the clinical parameters were equally useful in diagnosis when compared with cytology.

Meira *et al.* (2012) on study of the histological changes associated with endometritis noticed distorted surface epithelium, vascular congestion, stromal oedema, with varying degrees infiltration with inflammatory cells comprising lymphocyte and plasma cells in the superficial layers.

In a recent study, results of three diagnostic methods (vaginoscopy, gloved hand and Metricheck) were compared for identifying animals with CE between days 21 to 27 post calving and Metricheck was found to be more efficient (47.5% than the other two methods (vaginoscopy: 36.9%, gloved hand: 36.8%). Thus the authors concluded that in the absence of a golden standard diagnostic procedure, Metricheck followed by vaginoscopy was used as a cow-side diagnostic tool for diagnosing clinical endometritis (Szenci, 2016).

2.5.3 Pyometra – signs and symptoms

Pyometra was identified by a distended uterus with collection of purulent material within the lumen in the presence of a persistent CL and a closed cervix (Sheldon *et al.,* 2009).

2.6 TREATMENT

Griffin *et al*. (1974) stated that at 35 days after calving, 46 per cent of animals had no evidence of endometritis though at times non-treatment leads to pyometra. Steffan *et al*. (1984) had reported that the self-cure rate of endometritis was estimated to be 33 per cent.

LeBlanc *et al*. (2002) stated that the treatment for endometritis should be delayed until at least four weeks post calving. The authors further expressed that intrauterine infusion of antiseptics was at best useless and at worst deleterious to the uterus and that a rational therapy for the uterine infection should be aimed at either hormonal instigation of uterine defences or the use of antibiotics (Parkinson, 2009).

2.6.1 Antimicrobial Therapy

Masera *et al.* (1980) and Bretzlaff *et al*. (1983) compared systemic administration and intrauterine infusion and found that the latter achieved higher drug concentration in endometrium, but its penetrability to deeper layers of uterus or other genital tissues was little.

Bretzlaff (1987) also recommended that to reach higher antibiotic concentrations throughout the genital tract systemic administration was better.

Gilbert and Schwark (1992) infused oxytetracycline and Lugol's iodine into the uterus and found that though effective they acted as irritants causing coagulation and necrosis of endometrium.

Dohmen *et al*. (1995) reported that with intrauterine infusion of cephapirin in clinically affected cows, within two weeks 80 per cent clinical cure and 60 per cent bacterial clearance rates were achieved.

Cephapirin is a first generation cephalosporin; a broad-spectrum antibiotic effective against both gram-positive and gram-negative bacteria and can act on anaerobic environment which makes it valuable for treatment of the infected uterus (Adams 2001).

McDougall (2001) concluded that intrauterine treatment with 0.5g cephapirin enhanced the fertility of dairy cattle, especially in those cases involving RFM, still born calf or death within 24 hour of calving and cows with a vulval discharge.

When cows with endometritis were treated with cephapirin IU, the time for calving to conception was considerably reduced (LeBlanc *et al.,* 2002).

Okker *et al.* (2002) opined that systemic treatment would be best if the antibiotics infused into the uterus were subjected to degradation by conditions in the uterine lumen. The authors also opined that if a single dose of ceftiofur (1mg/kg) was administered subcutaneously 24 hours after calving, it would reach its peak concentrations within two hours of administration and this concentration in uterine tissues exceeded the minimal inhibitory concentration for common uterine pathogens thereby destroying them.

Kasimanickam *et al*. (2005b) observed that as endometritis was mainly caused by bacteria, it was reasonable to use antibiotics for the therapy. But antibiotic administration had to be done carefully so as to avoid destruction of the normal reproductive tract flora and uterine defence mechanism besides taking all the measures to ensure the absence of milk or meat residue. The authors additionally mentioned that the drugs should be effective in a pyogenic environment, achieving adequate minimum inhibitory concentration (MIC), be cost effective and be easy to administer.

Kaufmann *et al.* (2010) and Bartolome *et al.* (2014) demonstrated that the administration of ceftiofur consecutively for 3 days at the rate of 1mg/kg IM to cows with clinical endometritis at 21-27 DPP resulted in clearing of the vaginal discharge at 41-42 DPP.

Administration of cephapirin post AI reduced the CCI (Mosaferi *et al*., 2013).

2.6.2 Hormones

Hormones acted by stimulating uterine immunity either by activating oestrus with administration of $PGF_{2\alpha}$ in animals with an active CL who did not show any oestrus signs over a long time or administration of low doses of estradiol (Parkinson, 2009).

2.6.2.1 *$PGF_{2\alpha}$*

Nakao *et al.* (1997) and Hirsbrunner *et al.* (2003) reported that $PGF_{2\alpha}$ enhanced the immune functions or increased the uterine contractility facilitating the expulsion pathogenic bacteria along with the purulent content in animals that do not have an active CL.

Administration of exogenous $PGF_{2\alpha}$ was done to stimulate luteolysis in cows where there was an active CL. This reduced the level of progesterone and increased the concentration of oestrogen, inducing oestrus and resolve uterine infections (Heuwieser *et al.,* 2000 and Laven, 2003).

Mansour *et al.* (2003) proved that the $PGF_{2\alpha}$ had the least harmful effects among the hormones and this intervention came with the advantage that withdrawal period was unnecessary.

2.6.2.2 *Oestradiol*

The use of oestradiol to stimulate the myometrial contractions, phagocytosis and mucous protection was studied extensively (Bretzlaff, 1987 and Hussain, 1989).

The use of oestradiol for stimulation of uterine defence was prohibited due to higher residues in milk (Bretzlaff, 1987).

Sheldon and Noakes (1998) had established the use of oestradiol at doses of five to ten mg per animal therapeutically for postpartum endometritis and opined that it was as effective as $PGF_{2\alpha}$.

Oestradiol could also suppress milk production in lactating cows and ovarian cysts could be formed as a consequence (Gumen *et al.,* 2002 and Jeengar *et al.,* 2014).

2.6.3 Immunomodulators and antioxidants

Singh *et al.* (2000), Methai *et al.* (2005) and Sarma *et al.* (2010**)** experimented with substances (infused into uterus as a single dose) that stimulated the uterine defence mechanism to combat infection by acting as potent chemoattractants causing influx of PMN cells. These substances mainly constituted of *E coli* lipopolysaccharides (LPS) oyster glycogen and autologous plasma.

The levels of free radicals like nitric oxide were found to be high in cows affected with endometritis (Li De Jun *et al.,* 2010).

The free radicals could be counteracted by infusion of enzymes such as EDTA and Tris or lysosome (Ravikumar *et al.,* 2006) antioxidants like vitamin A, E and selenium (Sengupta and Nandi, 2013).

Ahmadi *et al.* (2014) studied the effects of hyper immune serum but mostly they were hard to come by and expensive.

Purohit *et al*. (2015) stated the efficiency of vitamin C against free radicals.

E. coli LPS can be considered as an alternative to antibiotics and/or antiseptics in the treatment of endometritis with reasonable conception rate (Robert, 2016).

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

The study was conducted at University Livestock Farm and Fodder Research and Development Scheme, Mannuthy. Postpartum dairy cows in their first to seventh parity, which were clinically normal and without any postpartum complications were selected for the study. Animals were screened on 20, 30 and 40 Days postpartum (DPP) for subclinical endometritis by transrectal ultrasonography (TRUS), endometrial cytology (EC) (by both bovine cytobrush and modified cytobrush technique) and uterine biopsy (UB) (the latter two techniques were done on only 30 and 40 DPP). A total of 24 cows (six primiparous, 18 pluriparous cows) that calved between September 2018 and June 2019 were enrolled in the study.

The crossbred cows were maintained under uniform management conditions and a balanced feeding protocol was followed consisting of green and dry fodder along with concentrates and mineral mixture as per the recommendations by Nutrient Requirements of Animals – Cattle and Buffalo (ICAR-NIANP), (2013). These animals were fed accordingly so as to meet the require ments of growth, maintenance, pregnancy and lactation.

Pregnant animals were dried off at seventh month of gestation and maintained in a separate shed. The practice of weaning of calves immediately after calving was followed in the farm and cows were milked twice daily.

3.2 HISTORY

The data pertaining to age, breed, parity, details of calves born, postpartum complications like retention of foetal membranes, metritis, pyometra as well as the incidence of any metabolic disorders like milk fever and ketosis were recorded in the study.

3.2.1 Body condition score (BCS)

The BCS of all the animals was calculated as reported by the score card set by Ferguson *et al*. (1994) on day of calving. The areas observed for determining BCS were the thurl region, ischial and ileal tuberosities, ilio-sacral and ischio-coccygeal ligaments, transverse processes of the lumbar vertebrae and spinous processes of the lumbar vertebrae. An absolute body condition score was derived for each cow and the scores ranged from 1 to 5.0.

3.3 UTERINE TONICITY AND TEXTURE

All the animals were subjected to rectal examination wherein the uterus was palpated so as to assess its consistency, presence of uterine fluid and status of the postpartum uterus such as symmetry of the uterine horns and location of uterus on 20, 30 and 40 DPP.

3.4 UTERINE INVOLUTION

Uterine involution in all the experimental animals were assessed by rectal palpation and TRUS on 20 DPP and subsequently at 10 days interval till 40 DPP.

3.5 ULTRASONOGRAPHIC EXAMINATION

Postpartum cows were subjected to ultrasonography on the 20, 30 and 40 DPP to diagnose possible delay in uterine involution, resumption in ovarian cyclicity and likelihood of any infection based on presence and nature of fluid in the uterus, diameter of the cervix and uterine horn, endometrial thickness and structures in both the ovaries. Presence of fluid in uterus (FIU) at three weeks postpartum in cows could be considered as a reliable indicator for endometritis, with good sensitivity and specificity (Mateus *et al.* 2002; Kasimanickam *et al.* 2004; Barlund *et al.* 2008 and Oral *et al.* 2009). On transrectal ultrasonography FIU could be seen as either anechoic or echogenic areas within the uterine lumen.

3.5.1 Description of the ultrasound scanner

Transrectal ultrasonography (TRUS) of uterus was done with a real time colour Doppler ultrasound scanning machine (MyLab™ Gamma, Esaote SpA, Italy) equipped with linear array, 5-10 MHz frequency trans-rectal transducer (SV3513, Esaote Europe B. V, Netherlands). The gain, brightness and contrast were set optimally for each examination. (Plate 3.1)

3.6 ENDOMETRIAL CYTOLOGY

In all the cows, cytological studies of the uterus were carried out on 30 and 40 DPP. After restraining the cow, the vulva and perineum were cleansed with tissue paper and povidone iodine to minimize contamination.

3.6.1 Cytobrush technique

In pluriparous cows, the endometrial samples were collected by a bovine cytobrush (Cytology Brush, Minitübe GmbH Tiefenbach, Germany), approved for use in large animals (Plate 3.2.1).

A sleeved arm after lubrication was introduced into the rectum to empty dung. The vulva was cleansed with cotton and painted with povidone iodine. A lubricant jelly (Lubic®, Lubricating jelly, Neon laboratories limited, Mumbai) was applied to the tip of the plastic sheath, the labia were gently parted and the cytobrush was inserted into the vagina to a point approximately half way between the vulva and the cervix. The cytobrush was manipulated per rectally so as to be introduced into the anterior vagina and through the cervix. The guarded cytobrush was passed through the cervix. Upon reaching the innermost ring of the cervix, the cytobrush was advanced approximately 1 cm beyond the guard into the lumen of the uterine body. The cytobrush was rotated a full 360 degrees clockwise in order to obtain cellular material from the endometrium. The cytobrush and rod were retracted inside the guard and carefully removed from the reproductive tract.

3.6.2 Modified cytobrush technique

In primiparous cows, endometrial samples for cytological examination were collected using an endocervical cytobrush (Medigold pap smear kit), modified for use in large animals The normal endocervical cytobrush handle was cut to approximately 2 cm in length and heat fixed on to a 45-cm solid stainless steel artificial insemination (AI) stylet. The instrument was prepared in lamiar airflow chamber and sterilised by ultraviolet radiation for 45 to 60 minutes before being used for sample collection. The brush with the fixed handle was then covered with a sanitary plastic sleeve to avoid vaginal contamination (Madoz *et al*., 2013 and Robert, 2016) (Plate 3.2.2).

The vulva was cleansed with cotton, scrubbed with povidone iodine and the instrument was passed upto the external os of cervix; the sanitary sleeve was punctured and the instrument was traversed through the cervix and into the body of the uterus, after which the stylet was pushed to expose the cytobrush.

The cytobrush was then rotated a full 360 degrees clockwise to obtain cellular material from the endometrium. The cytobrush was retracted into the artificial insemination sheath prior to removal from the uterus. Care was taken to ensure that the cytobrush did not damage the endometrial surface (Dhanusree, 2016).

3.6.3 Preparation of smear

Preparation of the smear was done in the barn itself. The cytobrush was gently rolled on to a clean glass slide. The slides were then air-dried and kept in transport box for further investigations in the laboratory (Plate 3.3 and Plate 3.4).

3.6.4 Staining and examination

3.6.4.1 *Modified Wright-Giemsa staining method*

The slides were air dried and flooded with modified Wright-Giemsa stain for 30 seconds. Equal amount of distilled water was then added and the slide was left undisturbed for two minutes. The slides were then washed in running tap water, dried and examined under the microscope.

A few slides were stained using Field Stain for the comparison of clarity of the slides and ease in identification of the cells.

3.6.4.2 *Cytological assessment*

Cytological assessment was performed using a binocular microscope at 400X magnification in order to identify individual cell types, including endometrial epithelial cells and polymorphonuclear (PMN) cells. PMN cells count were expressed as the proportion of PMN cells counted out of the total cells. A total of 100 cells were counted. Cows were categorized into two groups *viz*., subclinical endometritis (SCE) - positive or negative on the basis of the percentage of PMN cells. The ratio of PMNLs to the endometrial epithelial cells were evaluated and animals with more than 18 per cent PMN on days 21- 34 and more than 10 per cent on days 33- 47 were considered positive for SCE.

3.7 HISTOPATHOLOGICAL EXAMINATION OF THE ANIMALS

Representative endometrial samples were collected using Jacksons uterine biopsy forceps (Jorvett, USA) (Plate 3.5.a) in pluriparous animals and a stainless steel fabricated biopsy forceps similar to human bronchoscopy biopsy device (10370L Karl Storz®, Germany) with a circular cup for CB cows and heifers with smaller girth for cervical canal which had cupped jaw was also fabricated for the research (Plate 3.5b). The samples were kept in 10 per cent neutral buffered formalin, and sent for histopathological slide preparation. Tissue sections were stained with hematoxylin and eosin (Bancroft and Gamble, 2002) were examined

for any pathological changes in the endometrium involving surface epithelium, lamina propria, endometrial glands and vascular inflammatory status and scored according to the score card (Table 3.1) developed by Meira *et al.* (2012) the cut-off point of 15 was defined using SPSS 16.0.

Table 3.1 Histopathological Score card

Variable		**Category**	**Point**
Epithelium	Height	Columnar	1
		Cuboidal	2
		Flattened	3
	Damage	Absent	0
		Mild	1
		Moderate	2
		Ulcer	3
	Inflammatory cell type	Absent	0
		Mononuclear	1
		Polymorphonuclear	2
	Infiltrate intensity	Absent	0
		Mild (≤5 cells/hpf x 40[1])	1
		Moderate(≥6–10 cells/hpf×40)	2
		Severe (>10 cells/hpf; ×40)	3
Lamina propria	Inflammatory cell type	Absent	0
		Mononuclear	1
		Polymorphonuclear	2
	Infiltrate intensity	Normal (≤ 20 cells/hpf x 40)	0
		Mild (≥21–40 cells/hpf×40)	1
		Moderate (≥41–70 cells/hpf×40)	2
		Severe (>70 cells/hpf; ×40)	3

	Lymphocyte aggregate	Absent	0
		Mild (≤3 aggregates/hpf; ×10)	1
		Moderate (≥4–5 aggregates /hpf; ×10)	2
		Severe (≥6 aggregates/hpf; ×10)	3
Endometrial gland	Atrophy or dilation	Absent	0
		Present	1
	Fibrosis	Absent	0
		Mild (1–3 layers/hpf; ×40)	1
		Moderate (4–5 layers/hpf; ×40)	2
		Severe (≥6 layers/hpf; ×40)	3
Vascular	Vessel degeneration	Absent	0
		Present	1
	Haemorrhage	Absent	0
		Present	1
	Haemosiderin macrophages	Absent	0
		Present	1
Total			1-26

3.8 TREATMENT OF ANIMALS

Cows affected with SCE were treated with Cephapirin, containing a first generation cephalosporin antibiotic and a regeneration prompting agent (methyl uracil) (Metricef IU®, MSD) which was infused aseptically on the day of subsequent oestrus following artificial insemination (Mosaferi *et al.,* 2013). The product does not require withdrawal period of milk. The content of one Metricef syringe was introduced into the lumen of the uterus by using the disposable catheter. The syringe was fixed to the catheter. The catheter was introduced

through the cervix into the lumen of the uterus, by gentle oscillating movements of the cervix (Plate 3.6).

3.9 DAY OF FIRST POST PARTUM OESTRUS

The onset of first observed oestrus after calving was detected by observing behavioural signs, clinico-gynaecological examination, rectal palpation (for recording the uterine tonicity and intensity of oestrus) and ultrasonographic examination of uterus and ovary.

3.10 ARTIFICIAL INSEMINATION

All the animals were observed for spontaneous oestrus and inseminated on the detected oestrus. The animals that did not conceive to the first AI were re-inseminated for up to two consecutive oestrus.

3.11 PREGNANCY DIAGNOSIS BY TRANSRECTAL ULTRASONOGRAPHY

Pregnancy diagnosis was carried out on 28^{th} day post AI using real time B-mode ultrasonography. With a gloved, lubricated hand, the transducer was inserted into the rectum to visualize longitudinal section of uterus. Image of distended uterus with anechoic fluid and embryonic vesicle were confirmed positive as pregnant. Per-rectal examination was done on 45^{th} day post AI to confirm pregnancy.

3.12 PRINCIPLE OF ULTRASONOGRAPHY

Ultrasonography is based on the pulse-echo principle. A pulse of high-frequency sound (ultrasound) is transmitted into the body. Ultrasound with a frequency 1-10 MHz is used in animal diagnostics. Piezoelectric crystals in the transducer of real-time ultrasound machine transforms electric energy into sound waves and vice-versa. When piezoelectric crystals are stimulated electrically, it causes alteration in the crystal lattice structure causing it to vibrate and emit sound waves of high frequency. This high-frequency sound travels through the body

until it reaches a reflecting surface, at which point a portion of the ultrasound pulse (echo) is reflected back towards the source of the pulse. The time it takes to be reflected, allows determination of the reflecting surface's position. If an adequate number of points can be transmitted and received, a composite image of the reflecting surfaces can be displayed. The amount of reflected ultrasound pulse determines the brightness of the image (Burk and Feeney, 2003).

Tissues have different abilities to either propagate or reflect sound waves. The reflecting proportion will be represented on the ultrasound image by shades of grey, extending from black to white. Since liquids do not reflect sound waves, they appear non-echogenic or anechoic and appear black on screen. Dense tissues like bone reflect much of the sound waves and appear white or hyper-echogenic. Other tissues are seen in various shades of grey depending on their echogenicity (Ginther, 1995).

3.13 STATISTICAL ANALYSIS

The data obtained were analyzed using IBM SPSS statistics (version 24.0) software programme based on Snedecor and Cochran (1994).

Plates

Plate 3.1 Ultrasound scanning machine MyLab™ Gamma, Esaote SpA, Italy with transrectal transducer (SV3513, Esaote Europe B. V, Netherlands)

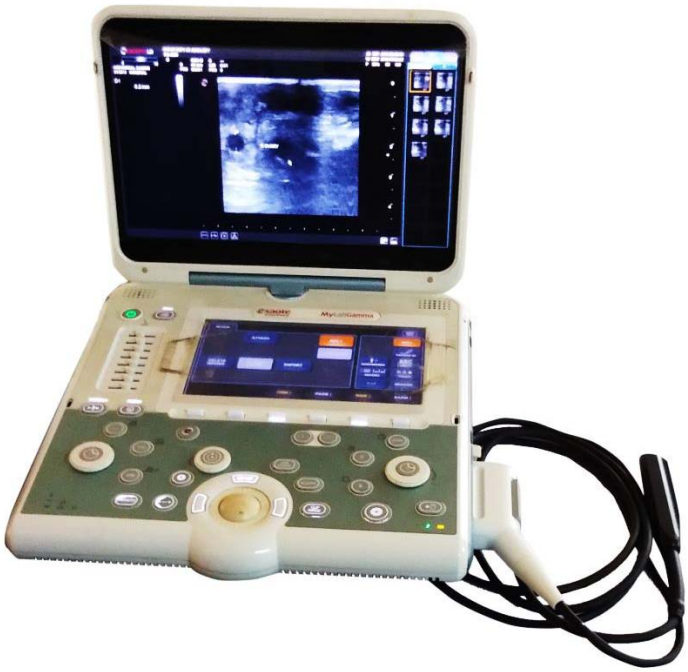
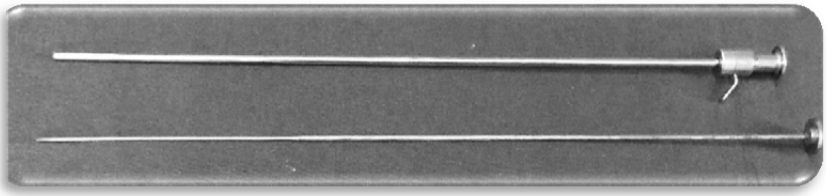


Plate 3.2.1 Bovine cytobrush, Minitübe GmbH Tiefenbach, Germany

Plate 3.2.2 Assembly of modified cytobrush

a. Human papsmear kit



b. AI gun

c. Cytobrush attached to stylet of AI gun

d. Cytobrush withdrawn inside the AI sheath

Plate 3.3 Modified cytobrush after taking sample

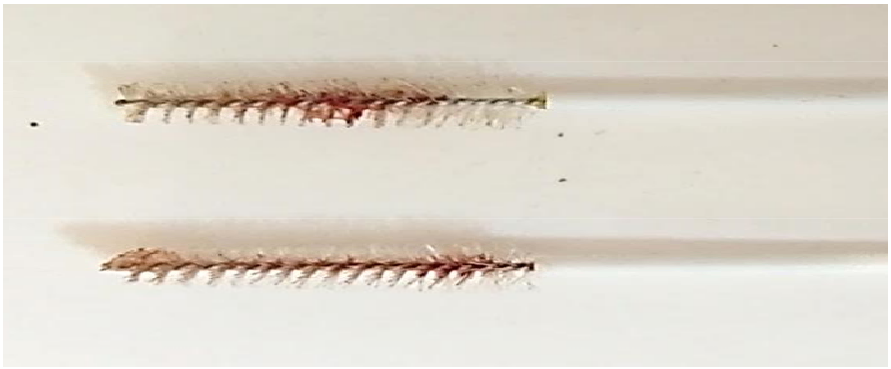


Plate 3.4 Rolling the modified cytobrush into sterile glass slides

Plate 3.5.a. Jackson uterine biopsy forceps with cutting jaws (Jorvett, USA)

3.5 b. Specially fabricated uterine biopsy forceps similar to human bronchoscopy biopsy device. (10370L Karl Storz®, Germany)

Plate 3.6 METRICEF ® (500 mg Cephapirin benzathine intrauterine suspension)

Results

4. RESULTS

The present study was conducted in a total of 24 postpartum dairy cows belonging to University Livestock Farm and Fodder Research and Development Scheme, Mannuthy from September 2018 – June 2019. On the basis of histopathological studies, 13 animals were found to be positive for SCE and 11 animals were found to be negative.

4.1 HISTORY

The cows included in the study were between three to twelve years of age and had not suffered from any periparturient complications.

4.2 AGE OF COWS

The mean (±SE) age of the cows calculated on the day of calving in normal and SCE groups were 5.91 ± 0.75 and 5.85 ± 0.58, respectively (Table 4.1). Statistical analysis of data revealed no significant difference between the groups ($p > 0.05$).

4.3 PARITY OF COWS

The parity of the cows in the study ranged from one to seven. Statistical analysis of the data has been done using Fisher's exact test to find whether there was any association between parity and occurrence of infection. There was no significant association ($p > 0.05$) between parity and occurrence of uterine infection.

4.4 WEIGHT OF CALVES BORN

The mean (±SE) birth weight of the calves (kg) born in normal and SCE groups were, 28.82 ± 1.75 and 28.62 ± 1.24, respectively (Table 4.1). Statistical analysis of data revealed no significant difference ($p > 0.05$) between the groups.

Table 4.1 Age of cows and birth weight (kg) of calves

Group	Normal (n = 11)	SCE (n = 13)	p-value
Age (year)	5.909 ± .75	5.85 ± 0.58	> **0.05**
Weight of calf (kg)	28.82 ± 1.75	28.62 ± 1.24	> **0.05**

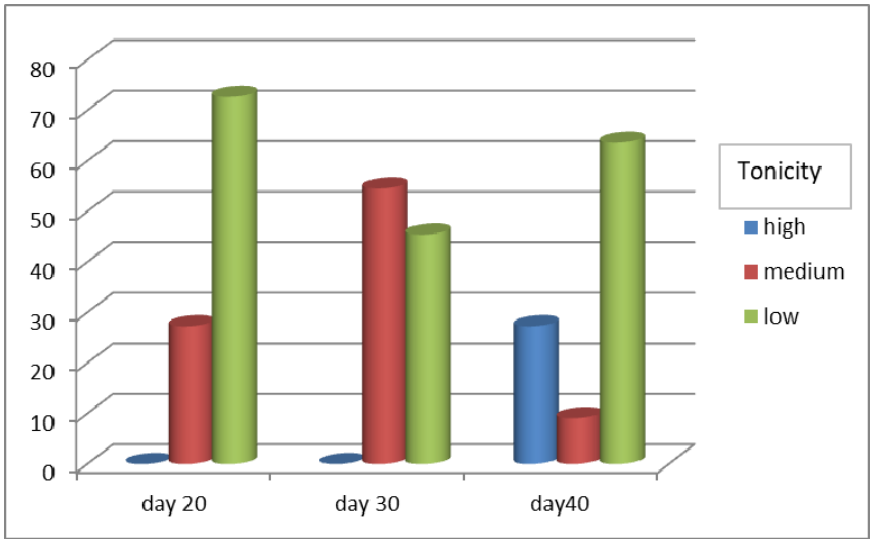
4.5 BODY CONDITION SCORE (BCS)

The body condition score for the animals ranged between 2.5 to 3.5 and statistical analysis using Fisher's exact test revealed that there was no significant association between parity and occurrence of infection.

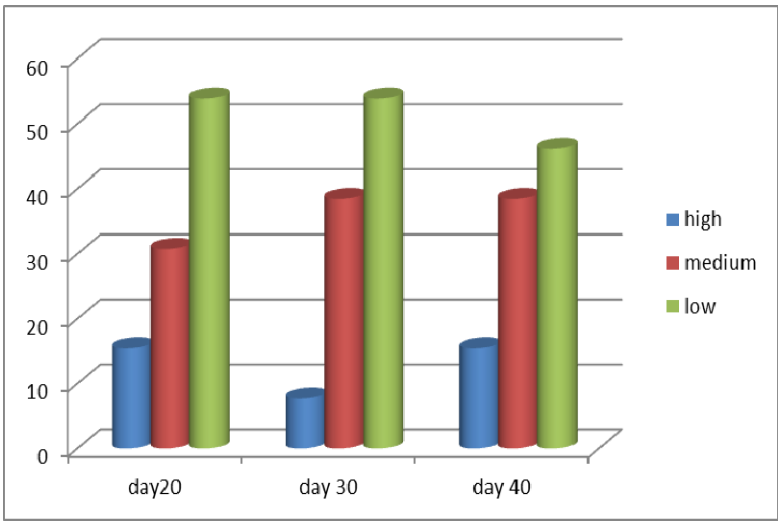
4.6 UTERINE TONICITY DURING DAYS OF OBSERVATIONS

The uterine tonicity expressed by the animals in normal and SCE groups were classified as high, medium and low tonicity during the days of observations and are presented in Fig. 4.1. Very few animals showed high uterine tonicity during the days of observation both in normal and SCE groups. Uterine tonicity varied with the ovarian status during the days of observation.

Fig. 4.1 Uterine tonicity by per rectal examination in normal and SCE groups



Normal group (n=11)



SCE group (n=13)

4.7 TRANSRECTAL ULTRASONOGRAPHY (TRUS)

TRUS was done on 20, 30 and 40 days postpartum (DPP) and the status of ovary and uterus was evaluated.

4.7.1 Ovarian status

The number of animals with follicles in dominant, recruiting and developing phase and presence of corpus lutem (CL) was noted in both normal and SCE groups (Table 4.2) (Plate 4.1).

By day 20, three animals in normal group and six animals in SCE group had developed dominant follicle (DF). Seven and six animals out of the normal group had developed DF on 30 and 40 DPP respectively. The number of cows with DF remained constant (six) in endometritis group.

When the cumulative total of DF was calculated in the normal group (n=11), three, eight and nine animals had DF respectively on days 20, 30 and 40. The same in the SCE group was six, nine and 12 respectively.

Table 4.2 Follicular dynamics during period of observation by TRUS examination in normal and SCE groups (%)

Day postpartum	Day 20		Day 30		Day 40	
Follicular dynamics	**Normal (n=11)**	**SCE (n=13)**	**Normal (n=11)**	**SCE (n=13)**	**Normal (n=11)**	**SCE (n=13)**
Developing follicle (<4mm) (%)	1 (9.09)	1 (7.69)	0 (0)	0 (0)	2 (18.18)	1 (7.69)
Recruiting follicle (4-9mm) (%)	7 (63.63)	6 (46.15)	4 (36.36)	7 (53.85)	3 (27.27)	6 (46.15)
Dominant Follicle (>9mm) (%)	3 (27.27)	6 (46.15)	7 (63.63)	6 (46.15)	6 (54.54)	6 (46.15)

4.7.1.1 *Resumption of ovarian cyclicity as assessed by the detection of active CL on TRUS*

Detection of active CL based on ultrasonographic evaluation is presented in Table 4. 3.

By day 20, three out of 11 animals in the normal group had active CL, whereas in the SCE group, six out of 13 animals had developed in CL. By day 30, eight animals in the normal group and seven in SCE group had active CL and by day 40, nine animals in both the groups had active CL.

When the cumulative total of CL was calculated in the normal group (n = 11), three, nine and eleven animals had CL respectively on days 20, 30 and 40. The same in the SCE group was six, eight and eleven, respectively. Two cows in SCE group did not show a CL during any days of observation.

Table 4.3 Ultrasonographic detection of functional CL in cows during the days of observation (%)

Group	Presence of CL by TRUS during the days of observation		
	Day 20	**Day 30**	**Day 40**
Normal (n = 11)	3 (27.27)	8 (72.72)	9 (81.81)
SCE (n = 13)	6 (46.15)	7 (53.84)	9 (69.23)

4.7.2 Cervix

The mean (± SE) diameter of the cervix (CD) was 30.92 ± 0.64, 29.63 ± 0.71 and 28.68 ± 0.76 in normal group and 30.47 ± 0.59, 29.02 ± 0.65 and 28.89 ± 0.69 in SCE group, respectively, on 20, 30 and 40 DPP (Table 4.4) (Plate 4.2).

Statistical analysis of the data revealed no significant difference ($p > 0.05$) in the CD between the normal and SCE groups on the given days of observation.

In the normal group the diameter of cervix gradually reduced from days 20 to 30. While in SCE animals there was significant reduction in the size of cervix from days 20 to 30.

Table 4.4 Cervical diameter (CD) in normal and SCE groups by TRUS

Group	Mean (± SE) CD in mm by echobiometry		
	Day 20	**Day 30**	**Day 40**
Normal (n = 11)	30.92 ± 0.64^{a}	29.63 ± 0.71^{ab}	28.68 ± 0.76^{b}
SCE (n = 13)	30.47 ± 0.59^{a}	29.02 ± 0.65^{b}	28.89 ± 0.69^{b}

Mean with common superscript (a-b within rows) does not differ significantly ($p > 0.05$)

4.7.3 Endometrial thickness

The mean (±SE) endometrial thickness was 7.19 ± 0.45, 6.62 ± 0.42 and 6.67 ± 0.45 in normal group and 7.29 ± 0.41, 7.45 ± 0.39 and 7.26 ± 0.42 in SCE group, respectively, on 20, 30 and 40 DPP (Table 4.5) (Plate 4.3).

Statistical analysis of the data revealed no significant difference between ($p > 0.05$) the normal and SCE groups on days 20, 30 or 40 postpartum. Also Statistical analysis within a particular group revealed no significant difference ($p > 0.05$) between the days 20, 30 and 40.

Table 4.5 Endometrial thickness in normal and SCE groups by TRUS

Group	**Mean (± SE) endometrial thickness in mm by echobiometry**		
	20	**30**	**40**
Normal (n = 11)	7.19 ± 0.44	6.62 ± 0.42	6.67 ± 0.45
SCE (n = 13)	7.29 ± 0.41	7.45 ± 0.39	7.26 ± 0.41

Measured at 5% level of significance

4.7.4. Uterine horn diameter

The mean (±SE) uterine horn diameter (UD) was 15.33 ± 0.47, 14.77 ± 0.57 and 14.83 ± 0.64 in normal group and 15.24 ± 0.43, 15.15 ± 0.52 and 15.05 ± 0.59 in SCE group on 20, 30 and 40 DPP, respectively (Table 4.6) (Plate 4.3).

Statistical analysis of the data revealed no significant difference ($p > 0.05$) between the normal and SCE groups on 20, 30 or 40 DPP. Also Statistical analysis within a particular group revealed no significant difference ($p > 0.05$) between days 20, 30 and 40 DPP.

Table 4.6 Uterine horn diameter (UD) in normal and SCE groups by TRUS

Group	Mean (± SE) UD in mm by echobiometry		
	20	**30**	**40**
Normal (n = 11)	15.33 ± 0.47	14.77±0.57	14.83±0.64
SCE (n = 13)	15.24 ±0.43	15.15±0.52	15.05±0.59

Measured at 5% level of significance

4.7.5 Fluid in uterus (FIU)

The details of animals with FIU are given in Table 4.7. At day 20 in the normal group about 81.81 per cent of animals had FIU, while it was only 30.76 per cent in SCE animals. The percentage of animals in the normal group with FIU reduced from days 20 to 30 and there was further reduction on day 40. While in SCE animals the percentage increased from day 20 to 30 and remained the same on day 40. The presence of FIU also depended on the ovarian status.

The percentage of animals with echogenic contents changed from 4 to nil in the normal group from days 20 to 30. Another animal had developed echogenic content after day 20 examination and it persisted on day 40 examination also.

In SCE group the echogenicity was observed in four animals on day 20 and two other animals developed echogenicity by day 30. All the animals except one had clear uterine fluid by day 40 (Plate 4.3).

Table 4.7 Number of animals (%) with fluid in uterus (FIU) in normal and SCE groups during days of observation by TRUS

Parameter	Normal group (n = 11)			SCE group (n = 13)		
	Day 20	**Day 30**	**Day 40**	**Day 20**	**Day 30**	**Day 40**
FIU (%) Echogenic & anechoic	9 (81.81)	5 (45.45)	6 (54.54)	4 (30.77)	9 (69.23)	9 (69.23)
Echogenic content (%)	4 (36.36)	1 (9.09)	1 (9.09)	4 (30.77)	6 (46.15)	2 (15.38)

4.8 ENDOMETRIAL CYTOLOGY USING CYTOBRUSH TECHNIQUE

Endometrial cytology was carried out to diagnose SCE in all the twenty-four cows by using uterine cytobrush (EC) technique on 30 and 40 DPP.

A total of 96 slides were prepared, stained and examined under 400X magnification. Samples in which slides more than 18 per cent and 10 per cent of polymorphonuclear (PMN) cells, respectively, were recorded on 30 and 40 DPP were considered as positive for subclinical endometritis. Out of 24 cows in the present study by endometrial cytology, eight cows and seven cows were diagnosed as positive for SCE, respectively, at 30 and 40 DPP (Plate 4.4).

4.8.1 Comparison between the normal and SCE groups

The mean (±SE) percentage of PMN cells by EC technique was 1.81 ± 0.88 and 1 ± 0.64 in normal group and 18 ± 0.81 and 9.92 ± 0.59 in SCE group, on 30 and 40 DPP, respectively (Table 4.8).

Statistical analysis of the data revealed significant difference between the percentage of PMN cells between the normal and SCE groups on both 30 and 40 DPP ($p < 0.05$).

Also there was significant reduction in PMN percentage in the SCE group between days 30 and 40 postpartum ($p < 0.05$).

Table 4.8 PMN (%) in normal and SCE groups

Group	**Day 30**	**Day 40**
Normal (n = 11)	1.81 ± 0.88^{B}	1 ± 0.64^{B}
SCE (n = 13)	18 ± 0.81^{Aa}	9.92 ± 0.59^{Ab}

Means with different superscripts (a-b in rows and A-B in columns) differ significantly (P<0.05).

4.9 HISTOPATHOLOGY EVALUATION

The endometrial sample was analysed on the basis of four different characters i.e. the surface epithelium, lamina propria, endometrial glands and vascular inflammatory status and a total score was assigned for the analysis (Plate 4.5).

4.9.1 Comparison based on surface epithelium

The mean (±SE) score of the surface epithelium were 4.90 ± 0.34 and 4.64 ± 0.34 in normal group and 7.77 ± 0.31 and 6.46 ± 0.31 in SCE group, on 30 and 40 DPP, respectively (Table 4.9).

Statistical analysis of the data revealed significant difference in the inflammation intensity and damage of the surface epithelium between the normal and SCE groups on both 30 and 40 DPP ($p < 0.05$). There was also significant reduction of the same parameter in the SCE group from days 30 to 40 postpartum ($p < 0.05$).

Table 4.9 Histopathological score of surface epithelium in normal and SCE groups

Group	Day 30	Day 40
Normal (n = 11)	4.91 ± 0.34^{B}	4.63 ± 0.34^{B}
SCE (n = 13)	7.77 ± 0.31^{Aa}	6.46 ± 0.31^{Ab}

Means with different superscripts (a-b in rows and A-B in columns) differ significantly (P<0.05)

4.9.2 Comparison based on lamina propria

The mean (±SE) score of the lamina propria were 2.64 ± 0.22 and 2.46 ± 0.18 in normal group and 4.62 ± 0.20 and 3.69 ± 0.16 in SCE group, on 30 and 40 DPP, respectively (Table 4.10).

Statistical analysis of the data revealed significant difference between the normal and SCE groups on both 30 and 40 DPP on comparing the inflammatory status of lamina propria ($p < 0.05$). There was also significant reduction within the SCE group between days 30 and 40 postpartum ($p < 0.05$).

Table 4.10 Histopathological score of lamina propria in normal and SCE groups

Group	30	40
Normal (n = 11)	2.64 ± 0.22^{B}	2.46 ± 0.18^{B}
SCE (n = 13)	4.62 ± 0.20^{Aa}	3.69 ± 0.16^{Ab}

Means with different superscripts (a-b in rows and A-B in columns) differ significantly ($p<0.05$).

4.9.3 Comparison based on endometrial gland

The mean (±SE) score of the endometrial glands were 1.90 ± 0.21 and 1.54 ± 0.15 in normal group and 3.15 ± 0.19 and 2.31 ± 0.14 in SCE group, on 30 and 40 DPP, respectively (Table 4.11).

Statistical analysis of the data revealed significant difference between the normal and SCE groups on both 30 and 40 DPP ($p <0.05$) with regard to degeneration and fibroplasia of gland. There was significant difference within the SCE group between days 30 and 40 postpartum ($p < 0.05$).

Table 4.11 Histopathological score of endometrial gland in normal and SCE groups

Group	Day 30	Day 40
Normal (n = 11)	1.91 ± 0.21^{B}	1.55 ± 0.15^{B}
SCE (n = 13)	3.15 ± 0.19^{Aa}	2.31 ± 0.14^{Ab}

Means with different superscripts (a-b in rows and A-B in columns) differ significantly (p<0.05).

4.9.4. Comparison based on vascular inflammation

The mean (±SE) score of the vessel were 1.54 ± 0.19 and 0.91 ± 0.13 in normal group and 2.15 ± 0.17 and 1.54 ± 0.12 in SCE group, on 30 and 40 DPP, respectively (Table 4.12).

Statistical analysis of the data revealed significant difference between and within the groups on 30 and 40 DPP regarding the vessel degeneration and haemorrhage ($p < 0.05$).

Table 4.12 Histopathological score of vessel in normal and SCE groups

Group	30	40
Normal (n = 11)	1.55 ± 0.19^{Ba}	0.91 ± 0.13^{Bb}
SCE (n = 13)	2.15 ± 0.17^{Aa}	1.54 ± 0.12^{Ab}

Means with different superscripts (a-b in rows and A-B in columns) differ significantly (p<0.05).

4.9.5 Comparison based on total Score

The mean (±SE) total score were 11.09 ± 0.59 and 9.64 ± 0.51 in normal group and 17.69 ± 0.55 and 14.00 ± 0.47 in SCE group, on 30 and 40 DPP, respectively (Table 4.13).

Statistical analysis of the data revealed significant difference between the normal and SCE groups on both 30 and 40 DPP ($p < 0.05$).

Table 4.13 Total histopathological score in normal and SCE group

Group	Day 30	Day 40
Normal (n = 11)	11.09 ± 0.59^{Ba}	9.64 ± 0.51^{Bb}
SCE (n = 13)	17.69 ± 0.55^{Aa}	14.00 ± 0.47^{Ab}

Means with different superscripts (a-b in rows and A-B in columns) differ significantly (P<0.05).

4.10 COMPARISON OF THE DIAGNOSTIC TECHNIQUES

The efficacy of histological findings (UB, cut off value for total score ≥ 15), TRUS findings (CD and FIU) and EC (positive or negative), for the diagnosis of SCE were analysed by means of Receiver Operating Characteristics (ROC) curve and Cochran's Q test separately for day 30 and day 40.

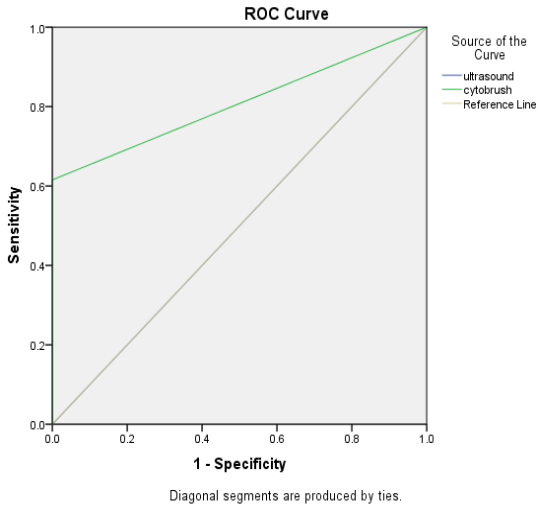
First, the cervical diameter (CD) on day 30 was compared with endometrial cytology (EC) and uterine biopsy (UB) on day 30 by keeping UB as gold standard.

Statistical analysis using Cochran's Q test revealed that TRUS findings based on CD for detection of SCE was significantly different ($p < 0.05$) from that of EC and UB, while diagnosis results of EC and UB showed no significant difference for diagnosis on day 30 ($p > 0.05$).

On ROC curve biopsy was set as gold standard and EC had a sensitivity of 61.5 per cent and 100 per cent specificity. Area under curve (AUC) was 80.8 per cent which indicates that EC has high predictability and can be a good alternative to UB for diagnosing SCE.

CD had very poor sensitivity and high specificity (100 %) and AUC was 50 per cent indicating that CD is a poor parameter for diagnosing SCE at day 30 (Fig.4.2).

Fig. 4.2 ROC curve of CD and EC with UB as standard on day 30



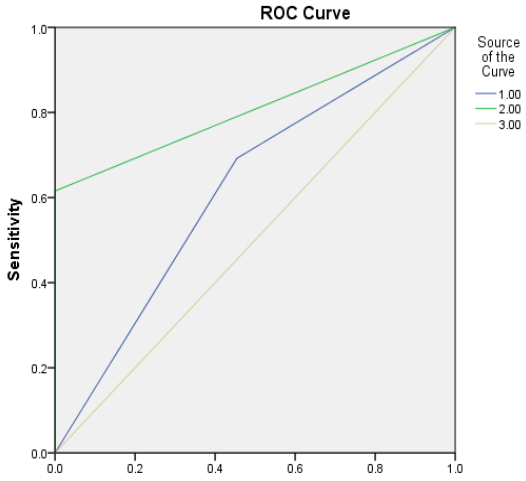
Blue line – CD (close to random, hence not distinguishable),
Green line – EC,
Grey line - random

FIU on day 30 was compared with EC and UB at day 30 by keeping UB as gold standard.

Statistical analysis using Cochran's Q test revealed that TRUS findings based on FIU for detection of SCE was similar to that of EC and UB.

On ROC curve analysis, FIU had 69.2 per cent sensitivity and 54.5 per cent specificity. AUC is 61.9 per cent and is a better parameter than CD at day 30 for detecting SCE (Fig. 4.3).

Fig. 4.3 ROC curve of FIU and EC with UB as standard on day 30



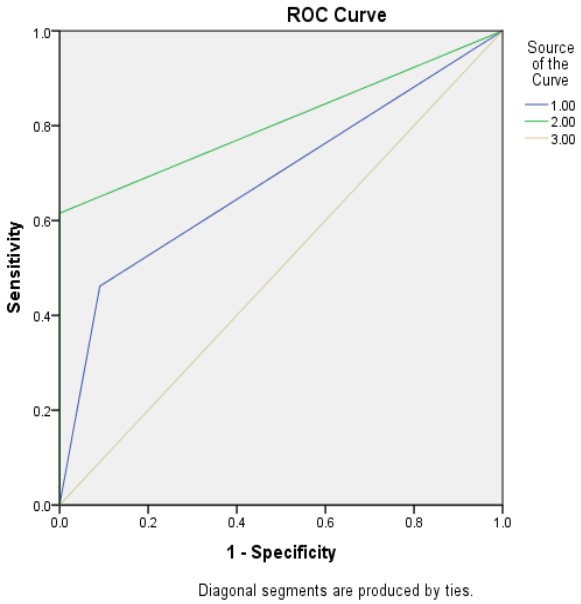
Blue line – FIU,
Green line – EC,
Grey line - random

Echogenic content on day 30 was compared with EC and UB at day 30 by keeping UB as gold standard.

Statistical analysis using Cochran's Q test revealed that TRUS findings based on echogenicity for detection of SCE was significantly different ($p < 0.05$) from that using UB, while diagnosis results of EC and echogenicity showed no significant difference for day 30 ($p > 0.05$).

On ROC curve analysis with UB as gold standard, echogenicity had 46.2 per cent sensitivity and 90.9 per cent specificity. AUC was 68.5 per cent indicating that the echogenicity has a moderate predictability (Fig.4.4).

Fig. 4.4 ROC curve of echogenicity of the contents and EC with UB as standard on day 30



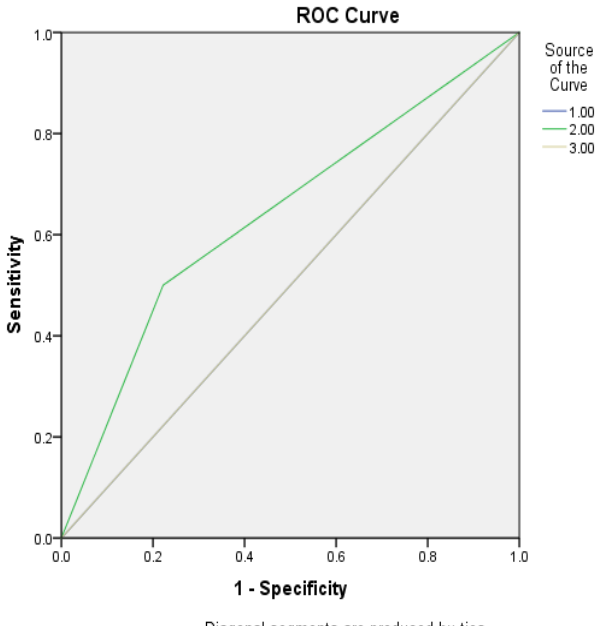
Blue line – Echogenicity of contents
Green line – EC
Grey line - random

The CD on day 40 was compared with EC and UB at day 40 by keeping UB as gold standard.

Statistical analysis using Cochran's Q test revealed that TRUS findings based on CD for detection of SCE was significantly different ($p < 0.05$) from that using EC, while diagnosis results of CD and UB showed no significant difference for day 40. Also diagnosis results of EC and UB showed no significant difference ($p > 0.05$).

On ROC curve, where UB was set as standard, EC had a sensitivity of 50 per cent and 78 per cent specificity. AUC was 63.9 per cent. CD had very poor sensitivity, high specificity and very low AUC equal to random indicating that CD had very poor predictability (Fig.4.5).

Fig. 4.5 ROC curve of CD and EC with UB as standard on day 40



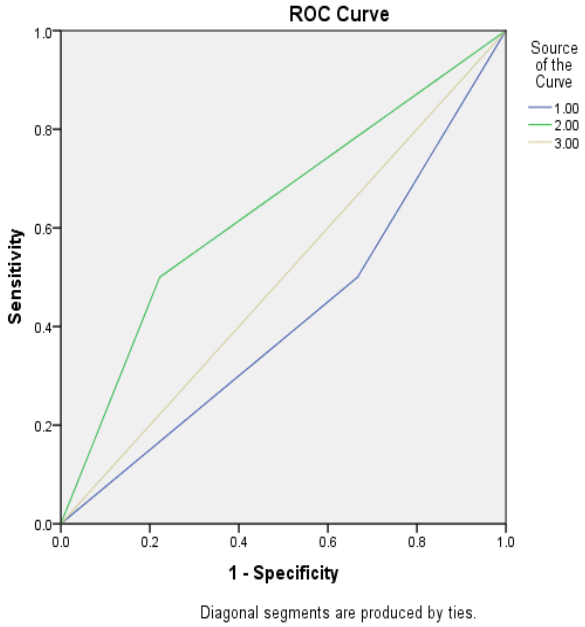
Blue line – CD not distinguishable from random
Green line – EC
Grey line - random

FIU on day 40 was compared with EC and UB at day 40 by keeping UB as gold standard.

Statistical analysis using Cochran's Q test revealed that TRUS findings based on FIU for detection of SCE was significantly different ($p < 0.05$) from that using UB, while diagnosis results of FIU and EC showed no significant difference for day 40 ($p > 0.05$). Also diagnosis results of EC and UB showed no significant difference ($p > 0.05$)

ROC curve with UB as standard, FIU had 50 per cent sensitivity and 33.3 per cent specificity. AUC was (41.7 per cent) below the random level, hence has very low predictability (Fig.4.6).

Fig. 4.6 ROC curve of FIU and EC with UB as standard on day 40



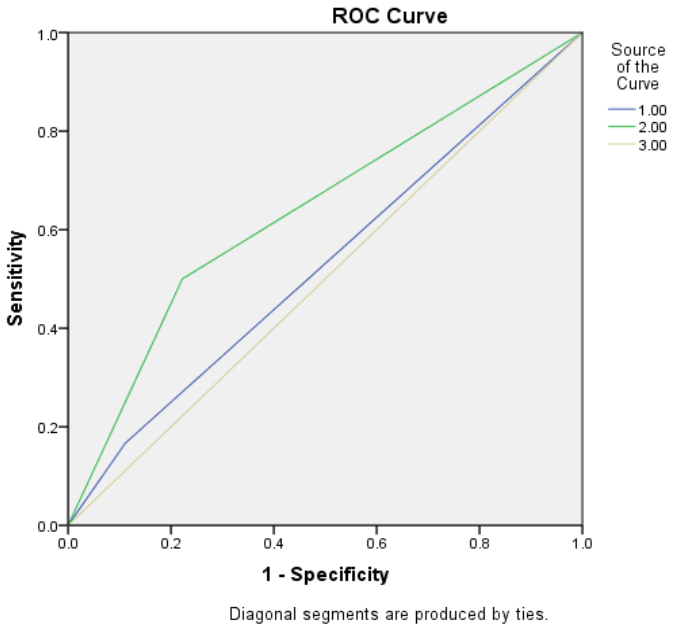
Blue line – FIU
Green line – EC
Grey line - random

Echogenic content on day 40 was compared with EC and UB at day 40 by keeping UB as gold standard.

Statistical analysis using Cochran's Q test revealed that diagnostic results of ultrasonography findings based on echogenicity of content, EC and UB for detection of SCE had no significant difference ($p > 0.05$).

On ROC curve with UB as standard, echogenic content had 16.7 per cent sensitivity and 88.9 per cent specificity with a low AUC of 52.8 having very low predictability (Fig. 4.7).

Fig. 4.7 ROC curve of echogenicity of the contents and EC with UB as standard on day 40



Blue line – Echogenicity of content
Green line – EC
Grey line - random

4.11 DAY OF FIRST POSTPARTUM OESTRUS

The mean onset of first observed oestrus after calving was respectively, 27.36 ± 1.38 and 29.38 ±1.37 in the normal and SCE group. Statistical analysis of data revealed no significant difference ($p < 0.05$) in both the groups (Table 4.14).

Table 4.14 Time interval from calving to first oestrus

Groups	Interval from calving to first oestrus (d)	p-value
	Mean (±SE)	
Normal (11)	27.36 ± 1.38	> 0.05
SCE (13)	29.38 ± 1.36	

4.12 UTERINE TONICITY AT DAY OF FIRST POSTPARTUM OESTRUS

The uterine tonicity expressed by the animals in normal and SCE animals were classified as high, medium and low tonicity (Table 4.15). Five cows in both the SCE and normal groups showed high intensity of oestrus. Medium tonicity was observed in seven animals (53.8%) and four animals (36.36%) in the SCE and in the normal group, respectively.

Table 4.15 Intensity of oestrus in normal and SCE groups

Intensity of oestrum	Normal Group (n=11)		Endometritis Group (n=13)	
	Number of Animals	%	Number of Animals	%
High	5	45.45	5	38.5
Medium	4	36.36	7	53.8
Low	2	18.18	1	7.69

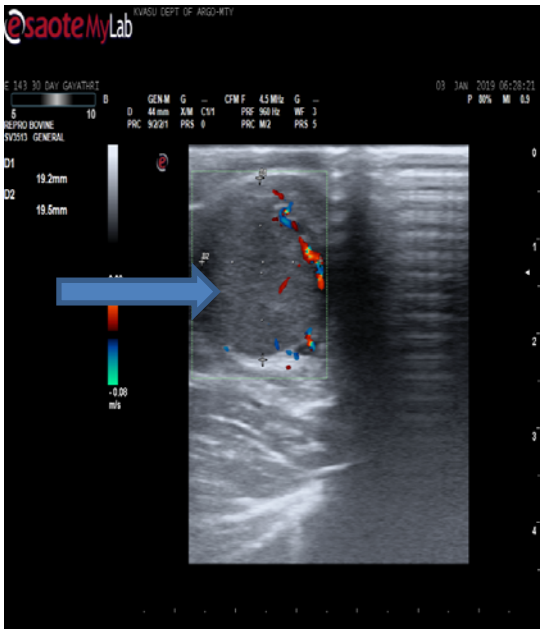
4.13 CONCEPTION RATE

The diagnostic procedures were conducted on 24 postpartum crossbred cows and 13 animals were found SCE positive. Treatment was done with Cephapirin (Metricef IU®, MSD) during the subsequent oestrus, 12 hours after AI was done. The percentage of animals conceived in the normal and SCE animals were 63.63 and 53.85 respectively (Table 4.16). Statistical analysis of data was using Fisher's exact test and there was no significant association ($p > 0.05$) between conception rate and occurrence of infection (Plate 4.6).

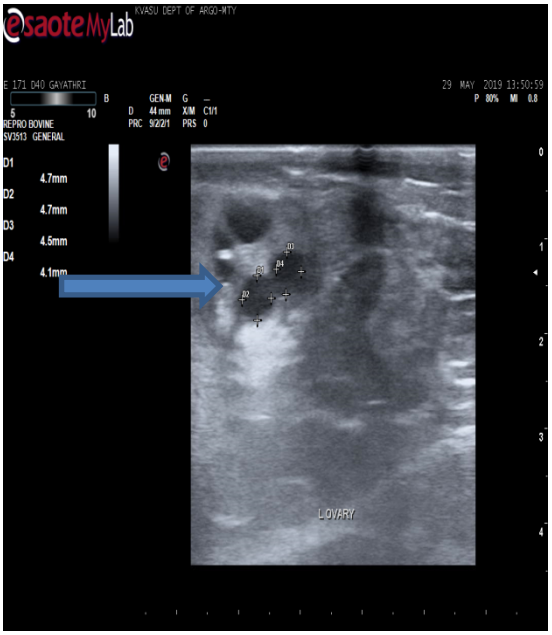
Table 4.16 Conception rate in normal and SCE groups

Group	Number of pregnant animals	Per cent
Normal (n = 11)	7	63.63
SCE (n = 13)	7	53.85

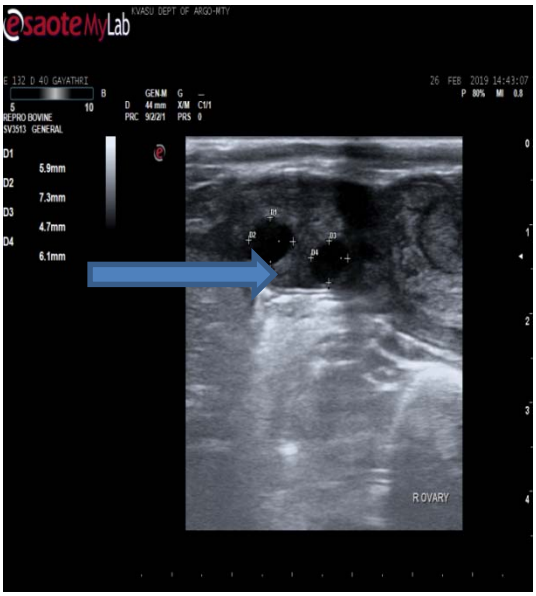
Plate 4.1 Ovarian structures on transrectal ultrasonography (TRUS)



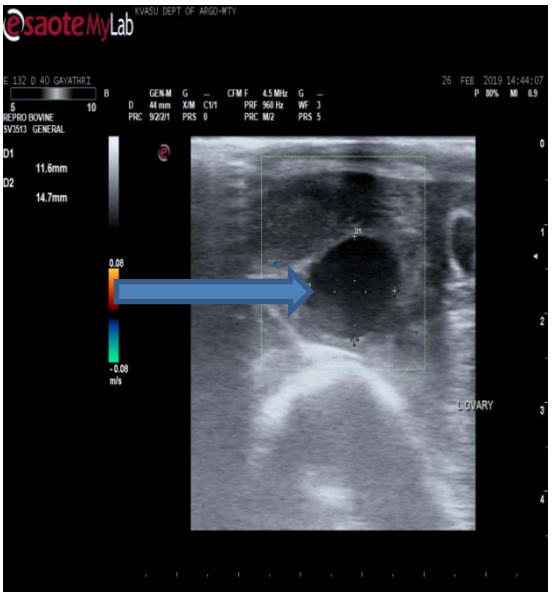
a. Active CL



b. Developing stage of follicles

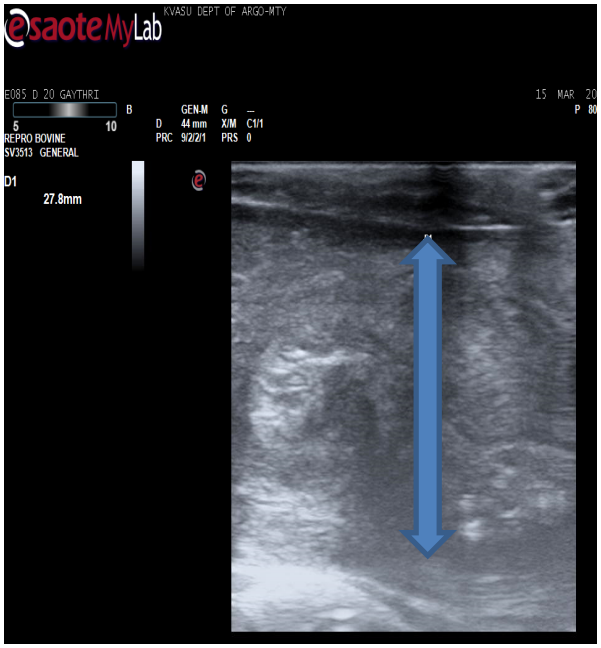


c. Developing and recruiting stage of follicles

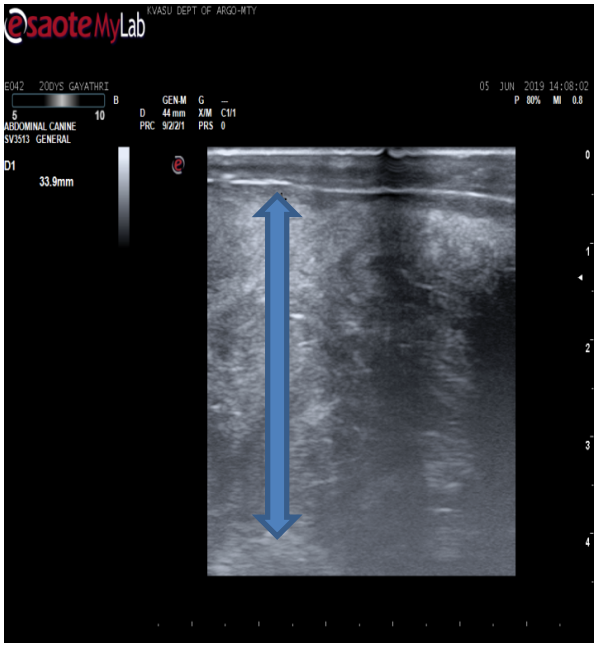


d. Dominant follicles

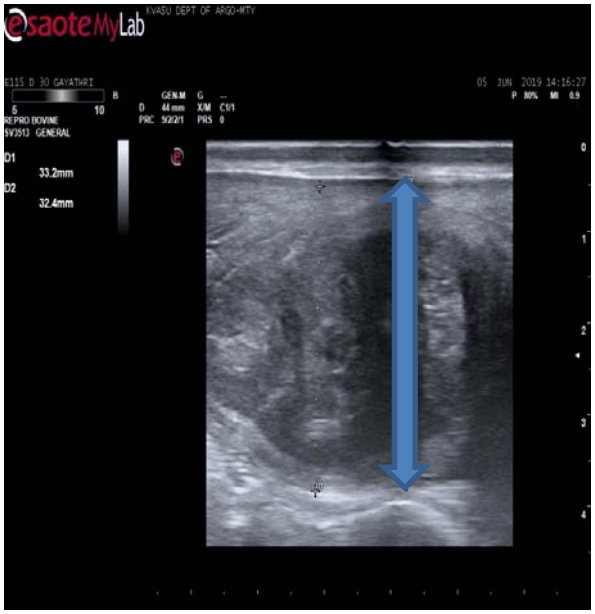
Plate 4.2 Cervical diameter (CD) on TRUS



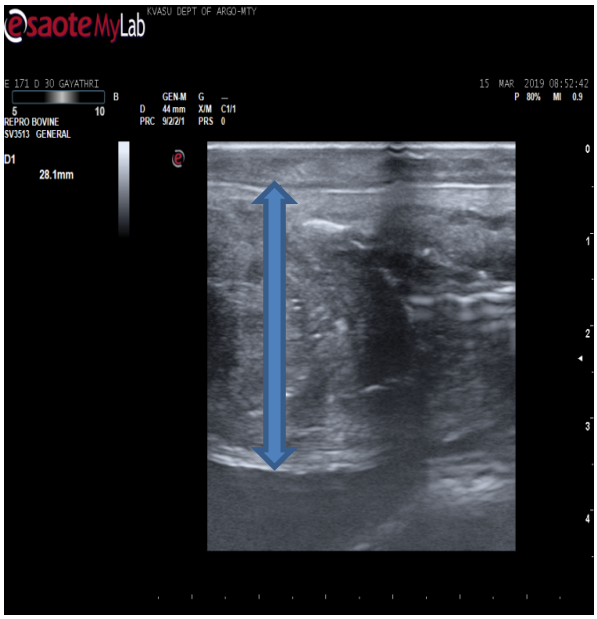
a. CD in normal animal on day 20



b. CD in SCE animal on day 20

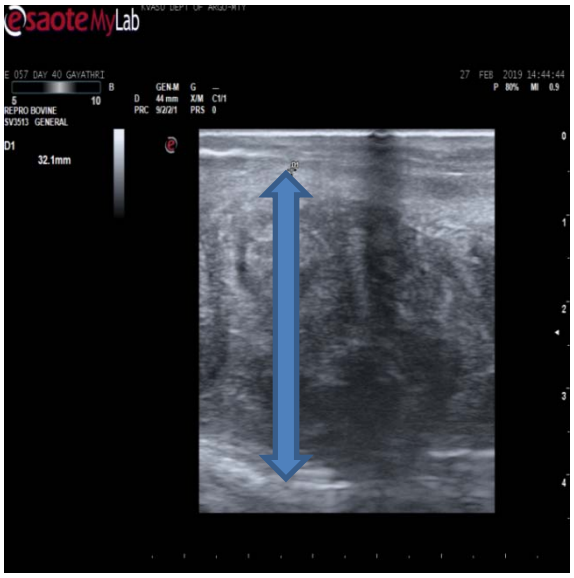


c. CD in normal animal on day 30

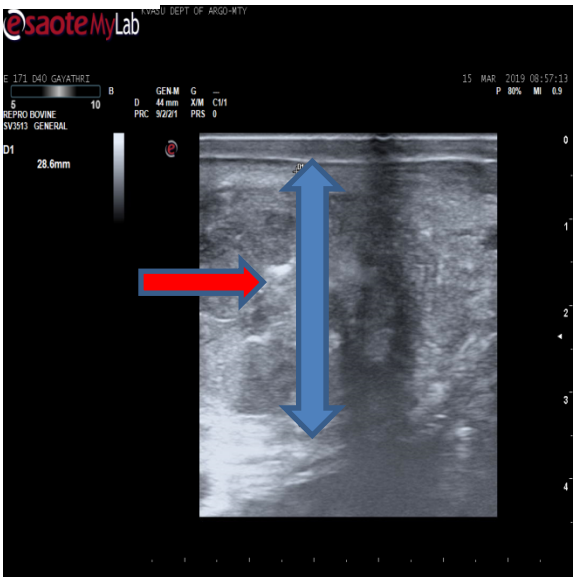


d. CD in SCE animal on day 30

Plate 4.2 Cervical diameter (CD) on TRUS (*continued*)

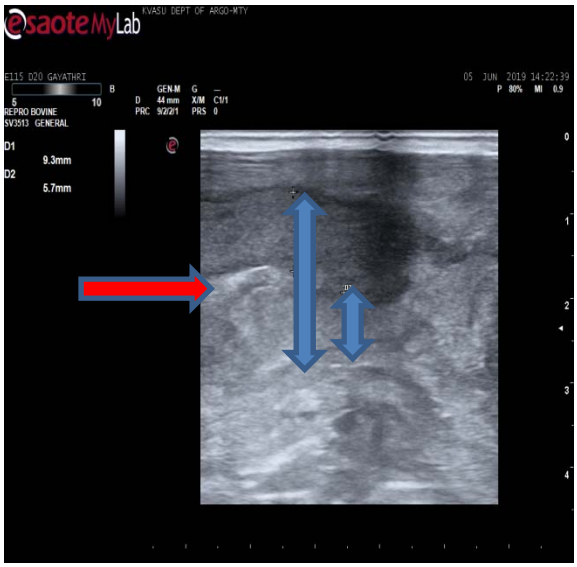


e. CD in normal animal on day 40

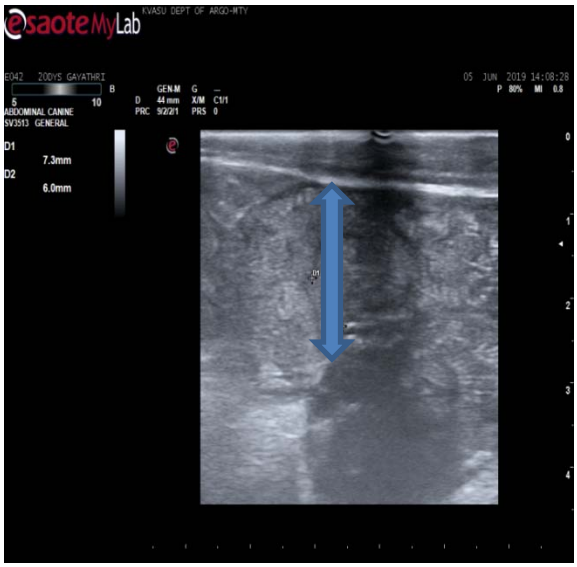


f. CD in SCE animal, with slight echogenicity (red arrow) on day 40

Plate 4.3 Uterine horn diameter (UD) and endometrial thickness on TRUS

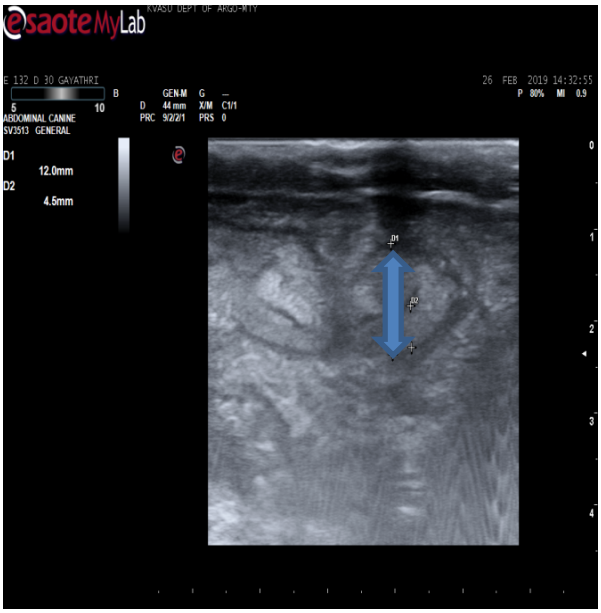


a. UD and ET in normal group, with slight echogenic content (red arrow) on day 20

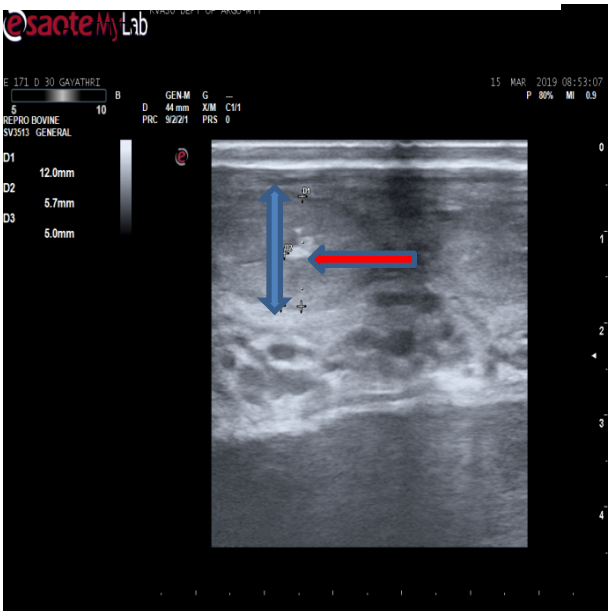


b. UD and ET in SCE group on day 20

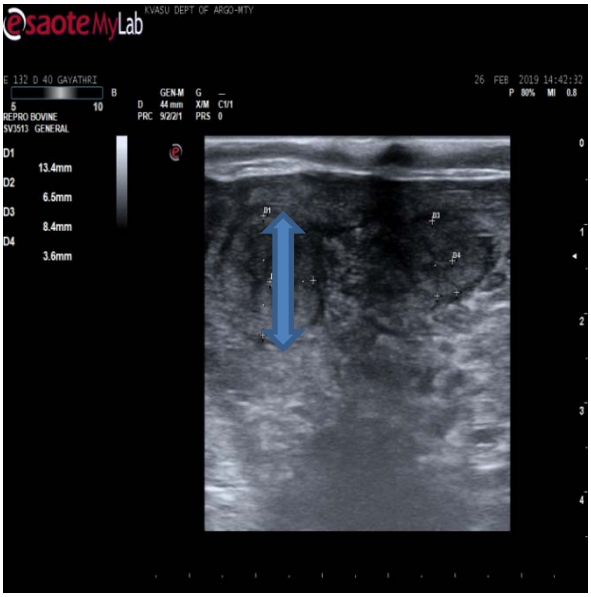
Plate 4.3 Uterine horn diameter (UD) and endometrial thickness on TRUS (*continued*)



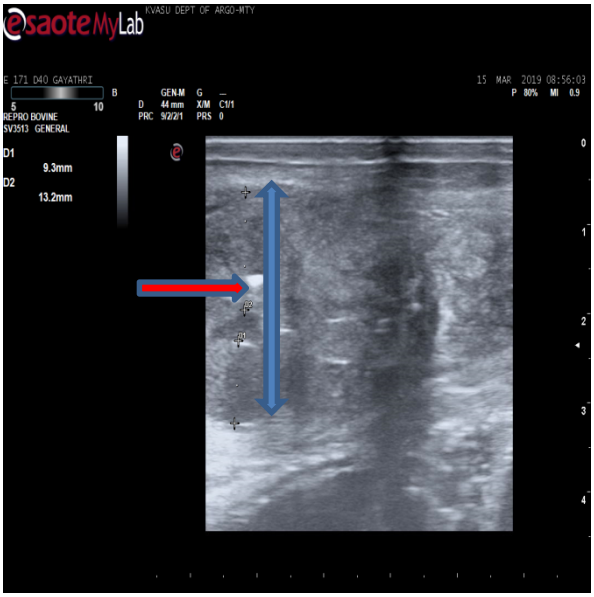
c. UD and ET in normal group on day 30



d. UD and ET in SCE group, with echogenic fluid content (red arrow) on day 30

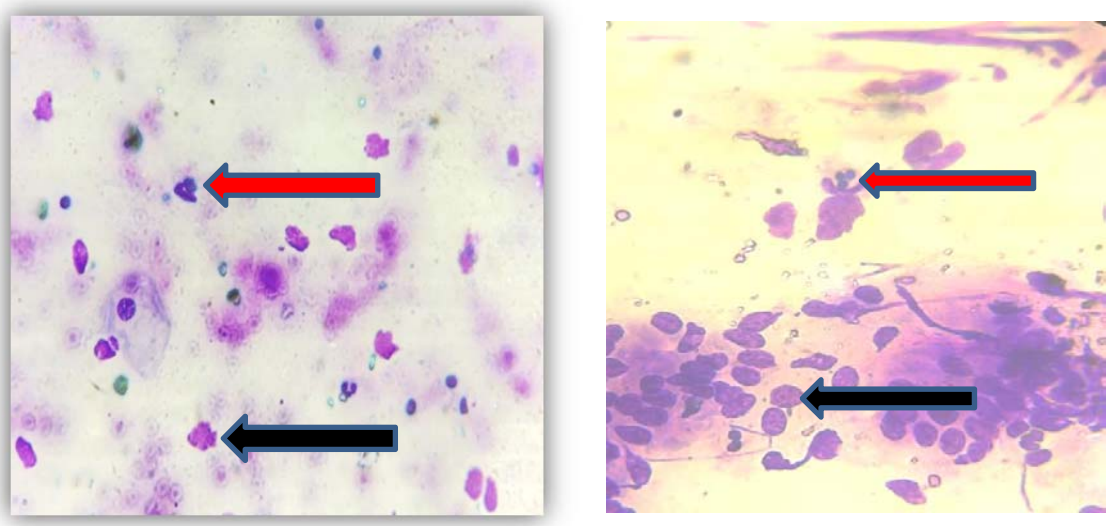


e. UD and ET in normal on day 40



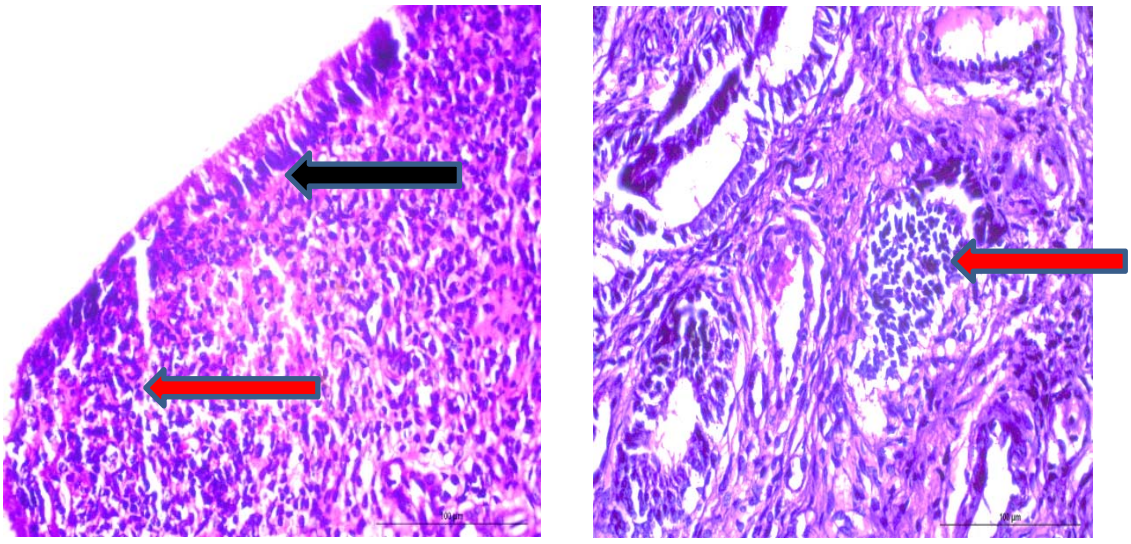
f. UD and ET in SCE group, with echogenic fluid content (red arrow) on day 40

Plate 4.4 Microphotograph of endometrial cytology at 400X magnification



a. Endometrial cells (black arrow) and PMN cells (red arrow)

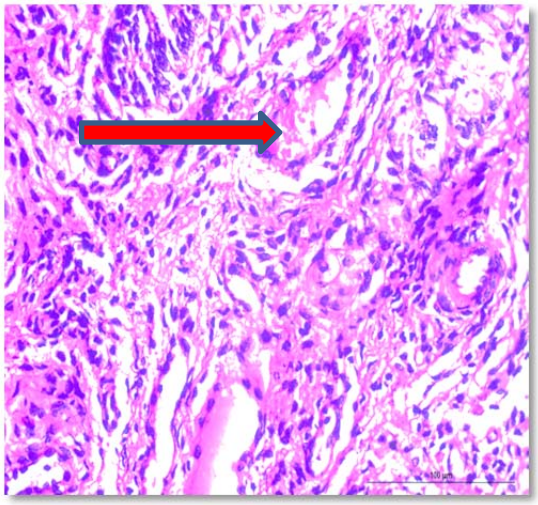
Plate 4.5 Microphotograph of histopathological sections of endometrium at 400X magnification



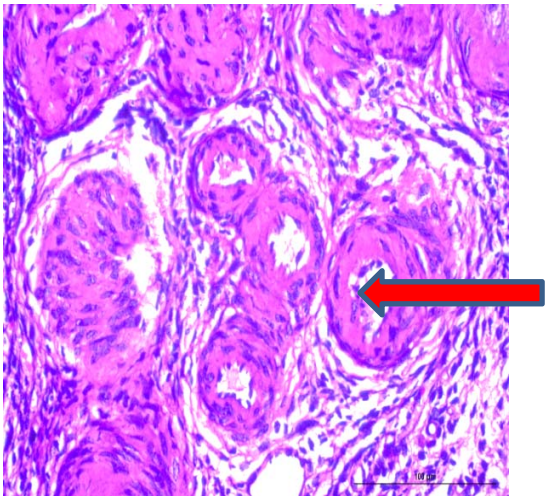
a. Intact surface epithelium, (columnar cells, black arrow) and PMN cells at surface epithelium (red arrow)

b. Atrophied glands (red arrow) with desquamated epithelial cells and PMN infiltration

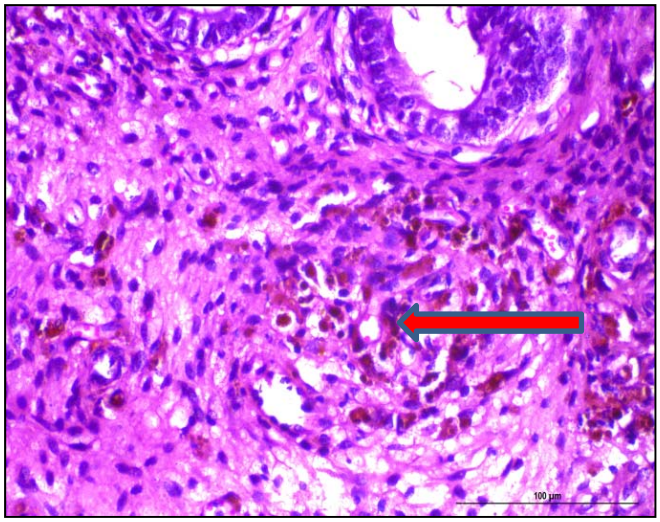
Plate 4.5 Microphotograph of histopathological sections of endometrium at 400X magnification (*continued*)



c. Congested blood vessels with RBCs visible (red arrow)

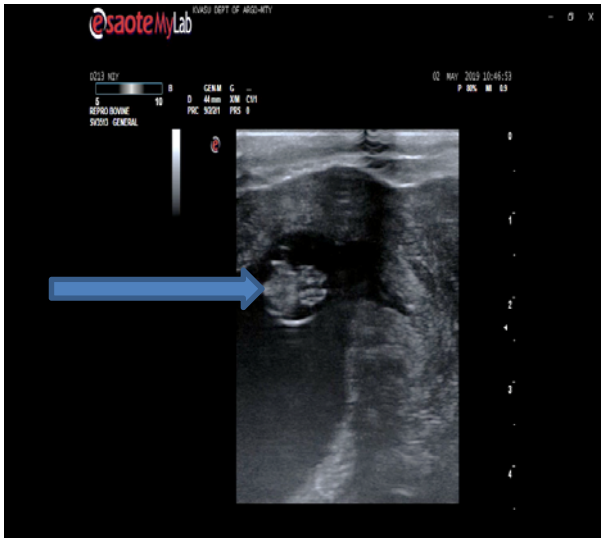


d. Perivascular fibroplasia (red arrow)

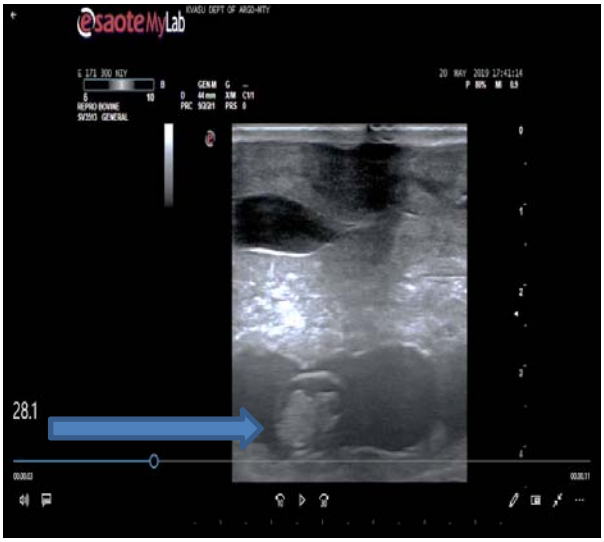


e. Hemosiderin laden macrophage (red arrow)

Plate 4.6 Pregnancy diagnosis by TRUS – after 28 days of AI



a. Bovine embryo in normal animal after 28 days of gestation (blue arrow)



b. Bovine embryo in SCE animal after 28 days of gestation (blue arrow)

Discussion

5. DISCUSSION

The present research work was carried out to compare the efficacy of ultrasonogrophic echobiometry of uterus, uterine cytology and uterine biopsy in the diagnosis of postpartum subclinical endometritis (SCE) in apparently normal crossbred dairy cows.

5.1 HISTORY AND AGE OF THE COWS

The 24 animals included in the study were three to 12 years of age with a mean age of 5.909 ± 0.75 in normal and 5.85 ± 0.58 in SCE groups and had not suffered from any periparturient complications. Analysis of data revealed that there was no significant difference between the two groups with regard to age of the cows ($p > 0.05$). Hence in this study there was no correlation between age and occurrence of infection. Contrary to this, Smith and Risco, (2002) reported a positive correlation between age and occurrence of infection. Bedewy and Rahawy (2019) also reported that there was a significant correlation between age and SCE

5.2 PARITY

The parity of cows in the present study ranged from one to seven. Statistical analysis of data using Fishers exact test did not find any association between parity and occurrence of infection ($p > 0.05$). This was in accordance with Gilbert *et al*. (2005) who found no significant effect of parity on cytological endometritis prevalence. Giuliodori *et al*. (2013) and Carneiro *et al.* (2014) also reported that parity did not affect the incidence of SCE.

Similarly, Chaffaux *et al.* (1991) found no significant association between clinical endometritis and parity, except for primiparous cows and those with more than five calving. The authors reasoned that the exception for primiparous cows may be due to increased likelihood of dystocia, endometrial lesions subsequent

clinical endometritis, whereas in older cows it could be due to reduced uterine elasticity and slower uterine involution.

5.3 WEIGHT OF THE CALVES BORN

In the present study the mean weight of the calves born to the cows calculated on the day of calving in normal and SCE animals were 28.82 ± 1.75 and 28.62 ± 1.24, respectively. Statistical analysis of data revealed no significant difference between the groups ($p > 0.05$). Potter *et al*. (2010) reported that a male calf usually weighs more than a female and increases the risk of dystocia and thereby the risk of endometritis. But in the current study male calves and female calves had similar body weight and also cows with no complications were selected for the study. Hence, such an association could not be made.

5.4 BODY SCORE CONDITION

The body condition score (BCS) for the animals ranged between 2.5 to 3.5 and statistical analysis using Fisher's exact test revealed that there was no significant association between BCS and occurrence of infection ($p > 0.05$). Bacha and Ragassa (2010) and Carneiro *et al.* (2014) had stated that cows with a low BCS (≤2.5) at 30 days postpartum had significantly higher prevalences of subclinical endometritis (SCE). However, the animals selected in the present study were within a good plane of nutrition since those with low BCS were excluded from the study, hence, no such associations with BCS and incidence of SCE could be made.

5.5 UTERINE TONICITY DURING DAYS OF OBSERVATIONS

Very few animals showed high uterine tonicity during the days of observations both in normal and SCE groups. Uterine tonicity also varied with the ovarian status during the days of observation. None of the animals had abnormal texture or doughy feel on palpation which is a common finding in clinical endometritis. According to a previous research conducted in the same farm (ULF

& FRDS) either doughy or low uterine tonicity was associated with cows having endometritis (Robert, 2016). None of the animals under study had abnormal texture or doughy feel on palpation which is a common finding in clinical endometritis. Hence it could be inferred from the present study that no correlation could be drawn from the uterine tonicity and SCE which was in accordance with Arias *et al*. (2018) who stated that in SCE no clinico-gynaecological abnormalities were readily recognizable and that special diagnostic techniques were required for diagnosis of SCE.

5.6 TRANSRECTAL ULTRASONOGRAPHY (TRUS)

TRUS was performed on days 20, 30 and 40 DPP and the status of ovary and uterus was evaluated.

5.6.1 Ovarian status

By day 20, three animals in normal group and six animals in the endometritis group had developed dominant follicle (DF). Three more animals in the SCE group and five more in normal group had developed DF by day 30. And by day 40, except for one animal in the SCE group and two animals in normal group, all the other animals under study had developed DF. No particular difference in the development of ovarian follicles could be discerned in between the groups under the present study. Thus, there was no delay in resumption of ovarian activity in the SCE group.

This finding was contrary to the report by Williams *et al*. (2005) and Williams *et al*. (2007) that growth and development of dominant follicle is suppressed by uterine infection during early postpartum period. This was attributed to the fact that the aromatization of androgens was inhibited, ergo, the oestradiol production affecting the granulosa cells and altering the lifespan of the follicle at recruitment and selection, and consequently ovulation (Herath *et al*., 2007).

However, in the present study all the animals had resumed ovarian cyclicity without delay. This could be due to the fact that there was reduction in the inflammation and infiltration by day 40 of observation and that the present study was conducted in SCE animals.

5.6.1.1 ***Resumption of ovarian cyclicity as assessed by the detection of CL on TRUS.***

By day 20, six animals in SCE group and three in normal group had CL. By day 40, all the animals except, two cows SCE group had developed CL during the period of study. There were no significant alterations in the pattern of CL development between the two groups.

This was in accordance with Carneiro *et al.* (2014) who reported that the presence or absence of CL had no effect on the incidence of subclinical endometritis**.**

In the present study there was no difference in the conception rates between the groups which could be attributed to the prompt resumption of cyclicity in both the groups. This was similar to the report by LeBlanc *et al.* (2002) where the evidence of ovarian activity (presence of a CL or follicle) was found to be associated with increased pregnancy rates.

5.6.2 Cervical diameter

The mean (±SE) diameter (in mm) of the cervix (CD) was 30.92 ± 0.64, 29.63 ± 0.71 and 28.68 ± 0.76 in normal group and 30.47 ± 0.59, 29.02 ± 0.65 and 28.89 ± 0.69 in SCE group, on 20, 30 and 40 DPP, respectively.

Statistical analysis of the data revealed no significant difference in the cervical diameter between the normal and SCE groups on the given days of observation ($p > 0.05$).

In the normal group the CD reduced gradually from days 20 to 40. While in SCE animals there was significant reduction in the size of cervix from days 20 to 30. By day 30, most of the animals, irrespective of the uterine infection, had a CD less than 3cm.

The cut off point for diagnosing animals as positive for SCE was taken according to the study by Kasimanickam *et al.* (2004) where a CD greater than 3 cm was considered positive for cytological endometritis. However, the present study was conducted in CB cows and the previous studies were done in pure bred cows where the size of cervix could be considerably large.

LeBlanc *et al*. (2002) also reported that cows with clinical endometritis had purulent uterine discharge or cervical diameter >7.5 cm after 20 DPP, or mucopurulent discharge after 26 DPP and that a CD >7.5 cm during the period of 27-33 DPP was significantly associated with reduced pregnancy rate.

In normal pure bred cows the the cervix normally involutes at a slower rate than the uterus, but both organs are expected to reach a diameter of <5 cm by 25 DPP (Morrow *et al.,* 1966).

However the present study was conducted in cross bred dairy cows where the average size of cervix is notably less than that mentioned in the reference articles. Hence a correlation between the CD and SCE could not be established statistically.

5.6.3 Endometrial thickness

The mean (±SE) endometrial thickness was 7.19 ± 0.45, 6.62 ± 0.42 and 6.67 ± 0.45 in normal group and 7.29 ± 0.41, 7.45 ± 0.39 and 7.26 ± 0.42 mm in SCE group on 20, 30 and 40 DPP.

Statistical analysis of the data revealed no significant difference ($p > 0.05$) in the endometrial thickness between the normal and SCE groups on days 20, 30

or 40 postpartum. Also Statistical analysis within a particular group revealed no significant difference between the days 20, 30 and 40.

Contrary to this, Pierson and Ginther (1987) and Kahn and Volkmann (2004) deduced that on the basis of ultrasonography, an increase in endometrial thickness is a typical finding in bacterial growth and delayed involution although these findings may be applicable mostly in clinical endometritis.

In the present study all the animals had undergone uterine involution within 30 DPP and the study was done in crossbred dairy cows and hence, no changes were noticed in terms of endometrial thickness.

5.6.4 Uterine horn diameter

The mean (±SE) uterine horn diameter was 15.33 ± 0.47, 14.77 ± 0.57 and 14.83 ± 0.64 in normal group and 15.24 ± 0.43, 15.15 ± 0.52 and 15.05 ± 0.59 in SCE group on 20, 30 and 40 DPP, respectively.

Statistical analysis of the data revealed no significant difference between the normal and SCE groups on days 20, 30 or 40 of postpartum ($p > 0.05$).

Also Statistical analysis within a particular group revealed no significant difference between days 20, 30 and 40 PP ($p > 0.05$)..

These findings are in accordance with Mateus *et al*. (2002) who reported that changes in uterine diameter are identifiable only in severe endometritis and such changes are negligible in SCE.

5.6.5 Fluid in uterus and echogenicity

At day 20, in the normal group about 81.81 per cent of animals had FIU, while it was only 30.77 per cent in SCE group. The percentage of animals in the normal group with FIU reduced from day 20 to 30 and there was further reduction on day 40. While in SCE animals the percentage increased from days 20 to 30 and remained same on day 40. The presence of FIU depends on the ovarian status.

This was in accordance with Dourey *et al*. (2011) who reported that there was a positive correlation between the quantity of uterine fluid and PMN percentage at four weeks postpartum, but it did not affect the interval from calving to first ovulation and speculated that it was very likely that the presence of uterine fluid indicates an active inflammatory process as evidenced by increased PMN.

The percentage of animals with echogenic content changed from four to nil in the normal group from days 20 to 30. But a new animal had developed very slight echogenic content after the day 20 examination and that persisted on day 40 examinations also.

In SCE group the echogenicity was observed in four animals on day 20 and two more animals developed echogenicity by day 30. All animals except for one had clear fluid by day 40.

The percentage of animals with echogenic content in the uterus was almost same in both the groups on day 20. This indicated that the echogenicity of the contents was not a reliable indicator of SCE. However, by day 30, there was an increase in echogenicity in the SCE group, while in the normal group there was a steady reduction. This was in accordance with Lenz *et al.* (2007) who reported that there was significant difference between healthy cows and cows with SCE regarding the echogenicity, during 21 to 27 DPP. However the echogenic content in both groups had been almost cleared by day 40. This reduction could be attributed to the resumption of ovarian cyclicity and the clearance of the uterine contents during the oestrus stage.

5.7 ENDOMETRIAL CYTOLOGY USING CYTOBRUSH TECHNIQUE (EC)

On EC at 30 DPP eight cows were found to be SCE positive and by 40 DPP, out of those eight, seven cows were diagnosed as positive for SCE using cytology alone.

The mean (±SE) percentage of PMN cells by UC technique were 1.81 ± 0.58 and 1 ± 0.47 in normal group and 18 ± 0.98 and 9.92 ± 0.69 in SCE group on 30 and 40 DPP, respectively.

Statistical analysis of the data revealed significant difference between the percentage of PMN cells between the normal and SCE groups on both 30 and 40 days postpartum ($p < 0.05$). Also there was significant reduction in PMN percentage in the SCE group between days 30 and 40 postpartum ($p < 0.05$).

This was in par with the studies by Kasimanickam *et al*. (2004) who used ROC analysis to identify the PMN per cent above which fertility was significantly reduced which was 18 per cent for 20–33 days postpartum and 10 per cent for 34–47 days postpartum. Different studies have used different threshold level in their studies. Arias *et al*. (2018) observed that the stage of oestrous cycle may affect the PMN infiltration.

The reduction in PMN percentage from days 30 to 40 was similar to the findings by Gilbert *et al.* (2005) and Kasimanickam *et al.* (2005a) and this could be explained by the completion of the process of uterine involution as similarly stated by Gilbert *et al.* (2005) and Senosy *et al.* (2009).

5.8 HISTOPATHOLOGY EVALUATION OF ENDOMETRIUM

The endometrial sample collected by UB was analysed on 4 different characters (surface epithelium, lamina propria, endometrial glands and vascular inflammatory status) and a total score was assigned for the analysis.

5.8.1 Comparison based on endometrial surface epithelium

The mean (±SE) score of the endometrial surface epithelium was 4.90 ± 0.34 and 4.64 ± 0.34 in normal group and 7.77 ± 0.31 and 6.46 ± 0.31 in SCE group on 30 and 40 DPP, respectively.

Statistical analysis of the data revealed significant difference in the inflammation intensity and damage of the surface epithelium between the normal and SCE groups on both 30 and 40 days postpartum ($p < 0.05$). There was also significant reduction of the same parameter in the SCE group from days 30 to 40 postpartum ($p < 0.05$).

This could be due to a higher degree of inflammatory infiltration mainly of PMNs cells (indicating an acute infection) in the surface epithelium and damage to the surface epithelium.

By day 40, the damage to the epithelium is resolved and there is clearance of the PMNs cells. This finding is similar to that reported by Meira *et al*. (2012).

Arias *et al.* (2018) also noted that higher infiltration could be detected during the follicular phase of the cycle.

5.8.2 Comparison based on lamina propria

The mean (±SE) score of the lamina propria was 2.64 ± 0.22 and 2.46 ± 0.18 in normal group and 4.62 ± 0.20 and 3.69 ± 0.16 in endometritis group on 30 and 40 DPP, respectively.

Statistical analysis of the data revealed significant difference between the normal and SCE groups on both 30 and 40 days postpartum on comparing the inflammatory status of lamina propria ($p < 0.05$). There was also significant reduction in the inflammatory status in the SCE group between days 30 and 40 postpartum ($p < 0.05$). By day 40, the score had reduced which indicated a reduced inflammatory status.

This was also in accordance with the study by Chethan *et al*. (2015) who reported that there was uniform distribution of inflammatory cells which included PMNs, macrophages and lymphocytes in the lamina propria and stratum compactum.

5.8.3 Comparison based on endometrial glands

The mean (±SE) score of the endometrial glands was 1.90 ± 0.21 and 1.54 ± 0.15 in normal group and 3.15 ± 0.19 and 2.31 ± 0.14 in SCE group on 30 and 40 DPP, respectively.

Statistical analysis of the data revealed significant difference between the normal and SCE groups on both 30 and 40 days postpartum ($p < 0.05$) regarding the degeneration and fibroplasia of gland. There was significant difference in the SCE group between days 30 and 40 postpartum ($p < 0.05$).

This was in accordance with Chethan *et al*. (2015) where there was periglandular infiltration of PMNs and lymphocytes along with atrophy and degeneration of glands.

But these findings were in disagreement with that reported by Meira *et al.* (2012) and Madoz *et al*. (2013) where they could not find any agreement with the status of endometrial glands and cytology studies, and these authors attributed that the glands are little affected in the case of SCE.

5.8.4 Comparison based on vascular inflammatory status

The mean (±SE) score of the vascular inflammatory status was 1.54 ± 0.19 and 0.91 ± 0.13 in normal group and 2.15 ± 0.17 and 1.54 ± 0.12 in SCE group on 30 and 40 DPP, respectively.

Statistical analysis of the data revealed significant difference between the normal and SCE group on 30 and 40 days postpartum regarding the vessel degeneration and haemorrhage ($p < 0.05$).

These findings were also similar to that by Chethan *et al*. (2015) who observed that dilation and congestion of blood vessels accompanied by haemorrhage in the lamina propria was observed.

5.8.5 Comparison based on total endometrial biopsy score

The mean (±SE) total endometrial biopsy score was 11.09 ± 0.59 and 9.64 ± 0.51 in normal group and 17.69 ± 0.55 and 14.00 ± 0.47 in endometritis group on 30 and 40 DPP, respectively.

Statistical analysis of the data revealed significant difference between the normal and endometritis group on 30 and 40 days postpartum ($p < 0.05$).

A total score of above 15 was taken as positive for defining SCE. In the SCE group the mean was greater than 15, which meant that all the four parameters taken for histopathological scoring was high in the SCE animals. This was in accordance with reports by Meira *et al*. (2012) that the animals with SCE had a higher inflammatory status in all the four layers.

Similarly Madoz *et al*. (2013) noted that in animals with SCE there was reduced likelihood of having a normal tissue biopsy and this chance decreased 2.1 percent for every increasing percentage point in PMNs.

5.9 COMPARISON OF EFFICACY OF DIAGNOSTIC TECHNIQUES

The efficacy of histological findings by UB (cut off value for total score ≥ 15), TRUS findings (CD and FIU along with the echogenicity of the content) and EC (positive or negative), for the diagnosis of SCE, were analysed by means of Receiver Operating Characteristics (ROC) curve and Cochran's Q test separately for day 30 and day 40.

On day 30, statistical analysis using Cochran's Q test revealed that TRUS findings based on CD for detection of SCE was significantly different from that of EC and UB. While diagnosis results of EC and UB showed no significant difference for day 30. While the analysis based on FIU for detection of SCE was similar to that of EC and UB. Hence all the three parameters could be effectively used for detection of SCE. Analysis based on echogenicity for detection of SCE

was significantly different from that using UB. While results of EC and echogenicity showed no significant difference for day 30.

These findings were also verified with ROC curve analysis. UB at day 30 was set as gold standard and EC, CD, FIU and echogenicity of the content were compared.

EC had a sensitivity of 61.5 per cent and specificity of 100 per cent. Area under curve (AUC) was 80.8 per cent which indicates that EC has high predictability and can be a good alternative for UB for diagnosing SCE.

CD had very poor sensitivity and high specificity and AUC was 50 per cent (worse than random), indicating that CD was a poor parameter for diagnosing SCE at day 30.

FIU had 69.2 per cent sensitivity and 54.5 per cent specificity. AUC is 61.9 per cent and is a better parameter than CD at day 30 for predicting SCE.

Echogenicity at day 30 had 46.2 per cent sensitivity and 90.9 per cent specificity. AUC was 68.5 per cent indicating that the parameter has a moderate predictability better than FIU and CD.

On day 40, Statistical analysis using Cochran's Q test revealed that TRUS findings based on CD for detection of SCE was significantly different from that using EC. While results of CD and UB showed no significant difference for day 40. Also results of EC and UB showed no significant difference.

Analysis based on FIU for detection of SCE was significantly different from that using UB. While results of FIU and EC showed no significant difference for day 40.

Analysis based on echogenicity of content, EC and UB for detection of SCE had no significant difference.

These findings were also verified with ROC curve analysis. UB at day 40 was set as gold standard and EC, CD, FIU and echogenicity of the content were compared.

EC had a sensitivity of 50 per cent and specificity of 78 per cent. AUC was 63.9 per cent. The efficiency of EC in diagnosing SCE was low when compared to day 30.

CD had very poor sensitivity, high specificity and very low AUC equal to random, indicating that CD was a poor parameter for diagnosing SCE at day 40.

FIU had 50 per cent sensitivity and 33.3 per cent specificity. AUC was (41.7 per cent) worse than the random, hence has very low predictability in diagnosing SCE.

Analysis based on echogenic content had 16.7 per cent sensitivity and 88.9 per cent specificity with a low AUC of 52.8 per cent (near to random) and hence having very low predictability on diagnosing SCE.

When comparing days 30 and 40 observations, it could be inferred that on day 30, EC was the best parameter for diagnosing SCE, followed by echogenicity, FIU and CD. And on day 40, the best one was EC and all the other parameters where of very poor use in diagnosing SCE.

The use of CD for diagnosis was very poor in the present study which was in contrast to the reports by Meira *et al*. (2012) that CD was found to be an intriguing tool for the diagnosis of cytological endometritis, and that its efficiency was better when combined with other observations. This could be due to the fact that the present study was conducted in cross bred dairy cows where the uterine and cervical size was considerably less compared to the reference articles in which pure bred cows were used.

However the current study was in agreement with Mateus *et al*. (2002) who reported that similar uterine diameters were observed in the cows with a

healthy uterus and mild endometritis (SCE) cases five weeks after calving. Polat *et al*. (2015) also reported similar cervical diameter in healthy and SCE animals.

The predictability of FIU for diagnosis of SCE in the present study was moderate on day 30 while it was low on day 40.This was contradictory with Meira *et al*. (2012) where FIU had good specificity (94%), and a similar observation was found in other studies (Kasimanickam *et al*., 2004; Oral *et al.,* 2009). This could be due to the fact that fluid in uterus was influenced by the stage of oestrus.

A similar observation was reported by Polat *et al*. (2015) that the sensitivity and specificity of diagnosing subclinical endometritis by using ultrasonography is lower than cytology examinations and that fluid accumulation could be observed when cows were in oestrus and was likely to give false positives.

The presence of echogenic content in the uterus as a parameter for diagnosis of SCE had poor sensitivity but very high specificity on both the days of examination. It indicated that animals with echogenic content were mostly positive for SCE, but not all animals having SCE had echogenic content in the uterus.

According to previous studies (Meira *et al*., 2012 and Madoz *et al*., 2013) in SCE, endometrial glands were rarely affected and only when glands were involved, abnormal uterine contents were observed. Hence parameters excluding the status of glands were more useful.

Contrary to the previous studies, there were involvement of the endometrial glands, with atrophy and inflammatory infiltration which could have resulted in the echogenicity. But by day 40, due to the expression of oestrus by almost all the animals, the uterine contents was evacuated and the echogenicity was cleared leading to its lower predictability.

On a similar note Mariño *et al*. (2017) found a significant relationship between presence of abnormal intrauterine fluid and SCE diagnosed by biopsy but not by cytology.

On comparing EC and UB, in the present study it could be noted that EC was a moderately good alternative for UB on both days of observation. This was in discordance with Meira *et al*. (2012) who reported that there was poor agreement between biopsy and cytology and the reason was attributed to the detailed information (dergree and extent of infiltration) obtained using histopathological studies, whereas cytology gives information about the superficial layers of endometrium.

There were other works where uterine biopsy and cytology was compared for the diagnosis of SCE in dairy cattle and the two diagnostic methods showed poor agreement (Madoz *et al*., 2013 and Marino *et al*., 2017).

5.10 DAY OF FIRST POSTPARTUM OESTRUS

The onset of first observed oestrus after calving was respectively, 27.36 ± 1.38 and 29.38 ±1.37 in the normal and SCE groups. Statistical analysis of data revealed no significant difference in both the groups.

This is in accordance with the findings that on average, dairy cows are expected to have their first postpartum ovulation between 14 and 28 DPP (Morrow *et al.,* 1966). However, contrary to the present study, Dourey *et al*. (2011), had observed that the interval from calving to first ovulation and the exhibition of oestrus signs were significantly shorter in cows with low PMN and most of them ovulated ovulated before 25 DPP than high PMN cows. Similar observation was also noted by Sheldon *et al*. (2002) and reported that uterine inflammation, suppressed LH secretion and delayed the postpartum resumption of cyclicity.

5.11 CONCEPTION RATE

The diagnostic procedures were conducted on 24 postpartum crossbred cows and 13 animals were found SCE positive. All the animals under the study were inseminated during the next observed oestrus. Treatment was done on the SCE positive animals in the subsequent oestrus using cephapirin 12 hour after artificial insemination was done. The percentage of animals conceived in the normal and SCE groups were 63.63 and 53.85 respectively. Statistical analysis of data revealed no significant difference in both the groups. This could be due to either the beneficial effect of treatment with cephapirin post AI or a high self-cure ability of SCE.

The efficiency of cephapirin for the treatment of endometritis was demonstrated by Leblanc *et al.* (2002) who stated that there was reduced time to pregnancy in treated animals than in untreated cows and Kasimanickam *et al.* (2005b) also demonstrated the effectiveness of single treatment with cephapirin which significantly improved the reproductive performance of SCE cows.

Mosaferi *et al*. (2013) demonstrated that the administration of cephapirin in cows with clinical endometritis in the first observed oestrus following a voluntary wait period, had reduced calving to conception interval and calving to the first service interval.

However, this was contradictory to the work by Gumen *et al*. (2012) who observed that the intrauterine cephapirin administered 24 hours post AI was not successful for the treatment of potential subclinical endometritis.

Similar to the present study Dourey *et al*. (2011) reported that there was no significant difference between low and high PMN groups in the interval from calving to pregnancy though this could have been die to extended interval to first service period.

However, there was a study by Green *et al.* (2011) that focuses on the self-cure rate of SCE, with a high self-cure rate in animals which had >18 percent PMNs at 21 days in which 81 percent had been cleared by 42 days postpartum.

In contrast the works by Okawa *et al*. (2017) demonstrated that the animals with mild endometritis if left untreated even after 40 DPP had a reduced reproductive efficiency.

In the present study, similar conception rates in both the groups could have been due to a mixture of factors, fast clearance rate of the herd and treatment with cephapirin.

Summary

SUMMARY

The study was conducted in 24 crossbred dairy cows belonging to the University Livestock Farm and Fodder Research and Development Scheme, Mannuthy from September 2018 to June 2019.

The history of the animals under study including age, parity, BCS and details of calves born were recorded. The animals with periparturient complications were excluded from the study. Clinico-gynaecological examinations were conducted daily from day 20 onwards to assess the reproductive status of the animals.

Tonicity and involution of the uterus were recorded by per rectal examination during days 20, 30 and 40 postpartum. Transrectal ultrasonography (TRUS) was done during the days of observation to analyse the uterine echobiometry and ovarian status.

The parameters recorded under echobiometry included cervical and uterine horn diameter, endometrial thickness, presence of fluid in uterus and the nature of fluid. The follicular dynamics along with development of CL was recorded by TRUS under the ovarian status.

Endometrial cytology (EC) samples were obtained on the days 30 and 40, by using bovine cytobrush in pluriparous cows and by using modified cytobrush in primiparous cows and in cows with smaller girth for the cervix. The cytobrush was rolled on to clean microscope slides and stained with modified Wright-Giemsa and Field Stain. The slides were observed under high power magnification of microscope and the PMNs cells were counted.

The histopathological samples were obtained using bovine Jacksons uterine biopsy forceps and a modification of the biopsy forceps reducing the bore size was fabricated with stainless steel to suit for animals with smaller girth for the cervix. The samples were kept in neutral buffered formalin, histological

sections were made and stained with Hematoxylin & Eosin stain. A score was assigned based on the pathological changes in the surface epithelium, lamina propria, endometrial glands and endometrial vessels with a cutoff point of 15 for SCE.

Day of first observed oestrus after calving was detected by clinico-gynaecological examination and confirmed by rectal palpation and TRUS.

All the animals were inseminated in the next heat observed after a voluntary wait period. The animals diagnosed as SCE positive were treated with Cephapirin intrauterine infusion as a single dose 12 hours after AI. All the animals were subjected to pregnancy diagnosis using TRUS on 28^{th} day.

Among 24 animals included in the study, using the three diagnostic techniques 13 cows were found to be positive for SCE by histopathological examination.

The animals under study were aged between three and 12 years. The mean (±SE) age of the cows was 5.91 ± 0.75 and 5.85 ± 0.58. The age did not seem to affect the occurrence of infection.

The parity of the animals ranged from one to seven. On statistical analysis using Fisher's exact test, there was no correlation between the parity and occurrence of infection.

The mean weight (kg) of the calves born to the cows under the study was 28.82 ± 1.75 and 28.62 ± 1.24, respectively in the normal and SCE groups. On statistical analysis there was no significant difference between the groups.

The mean BCS of cows at time of calving irrespective of groups ranged from 2.5 to 3.5. On statistical analysis using Fisher's exact test, there was no correlation between the BCS and occurrence of infection.

On recording the uterine tonicity expressed by the animals in normal and SCE groups, it could be noted that very few animals showed high uterine tonicity during the days of observation, which could be influenced by the ovarian status.

TRUS examination of the ovary during the days of observation revealed that by day 20, six animals in the SCE group and three in normal groups had developed a DF, which indicated that in these animals the follicular dynamics began by day 20. Only one cow in SCE group and two in normal group did not show a DF during any days of observation.

Similarly, by day 20, six animals in SCE group and three in normal group had a functional CL and this was taken as proof for ovulation in these animals before day 20. After conclusion of the study only two cows in SCE group did not show a CL during any days of observation.

The mean (± SE) diameter of the cervix (CD) was 30.92 ± 0.64, 29.63 ± 0.71 and 28.68 ± 0.76 in normal group and 30.47 ± 0.59, 29.02 ± 0.65 and 28.89 ± 0.69 in SCE group, respectively, on 20, 30 and 40 DPP. Statistical analysis of the data revealed no significant difference in the cervical diameter between the normal and SCE groups on the given days of observation. However, there was significant reduction in CD from days 20 to 30 in SCE group.

The mean (±SE) endometrial thickness was 7.19 ± 0.45, 6.62 ± 0.42 and 6.67 ± 0.45 in normal group and 7.29 ± 0.41, 7.45 ± 0.39 and 7.26 ± 0.42 in SCE group, respectively, on 20, 30 and 40 DPP. Statistical analysis of the data revealed no significant difference between and within the groups on days 20, 30 or 40 postpartum.

The mean (±SE) uterine horn diameter (UD) was 15.33 ± 0.47, 14.77 ± 0.57 and 14.83 ± 0.64 in normal group and 15.24 ± 0.43, 15.15 ± 0.52 and 15.05 ± 0.59 in SCE group on 20, 30 and 40 DPP, respectively. Statistical analysis of the data revealed no significant difference between and within the groups on days 20, 30 or 40 postpartum.

The number of animals with FIU was high in normal group on the first day of observation and there was steady reduction in this number during the subsequent days of observation. But in SCE group, initially the number was less, then there was a rise in the number and remained constant during the next two days of observation.

However, the animals with echogenic content were same in both the groups on first day of observation. In the normal group, the echogenicity had cleared faster. But in SCE group, the animals with echogenicity increased from days 20 to 30. On day 40, the number of animals with echogenic content was one in both the groups.

The mean (±SE) percentage of PMN cells by EC technique was 1.81 ± 0.88 and 1 ± 0.64 in normal group and 18 ± 0.81 and 9.92 ± 0.59 in SCE group, on 30 and 40 DPP, respectively. Statistical analysis revealed significant difference between the percentage of PMN cells between the groups on both 30 and 40 DPP ($p < 0.05$). There was also significant difference within the SCE group during both the days of study.

The mean (±SE) score of the inflammatory status of endometrial surface epithelium were 4.90 ± 0.34 and 4.64 ± 0.34 in normal group and 7.77 ± 0.31 and 6.46 ± 0.31 in SCE group, on 30 and 40 DPP, respectively. Statistical analysis of revealed significant difference between the normal and SCE groups on both 30 and 40 DPP. There was also significant reduction of the same parameter in the SCE group from days 30 to 40 postpartum ($p < 0.05$).

The mean (±SE) score of the inflammatory status of lamina propria were 2.64 ± 0.22 and 2.46 ± 0.18 in normal group and 4.62 ± 0.20 and 3.69 ± 0.16 in SCE group, on 30 and 40 DPP, respectively. Statistical analysis revealed significant difference between the normal and SCE groups on both 30 and 40 DPP ($p < 0.05$). There was also significant reduction within the SCE group between days 30 and 40 postpartum ($p < 0.05$).

The mean (±SE) score of the endometrial glands were 1.90 ± 0.21 and 1.54 ± 0.15 in normal group and 3.15 ± 0.19 and 2.31 ± 0.14 in SCE group, on 30 and 40 DPP, respectively. Statistical analysis revealed significant difference between the normal and SCE groups on both 30 and 40 DPP ($p < 0.05$) with regard to degeneration and fibroplasia of gland. There was significant difference within the SCE group between days 30 and 40 postpartum ($p < 0.05$).

The mean (±SE) score of the vascular inflammatory status were 1.54 ± 0.19 and 0.91 ± 0.13 in normal group and 2.15 ± 0.17 and 1.54 ± 0.12 in SCE group, on 30 and 40 DPP, respectively. Statistical analysis of the data revealed significant difference between and within the groups on 30 and 40 DPP regarding the vessel degeneration and haemorrhage ($p < 0.05$).

The mean (±SE) total histopathological score were 11.09 ± 0.59 and 9.64 ± 0.51 in normal group and 17.69 ± 0.55 and 14.00 ± 0.47 in SCE group, on 30 and 40 DPP, respectively. Statistical analysis of the data revealed significant difference between and within the groups on 30 and 40 DPP regarding the vessel degeneration and haemorrhage ($p < 0.05$).

The efficacy of histological findings by UB (cut off value for total score ≥ 15), TRUS findings (CD and FIU along with the echogenicity of the content) and EC (positive or negative), for the diagnosis of SCE, were analysed by means of Receiver Operating Characteristics (ROC) curve and Cochran's Q test separately for days 30 and 40.

On day 30, statistical analysis using Cochran's Q test revealed that TRUS findings based on CD for detection of SCE was significantly different from that of EC and UB. While results of EC and UB showed no significant difference. The result based on FIU was similar to that of EC and UB. The result of echogenicity was significantly different from that using UB, while results of EC and echogenicity showed no significant difference for day 30.

These findings were also verified with ROC curve analysis. UB at day 30 was set as gold standard and EC, CD, FIU and echogenicity of the content were compared. EC had a sensitivity of 61.5 per cent and specificity of 100 per cent. Area under curve (AUC) was 80.8 per cent which indicates that EC has high predictability and can be a good alternative for UB for diagnosing SCE. CD had very poor sensitivity and high specificity and AUC was 50 per cent (worse than random), indicating that CD was a poor parameter.

FIU had 69.2 per cent sensitivity and 54.5 per cent specificity. AUC was 61.9 per cent and was a better parameter than CD. Echogenicity had 46.2 per cent sensitivity and 90.9 per cent specificity. AUC was 68.5 per cent indicating that the parameter has a moderate predictability better than FIU and CD.

On day 40, statistical analysis using Cochran's Q test revealed that TRUS findings based on CD for detection of SCE was significantly different from that using EC, while results of CD and UB showed no significant difference. Also results of EC and UB showed no significant difference. The result of FIU was significantly different from that using UB while results of FIU and EC showed no significant difference. The results of EC and UB had no significant difference.

These findings were also verified with ROC curve analysis. UB at day 40 was set as gold standard and EC, CD, FIU and echogenicity of the content were compared.

EC had a sensitivity of 50 per cent and specificity of 78 per cent. AUC was 63.9 per cent. The efficiency of EC in diagnosing SCE was low when compared to day 30. CD had very poor sensitivity, high specificity and very low AUC equal to random, indicating that CD was a poor parameter. FIU had 50 per cent sensitivity and 33.3 per cent specificity. AUC was (41.7 per cent) worse than the random, hence has very low predictability.

The results of echogenic content had 16.7 per cent sensitivity and 88.9 per cent specificity with a low AUC of 52.8 per cent (near to random) and hence having very low predictability.

The mean (± SE) day of first observed oestrus after calving was respectively, 27.36 ± 1.38 and 29.38 ±1.37 in the normal and SCE group. Statistical analysis of data revealed no significant difference between the groups.

On the day of observed oestrus five cows in both the SCE and normal groups showed high intensity of oestrus.

Animals positive for SCE was treated with Cephapirin after AI was done and the percentage of animals conceived in the normal and SCE groups were 63.63 and 53.85 respectively. Statistical analysis using Fisher's exact test revealed no significant association ($p > 0.05$) between conception rate and occurrence of infection.

Based on the present study for comparison of the three diagnostic aids for detecting SCE, following conclusions could be made

1. Uterine biopsy (UB) was the best tool and gave a detailed idea about the extent and severity of the infection.
2. Endometrial cytology (EC) also had a high sensitivity, specificity and a high predictability and could be used as an alternative for UB.
3. The TRUS parameters (presence of FIU, echogenic content and CD) alone are of low value in the diagnosis of SCE. However, these could be beneficial when used as a supporting aid along with EC and UB.
4. The conception rates in both the groups were same which could be due to either the fast clearance rate of the infection in the herd or the effectiveness of the treatment or both.

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ENDOMETRIAL ECHOTEXTURE, CYTOLOGY AND BIOPSY IN POSTPARTUM SUBCLINICAL ENDOMETRITIS OF CROSSBRED DAIRY COWS

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(17-MVM-35)

ABSTRACT

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MASTER OF VETERINARY SCIENCE

(Animal Reproduction, Gynaecology and Obstetrics)

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ABSTRACT

Abstract

The study was undertaken at University Livestock Farm and Fodder Research and Development Scheme, Mannuthy with the objective of comparing the efficacy of endometrial cytology, biopsy and ultrasonography in crossbred postpartum dairy cows for the diagnosis of subclinical endometritis (SCE). Twenty-four apparently normal cows without any periparturient complications were randomly selected so that at least 12 animals with SCE could be obtained. Transrectal ultrasonography (TRUS) was performed on days 20, 30 and 40 to record the ovarian status and echobiometry of uterus and cervix. Endometrial cytology (EC) and uterine biopsy (UB) samples were collected on days 30 and 40. The mean (±SE) percentage of polymorphonuclear (PMN) cells by EC were 1.81 ± 0.88 and 1.00 ± 0.64 in normal group and 18.00 ± 0.81 and 9.92 ± 0.59 in SCE group, respectively, on 30 and 40 days postpartum (DPP). The mean (±SE) percentage of total histopathological score were 11.09 ± 0.59 and 9.64 ± 0.51 in normal group and 17.69 ± 0.55 and 14 ± 0.47 in SCE group, respectively, on 30 and 40 DPP. Significant difference was noticed between EC and UB in normal and SCE groups both on 30 and 40 DPP. The SCE positive animals also had a fast clearance rate which was evidenced by the reduction in the inflammatory status between the days of 30 and 40 DPP in both EC and UB. Among the TRUS parameters cervical diameter was the least valuable one, though this could be due to smaller girth for cervix in crossbred cows used for study. It could be inferred that ultrasound findings alone has poor efficacy in diagnosing SCE but it could be used as a supporting tool along with cytological or histopathological studies. Both cytology and biopsy could be used as valuable diagnostic techniques in detecting SCE. Biopsy allowed more detailed information about the deeper layers of uterus and if samples are collected with care the future fertility is not affected. All the animals were inseminated during next observed heat. Animals found to be positive for SCE was treated with intrauterine infusion of 500 mg Cephapirin Benzathine 12 hours after insemination was done. There was no significant difference in the conception rates in SCE and normal groups in the present study.

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KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

Faculty of Veterinary and Animal Sciences

PROGRAMME OF RESEARCH WORK FOR THESIS FOR MASTERS DEGREE

1. Title of thesis

Endometrial echotexture, cytology and biopsy in postpartum subclinical endometritis of crossbred dairy cows

2. (a) Title of the departmental/ KVASU research project of which this forms a part

Not applicable

(b) Code no. if any and order by which departmental /KVASU research project is approved

Not applicable

3. a) Name of student

Gayathri Prathap

b) Admission no

17-MVM-35

c) Name of the Discipline

Animal Reproduction, Gynaecology and Obstetrics

4. a) Name of Major Adviser (Guide)

Dr. Shibu Simon

b) Designation

Assistant Professor and Head
University Livestock Farm and Fodder Research and Development Scheme
Mannuthy

5. Objective of the study

Comparison of the efficacy of endometrial ultrasonography, cytology and biopsy in postpartum dairy cows for the diagnosis of subclinical endometritis

6. Practical / Scientific utility

Postpartum uterine infections are very common in dairy cattle and can cause severe economic loss to the dairy farmer due to embryonic mortality, abortions and infertility. Subclinical endometritis (SCE) is one of the major causes of infertility but is unlikely to have a profound effect on the general health of the cow. It is defined as superficial inflammation of the endometrium and is characterised by the presence of greater than 18 per cent polymorphonuclear (PMN) cells in uterine cytology samples collected 21-33 days postpartum or greater than 10 per cent PMN cells in samples collected at days 34-47 postpartum (Kasimanickam *et al.*, 2004).

Although endometrial cytology is considered as the preferred method for diagnosis of subclinical endometritis, uterine biopsy provides accurate information about uterine health status and if properly used does not compromise fertility. Ultrasound scanning is popular

due to its non-invasive nature and changes in endometrial echotexture can be used as potential diagnostic indicators for subclinical endometritis. The present study is undertaken with a view to compare ultrasonography, endometrial cytology and uterine biopsy in the diagnosis of SCE.

7. Important publications on which study is based

DeBois and Manspeaker (1986) composed uterine biopsy score by summing up 4 scores that reflected the surface epithelium, lamina propria, the endometrial glands and the vascular inflammatory status respectively.

LeBlanc *et al.* (2002) stated that those animals with purulent discharge and cervical diameter greater than 7.5 cm after 20 days in milk and mucopurulent discharge after 26 days in milk had endometritis. They concluded that the sizes of uterus and cervix in cows with clinical endometritis were associated with a decrease in pregnancy rate if the size of uterus was greater than 8cm in diameter and cervix was greater than 7.5 cm in diameter between 20 and 33 Days in Milk

Kasimanickam *et al.* (2004) studied the relationship between endometrial cytology and ultrasonography in detection of subclinical endometritis in apparently normal cows. The authors concluded that ultrasonographic method and cytological method pertained to different causative factors, the former measuring the clearance mechanism and the latter the cellular response of the uterus.

Barlund *et al.* (2008) compared the various diagnostic methods for endometritis in postpartum cows which included vaginoscopy, ultrasonographic assessment of endometrial thickness and uterine fluid volume, endometrial cytology collected by either uterine lavage or cytobrush and concluded that cytobrush cytology was the most reliable method of diagnosing endometritis in cattle.

Oral *et al*. (2009) opined that a combination of vaginal discharge score, transrectal ultrasonography methods and cytobrush technique were useful for the diagnosis of subclinical endometritis.

Chapwanya *et al*. (2010) described a safe and reliable method for conducting endometrial biopsy without deleteriously affecting fertility. It provided excellent tissue sample for evaluating the soundness of endometrium.

Meira *et al*. (2012) attempted the comparison of ultrasonographic and histopathological examinations, their effectiveness in combination, for the diagnosis of endometritis and found that ultrasonography was the most practical method and the combination study with cervical diameter and intrauterine fluid increases its specificity and sensitivity.

Uterine biopsy score was composed by summing up 4 scores that reflected the surface epithelium, the lamina propria, the endometrial glands, and the vascular inflammatory status, respectively.

Madoz *et al.* (2014) assessed the agreement between uterine biopsy and endometrial cytology in diagnosis of subclinical endometritis. However, they could not find a positive correlation for uterine biopsy to cytological evaluation. Nevertheless, uterine biopsy is found to be imperative in evaluating the health of uterus. Cut off values for diagnosis of SCE are greater than 8 per cent PMN cells at 21 to 33 DIM, greater than 6 per cent PMNL at 34 to 47 DIM, and greater than 4 per cent at 48 to 62 DIM. Cows with subclinical endometritis had a uterine biopsy score of 1 or 2 respectively.

Robert (2016) deduced that endometrial cytology could be used for a presumptive diagnosis of SCE and that *E.coli* Lipopolysaccharide had a reasonable therapeutic effect on the management of SCE

Salah and Yimer (2017) established that for the diagnosis of endometritis in beef cattle at 4 to 5 weeks postpartum, ultrasound method was most useful and practical with a 60 per cent sensitivity, 93.8 per cent specificity, especially when the parameters of intrauterine fluids and measurement of cervix diameter are used in combination.

8. Outline of technical programme

The study will be conducted at University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS), Mannuthy. Apparently healthy postpartum crossbred dairy cows will be selected for the study so as to obtain at least 12 cows with subclinical endometritis. Body condition score, parity, details of calf born, postpartum complications and number of inseminations per conception, will be recorded. From 20 days postpartum, the health condition and reproductive status of the animals will be monitored on a daily basis. The day on which the animal returns to cycle was recorded based on exhibition of heat signs and confirmed by clinico-gynaecological examination will be recognised.

Transrectal ultrasonography using a 5 to 10 MHz linear array transducer will be conducted on days 20, 30 and 40 postpartum to measure cervical diameter, fluid accumulation in the uterus and diameter of the base of uterine horn. Clinical samples for endometrial cytology and tissue samples for uterine biopsy using Jacksons uterine biopsy forceps will be collected on days 30 and 40 postpartum.

Cytological samples will be stained with modified Wright-Giemsa stain and examined under bright-field microscopy at 400X magnification, to

determine the percentage of polymorphonuclear cells (PMNs). The animals found to be positive for endometritis will be treated with intrauterine administration of cephapirin (19g total dose). Animals in oestrus will be inseminated with frozen thawed semen after 60 days postpartum. Pregnancy will be confirmed at 28 days after AI by transrectal ultrasonography. The data will be subjected to statistical analysis as per SPSS (version 24).

9. Main items of observation

a. Body condition score
b. Uterine tonicity and texture
c. Duration and intensity of oestrus
d. Day of first postpartum oestrus
e. Cervical diameter
f. Endometrial thickness
g. Fluid accumulation
h. Uterine horn diameter
i. Histological changes assessed and scored
j. Ovarian status
k. Cytological picture

10. Facilities

(a) Existing:

The existing facilities at ULF & FRDS, Dept of ARGO and the College of Veterinary and Animal Sciences, Mannuthy

(b) Additional facilities required:

Chemicals, biological and diagnostic aids

11. Duration of study

Four semesters

12. Financial estimate

Chemicals, diagnostic aids and biologicals	: Rs 20000/-
Contingencies	: Rs 5000/-
Total	**: Rs 25000/-**

13. Signature of Student:

14. Signature of Major Adviser:

Place: Mannuthy

Date: 02/07/2018

Name, Designation and signature of members of the Advisory Committee

Chairman

Dr. Shibu Simon
Assistant Professor and Head,
University Livestock Farm and Fodder Research and Development Scheme,
Mannuthy, Thrissur – 680 651.

Members

1. Dr. M.O. Kurien
Professor and Head,
Department of Animal Reproduction Gynaecology and Obstetrics,
College of Veterinary Animal Sciences, Mannuthy

2. Dr. B. Bibin Becha
Assistant Professor,
Livestock Research Station,
Thiruvazhamkunnu

3. Dr. Surej Joseph Bunglavan
Assistant Professor,
Department of Animal Nutrition,
College of Veterinary and Animal Sciences, Pookode

APPENDIX – I

References:

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APPENDIX-II

Time frame of work

Semester I

1. Planning of programme for research work
2. Collection of literature
3. Preparation of synopsis

Semester II

1. Procurement of research material
2. Review of literature
3. Standardization of Procedure

Semester III

1. Research work

Semester IV

1. Continuation of Research work
2. Statistical analysis of data
3. Preparation and Submission of thesis

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the prevention of cruelty to animals act (amendment, 1998).

Place: Mannuthy
Date: 02/07/2018

Dr. Shibu Simon
(Major advisor)

Appendix

CURRICULUM VITAE

Name of the Candidate	:	**GAYATHRI PRATHAP**
Date of Birth	:	31.03.1992
Place of Birth	:	Muvattupuzha, Ernakulam, Kerala
Marital Status	:	Married
Permanent Address	:	Swasthi (H), Kizhakkekara, Muvattupuzha P.O, Ernakulam, Kerala. Pincode: 686661
Major Field of Specialization	:	Animal Reproduction, Gynaecology and Obstetrics
Educational Status	:	Completed B.V.Sc.&A.H., in 2017 Joined M.V.Sc., Programme in September 2017
Publication made	:	2
Membership of Professional Society	:	Life member of Kerala State Veterinary Council Life member of Indian Society for study of Animal Reproduction Life member of Indian Society for Advancement of Canine practice