NON-ANTIBIOTIC THERAPY FOR SUB CLINICAL ENDOMETRITIS IN REPEAT BREEDING CATTLE

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled **"NON-ANTIBIOTIC THERAPY FOR SUB CLINICAL ENDOMETRITIS IN REPEAT BREEDING CATTLE"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"NON-ANTIBIOTIC THERAPY FOR SUB CLINICAL ENDOMETRITIS IN REPEAT BREEDING CATTLE"** is a record of research work done independently by **Safna Isaac. M.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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I shall not want"

Psalms 23: 1.

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Introduction

1. INTRODUCTION

Livestock sector is an integral part of our agrarian economy not only by supplying valuable animal proteins, contributing vital power for agricultural operations and organic manure to enhance soil fertility but also by providing a steady income for millions of poor farmers. Implementations of intensive cross breeding programme among cattle have definitely made an impact on rural economy by enhanced milk production. Crossbreeding also tremendously increased the value of our local animals, leading to greater awareness on the part of farmers about the economic loss associated with infertility in cattle. The success of dairy cattle rearing lies in ensuring proper and optimal reproductive rhythm of each cow in the herd. Any deviation in the breeding rhythm results in progressive economic loss through reduced calf crops and milk production.

Despite the massive emphasis bestowed on preserving fertility in dairy cattle, it seems to be going down progressively for the last 50 years due to various factors associated with high milk production. So the need for improving fertility in dairy cows becomes much more pertinent today than ever before.

Infertility denotes a degree of reduced fertility which results in failure to produce or delay in producing the annual live calf. Risk factors for reduced fertility vary among different regions or countries because of difference in the general management, environment and herd health control programme.

Uterine contamination following calving is common, but most healthy cows are able to clear off the uterine microorganisms within first two to three weeks after calving (Bondurant, 1999). The severity of uterine infection varies from a mild

superficial endometritis to an acute septic metritis involving the entire thickness of uterine wall.

Endometritis is the common reproductive disorder in cattle and its incidence vary between 16.9 per cent to 53 per cent. The common pathogens isolated from endometritis cases are *Coliforms (E. coli), Actinomyces pyogenes, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas, Bacillus* species of bacteria, viruses, fungi and mycoplasma. Uterine infection is eliminated by the natural defense mechanism of the host, which is activated during oestrus by the margination of host defense cells in the endometrium, active phagocytosis and higher concentration of immunoglobulins in the luminal secretion. Even without treatment, elimination of *E. coli* and *streptococci* occurs within two to three weeks, whereas *A. pyogenes* and *F. necrophorum* are eliminated within three to five weeks postpartum. However, cows that cannot eliminate infection may subsequently develop endometritis.

The uterus is highly resistant to infection during the oestrogenic phase, but very susceptible during the period of progesterone dominance allowing greater bacterial growth and the appearance and activity of leukocytes are decreased. Generally, the concentrations of immunosuppressant proteins present in the uterine lumen increase under progesterone dominance and these inhibit lymphocyte proliferation making the uterus more susceptible to infection (Dhaliwal *et al.*, 2001).

Endometritis can be diagnosed on the basis of history, clinical symptoms in the form of uterine changes and nature of discharges and can be confirmed by bacteriological and histopathological studies. Cows that developed endometritis showed greater total leukocyte numbers, elevated neutrophils, lymphocyte and monocyte counts and also serum TNF alpha levels. The treatment of chronic

endometritis in dairy cattle has been under discussion for several years. The general principle of therapy is to halt and reverse inflammatory changes that impair fertility and enhance the process of uterine defense and repair. Some authors stated that chronic endometritis has a tendency to recover automatically with no negative impact on reproductive performance while others described a depressed fertility even for cases of sub clinical endometritis.

For the treatment of endometritis, proper selection of antibiotics is essential to prevent development of resistant strains of organisms and clearing off infection as quickly as possible. An antibacterial drug to be used in treating infection must be effective against the primary uterine pathogens, it must reach above the minimum inhibitory concentration (MIC) for infecting agents at the site of infection, and must be active in the presence of organic debris and in the anaerobic environment of the postpartum bovine uterus (Shah and Dhami, 2004). Because the uterine flora changes over time, the safest strategy is to determine the microbiologic status, including the MIC test. The cost benefit of antimicrobial susceptibility testing seems unlikely to be favorable, particularly for large dairy herds.

Inspite of several decades experience with a variety of antibiotic treatment regimens, it is clear that it does not alleviate infertility associated with uterine infections. The possible reasons for lack of efficacy may include use of an inappropriate antibiotic agent, inadequate dose or insufficient number of treatments. Increased awareness of the risk of bacterial resistance and for tissue residues necessitating with holding times for milk and meat has made it clear that non-antibiotic alternatives are needed for treating uterine infections.

In terms of fertility, the therapeutic and prophylactic use of prostaglandin F_2 alpha and its analogues in the postpartum period show results equal to or better than intrauterine antibacterial therapy. If prostaglandin is administered during the luteal

phase of the oestrous cycle, it hastens the return of oestrus and at the same time reduces the period during which the genital system is under the influence of progesterone. It also stimulates the myometrial contraction of uterus thereby helps in the expulsion of debris and microorganisms and also have a stimulatory effect on the phagocytic activity of uterine polymorphonuclear cells (PMNs). Because prostaglandin F_2 alpha has the advantage of systemic versus intrauterine administration and no requirement of milk withdrawal, it should be recommended as the initial treatment regimen for the postpartum uterine disorders (Paisely *et al.*, 1986).

Intrauterine infusions with various antiseptic solutions like lugol's iodine have also been proved beneficial for the treatment of postpartum infections. It produced local irritation and leucocytosis, there by enhancing phagocytosis and helped in curing the infection (Roberts, 1986). Moreover it produces uniform suppression of microorganisms without development of resistance and is less expensive. So it is better to strengthen the natural defense mechanisms and self cleansing of infected uterus by using alternative hormones or chemotherapeutic agents rather than going for antibiotic preparations.

The present study was aimed to improve conception rate of repeat breeder cattle with sub clinical endometritis by formulating an effective and economic therapy. Lugol's iodine therapy was commonly practiced either alone or in association with insemination with varying results. A good percentage of animals brought for insemination are in early stages of oestrus and so lugol's iodine can be given intrauterine before insemination. Intrauterine medication of lugol's solution 24 hours after insemination will help to control the infection and give better result in endometritis. Hence therapeutic approaches viz., pre insemination and post insemination intrauterine infusion of one per cent lugol's iodine 24 hours prior to and

after service and single and double regime intramuscular administration of prostaglandin F_2 alpha during the luteal phase followed by timed insemination at 72 and 96 hours were undertaken, and their efficacy and economic viability were compared.

Review of Literature

2. REVIEW OF LITERATURE

Bovine endometritis is a major cause of reduced reproductive efficiency of dairy herds that adversely affects the production potential. Depending upon the management and environmental conditions such as deficiencies in hygiene at calving, dystocia, inadequate feeding of dry cows and infectious diseases, the average incidence of chronic endometritis may go up to 37.5 per cent (Drillich *et al.*, 2005). Decreased productivity, increased treatment, labor costs and increased culling rates have serious economic impact on dairy operations.

2.1 OCCURRENCE OF ENDOMETRITIS

Among the five Holstein herds in United Kingdom, the incidence of milk fever followed by endometritis, endometritis after retained fetal membranes and endometritis alone were 1.4 percent, 3.3 per cent and 10.1 per cent respectively (Borsberry and Dobson., 1989). In a study conducted, Glanvil and Dobson (1991) reported an incidence of 61 per cent of endometritis in UK. Similarly Gilbert *et al.* (1998) reported an incidence of 61.6 per cent of endometritis in Central New York.

On examination of 1865 Holstein cows in Canada, Le Blanc *et al.* (2002) reported an incidence of 16.9 per cent of endometritis while Kim and Kang (2003) observed an incidence of 36.6 per cent endometritis in Central Korea.

According to Kasimanickam *et al.* (2004), the prevalence of sub clinical endometritis was 45.1 per cent in Holstein dairy farms of Canada. Gilbert (2005) also reported that the herd prevalence varied from 37 to 74 per cent, with an average of 53 per cent in USA. However, Mejia *et al.* (2005) recorded that the prevalence varied from 18 to 22 per cent in dairy herds of Argentina.

Quite a few scientific reports were documented on the prevalence of endometritis from different parts of India. Rao and Kotayya (1976) reported that in Andhra Pradesh endometritis was prevalent to an extent of 30.77 per cent in cross breds and 19.41 per cent in indigenous cattle. Similar incidence of 32.86 per cent has been reported from Karnataka by Rao *et al.* (1983). Varadarajan and Nair (1990) recorded the incidence of endometritis among crossbred cows of Kerala as 9.66 per cent while Rehman *et al.* (1990) observed an incidence of 16 per cent metritis in the Kashmir Valley.

A higher incidence of 20 per cent endometritis was reported by Iyer *et al.* (1992) in Kerala and Mohanty *et al.* (1992) also reported the incidence as 29.38 per cent and 27.09 per cent respectively in cows and heifers of Orissa. However, Vahida (1992) reported an incidence of only 7.95 per cent in Kerala. An incidence of 17.64 per cent uterine infections was reported by Dhami *et al.* (1993) in Gujarat.

Of the studies conducted by Satheshkumar and Punniamurthy (2003), the incidence of repeat breeding contributed to 24.60 per cent and infectious conditions predominantly endometritis were apparent in 19.36 per cent of the repeat breeding heifers. Velayudakumar (2003) reported an incidence of 34.97 per cent of repeat breeding of which 8.69 per cent were due to uterine infections. But according to Selvaraju *et al.* (2005), the over all incidence of repeat breeding and endometritis were found to be 5.59 and 7.10 per cent respectively.

2.2 ETIOLOGY OF ENDOMETRITIS

Studies on the microbial pattern by Steffan *et al.* (1984) revealed *Corynebacterium pyogenes* in more than 50 per cent of isolates either alone or in combination with other organisms and gram negative organisms like *E.coli* were

found in small proportion (12 per cent). But according to the studies conducted by Bonnett *et al.* (1991) *Streptococci, E.coli* and *Actinomyces pyogenes* were the most common isolates among the endometrial biopsy samples.

All cows with retained fetal membranes had bacterial endometritis and the predominant bacteria were *E.coli, Streptococci, Fusobacterium necrophorum, Arconobacterium pyogenes, Bacteroides* species, *Pasturella* species and *Proteus* species (Konigsson *et al.*, 2001). Also Mateus *et al.* (2003) isolated *E. coli, Arcanobacterium pyogenes* and gram negative anaerobes (*Fusobacterium necrophorum, Bacteroides melaninogenicus*) from abnormal lochial discharge.

According to work done by Singla *et al.* (2004) at Ludhiana, microorganisms isolated from uterine swabs of cows with sub clinical endometritis were mainly *E.coli, Staphylococcus aureus* and *Proteus* species where as the incidence of *Streptococci* species, *Klebsiella* species, *Pseudomonas* species, *Corynebacterium* species and *Bacillus* species were relatively less. Similarly Zilaitis *et al.* (2004) also found that *E. coli* were the most prominent bacteria among microorganisms isolated from cows with clinical endometritis.

2.3 DETECTION OF ENDOMETRITIS

According to Nampoothiripad (1971), abnormal colour of uterine mucus is not specific to repeat breeding, but mucus with thick consistency was found in higher per cent in them. Uterine mucus was collected from the repeaters and then incubated in suitable agar plates for the identification of bacterial growth and detection of endometritis. Similarly Steffan *et al.* (1984) advanced sterile catheter into the uterus and aspirated the uterine contents and Bonnett *et al.* (1991) collected endometrial biopsy samples for identifying the organisms associated with endometritis.

Sinha *et al.* (1984) used lymphocytic infiltration score (LIS) technique for the diagnosis of endometritis and opined that the fertility of the animal reduced when the LIS value was above 0.5 and increased significantly when it was below that level and also found that 58.55 per cent of endometritis cases showed the value above 0.5. Umashankar *et al.* (1984) studied the physio-biochemical characteristics of cervical mucus in normal and repeat breeding cattle and opined that the discharge was slightly cloudy in repeat breeding cattle even though the consistency was same in both groups. Also the infiltration of lymphocytes was less in normal animals in comparison to repeat breeders.

Pateria and Rawal (1990) carried out white side test on uterine discharge of buffaloes to detect sub clinical cases of metritis. Out of 100 animals, 25 showed light yellow colour, 50 yellow colour and 25 dark yellow colour and the animals were categorized as having slight, moderate and intense sub clinical metritis respectively.

According to Konigsson *et al.* (2002), the blood samples of cows with retained fetal membranes had high levels of 15-ketodihydro-PGF_2 alpha, a prostaglandin metabolite, immediately after parturition and the levels fell rapidly within two weeks postpartum. The prostaglandin metabolite levels increased again and remained elevated during the period of uterine infection. Seals *et al.* (2002) also studied the relationship between postpartum changes in 13, 14-dihydro, 15-keto-PGF2 alpha (PGFM) concentrations in Holstein cows and their susceptibility to endometritis and concluded that PGFM measures had the potential to be used in the diagnosis of endometritis.

White side test could be used as a cost effective spot test in the diagnosis of sub clinical endometritis and the intensity of colour changes could be explained on

the basis of leukocytes present in the uterine discharges. The normal discharge had low number of leukocytes compared to mild, moderate and severe degrees of sub clinical endometritis (Krishnakumar *et al.* 2003). In a study conducted by Velayudakumar (2003), higher per cent of repeat breeding animals showed clear mucus discharge, but with thicker consistency and ruled out the possibility of sub clinical endometritis in repeat breeders by doing white side test. The appearance of yellow colour was taken as a positive indication while those which were negative for colour reactions were considered as devoid of uterine infections.

In cows with endometritis, uterine biopsies were obtained and plasminogen activator activities (PAA) were determined by spectrophotometric method. Both plasminogen activators (PAs) *i.e.* tissue type (t-PA) and urokinase (u-PA) were immunologically identified in all uterine biopsies. Plasminogen activator activity increased, where as plasminogen activator inhibition (PAI) and plasmin inhibition (PI) were decreased in proportion to the degree of inflammation (Moraitis *et al.*, 2003). A study was conducted by Sattler *et al.* (2004) to prove the correlation between creatine kinase (CK) and aspartate aminotransferase (AST) activities in serum and the severity of endometritis. These enzyme activities were found to be higher in accordance with the degree of pathological changes in the uterus. There was a positive correlation between CK and AST in serum and degree of damage to uterine wall and so can be used as a screening parameter for the detection of endometritis.

Kasimanickam *et al.* (2004) studied the use of endometrial cytology (EC) and ultrasonography (US) to diagnose sub clinical endometritis in clinically normal postpartum dairy cows. Its impact on reproductive performance was measured and concluded that sub clinical endometritis diagnosed by EC or US was associated with reduced pregnancy rate.

Methai *et al.* (2005) carried out white side test in eight crossbred Jersey cows with endometritis and eight apparently healthy cows. Discharges of all infected cows showed positive response by turning the colour to yellow while the healthy animals were negative for colour reaction. Similarly the uterine discharges of 105 buffaloes were subjected to white side test and the percentage of sub clinical and clinical metritis were found to be 45.72 per cent and 13.33 per cent respectively based on the severity of colour reaction (Mohankumar *et al.*, 2006).

2.4 EFFECT OF ANTIBIOTIC THERAPY

An intrauterine infusion of chloramphenicol and framycetin were given in three doses at one week interval for cows with clinical metritis and obtained a recovery rate of 49 per cent with culling rates and fertility similar in both treatment and control groups (Steffan *et al.*, 1984). This may be attributed to the fact that the minimum inhibitory concentration of the antibiotics might not be reached for a sufficient duration or uterine catheterization might have been performed in luteal phase with obturated cervix in cycling cows.

A single intrauterine infusion with 0.8 to one million units of procaine penicillin or 500 milligram of oxytetracycline was tried for cows with endometritis and no difference on time of treatment to pregnancy was found between the treated and control groups (Thurmond *et al.*, 1993). The possible reasons for the lack of efficacy include use of an inadequate dose of antimicrobial agent, an inadequate number of treatments or an inappropriate antimicrobial agent. Sheldon and Noakes (1998) obtained a success rate of 73 per cent by using 1500 mg of oxytetracycline intrauterine for the treatment of endometritis. However, Brooks (2000) obtained a success rate of only 63 per cent by using the same therapeutic agent.

Dhaliwal *et al.* (2002) stated that intrauterine antibiotic administration suppressed uterine leukocytic activity and could have serious implications in the treatment of metritis, if the bacteria involved be resistant to the particular antibiotic. Also LeBlanc *et al.* (2002) studied the effect of intrauterine antibiotic (cephapirin benzathine 500 mg) in dairy cows with clinical endometritis and opined that the results were consistent with many of the published reports which had failed to document significant improvements in reproductive performance associated with antibiotics. On using Cefquinone for treatment and prevention of bovine endometritis, Amridis *et al.* (2003) opined that it was useful in the control of disease condition especially in reduced efficacy of older generation antimicrobial agents.

Dhillon *et al.* (2005) treated repeat breeding cows having uterine infection with Tinidazole and Ciprofloxacin combination for three consecutive days. On examination of uterine swabs taken at subsequent oestrus, 60 per cent were completely cured off infection, but in only 13.33 per cent first service pregnancy was achieved with an overall pregnancy rate of 40 per cent within three inseminations and it was concluded that this combination was effective against microbes, but results in terms of pregnancy were not encouraging.

2.5 EFFECT OF LUGOL'S IODINE THERAPY

According to Grunert *et al.* (1973), intrauterine infusion of lugol's iodine from day four to day 12, on day 17 and during oestrus did not affect the oestrus cycle length. But cows with normal oestrous cycle showed a significant increase in the duration of cycle following a single intrauterine infusion of iodine solution between day 13 and 16; with the most distinctive lengthening on day 15 with an average of 3.7 days. Seguin *et al.* (1974) carried out a study by giving dilute iodine solution intrauterine to cows at oestrus, on day four and day 15 of oestrous cycle and the

mean cycle length obtained were 22.4 ± 0.1, 10.6 ± 0.2 and 25.1 ± 0.6 days respectively. The results showed that the regression of corpus luteum was hastened by dilute iodine solution given on day four, delayed when given on day 15 and was not affected when given at oestrus.

On infusion of 250 ml of two per cent lugol's iodine intrauterine, Seguin *et al.* (1974) opined that the endometrium of cows recovered rapidly after intrauterine infusion of iodine solution and subsequent reproductive performance seemed to be normal. It was stated that irritant preparations should not be used for intrauterine infusion more than 48 hours after breeding unless abortion was intended.

Oxender *et al.* (1976) reported that biopsy specimen taken 24 hours after infusion of dilute iodine solution showed destruction of surface epithelium, oedema and superficial endometrial necrosis. The uterine irritation might be the mechanism by which dilute iodine solution altered the duration of the oestrous cycle and recommended that infusion of a mildly irritating solution might be beneficial, if luteolysis was desired. Similarly Gustafsson (1984) also documented that lugol's solution infused during the early part of oestrous cycle induced estrus within four to seven days of treatment. Infusion of lugol's solution was followed by PGF_2 alpha release and hence it might be an alternative therapy to prostaglandin for the treatment of endometritis and opined that it seemed to be logical to avoid a too strong solution and too large volume of lugol's iodine (minimum one per cent, Fifty to hundred millilitre).

Roberts (1986) documented the beneficial effects of lugol's solution due to its microbicidal activity, the local irritation, leucocytosis and hyperemia produced on endometrium, when given as intrauterine infusion. Gupta *et al.* (1989) also observed mild degenerative changes in surface epithelium, moderate to severe oedema,

congestion of blood vessels, hemorrhage and moderate neutrophilia, 24 hours after infusion of lugol's solution.

Uterine biopsies from repeat breeder cows were collected by Singh *et al.* (1988) after infusing five per cent and ten per cent lugol's solution at standing oestrus, the endometrial damage was studied by suitable staining methods and observed that the damage was nearly normal at 24 hours post infusion with five per cent solution while ten per cent caused severe irritation of endometrium. It was concluded that mild solution of lugol's iodine did not have any serious damaging effect on the endometrial structure and function.

The biopsy of endometrium was obtained from cows on the day of oestrus, just before and 24 h post infusion of 0.25 per cent of lugol's iodine by Singla *et al.* (1993). There was mild to moderate oedema of the stroma and blood vessels were congested at oestrus before infusion of lugol's iodine while severe oedema of stroma, congested blood vessels with neutrophils and inflammatory reactions of moderate intensity were noticed after infusion, which will be beneficial in eliminating the infection of uterus.

2.6 FERTILITY AFTER LUGOL'S IODINE THERAPY

Roberts (1956) treated repeat breeder cows with intrauterine administration of 1.5 to three per cent solution of lugol's iodine and obtained a result of 50 per cent and 52.4 per cent conception rates on artificial insemination and natural service respectively during the next oestrum. Intrauterine medication of lugol's solution 24 to 36 hours after service gave better result in endometritis, because three to four day were required for fertilized ovum to reach the uterus and the medication could act by this time to control infection.

On treating clinically normal repeat breeders with intrauterine infusion of dilute lugol's iodine solution, Mutiga (1978) obtained a conception rate of 62 per cent while that of control group was only 26 per cent. Gupta *et al.* (1983) treated 140 repeat breeder cows with 20 ml of five per cent lugol's iodine solution by intrauterine route 24 hours after insemination and obtained a conception rate of 44.28 per cent. Repeat breeder cows were treated by Pandey *et al.* (1983) with 0.1 per cent of lugol's iodine 10 ml intrauterine one hour after insemination and obtained a fertility rate of 61.54 per cent.

Patil *et al.* (1983) swabbed five per cent lugol's solution on os uteri externum of anoestrus cows and 40 per cent of them came to oestrus, out of which 71.42 per cent conceived and opined that the lugol's iodine paint caused local irritation and hyperemia of uterus, which in turn stimulated the hypophysis reflexly resulting in the release of gonadotropic hormones.

Cows with mycotic infection were treated by Pattnaik *et al.* (1992) using intrauterine infusion of 0.3 per cent lugol's iodine (50 to70 ml) depending up on the size of the uterus for three days. The treatment was repeated for the next two heat periods and then artificial insemination was performed. Out of the nine animals treated, five conceived and suggested that lugol's iodine could be effectively used to combat mycotic infection.

Rao *et al.* (1998) flushed one per cent lugol's iodine into uterus of repeat breeder cows during standing heat, five times by flushing 80 to 120 ml at a time by collecting the flushing in a measuring jar. During next heat all animals were inseminated twice with frozen semen and the conception rate was found to be 73.33 per cent by taking 1.42 services per conception. However, Ramoun *et al.* (2002) treated repeat breeder cows with 1.33 per cent lugol's iodine solution intrauterine on

day four of metoestrus of one spontaneous cycle and two induced cycles and by inseminating on next spontaneous heat obtained a first service conception rate of only seven per cent.

Rathour *et al.* (2005) treated anoestrus Murrah buffaloes with 40 ml lugol's iodine solution intrauterine and noticed that 50 per cent animals expressed oestrus and 66.66 per cent of them conceived. The action of lugol's iodine in the induction of oestrus was thought to be due to either stimulatory effect on the hypothalamus or by the release of uterine luteolytic factor acting via. the utero-ovarian and utero-pituitary-ovarian pathway.

2.7 EFFECT OF PROSTAGLANDIN THERAPY

Cooper and Furr (1974) used 500 microgram of synthetic analogue structurally related to "F" series of prostaglandins twice at an interval of ten to 12 days and observed that treated Friesian heifers showed oestrus 48 to 72 hours after the second treatment with a small number coming to heat between 72 and 96 hours. Similarly Johnson (1978) carried out a trial to find out the time of onset of oestrus after injections with cloprostenol in heifers. Among the group of animals that received two injections of cloprostenol (500 microgram) at 11 days interval and other group that received a single injection on day eight of oestrous cycle, the time of onset of oestrus after the first and second injection of double injection regime and single injection were found to be 68.6 ± 20.8, 59.9 ± 15.8 and 57.4 ± 12.5 hours respectively.

Singh *et al.* (1979) collected tissue strips from the ovary, oviduct and uterus of pregnant and nonpregnant cows and tested their contractile response to PGF_2 alpha. Results indicated that the bovine ovary contracted rhythmically and that its

sensitivity to PGF2 alpha decreased during pregnancy in contrast to the bovine uterus which became increasingly sensitive during pregnancy.

Of the animals treated with cloprostenol, 73 per cent came to oestrus in two to five days, out of which day three after treatment was the most common day for oestrus (Seguin *et al.*, 1983). Similarly Macmillan and Henderson (1984) treated cows at seven to 16 days of oestrous cycle and observed that over 70 per cent of the cows injected on day seven and 16 were in oestrus from 48 to 72 h post injection while cows injected on days 11 and 12 showed response four to five days post injection.

A luteolytic dose (500μg) of cloprostenol was given on day 12 of oestrous cycle and blood samples were collected five to 20 minutes interval from -6 to zero (control period), zero to 12 and 24 to 36 hours after PG injection. It was found that progesterone and oxytocin secretion from the CL was initially increased and then dramatically decreased by a luteolytic dose of prostaglandin (Schallenberger *et al.*, 1984). Similarly Harrison *et al.* (1985) also documented that cloprostenol caused rapid diminution of luteal function which was accompanied by a reduction in progesterone content 12 h following injections. Further more the serum oestrogen level was elevated until 24 h and then declined through 72 h after cloprostenol injection.

Two groups of animals bred by artificial insemination and natural service respectively were treated with 25 mg of PGF_2 alpha during the luteal phase and found that 46.3 per cent of animals came to oestrus 68.7 h after injection in one group, while in the other group 54.8 per cent of animals were detected in oestrus 59.5 h after injection (Landivar *et al.*, 1985).

Paisley *et al.* (1986) reported the beneficial effects of PGF_2 alpha in uterine infections by stimulating the phagocytosis by uterine leukocytes and making the discharge clear by evacuating the uterine contents and enhanced the healing of endometrium. Young and Anderson (1986) opined that the resumption of ovarian cyclicity after calving may be advanced by the administration of PGF_2 alpha or its synthetic analogue after calving. The advantage of postpartum prostaglandin administration was also reported by Etherington *et al.* (1988) who suggested that it reduced the occurrence of pyometra and improved fertility.

Incomplete luteal regression following PGF2 alpha might have resulted in elevated serum progesterone during oestrus, which altered oestrus and ovulation (Hansen *et al.*, 1987). Artificially shortening the oestrous cycle and time of exposure to progesterone with PGF_2 alpha could impair events associated with follicle selection, oestrus, ovulation and formation of a functional corpus luteum. Impaired corpus luteum function might be one explanation for the low fertility following PGF induced oestrus in cattle.

In a trial conducted by Jacob (1993), average time taken for induction of oestrus after prostaglandin therapy was found to be 58.95 h and the duration of induced estrus varied from 24 to 48 h (mean 29.98 h). The percentage of animals showing intense, medium and weak oestrum was 66.66, 19.04 and 14.24 respectively. The treated animals showed almost similar cyclical changes of the reproductive tract irrespective of induced and natural oestrum. However, according to Ajitkumar (1994), the physical changes in the reproductive tract such as intensity of vulval oedema, hyperemia of vestibular mucous membrane, tonicity of uterus and over all oestrus response showed a marginal increase in prostaglandin induced oestrum than during natural oestrum.

Ajitkumar *et al.* (1995) studied the effect of single and double dose of PGF_2 alpha (25 mg Dinoprost) in heifers and found that the time taken for induction of oestrus were 63.38 h and 67.50 h respectively. The duration of oestrus ranged from 18 to 48 h (mean 29.25 h) and 24 to 48 h (mean 33 h) respectively from single and double injection groups. According to Arthur *et al.* (1996), when PGF_2 alpha was administered during the luteal phase of estrous cycle, it hastened the return to oestrum. Administration of prostaglandin F_2 alpha at 11 days interval and timed AI at 72 and 96 hours after second injection gave comparable result in conception rate.

Senthilkumar and Rajasekar (1998) recorded the oestrus response and oestrual pattern in groups I and II by giving 25 mg and 15 mg of PGF_2 alpha analogue respectively at mid luteal phase of oestrous cycle and noticed 100 per cent oestrus response in group I, while only 59 per cent in group II. The mean onset of oestrus were 64.92 $\pm$ 2.17 h and 79.43 $\pm$ 3.54 h respectively in groups I and II and the respective mean duration of oestrus were 19.27 $\pm$ 1.57 and 22.29 $\pm$ 1.48 h.

Effect of two dosages of d-cloprostenol (0.15 mg and 0.3 mg) on intrauterine pressure and uterine motility were studied by Hirsbrunner *et al.* (1999). Intrauterine pressure was recorded during diestrum of lactating dairy cows, using a transcervically placed intraluminal pressure microtransducer. Significant difference was found on the area under the curve and mean amplitude, and it was concluded that the double luteolytic dose of d- Cloprostenol gave better action than single dose.

Oestrus was synchronized by the administration of 25 mg each of PGF_2 alpha in 10 cross bred heifers in two doses at 12 days interval. The mean interval between treatment and appearance of estrus following first and second dose were 64.00 $\pm$ 10.22 and 52.8 $\pm$ 4.07 h respectively (Reddy *et al.*, 2001). The injection of PGF_2 alpha at day five of oestrous cycle caused immediate regression of the corpus luteum

thereby causing sharp decline in progesterone concentration to basal level within 24 hours. This increased the LH pulse frequency causing a significant increase in oestradiol concentration from the dominant follicle and the induction of oestrus and ovulation (Diskin *et al.*, 2002).

According to Leeba (2003), higher intensity of oestrus was shown by animals in prostaglandin induced oestrus than during natural one and the percentage of animals showing high, medium and low intensity of oestrus were found to be 51.14, 28.57 and 14.2 respectively. But Jeba (2005) reported that the physical changes in the reproductive tract of repeat breeder were more pronounced during natural than prostaglandin induced oestrum. The average time taken for induction of oestrum and the duration of oestrum were 52 h and 38 h respectively in induced oestrum.

2.8 FERTILITY AFTER PROSTAGLANDIN THERAPY

Jackson (1977) conducted experiment in dairy cattle showing clinical postpartum endometritis by treating with intramuscular injection of 500 micro gram cloprostenol. All animals had an immediate reduction of plasma progesterone concentration and showed oestrus within two to three days. In 85.71 per cent cases, the clinical endometritis resolved with uterine involution within seven days and uterine mucus samples showed no specific microbial growth. Similarly Coulson (1978) treated cows with metritis using single intramuscular injection of 25 mg of Dinoprost and a recovery rate of 76.3 per cent were obtained and opined that luteolytic dose of prostaglandin produced regression of the CL, relaxation of the cervix and expulsion of the uterine contents.

A double injection scheme using PGF_2 alpha and timed insemination after second injection was conducted by Seguin (1980) and only seven out of 32 (22 per

cent) become pregnant at the controlled oestrus. However, Chauhan *et al.* (1983) treated endometritis with five milligram PGF_2 alpha twice during the luteal phase of oestrous cycle 11 to 12 days apart and sexual rest was given on the induced oestrus. Inseminations were done on exhibition of next natural oestrus and obtained an over all conception rate of 100 per cent. Mc Intosh *et al.* (1984) opined that cattle treated with cloprostenol and inseminated on detection of oestrus resulted in improved conception rates for the treated cows when compared with those in which oestrus had not been synchronized.

Pepper and Dobson (1987) treated cows with chronic endometritis using 25 mg Dinoprost intramuscularly at the luteal phase of oestrous cycle and a first service conception rate of 55 per cent were obtained. Different degrees of endometritis cases (such as severe, moderate and mild) were treated by Murray *et al.* (1990) with a single injection of alfaprostol and obtained a success rate of 74 per cent.

According to Glanvil and Dobson (1991) the administration of PGF_2 alpha between 14 and 28 days after calving had no beneficial effect upon the reproductive performance of 'problem cows' and the treatment apparently failed to improve the fertility of animals with endometritis. Similarly Gay and Upham (1994) also administered PGF_2 alpha 20 to 40 days after parturition and found that its use was associated with a significant decrease in conception rate at first breeding from 42 to 29.3 per cent.

Animals showing clinical endometritis were subjected to induction of oestrus using 25 mg Dinoprost at eight to 12 days of cycle and then inseminated twice at 24 hour interval and obtained a first insemination conception rate of 54.53 per cent with an over all conception rate of 81.80 per cent (Jacob *et al.*, 1995).

Ajitkumar *et al.* (1996) subjected cross bred heifers of breedable age maintained under identical conditions to single and double regime prostaglandin therapy at the luteal phase of oestrous cycle. In single prostaglandin therapy, the first insemination conception rate and overall conception rate were 33.33 per cent and 66.67 per cent when inseminated 72 h post treatment, while the respective values were 33.33 per cent and 50 per cent when inseminated 96 h post treatment. A marginal increase in overall conception rate was observed in heifers inseminated 72 hours after the administration of second dose of PGF_2 alpha, 12 per cent of animals conceived at first insemination while the overall conception rate was 62.5 per cent. The corresponding values at 96 h insemination were 25 per cent and 50 per cent respectively.

Sheldon and Noakes (1998) used 500 µg of cloprostenol intramuscularly for the treatment of cows with endometritis at the luteal phase of oestrous cycle and obtained a success rate of 67 per cent. Knutti *et al.* (2000) classified cows with endometritis into mild and severe based on rectal palpation and vaginoscopy and synthetic analogues of PGF_2 alpha were administered to them and obtained a conception rate of 84.2 per cent and 64.7 per cent respectively for mild and severe endometritic cases. Cows with endometritis were treated with 500 µg cloprostenol intramuscularly and observed that there were fewer number of services per conception (1.1 ± 1), shorter interval to oestrus (6.2 ± 1.1 days) and an over all conception rate of 77.4 per cent (Ghanem *et al.*, 2004).

Cows with sub clinical endometritis were treated by Kasimanickam *et al.* (2005) using cloprostenol and found that the relative pregnancy rate was 63 per cent higher when compared to control group which received no treatment. Similarly Kharche and Srivastava (2005) treated cows with different doses of Tiaprost through

various routes for oestrus synchronization and subsequent fertility were assessed and obtained 83.33 per cent of oestrus response and 60 percent conception rate when given intramuscularly. However, Mejia *et al.* (2005) treated cows with endometritis using Tiaprost and insemination was withheld until clinical signs abated. It was found that untreated cows were conceived 20 days earlier than treated cows and so the treatment impaired the reproductive performance and increased the costs.

Waldmann *et al.* (2006) studied the pregnancy status of cows treated with two doses of PGF_2 alpha 14 days apart and inseminated at fixed time 80 to 82 h later. The overall pregnancy rate was 38 per cent and 33.3 per cent respectively at 24 and 48 days after insemination and suggested that the primary reason for low pregnancy rate was inappropriate ovarian function such as failure of luteolysis following treatment or anovulation following luteolysis

Materials and Methods

3. MATERIALS AND METHODS

The material for the present study consisted of crossbred cows and heifers presented at AI centre and Bull station attached to the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy and also crossbred cows and heifers belonging to University Livestock Farm, Mannuthy. Cows and heifers which failed to conceive even with three or more inseminations were selected and screened for sub clinical endometritis by conducting detailed clinico-gynaecological examination and also by studying the characteristics of oestrual mucus including the white side test. Data regarding occurrence, onset, duration and intensity of oestrum were collected.

3.1 OCCURRENCE OF REPEAT BREEDING AND SUB CLINICAL ENDOMETRITIS

The breeding history of cows and heifers brought for insemination were collected during the period from July 2006 to February 2007 and the occurrence of repeat breeding was recorded. Among the repeat breeders, the presence of sub clinical endometritis was determined from the findings such as increase in the size and tonicity of uterus, colour of the mucus discharge and also by doing white side test as described by Pateria and Rawal, (1990) and the percentage of incidence was recorded.

3.2 CHANGES IN THE EXTERNAL GENITALIA DURING OESTRUS

Physical changes in the reproductive tract of all animals such as oedema of vulval lip and hyperemia of vestibular mucous membrane were noticed and graded as low, medium and high depending upon the degree of changes.

Cervical mucus samples were collected aseptically by aspiration using an AI pipette before insemination. Characteristics like colour and consistency were noted and compared with those of normal animals. All the samples were subjected to white side test for detection of sub clinical endometritis.

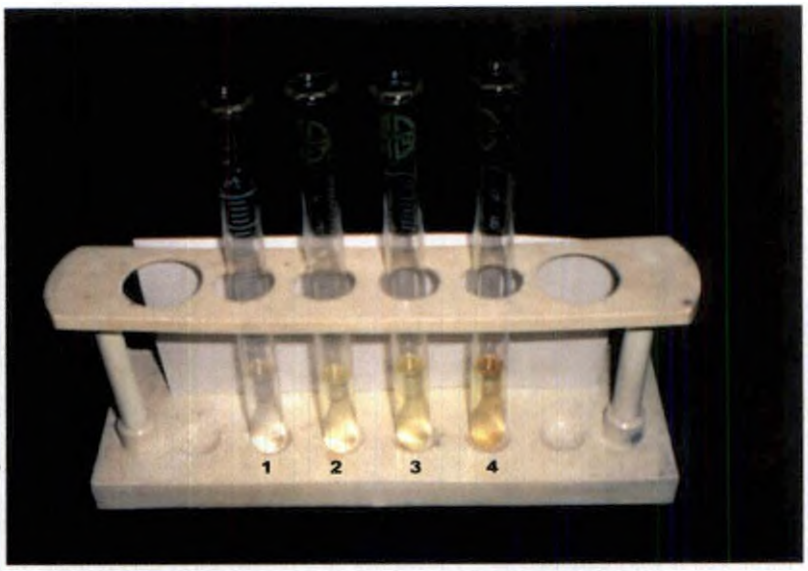
3.4 WHITE SIDE TEST

The uterine discharge was collected aseptically using sterile pipette and rubber adapter with a syringe attached to it. It was then mixed with an equal volume of five per cent sodium hydroxide. The mixture was heated up to the boiling point and intensity of colour change was studied and graded as follows.

1. Normal – Turbid or no colour.
2. Mild – Light yellow colour.
3. Moderate – Yellow colour.
4. Severe – Dark yellow colour.

Animals showing positive response to white side test were selected and randomly divided into five groups. They were subjected to following treatment regimes, with each group comprising of 10, 12, 12, 10 and 10 animals respectively from groups I to V.

Group I – Fifty millilitre of one per cent lugol's iodine in distilled water was administered intrauterine 24 hours prior to artificial insemination in animals found in early oestrum.



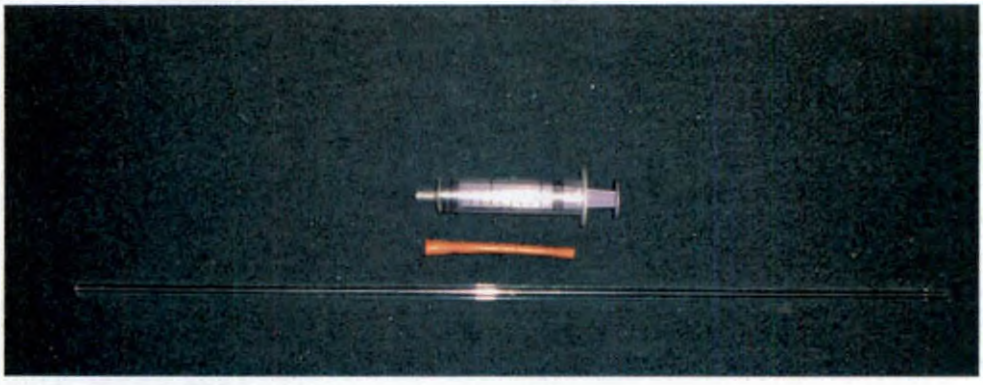
Response to white side test

1- Negative

2- Slight

3- Moderate

4- Intense



AI pipette used for aseptic collection of uterine discharge

Group II -- Fifty millilitre of one per cent lugol's iodine in distilled water was administered intrauterine 24 hours after artificial insemination.

Group III – A synthetic prostaglandin analogue, *CLOSTENOL two ml was given intramuscularly after 11 days of oestrum and timed inseminations were done at 72 and 96 hours.

Group IV -- A synthetic prostaglandin analogue, *CLOSTENOL two ml was given intramuscularly at 11 days interval on the luteal phase of oestrous cycle, followed by timed inseminations at 72 and 96 hours.

Group V – Animals showing positive response to white side test were subjected to artificial insemination at standing oestrum, without any treatment.

*(CLOSTENOL,2ml vial, cloprostenol sodium 263 µg per ml, synthetic prostaglandin F_2 alpha analogue, Sarabai Zydus).

Artificial insemination was done in apparently healthy animals brought for fresh insemination at AI centre, Mannuthy and the non-return rates were calculated during the period of study.

3.5 ASSESSMENT OF UTERINE TONICITY AND OVARIAN STATUS

The tonicity of uterus was palpated and recorded after grading as high, medium and low depending on the degree of hardness and texture. Both right and left ovaries were palpated for the presence of follicle and corpus luteum and their locations were noted.

Uterine catheter used for infusing Lugol's Iodine

Synthetic PGF_2 alpha analogue used for the study

3.6 INTENSITY OF OESTRUS

Intensity of oestrus was noticed and graded as high, medium and low from clinical and behavioral manifestations

3.7 OESTRUS RESPONSE AFTER INDUCTION USING PROSTAGLANDIN

The oestrus responses such as time taken for induction of oestrus and duration of oestrum after PGF2 alpha injection were observed in detail. The time taken for induction of oestrus was calculated by recording the interval from prostaglandin administration to the onset of oestrum. Duration of oestrum was monitored by the close observation of clinical signs like mucus discharge, vulval oedema, and hyperemia of vestibular mucous membrane, bellowing and mounting.

3.8 CONCEPTION RATES

Pregnancy was ascertained in all groups 60 days after artificial insemination by per rectal examination. Conception rates of all treatment groups were compared with each other and with control group.

3.9 STATISTICAL ANALYSIS

The data obtained were compiled and subjected to statistical analysis as per Snedecor and Cochran (1985).

Results

4. RESULTS

Results of the investigation on 'Non antibiotic therapy for sub clinical endometritis in repeat breeding cattle' are shown in tables 1 to 12 and figures 1 to 13.

4.1 OCCURRENCE OF REPEAT BREEDING AND SUB CLINICAL ENDOMETRITIS

In the present study, the occurrence of repeat breeding and sub clinical endometritis during the period from July 2006 to February 2007 was investigated in cows and heifers brought to Artificial Insemination centre and Bull station attached to the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy (Table 1 and Figures 1 & 2).

4.1.1 Occurrence of Repeat Breeding

A total of 576 cross bred animals consisting of 440 cows and 136 heifers were presented for insemination at AI centre, Mannuthy. Out of them, 110 animals were screened as repeat breeders showing an overall incidence of 19.01 per cent. Among the repeat breeders there were 32 heifers and 78 cows which constituted 23.53 per cent and 17.72 per cent respectively (Table 1 and Figure 1).

4.1.2 Occurrence of Sub Clinical Endometritis

On further detailed investigation of repeat breeders, 15 heifers and 32 cows were found to have sub clinical endometritis showing an occurrence of 46.88 per cent and 46.15 per cent respectively. The over all occurrence of sub clinical endometritis was found to be 46.36 per cent (Table 1 and Figure 2).

4.2 CHANGES IN THE EXTERNAL GENITALIA DURING OESTRUS

Changes in the reproductive tract such as oedema of vulval lips and hyperemia of vestibular mucous membrane were observed. Both of them were classified as high, medium and low depending on the degree of changes and are shown in tables 2 & 3 and figures 3 & 4 respectively.

4.2.1 Intensity of Vulval Oedema

The percentage of animals showing high, medium and low degree of vulval oedema respectively was 20, 30 and 50 in group I, 41.67, 33.33 and 25 in group II, 33.33, 41.67 and 25 in group III, 20, 40 and 40 in group IV and 20, 50 and 30 in group V.

4.2.2 Hyperemia of Vestibular Mucous Membrane

The corresponding data denoting hyperemia of vestibular mucous membrane were 30, 30 and 40 in group I, 33.33, 41.67 and 25 in group II, 33.33, 33.33 and 33.33 in group III, 30, 30 and 40 in group IV and 20, 30 and 50 in group V.

4.3 CHARACTERISTICS OF CERVICO VAGINAL MUCUS DISCHARGE

Cervical mucus samples were collected aseptically by aspiration using an AI pipette with syringe and adapter and colour and consistency were recorded.

4.3.1 Colour

The colour of the discharge was classified as clear and cloudy depending on

the transparency of mucus. The percentage of animals with clear and cloudy discharges respectively in each treatment group was 60 and 40 in group I, 58.33 and 41.67 in group II, 66.67 and 33.33 in group III, 50 and 50 in group IV and 70 and 30 in group V (Table 4 and Fig. 5).

4.3.2 Consistency

The consistency of mucus samples were scored as watery, thin and thick depending on the nature of discharge. The percentage of animals showing watery, thin and thick discharges in each group respectively was 40, 40 and 20 in group I, 33.33, 50 and 16.67 in group II, 25, 41.67 and 33.33 in group III, 20, 50 and 30 in group IV and 30, 30 and 40 in group V (Table 5 and Fig. 6).

4.4 RESPONSE TO WHITE SIDE TEST

The mucus samples of all repeat breeders were collected aseptically and subjected to white side test. The samples which turned yellow were classified as slight, moderate and intense depending on the intensity of colour change. The percentage of animals showing slight, moderate and intense reactions respectively in each group was 50, 30 and 20 in group I, 41.67, 41.67 and 16.67 in group II, 33.33, 33.33 and 33.33 in group III, 20, 30 and 50 in group IV and 30, 50 and 20 in group V (Table 6 and Fig. 7).

4.5 ASSESSMENT OF UTERINE TONICITY AND OVARIAN STATUS

The tonicity of uterus was classified as high, medium and low depending on the texture of uterus. Ovarian changes during oestrus such as presence of graafian

follicle (GF) and regressed corpus luteum (RCL) were palpated and recorded in each group.

4.5.1 Uterine Tonicity

The percentage of animals showing high, medium and low degree of uterine tonicity respectively was 30, 50 and 20 in group I, 41.67, 41.67 and 16.67 in group II, 50, 33.33 and 16.67 in group III, 50, 20 and 30 in group IV and 40, 40 and 20 in group V (Table 7 and Fig. 8).

4.5.2 Ovarian Status

In group I out of ten animals examined, GF and RCL were seen on right ovary in seven and six cases and on left ovary in three and four cases respectively. In group II, right ovary of seven cases and left ovary of five cases showed both GF and RCL. In group III, GF and RCL were seen on right ovary in seven and six cases and left ovary in five and six cases respectively. In group IV, right and left ovary of five cases each showed both GF and RCL and in group V, GF and RCL were seen on right ovary in six and four cases and in left ovary in five cases each (Table 8, Fig. 9).

4.6 INTENSITY OF OESTRUM AFTER INDUCTION USING CLOSTENOL

Oestrus intensity after induction using clostenol were noted and classified as high, medium and low intensities. The percentage of animals showing high, medium and low intensities respectively was 50, 33.33 and 16.67 in group III and 40, 30 and 30 in group IV (Table 9 and Fig. 10).

4.7 OESTRUS RESPONSE AFTER INDUCTION USING CLOSTENOL

Oestrus response such as time taken for induction of oestrum and duration of oestrus were recorded in groups III and IV after the administration of clostenol. All the animals in each group were responded to treatment by exhibiting oestrus signs and hence there was 100 per cent efficiency. The time taken for induction of oestrum in group III and IV were 59.38 $\pm$ 3.70 h and 58.88 $\pm$ 2.75 h respectively. The duration of oestrus in groups III and IV were 37.5 $\pm$ 3.70 h and 38.63 $\pm$ 3.65 h respectively. On analysis of data, it was found that there was no significant difference in the oestrus response in both groups after induction (Table 10, Fig. 11).

4.8 CONCEPTION RATES

The conception rates of animals obtained after treatment in groups I to V were 40, 50, 50, 40 and 20 respectively (Table 11 and Fig. 12).

Out of the 576 cross bred animals brought for fresh insemination, 210 animals were subjected to re-insemination. A non-return rate of 63.55 per cent was obtained during the period of study from July 2006 to February 2007.

Table 1. Occurrence of repeat breeding and sub clinical endometritis among cows and heifers presented at AI centre, Mannuthy.

Animals	No: of animals screened	Repeat breeding animals		Repeat breeding animals showing sub clinical endometritis	
		Number	Per cent	Number	Per cent
Cows	440	78	17.72	36	46.15
Heifers	136	32	23.53	15	46.88
Total	576	110	19.01	51	46.36

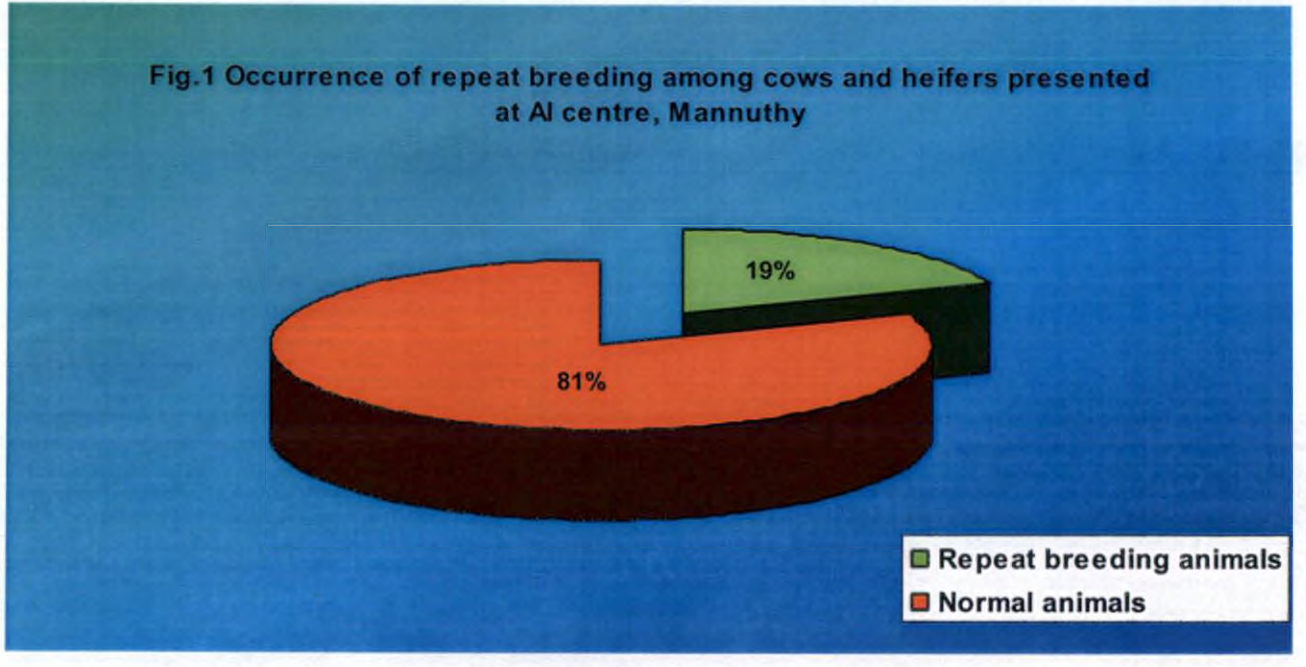


Fig.1 Occurrence of repeat breeding among cows and heifers presented at AI centre, Mannuthy
19%
81%
Repeat breeding animals
Normal animals

Fig.2 Occurrence of sub clinical endometritis among repeat breeders presented at AI centre, Mannuthy

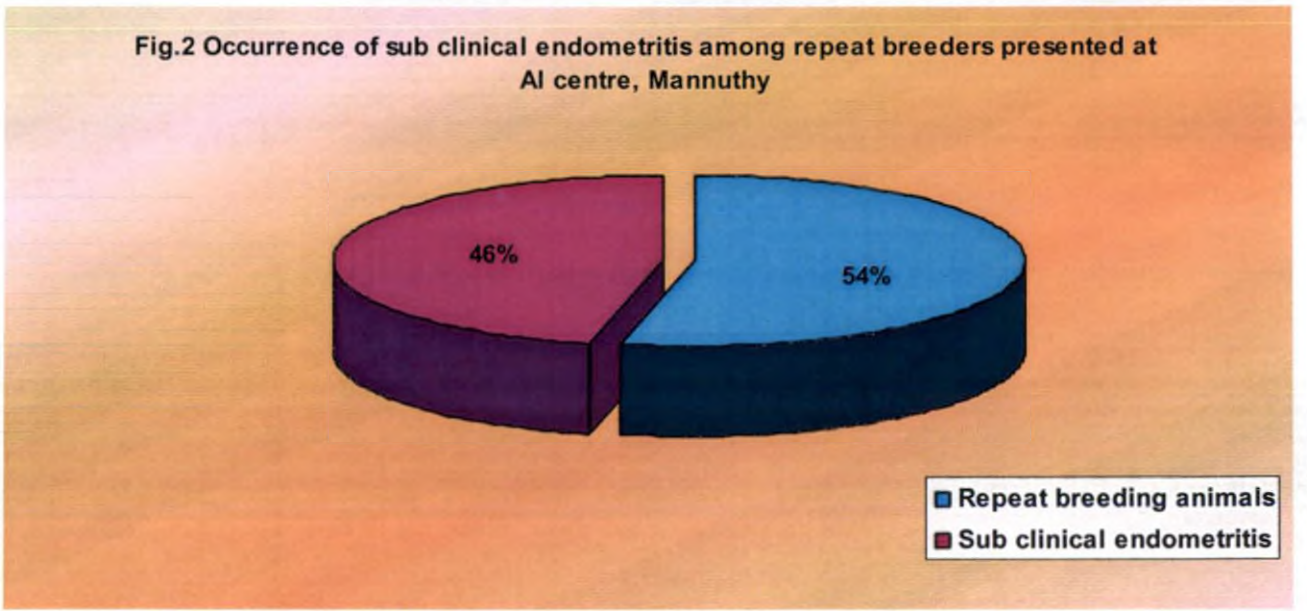


Table 2. Vulval oedema in repeat breeders with sub clinical endometritis

Groups	High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent
Group I	2	20	3	30	5	50
Group II	5	41.67	4	33.33	3	25
Group III	4	33.33	5	41.67	3	25
Group IV	2	20	4	40	4	40
Group V	2	20	5	50	3	30

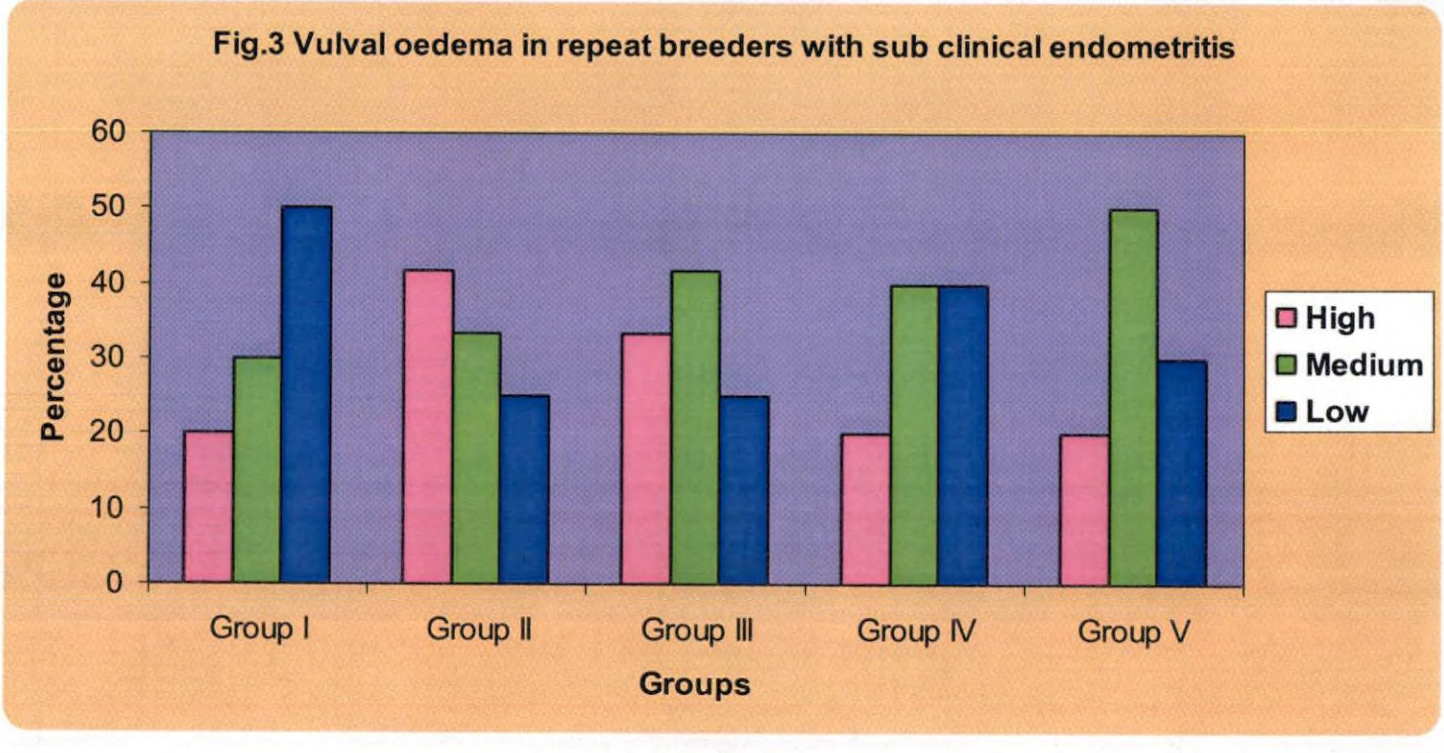


Fig.3 Vulval oedema in repeat breeders with sub clinical endometritis
60
50
40
30
20
10
0
Percentage
Group I
Group II
Group III
Group IV
Group V
Groups
High
Medium
Low

Table 3. Hyperemia of vestibular mucous membrane in repeat breeders with sub clinical endometritis

Groups	High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent
Group I	3	30	3	30	4	40
Group II	4	33.33	5	41.67	3	25
Group III	4	33.33	4	33.33	4	33.33
Group IV	3	30	3	30	4	40
Group V	2	20	3	30	5	50

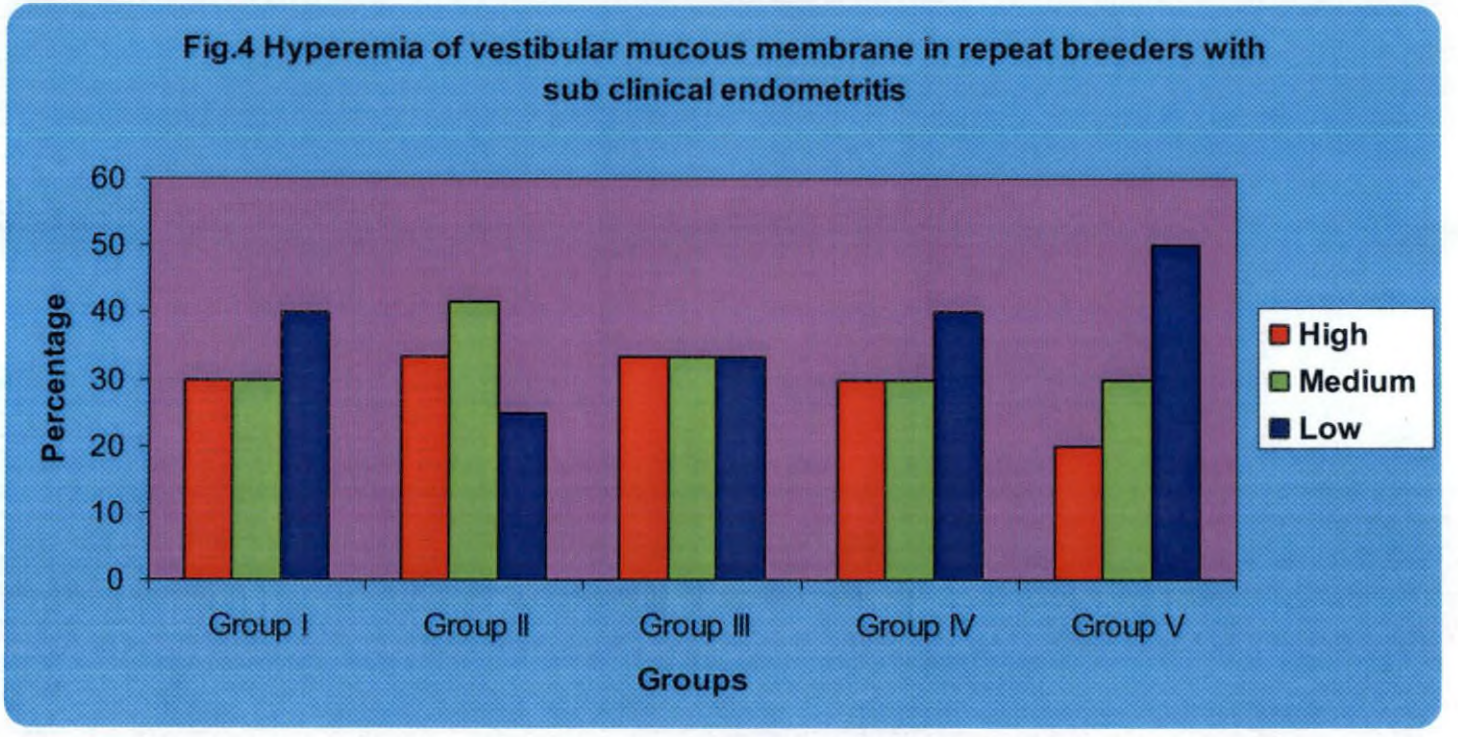


Fig.4 Hyperemia of vestibular mucous membrane in repeat breeders with sub clinical endometritis
Percentage
60
50
40
30
20
10
0
Group I
Group II
Group III
Group IV
Group V
Groups
High
Medium
Low

Table 4. Colour of mucus discharge in repeat breeders with sub clinical endometritis

Groups	Clear		Cloudy	
	Number	Per cent	Number	Per cent
Group I	6	60	4	40
Group II	7	58.33	5	41.67
Group III	8	66.67	4	33.33
Group IV	5	50	5	50
Group V	7	70	3	30

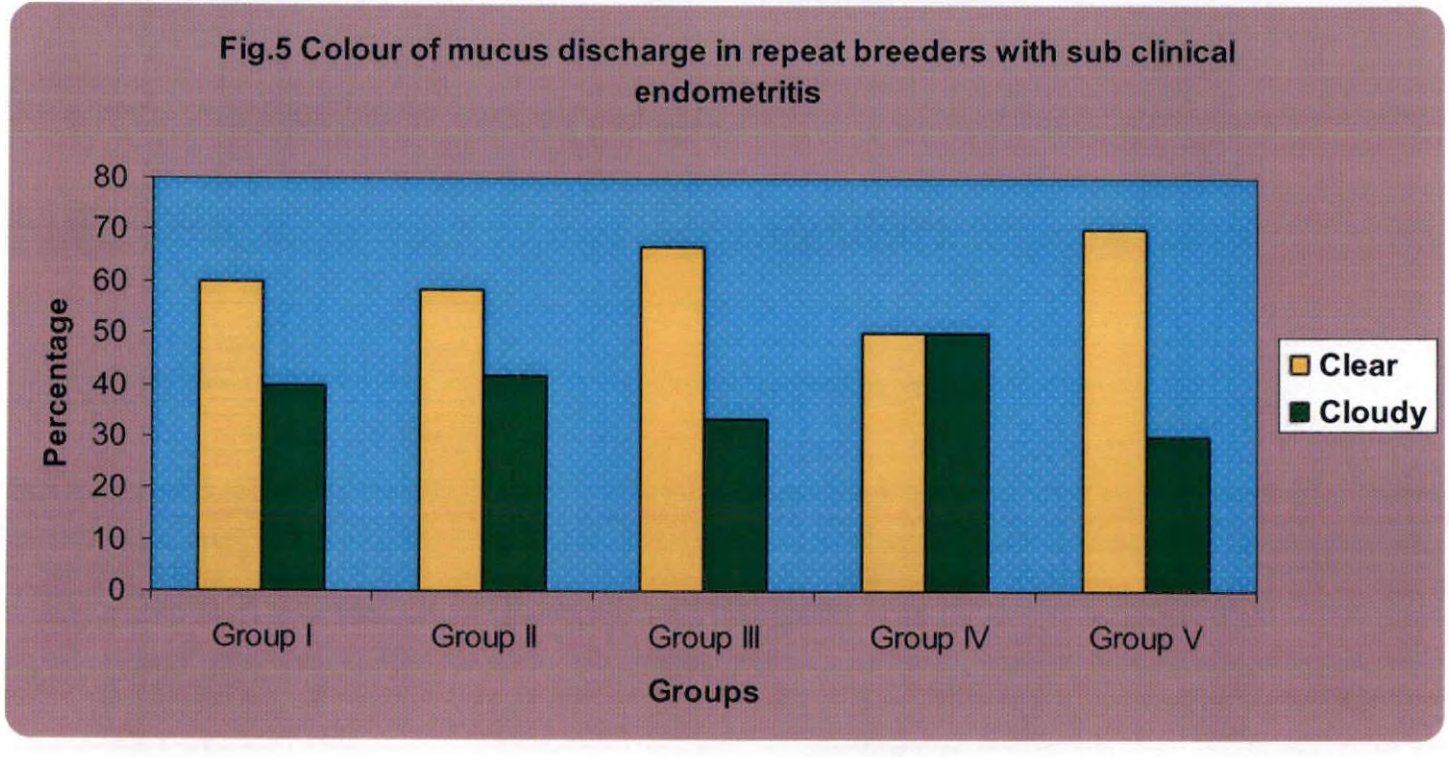


Fig.5 Colour of mucus discharge in repeat breeders with sub clinical endometritis
Percentage
80
70
60
50
40
30
20
10
0
Group I
Group II
Group III
Group IV
Group V
Groups
Clear
Cloudy

Table 5. Consistency of mucus discharge in repeat breeders with sub clinical endometritis

Groups	Watery		Thin		Thick	
	Number	Per cent	Number	Per cent	Number	Per cent
Group I	4	40	4	40	2	20
Group II	4	33.33	6	50	2	16.67
Group III	3	25	5	41.67	4	33.33
Group IV	3	20	5	50	3	30
Group V	3	30	3	30	4	40

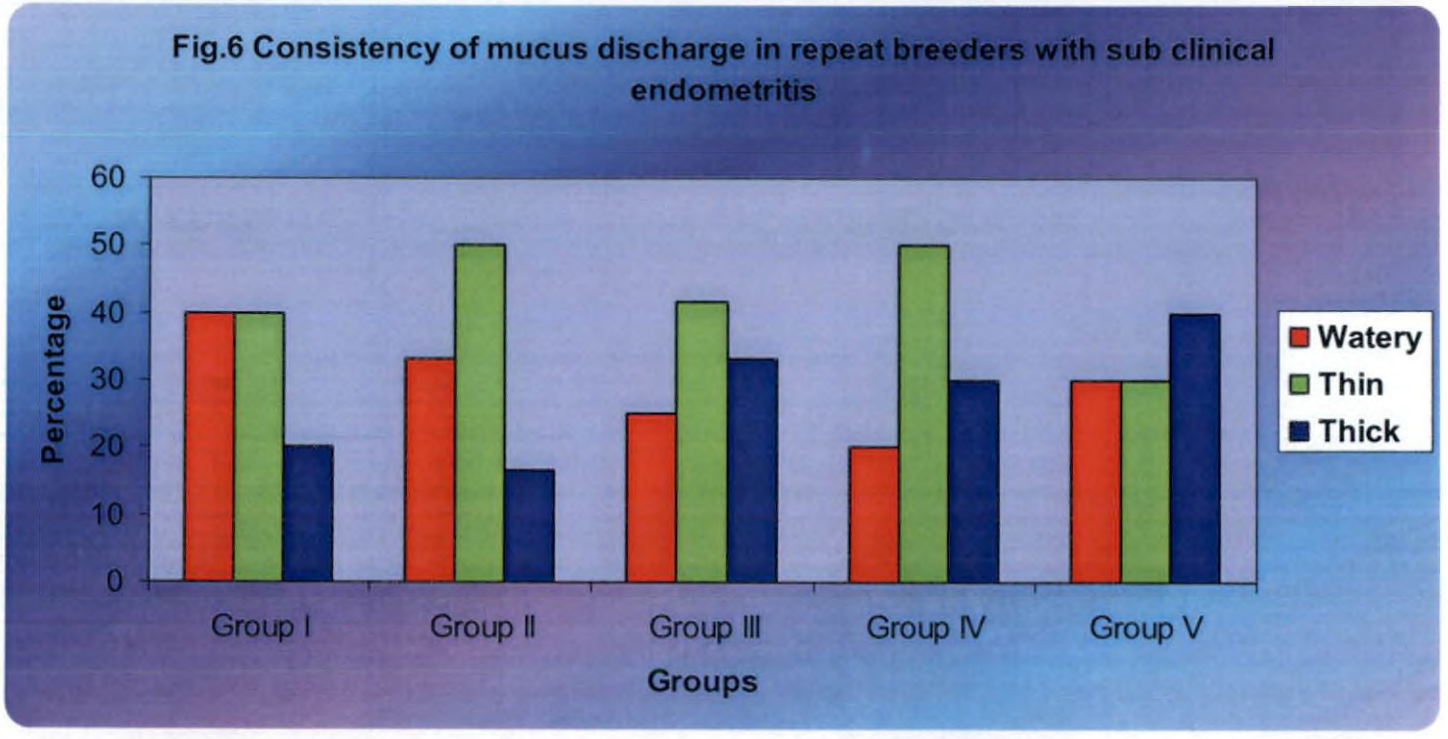


Fig.6 Consistency of mucus discharge in repeat breeders with sub clinical endometritis
60
50
40
30
20
10
0
Percentage
Group I
Group II
Group III
Group IV
Group V
Groups
Watery
Thin
Thick

Table 6. Response to white side test in repeat breeders with sub clinical endometritis

Groups	Slight		Moderate		Intense	
	Number	Per cent	Number	Per cent	Number	Per cent
Group I	5	50	3	30	2	20
Group II	5	41.67	5	41.67	2	16.67
Group III	4	33.33	4	33.33	4	33.33
Group IV	2	20	3	30	5	50
Group V	3	30	5	50	2	20

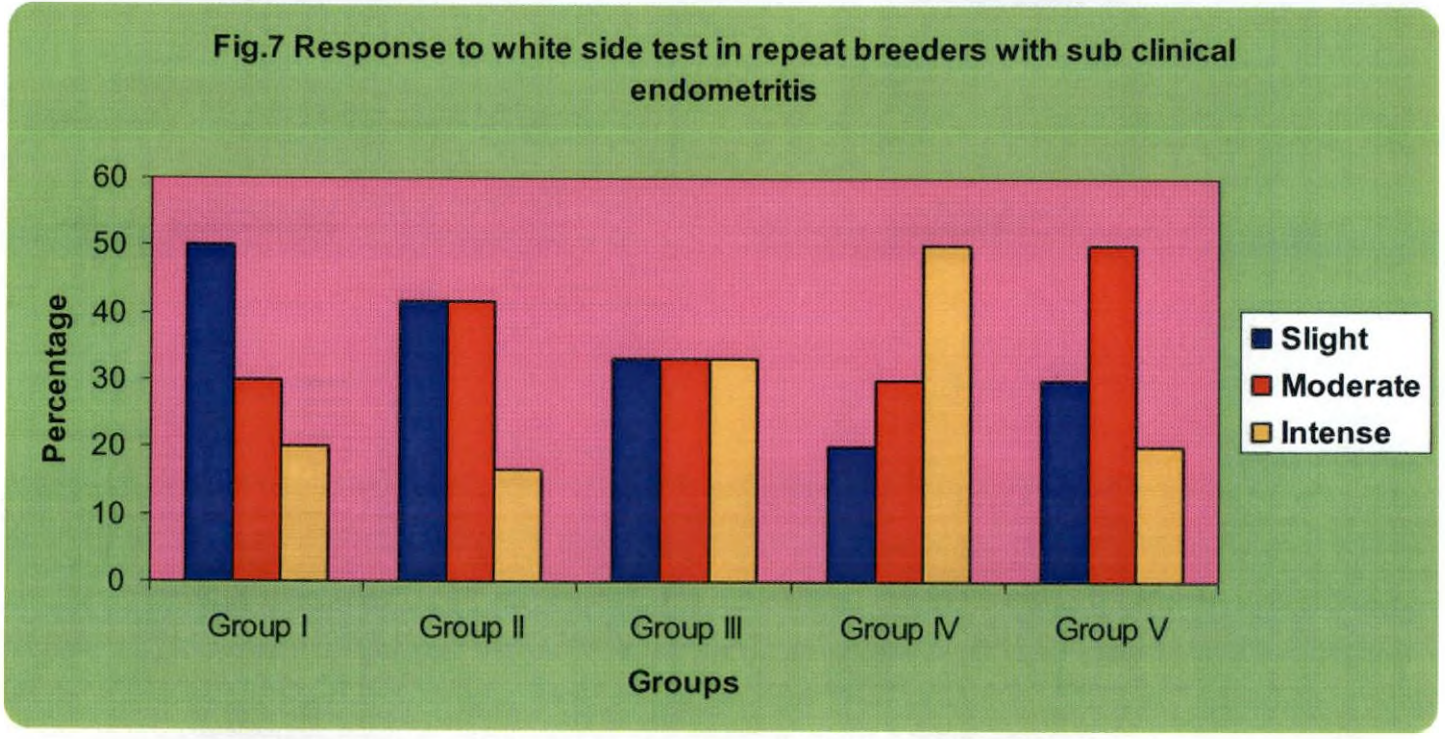


Fig.7 Response to white side test in repeat breeders with sub clinical endometritis
60
50
40
30
20
10
0
Percentage
Group I
Group II
Group III
Group IV
Group V
Groups
Slight
Moderate
Intense

Table 7. Tonicity of uterus in repeat breeders with sub clinical endometritis

Groups	High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent
Group I	3	30	5	50	2	20
Group II	5	41.67	5	41.67	2	16.67
Group III	6	50	4	33.33	2	16.67
Group IV	5	50	2	20	3	30
Group V	4	40	4	40	2	20

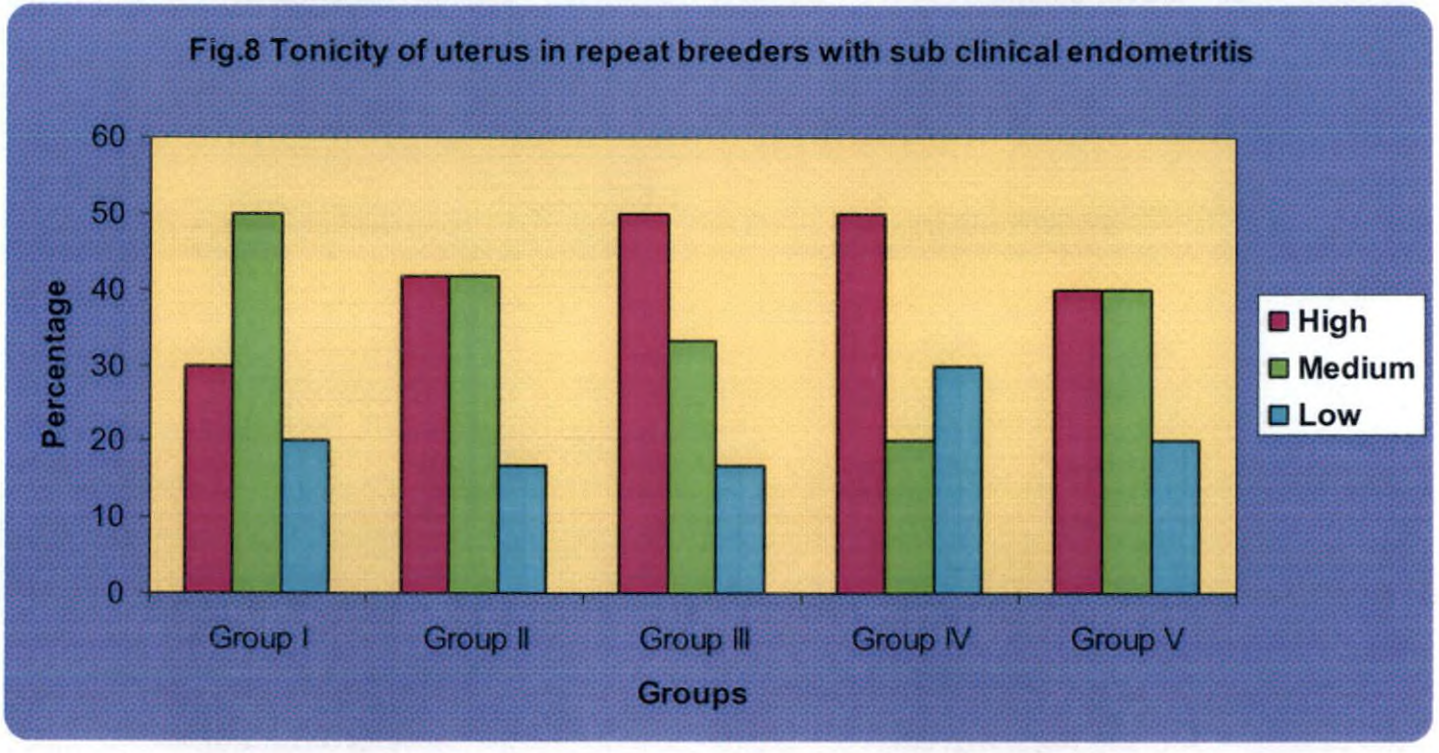


Fig.8 Tonicity of uterus in repeat breeders with sub clinical endometritis
60
50
40
30
20
10
0
Percentage
Group I
Group II
Group III
Group IV
Group V
Groups
High
Medium
Low

Table 8. Ovarian changes during oestrus in repeat breeders with sub clinical endometritis

Groups	No: of animals examined.	Presence of graafian follicle.		Presence of regressed Corpus luteum.	
		Right	Left	Right	Left
Group I	10	7	3	6	4
Group II	12	7	5	7	5
Group III	12	7	5	6	6
Group IV	10	5	5	5	5
Group V	10	6	4	5	5

Fig.9 Ovarian changes during oestrus in repeat breeders with sub clinical endometritis

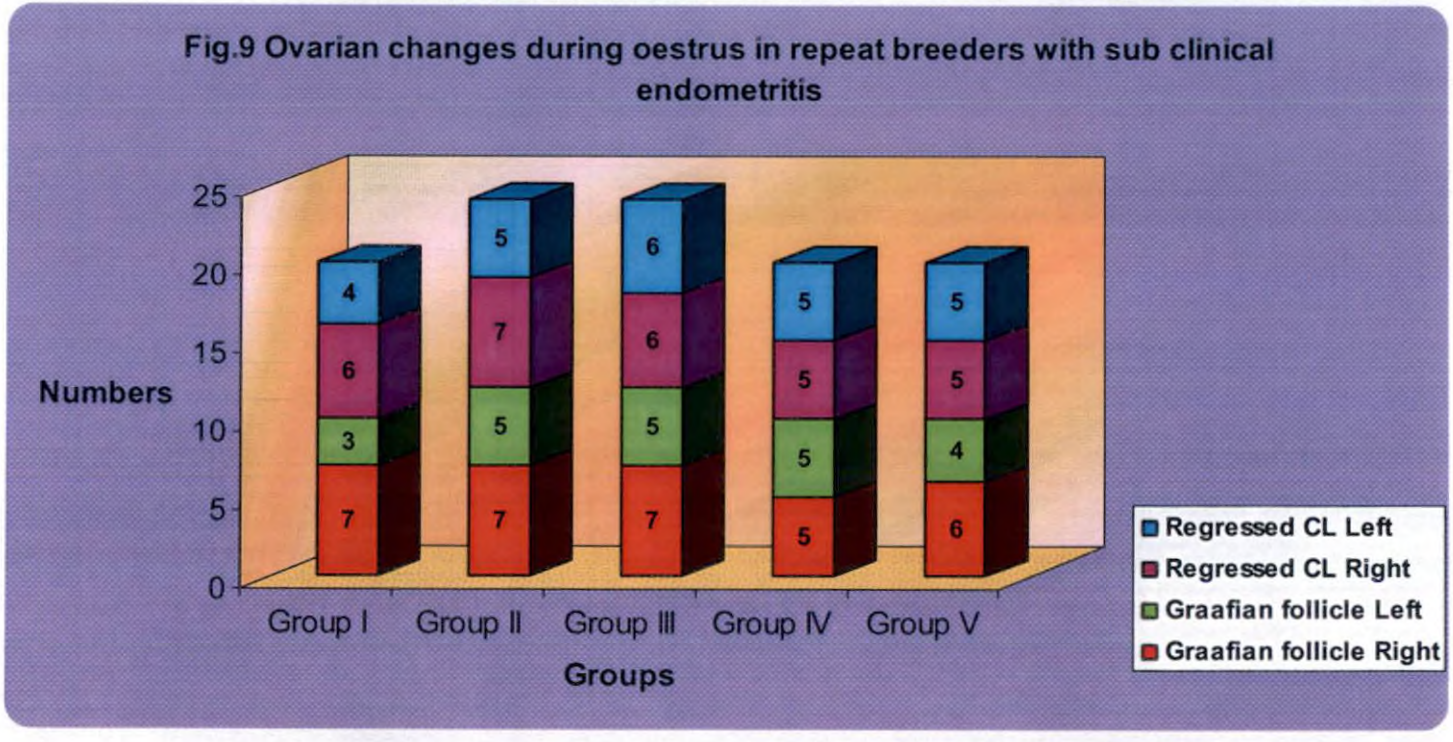


Table 9. Intensity of oestrum after induction using Clostenol

Groups	High		Medium		Low	
	Number	Per cent	Number	Per cent	Number	Per cent
Group III	6	50	4	33.33	2	16.67
Group IV	4	40	3	30	3	30

Fig.10 Intensity of oestrum after induction using Clostenol

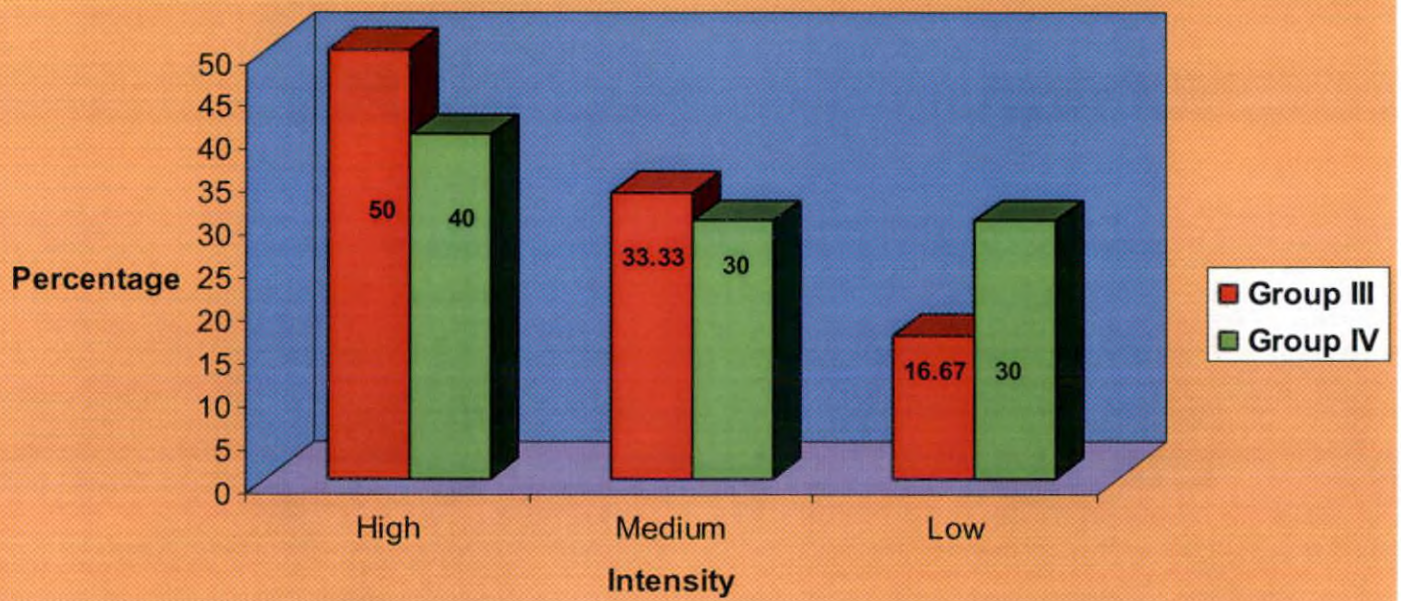


Table 10. Oestrus response after induction using Clostenol

Groups	Group III	Group IV
No: of animals treated	12	10
No: of animals responded to treatment	12 (100 %)	10 (100 %)
Time taken for induction of oestrum (Mean $\pm$ SE hours)	59.38 $\pm$ 3.70	58.88 $\pm$ 2.75
Duration of oestrum (Mean $\pm$ SE hours)	37.5 $\pm$ 3.70	38.63 $\pm$ 3.65

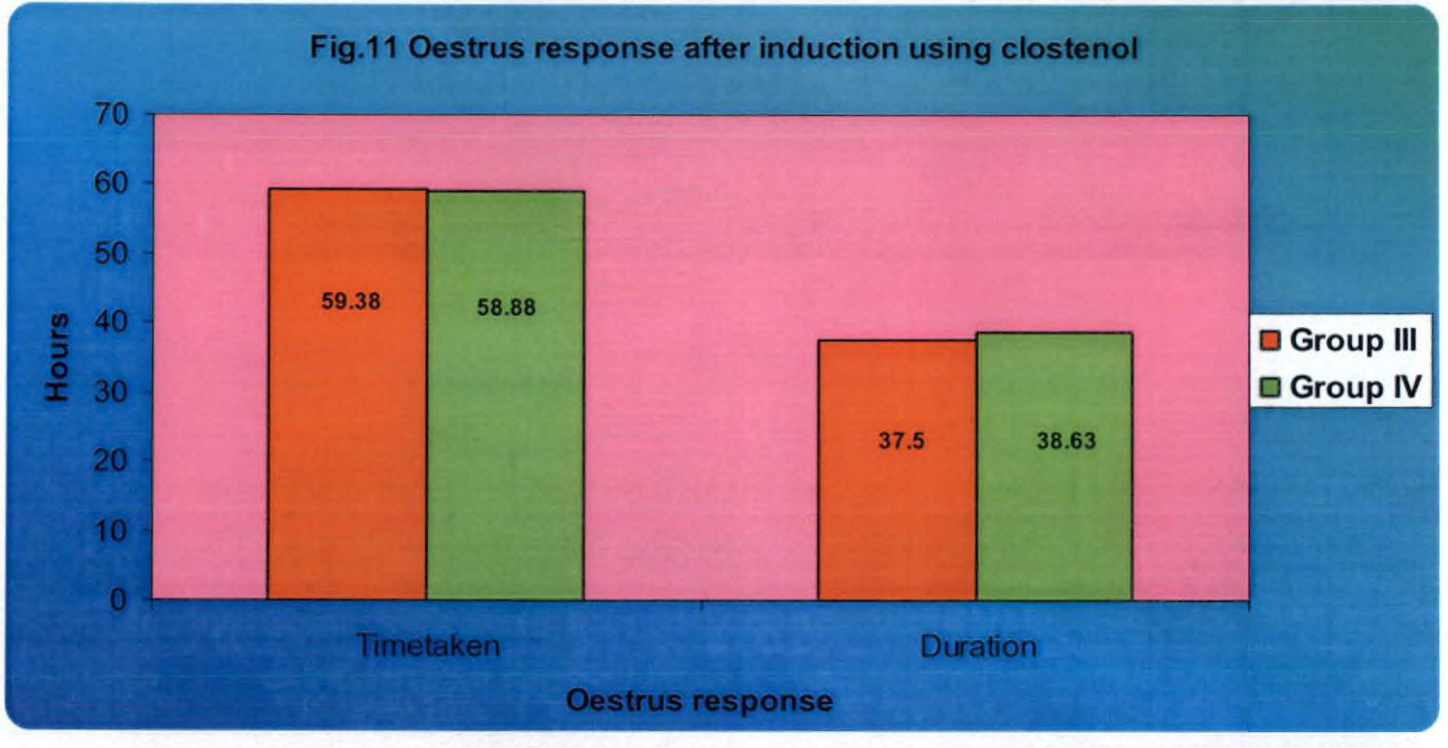


Fig.11 Oestrus response after induction using clostenol
70
60
50
40
30
20
10
0
Hours
59.38
58.88
37.5
38.63
Group III
Group IV
Timetaken
Duration
Oestrus response

Table 11. Fertility after different treatments in repeat breeders with sub clinical endometritis

Groups	No: of animals	Conception rate after therapy	
		Number	Per cent
Group I	10	4	40
Group II	12	6	50
Group III	12	6	50
Group IV	10	4	40
Group V	10	2	20

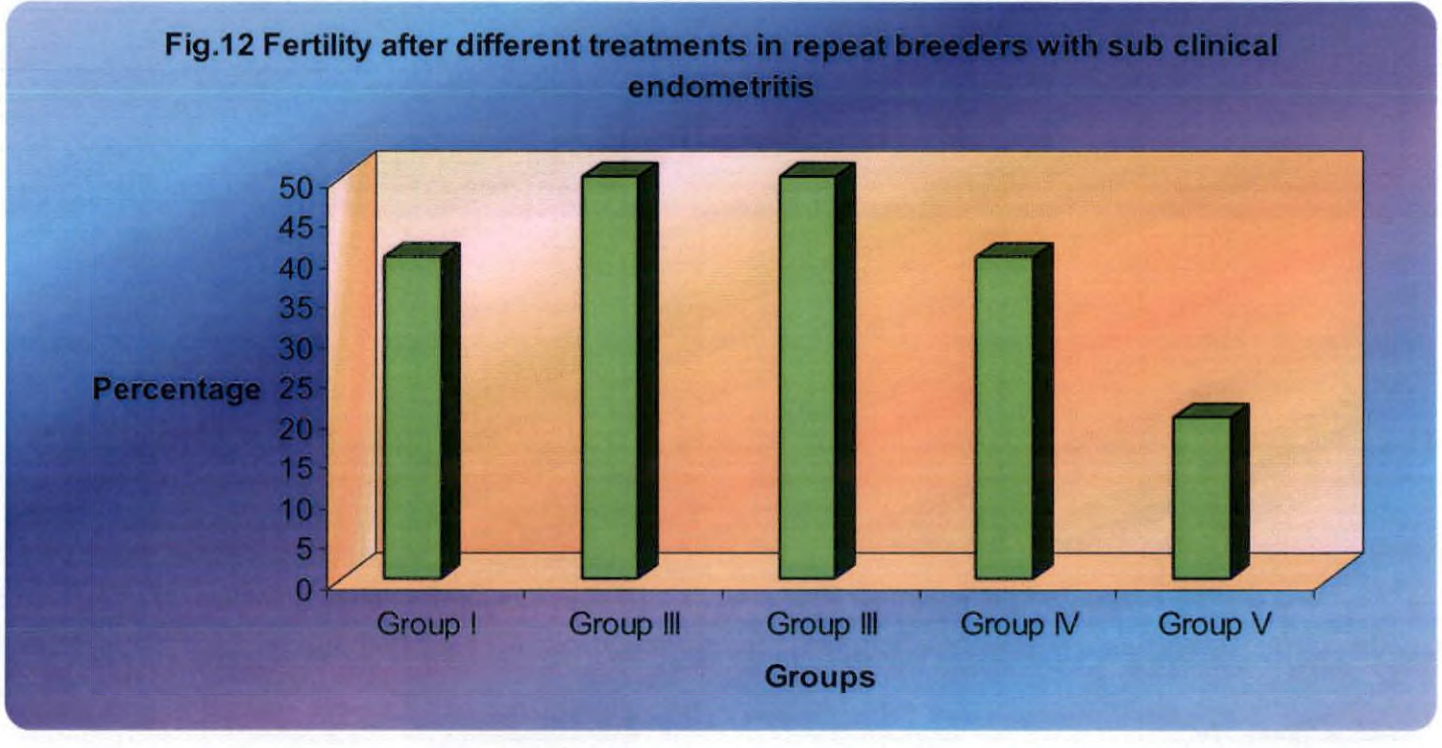


Fig.12 Fertility after different treatments in repeat breeders with sub clinical endometritis
Percentage
50
45
40
35
30
25
20
15
10
5
0
Group I
Group III
Group III
Group IV
Group V
Groups

Discussion

5. DISCUSSION

Sub clinical endometritis without any pronounced clinical symptom is a major cause for repeat breeding among cattle and its incidence was found to vary between 16.9 per cent (Le Blanc *et al.*, 2002) to 53 per cent (Gilbert, 2005). The most common method of treating endometritis with different types of antibiotic preparations were reported to give varying results (Le Blanc *et al.*, 2002, Amridis *et al.*, 2003, Dhillon *et al.*, 2005). The present study was aimed to evolve a suitable method of non-antibiotic therapy for sub clinical endometritis in cattle by comparing the fertility rate of different treatment regimes.

5.1 OCCURRENCE OF REPEAT BREEDING AND SUB CLINICAL ENDOMETRITIS

5.1.1 Occurrence of Repeat Breeding

The overall incidence of repeat breeding among the animals brought for artificial insemination at AI centre, Mannuthy was found to be 19.01 per cent. Almost similar incidences of repeat breeding were reported by Jeba (2005) and Satheshkumar and Punniamurthy (2003) which were found to be 21.91 per cent and 24.60 per cent respectively. However, a higher incidence of 34.97 per cent was reported by Velayudakumar (2003) where as Selvaraju *et al.* (2005) reported a lower incidence of only 5.59 per cent.

5.1.2 Occurrence of Sub Clinical Endometritis

Among the repeat breeders, the occurrence of sub clinical endometritis in the present study was found to be 46.36 per cent. This was almost similar to the findings

of Kasimanickam *et al.* (2004) and Gilbert (2005). However, lower incidences were reported by Vahida (1992), Iyer *et al.* (1992), Dhami *et al.* (1993) and Mejia *et al.* (2005).

5.2 CHANGES IN THE EXTERNAL GENITALIA DURING OESTRUS

Perusal of the data presented in tables 2 & 3 and figures 3 & 4 revealed the physical changes in the reproductive tract in animals of different treatment groups. It was found that all treatment groups showed almost similar cyclical changes of the reproductive tract irrespective of induced and natural oestrum as reported by Jacob *et al.* (1993). But Ajitkumar (1994) observed a marginal increase in the vulval oedema, hyperemia of vestibular mucosa and oestrual discharge in PGF_2 alpha induced oestrus, when compared to natural oestrum. While Jeba (2005) reported that the physical changes in the reproductive tract of repeat breeders with prolonged oestrum were found to be more pronounced during natural oestrum than during induced oestrum.

5.3 CHARACTERISTICS OF CERVICO-VAGINAL MUCUS DISCHARGE

5.3.1 Colour

The colour of the discharge was classified as clear and cloudy depending on the transparency of mucus. In the present study, almost equal per cent of animals showed both clear and cloudy discharge. The results were in agreement with the findings of Nampoothiripad (1971) who opined that abnormal colour was suggestive of repeat breeding but cannot be considered as specific to repeat breeders. Higher percentage of clear cervico-vaginal mucus in repeaters was reported by

Velayudakumar (2003) also. However, Umashankar *et al.* (1984) reported that more cloudy mucus was shown by repeat breeders when compared to normal animals.

5.3.2 Consistency

In the present study, animals in all the five groups exhibited almost equal per cent of watery, thin and thick discharge. This was in compliance with the report of Umashankar *et al.* (1984), who found similar consistency of oestrual mucus in normal and repeat breeding animals. But according to Nampoothiripad (1971) and Velayudakumar (2003), mucus with thick consistency was found in higher per cent in repeat breeders.

5.4 RESPONSE TO WHITE SIDE TEST

The mucus samples of all animals in the treatment and control groups were collected aseptically and subjected to white side test. All samples showed positive reaction by changing the colour to yellow and was classified as slight, moderate and intense depending on the intensity of colour change. The percentage of animals showing slight, moderate and intense reactions respectively in each group was 50, 30 and 20 in group I, 41.67, 41.67 and 16.67 in group II, 33.33, 33.33 and 33.33 in group III, 20, 30 and 50 in group IV and 30, 50 and 20 in group V. The present study confirmed with the findings of Pateria and Rawal (1990), Krishnakumar *et al.* (2003) and Mohankumar *et al.* (2006). The normal uterine discharge has too low number of leukocytes to cause any change in colour when compared to mild, moderate and severe degrees of colour changes in discharge, which has very high number of leukocytes.

5.5 ASSESSMENT OF UTERINE TONICITY AND OVARIAN STATUS

Data revealed that all treatment groups showed almost similar cyclical changes in the uterus and ovary irrespective of induced and natural oestrum. This is similar to the findings of Jacob *et al.* (1995). Jeba (2005) reported that the physical changes in the reproductive tract of repeat breeders were found to be more pronounced during natural oestrum than during induced oestrum. While Ajitkumar (1994) observed a marginal increase in the uterine tonicity in PGF_2 alpha induced oestrum. Ovarian changes in the animals revealed that all animals are cycling and coming to oestrum regularly, both in experimental and control groups.

5.6 INTENSITY OF INDUCED OESTRUM

The percentage of repeat breeding animals showing high, medium and low intensity of oestrum after PGF_2 alpha administration was 50, 33.33 and 16.67 in group III and 40, 30 and 30 in group IV respectively. In the present study, more number of animals was showing higher intensity of oestrus which could be compared to the findings of Jacob (1993), Ajitkumar (1994) and Leeba (2003). Jacob (1993) reported 66.66, 19.04 and 14.24 per cent of intense, medium and weak oestrum where as Leeba (2003) 51.14, 28.57 and 14.2 per cent of high, medium and low intensity after prostaglandin treatment. This could be attributed to the beneficial effects of PGF_2 alpha in detection of oestrus in a better and pronounced manner in sub oestrus cows.

5.7 OESTRUS RESPONSE AFTER INDUCTION USING PROSTAGLANDIN

Perusal of the data shown in table 10 revealed that all the animals in group III and IV which were treated with prostaglandin analogue responded to the treatment

by showing oestrum within two to four days and the efficacy was 100 per cent. The present study indicated that single as well as double injection schedule of PGF_2 alpha at luteal phase of oestrous cycle were effective in inducing oestrus. Cooper *et al.* (1974), Jackson (1977) and Jeba (2005) had also shown similar results. But Seguin *et al.* (1983) obtained a lowered oestrus response of 73 per cent and Landivar *et al.* (1985) only 54.8 per cent after the injection of prostaglandin. The 100 per cent efficacy in the present study confirmed that all animals in the experimental group were having a functional corpus luteum on the day of treatment and prostaglandin administration was effective in inducing oestrus by bringing the lysis of luteal tissue.

5.7.1 Time Taken for Induction of Oestrus

Time taken for induction of oestrus in animals belonging to group III and IV were 59.38 $\pm$2.81 h and 58.88 $\pm$ 2.75 h respectively. Similar observations were made by Johnson *et al.* (1978), Landivar *et al.* (1985) and Jacob (1993). But the values obtained for the mean onset of oestrus was above 63 h as per the findings of Ajitkumar (1994) and Senthilkumar and Rajasekhar (1998) which was comparatively higher. On contrary, Jeba (2005) obtained a lower value of 52 h only. The variation in the time taken for induction of oestrus observed by various researchers was probably due to varying efficacy of different prostaglandin analogues used in their experiments.

5.7.2 Duration of Oestrus after Induction

The mean duration of oestrum after induction using PGF_2 alpha analogue in group III and IV were 37.5 $\pm$ 3.70 h and 38.63 $\pm$ 3.65 h respectively. Analysis of data revealed that there was not much significant difference between values obtained in single and double regime prostaglandin therapy. A similar duration of oestrum

was reported by Jeba (2005). On the other hand, Ajitkumar *et al.* (1995), Jacob (1993) and Senthilkumar and Rajasekhar (1998) obtained lower values for the same. However, it was reported that the response of PGF_2 alpha in terms of the duration of oestrus and onset of oestrus was found to be variable especially in lactating cows irrespective of the schedule of administration. This was attributed to the ovarian status at the time of PGF_2 alpha administration and also due to variations in the prostaglandin analogues used in the experiments.

5.8 CONCEPTION RATES WITH LUGOL'S IODINE AND PROSTAGLANDIN THERAPY

The conception rates of animals in the experimental and control groups are presented in table 11 and figure 12. The conception rates in group I, II, III, IV and V were 40, 50, 50, 40 and 20 per cent respectively.

Animals in group I received 50 ml of one per cent lugol's iodine 24 h prior to insemination and obtained a conception rate of 40 per cent. Seguin *et al.* (1974) opined that endometrium of cows recovered rapidly after intrauterine infusion of iodine solution and subsequent reproductive performance seemed to be normal. Lugol's iodine produced mild degenerative changes in surface epithelium, moderate to severe oedema, congestion of blood vessels, hemorrhage and moderate neutrophilia (Gupta *et al.*, 1989) which was beneficial in eliminating the infection of uterus. Singh *et al.* (1988) studied endometrial damage after intrauterine infusion of 5 per cent lugol's iodine by suitable staining method and observed that the endometrium was nearly normal at 24 h post infusion. In the present study, the conception rate was slightly lowered in group I probably due to the irritation and subsequent inflammation of endometrium. This might have interfered the normal transport of spermatozoa from the site of deposition to the site of fertilization.

A fertility rate of 50 per cent was obtained in group II which was similar to the findings of Gupta *et al.* (1983) who reported a conception rate of 44.28 per cent in repeat breeders which received 20 ml of five per cent lugol's iodine intrauterine 24 h after insemination. Repeat breeder cows were treated by Pandey *et al.* (1983) with 0.1 per cent of lugol's iodine 10 ml intrauterine one hour after insemination and obtained a fertility rate of 61.54 per cent. Intrauterine medication of lugol's solution 24 to 36 hours after service gave better result in endometritis, because three to four days were required for fertilized ovum to reach the uterus and the medication could act by this time to control infection (Roberts, 1955). The better result obtained in this group might be attributed to the antimicrobial action of lugol's iodine, which helped to clear off the uterine infection by providing a favourable environment for the nidation of embryo.

Single and double regime prostaglandin therapy resulted in 50 and 40 per cent conception rates respectively in groups III and IV. The success rates of prostaglandin therapy for bovine endometritis were reported by Jackson (1977), Coulson (1978), Murray *et al.* (1990), Knutti *et al.* (2005), Ghanem *et al.* (2004) and Kasimanickam *et al.* (2005). Paisley *et al.* (1986) reported that the beneficial effects of PGF_2 alpha in overcoming the uterine infections was by stimulating the phagocytosis by uterine leukocytes and making the discharge clear. It also helped in evacuating the uterine contents and enhanced the healing of endometrium. But Waldmann *et al.* (2006) obtained an over all pregnancy rate of 38 per cent in double regime prostaglandin therapy and suggested that the primary reason for low pregnancy rate was inappropriate ovarian function such as failure of luteolysis following treatment or anovulation following luteolysis. Inspite of the beneficial effects of prostaglandin F_2 alpha, the reason for low fertility rate obtained in group IV might be attributed to the ovulatory disturbances due to the hormonal therapy, which interfered the natural oestrous cycle.

However, better conception rates could be obtained in all treatment groups when compared to control group in which the success rate was only 20 per cent. More over the non- return rates obtained in the AI centre during the period of study was 63.55 per cent, which revealed not much difference with the conception rates obtained in all the treatment groups. Both lugol's iodine and prostaglandin were effective in stimulating the natural defense mechanisms of uterus and helped to evacuate the various microbial agents from the uterus. There is no risk of bacterial resistance and requires no withdrawal time for milk and meat as in case of antibiotics. Hence both of them can be used effectively to improve the conception rates of repeat breeding cattle with sub clinical endometritis. However, treatment with lugol's iodine is comparatively less expensive and it is easily available also, so can be recommended as the drug of choice for tackling cases of repeat breeding with sub clinical endometritis.

Summary

6. SUMMARY

The present investigation was undertaken to formulate an effective and economic non-antibiotic therapy for sub clinical endometritis in repeat breeding animals. The material for the present study consisted of repeat breeder cows and heifers maintained at University Livestock Farm, Mannuthy and those presented at Artificial insemination centre and Bull station attached to the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy. The study was conducted during the period from July 2006 to February 2007. Repeat breeding cows and heifers were selected and screened for sub clinical endometritis by conducting detailed clinico-gynaecological examination and also by studying the characteristics of oestrual mucus including the white side test. Physical changes of reproductive tract including oedema of vulval lips, hyperemia of vestibular mucous membrane and tonicity of uterus were recorded and graded as low, medium and high. Both right and left ovaries were palpated for the presence of follicle and corpus luteum.

Out of the 576 cross bred cows and heifers presented for insemination, 110 animals (19.01 per cent) were screened as repeat breeders, which included 23.53 per cent and 17.72 per cent heifers and cows respectively. On further detailed investigation of repeat breeders, 15 heifers (46.88 per cent) and 32 cows (46.15 per cent) were found to have sub clinical endometritis, giving an over all occurrence of 46.36 per cent.

Repeat breeding animals with sub clinical endometritis were randomly selected and allotted to groups I to V comprising of 10, 12, 12, 10 and 10 animals respectively and following treatment regimes were undertaken. Group I animals were given 50 ml of one per cent lugol's iodine intrauterine 24 h prior to

insemination. Group II animals were given 50 ml of one per cent lugol's iodine intrauterine 24 h after insemination. In group III, a synthetic PGF_2 alpha analogue (CLOSTENOL) was given intramuscularly on eleventh day of oestrous cycle followed by timed inseminations at 72 and 96 h of injection. Group IV animals were subjected to prostaglandin injections at 11 days interval on the luteal phase of oestrous cycle followed by timed inseminations at 72 and 96 h of injection. Group V animals were artificially inseminated without any treatment, which formed the control group.

In repeat breeders with sub clinical endometritis, physical changes in the reproductive tract such as intensity of vulval oedema, hyperemia of mucous membrane and tonicity of uterus showed almost similar pattern in all treatment groups irrespective of induced and natural oestrum. The percentage of animals with clear and cloudy discharges respectively in each treatment group was 60 and 40 in group I, 58.33 and 41.67 in group II, 66.67 and 33.33 in group III, 50 and 50 in group IV and 70 and 30 in group V. The consistency of mucus samples were scored as watery, thin and thick depending on the nature of discharge. The percentage of animals showing watery, thin and thick discharges in each group respectively was 40, 40 and 20 in group I, 33.33, 50 and 16.67 in group II, 25, 41.67 and 33.33 in group III, 20, 50 and 30 in group IV and 30, 30 and 40 in group V. On conducting the white side test, the samples which turned yellow were taken as positive and they were further classified as slight, moderate and intense depending on the intensity of colour change. The percentage of animals showing slight, moderate and intense reactions respectively in each group was 50, 30 and 20 in group I, 41.67,41.67 and 16.67 in group II, 33.33 each in group III, 20, 30 and 50 in group IV and 30, 50 and 20 in group V. All treatment groups showed almost similar cyclical changes in the ovary including the control group.

All the animals in group III and IV treated with PGF_2 alpha analogue were responded to the treatment showing an efficacy of 100 per cent. Oestrus intensity after induction using clostenol was noted and the percentage of animals showing high, medium and low intensities respectively was 50, 33.33 and 16.67 in group III and 40, 30 and 30 in group IV. The time taken for the induction of oestrum in group III was 59.38 $\pm$ 3.70 h as against 58.88 $\pm$ 2.75 h in group IV. The mean duration of oestrus in groups III and IV were 37.5 $\pm$ 3.70 h and 38.63 $\pm$ 3.65 h respectively. On analysis of data, it was found that there was no significant difference in the oestrus response in both groups after induction

Conception rate was found to be 40, 50, 50, 40 and 20 per cent respectively in groups I, II, III, IV and V. In the present study, conception rate was found to be highest in groups II and III in which post AI lugol's iodine and single regime prostaglandin therapy were undertaken respectively. So it could be inferred that both prostaglandin and lugol's iodine can be effectively used for the treatment of sub clinical endometritis. At the same time it was found that administration of lugol's iodine 24 h prior to insemination and induction of oestrum with double regime prostaglandin were also beneficial in improving the conception rates in repeat breeding cattle with sub clinical endometritis.

The present study revealed that a comparatively less expensive, easily prepared dilute lugol's iodine if used judiciously can help in the treatment of repeat breeding cows with sub clinical endometritis effectively.

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NON-ANTIBIOTIC THERAPY FOR SUB CLINICAL ENDOMETRITIS IN REPEAT BREEDING CATTLE

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ABSTRACT

The present investigation "Non-antibiotic therapy for sub clinical endometritis in repeat breeding cattle" was conducted during the period from July 2006 to February 2007 in cross bred animals brought for artificial insemination. The material for the study consisted of repeat breeder cows and heifers maintained at University Livestock Farm, Mannuthy and those presented at Artificial insemination centre and Bull station attached to the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy. The incidence of repeat breeding, the occurrence of sub clinical endometritis and various aspects of oestrous cycle were studied. Repeat breeding animals with sub clinical endometritis were randomly selected and allotted to groups I to V comprising of 10, 12, 12, 10 and 10 animals respectively and following treatment regimes were undertaken. Group I animals were given 50 ml of one per cent lugol's iodine intrauterine 24 h prior to insemination. Group II animals were given 50 ml of one per cent lugol's iodine intrauterine 24 h after insemination. In group III, a synthetic PGF_2 alpha analogue (CLOSTENOL) was given intramuscularly on eleventh day of oestrous cycle followed by timed inseminations at 72 and 96 h of injection. Group IV animals were subjected to prostaglandin injections at 11 days interval on the luteal phase of oestrous cycle followed by timed inseminations at 72 and 96 h of injection. Group V animals were artificially inseminated without any treatment, which formed the control group.

The over all incidence of repeat breeding was found to be 19.01 per cent, out of which 46.36 per cent showed sub clinical endometritis. The characteristics of oestrual cervical mucus and physical changes in the genital tract were examined in detail and found that almost all treatment groups followed similar pattern irrespective of induced and natural oestrum.

The intensity of oestrum after induction using prostaglandin analogue was found to be high in most of the repeat breeders with sub clinical endometritis. Time taken for induction of oestrus in animals belonging to group III and IV were 59.38 $\pm$2.81 h and 58.88 $\pm$ 2.75 h respectively. The mean duration of oestrum after induction using PGF_2 alpha analogue in group III and IV were 37.5 $\pm$ 3.70 h and 38.63 $\pm$ 3.65 h respectively.

The conception rates of animals in different experimental groups were 40, 50, 50, 40 and 20 per cent in groups I, II, III, IV and V respectively. The conception rate was highest, 50 per cent in groups II and III, in which animals were subjected to post AI lugol's iodine and single regime prostaglandin therapy respectively. However, better conception rates could be obtained in all treatment groups when compared to control group in which the success rate was only 20 per cent. Hence it could be inferred that both lugol's iodine and prostaglandin can be used effectively in the treatment of sub clinical endometritis in repeat breeders. However, treatment with lugol's iodine is comparatively less expensive and it is easily available also, so can be recommended as the drug of choice for tackling cases of repeat breeding with sub clinical endometritis.