THERAPEUTIC MANAGEMENT OF REPEAT BREEDING DUE TO SUB-CLINICAL ENDOMETRITIS IN CATTLE USING HERBAL PLANTS

THESIS

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1. INTRODUCTION

India has about 190.8 million cattle population which includes 151.1 million indigenous and 39.7 million crossbreed and exotic cattle (Livestock Census, 2012).

Economy of dairy farming mainly depends on conception rate after insemination. Reproduction plays crucial role in entire economy of farm because ‘Reproduction is the basis of production. It is true that many bovine genital infections which may be specific or non-specific causes large number of pregnancy failure in cattle (Sirohi et al., 1989). Bacterial infections plays major role among various causes of the sub-fertility (Dholakia et al., 1987). There are various conditions like cervicitis and endometritis of various degree, which may lead to embryonic death and repeat breeding (Elliott et al., 1968).

Postpartum reproductive disorders causes heavy economic losses in dairy sector. The annual postpartum uterine infection ranges from 10-50 per cent in cattle (Lewis, 1997) and 20-75 per cent in buffalo (Usmani et al., 2001). Uterine infections include pyometra, mucometra, endometritis, metritis and tumors. Among all of these, repeat breeding due to endometritis is one of the major gynaecological problem affecting reproductive efficiency and economy of milk production in dairy animals. Repeat breeder cow is one that has normal or nearly normal oestrous cycles with apparently no palpable abnormalities of the reproductive tract, but fail to conceive to three or more AI with good quality semen.

Under normal conditions, the uterine defense mechanism (UDM) prevents invading bacteria from colonizing in uterus but when this mechanism get impaired or weakened, bacteria may colonize in uterus and lead to endometritis. Endometritis alter the physico-chemical properties of cervical mucus. Therefore, examination of cervical mucus for appearance, consistency and pH may be valuable in its diagnosis. The therapeutic approach to
endometritic cow involves either antibiotics and antiseptics or hormonal therapy (Vijayarajan et al., 2007).

The treatment of endometritis with antibiotics have met with varying degree of success, inconsistent recovery rate, high cost of treatment, milk disposal, immerge of microbial resistance and reduced phagocytic activity of polymorphonuclear (PMN) cells.

So, there is an urgent need to find out an alternative therapy for treatment of uterine infections by using natural substances as a means of activation of natural defense mechanism in the uterus. Use of certain plant products as a therapeutic agent has become a subject of recent scientific investigations.

In India, around 2000 medicinal plants have been recorded and more than 500 traditional communities are using about 800 plants for curing different diseases (Kamboj, 2000). Around 80 per cent people in world depend on plant derived medicines because it has no side effects (Verma and Singh, 2008).

Herbal medicine is becoming popular now a days because of heavy toxicity and side effects of Allopathic medicine. Herbal medicine plays crucial role to manage the various reproductive problems in past few years. Many herbal plants like Neem, Tulsi, Garlic, Ashwagandha etc. have been tried for the treatment of endometrities in cattle with varying success rate (Garg et al., 1983).

Neem (Azadirachta indica) has been extensively used in india as traditional ayurvedic medicine for treatment of various diseases (Bandyopadhyay et al., 2002). It has been demonstrated to exhibit immunomodulatory, anti-inflammatory, antifungal, antiviral, antibacterial and antioxidant properties (Subapriya and Nagini, 2005).

Keeping this in view, the present study was planned with following specific objectives:

1) To study the incidence of repeat breeding in cattle.
2) To study diagnostic efficacy of white side test and endometrial cytology in sub-clinical endometritis.

3) To evaluate the therapeutic efficacy of herbal extract for management of sub-clinical endometritis.

2. REVIEW OF LITERATURE

Under normal conditions, the uterine defense mechanism (UDM) prevents invading bacteria from colonizing in uterus. Bovine uterus remains sterile prior to calving. During gestation, the cervix provides protection against intrauterine invasion by pathogens; otherwise the developing foetus is at risk of getting aborted. A variety of organisms enter the uterus at the time of parturition resulting in uterine infection, inflammation and, finally uterine clearance.

2.1 Endometritis

Histologically, endometritis is defined as a disruption of the epithelium with the presence of inflammatory cells (Bondurant, 1999). Sub-clinical endometritis is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis is indicated by the presence of purulent/mucopurulent discharge (Sheldon et al., 2006). Postpartum endometritis has a negative effect on reproductive performance, causing an increase in the number of services per pregnancy and in the length of calving-conception interval (Bell and Roberts, 2007).

2.2 Incidence of repeat breeding

Saxena (2004) reported that the incidence of repeat breeding in cattle varied from 5.5 to 33.33 per cent. He conducted study on 314 animals to find out the incidence in cattle.

Bhat et al. (2012) reported the incidence of repeat breeding as 28.31 per cent in cattle. They conducted study on 1074 cattle, out of which 304 cattle were found to suffer from repeat breeding. They observed that the repeat breeding was due to breed, season, ovulatory disturbances and ovarian cyst.
Mohteshamuddin et al. (2012) analysed total of 1718 cattle in one year to calculate the incidence of repeat breeding. The overall incidence rate of repeat breeding was 6.64 per cent. The highest incidence was recorded at OPD, Veterinary College, Bidar (18.5%), followed by VHDDIC, APMC Hospital, Bidar (11.76%) and KVS Hospital, Bidar (4.76%).

Khan et al. (2016) reported the overall incidence of repeat breeding as 27.33 per cent (21.67% cattle and 33.04% buffalo). They conducted study on 1167 animals, out of which 319 animals were found positive for repeat breeding.

### 2.3 Incidence of endometritis

Lincke et al. (2007) reported the prevalence of sub-clinical endometritis between 16 to 90 per cent with significant decrease in conception rate, prolonged days to first service and number of days open and reduction in number of pregnancy in cattle.

Potter et al. (2010) reported 27 per cent incidence of endometritis in lactating postpartum dairy cattle while Plontzke et al. (2011) reported prevalence of clinical and sub-clinical endometritis as 35 and 38 per cent between 18-38 days postpartum.

LeBlanc et al. (2012) reported the incidence of sub-clinical endometritis as 15 per cent and also clinical endometritis as 15 per cent between 10 to 20 per cent in postpartum dairy cattle.

Pillai (2012) conducted study on twenty seven animals for diagnosis of endometritis. The incidence of sub-clinical and clinical endometritis in crossbred cattle was 29.69 per cent and 12 per cent, respectively.

Singh et al. (2016) reported the incidence of sub-clinical endometritis as 29.4 per cent. They conducted study on 170 repeat breeder crossbred cattle. All the cattle were examined through rectal palpation and cervico-vaginal discharge. They adopted more than or equal to 4 per cent PMN (%) to declare sub-clinical endometritis.
2.4 Physical properties of cervico-vaginal mucus

Tsiligianni et al. (2001) reported that the cervical mucus of cattle that conceived have significantly lower viscosity and significantly higher crystallization in comparison of cattle that did not conceive. Results for pH and spinbarkeit did not differ significantly between the two groups.

Kumar et al. (2007) reported that the mean Hydrogen ion concentration of uterine flushings was significantly (P<0.01) higher in endometritic cattle (7.88±0.11) than in healthy cattle (7.18±0.08).

Sahadev et al. (2007) also reported that the mean pH of uterine flushings of endometritis affected cattle (8.12±0.04) was significantly higher compared to that of healthy cattle (7.34±0.09). A number of workers have found that higher pH towards alkaline side is associated with uterine infection.

Dodamani et al. (2010) studied twenty four Deoni repeat breeder cattle which were randomly allocated into 4 groups of six each and reported that among physical characters of oestrual cervico-vaginal mucus, typical arborization pattern (80.95 % in pregnant vs.55.56 % in non-pregnant cattle) and marginally high spinnbarkeit readings (24.67+2.7cms in pregnant and 22.21+1.32 cms in non-pregnant cattle) favored better fertility, although the differences between the groups were statistically non-significant.

Kumar et al. (2010) reported that the conception rate was found to be 60.52 and 42.85 per cent for thin and thick consistency of cervical mucus. The result revealed that higher conception rate was found in animals showing thin consistency than thick consistency of cervical mucus.

Modi et al. (2011) studied cervical mucus samples from 20 repeat breeder and 10 normal cyclic cattle. Samples were examined for colour (clear or cloudy), consistency (thin or thick), fern pattern (typical, atypical or nil), pH and spinnbarkeit. The observations indicated that cattle with thin and clear discharge, alkaline pH and showing typical fern pattern were normal breeder, whereas, cloudy, thick, acidic cervical mucus with atypical fern pattern were repeat breeder cattle.
Tsilligianni et al. (2011) studied twenty one high producing Holstein cattle aged 3.5 to 5 years, from 2 farms in central Greece. Cervico-vagical mucus samples were collected at the beginning of oestrus. The pH, spinnbarkeit (spinability) and crystallization value were measured. The pH was lower at 8 hrs. (7.00 ± 0.24) in group A compared with group B (7.55 ± 0.12). Crystallization was significantly lower at 4 hrs. (2.00 ± 0.63) and 20 hrs. (1.50 ± 0.82) in group A compared with group B (3.13 ± 0.32) at 4 hrs. and 3.00 ± 0.41 at 20 hrs.). The pH, crystallization, estradiol, and progesterone differed within group.

Kumar et al. (2013) studied twenty four cattle which were selected on the basis of history, breeding records, trans-rectal examination and whiteside test. Cervical mucus samples were tested for appearance, pH, whiteside test and bacterial load. Significant decline in pH and bacterial load was observed in cervical mucus of the groups after treatment.

Zaman et al. (2013) reported that the examination of physical properties of cervical mucus in majority of the repeat breeding cattle showed that the colour was turbid (64.44%), consistency was thick (84.44%), elasticity was elastic (62.22%) and fern pattern was typical (60.00%). The pH value of cervical mucus in repeat breeding (7.95±0.096) cattle was significantly higher than the normal (7.35±0.167) cattle. The conception rate of 90 per cent was recorded in normal cattle and 100 per cent pregnancy rate was observed in clean, thin, elastic and typical fern pattern type of cervical mucus.

Lim et al. (2014) conducted studies on physical properties of oestrus mucus in relation to conception rates in 108 dairy cattle and reported that oestrus mucus of the dairy cattle was transparent in 58.3 per cent, turbid in 31.5 per cent and dirty in 10.2 per cent. It was further observed that the mucus consistency of the dairy cattle was thin in 74.1 per cent and thick in 25.9 per cent. In the pregnant group, 67.3 per cent mucus samples were found transparent, turbid in 23.6 per cent and dirty in 9.1 per cent. The consistency of cervical mucus was found to be thin in 74.1 per cent and thick in 25.9 per cent of dairy cattle. The conception rates of dairy cattle with thin
and thick consistency of cervical mucus were 81.8 per cent and 18.2 per cent, respectively. Pregnancy was associated with consistency of cervical mucus. Dairy cattle with thin consistency of cervical mucus and had clear discharge were pregnant cattle.

2.5 Etiology and predisposing factors of endometritis

After parturition animal get rid of uterine infection by rapid uterine involution by discharging components and natural defense mechanism (Hussain and Daniel, 1992). Many of the wrong practices like inserting hand by owners and quacks for stimulation of milk let down, excess uterine stretching due to dropsy of fetus and fetal membranes, rupture of genital tissue during dystocia and unhygienic managemental conditions during calving period diminishes uterine tone and decreases uterine ability to combat infections (Paisely et al., 1986). Uterine discharge acts as medium for the multiplication of many types of bacteria, which causes toxic puerperal metritis (Drillich et al., 2001) if not expelled at proper time and if it persists for a long time then it causes impaired fertility (Seals et al., 2002).

Due to presence of aerobic bacterial infection in uterus, there is decrease in intrauterine oxygen reductase potential because of either microorganism metabolism or increase on oxygen consumption by polymorphonuclear (PMN) cells, which creates an anaerobic environment in uterus. Severe endometritis and conception failure occurs if A. pyogens present in uterine fluid approximately 21 days postpartum (Dohmen et al., 1995).

2.6 Uterine defense mechanism against endometritis

There are various anatomical barriers like vulvar sphincters, vestibule and cervix which act as barrier between sterile uterus and infection. Cervico-vaginal mucus and cervical rings act as a physical barrier for organisms (Sheldon and Dobson, 2004).

The uterine defense mechanism were maintained in several ways; anatomically, by the columnar epithelium which may be simple or
pseudo-stratified covering to endometrium; immunologically, through the action of polymorphonuclear inflammatory cells and humoral antibodies; chemically, by mucus secretions from endometrial glands (Dhaliwal et al., 2001).

Under normal conditions, there are various anatomical and functional barriers, specific and non specific immune responses which prevent pathogenic microorganisms from colonizing in the uterus (Foldi et al., 2006). The presence of devitalized tissue, degree of uterine contamination, fluid for the growth of bacteria and defense mechanism determine the presence of infection in the uterus (Roberts, 1986 and Noakes et al., 2002).

2.7 Diagnosis of endometritis

Among all the postpartum uterine infections, clinical and subclinical endometritis are the major causes of infertility in dairy cattle, enhancing luteal phases, delaying the postpartum ovarian rebound and reducing pregnancy rates. So, early and efficient diagnosis is necessary for proper treatment to reduce the production losses. Different diagnostic methods commonly used for endometritis are as under:

2.7.1 Per-rectal examination

Rectal examination is the most commonly practiced diagnostic method in which cervical os, uterine horns and ovaries are palpated to know the status of the genital organs. It provides little and limited information for pregnancy failure. A systemic approach will be helpful in providing information about multi factorial disease. Trans-rectal palpation of uterus is not an accurate diagnostic method for diagnosis of all type of endometritis (Oral et al., 2009).

2.7.2 Vaginoscopy

Vaginoscopy is considered as a more accurate method than rectal palpation for diagnosis of uterine infections. Observation of vaginal
discharge are helpful for the diagnosis of clinical endometritis but not for sub-clinical endometritis.

LeBlanc *et al.* (2002) reported that vaginoscopic examination for detection of vaginal and cervical discharge is a very useful tool for diagnosis of endometritis.

**2.7.3 Whiteside test**

Whiteside (1939) first used this test for diagnosis of mastitis. In this test, cervico-vaginal mucus is collected aseptically from suspected animal with the help of syringe and pipette. Then cervico-vaginal mucus is boiled with equal amount of 5 per cent sodium hydroxide solution. This test is based on correlation between the number of polymorphonuclear cells present in the mucus and intensity of yellow colour (Pateria and Rawal, 1990).

**2.7.4 Endometrial cytology**

Various recent research workers have indicated that endometrial biopsy and endometrial cytology in postpartum dairy cattle is very useful and accurate procedures for detecting existence and severity of endometritis (Honparkhe *et al.*, 2014). Cytobrush technique and uterine lavage are best methods for uterine cytology and this method based on percentage of polymorphonuclear cells present in samples obtained.

Many research workers have used different cut off values of polymorphonuclear cells to declare sub-clinical endometritis. Kasimanickam *et al.* (2004) found more than 18 per cent neutrophils at 20-33 days postpartum or more than 10 per cent neutrophils at 34-47 days postpartum in uterine samples are indicative of sub-clinical endometritis whereas, Gilbert *et al.* (2005) found 5 per cent neutrophils at 40 to 60 days postpartum as an indicator of sub-clinical endometritis in cattle. Barlund *et al.* (2008) set a cut off value of 8 per cent at 28-41 days postpartum in cattle while Hammon *et al.* (2006) set a cut off value of 25 per cent neutrophils at 28 days postpartum to declare endometritis.
Ghasemi et al. (2012) found that cytobrush technique was simple and easy to handle, provide adequate uterine cells for uterine cytology and gene expression analysis can be done in a single sampling.

Honparkhe et al. (2014) diagnosed sub-clinical endometritis in buffaloes on the basis of percentage of polymorphonuclear cells (>5%) in uterine cytobrush samples which was later confirmed microbial assay and concluded that the technique is an efficient and early diagnostic method for sub-clinical endometritis.

2.8 Treatment of endometritis

Endometritis is often self-limiting with recovery occurring after subsequent oestrous cycles (Arthur et al., 1989). Under normal conditions, the uterine defense mechanism (UDM) prevents invading bacteria from colonizing in uterus but when this mechanism get impaired or weakened, bacteria may colonize in uterus and lead to endometritis. So an effective treatment is one which reduce the bacterial load and enhance uterine defense mechanism without hamper normal uterine defense mechanism and not cause adulteration of milk or meat for human consumption (Agarwal et al., 2013).

Better conception rates and shorter calving intervals can be achieved by early treatment of endometritis. A wide variety of therapies for endometritis have been used with variable success rate. Herbal medicine is becoming popular now a days because of heavy toxicity and side effects of Allopathic medicine. Herbal medicine plays crucial role to manage the various reproductive problems in past few years. The commonly used intrauterine and systemic therapies are as under

2.8.1 Herbal drugs

Bajaj (2002) studied effect of herbal medication containing Peganum harmala, Aristolochia bactrecta, Camiphora molmol, Rubia cardifolia and Lepadenia reticulate in buffaloes suffering from endometritis. He reported that the treated animals showed better conception rates in post-treatment first cycle as compared to other treatment cycle. Herbal ingredients
administered showed emmenagogue, uterotonic, anti-inflammatory and antibacterial actions histopathologically as well as clinically.

Sarkar et al. (2006) evaluated the efficacy of garlic extract and PGF$_2$α in the treatment of endometritis in cows. After treatment there was a significant reduction in bacterial load, whereas, it was increased in control group. The oestrous CVM turned clear in 70 per cent animals treated with garlic extract The overall conception rate was 50 per cent in treated groups as compared to nil pregnancy in the control.

Rathod et al. (2012) reported that the ethanolic extract of neem bark showed more significant activity at 250 μg concentrations than neem leaves at all the studied concentrations. The study indicated that neem bark was found to possess more significant antibacterial activity than neem leaves and tulsi leaves.

Kumar et al. (2013) studied immunomodulatory and therapeutic efficacy of neem on endometritis in repeat breeding crossbred cows. It was concluded that hydro-alcoholic extract of the neem has a better antibacterial and immunomodulation and can be used as a therapy for endometritis in repeat breeding crossbred cows.

Rahi et al. (2013) conducted studies on the comparative effect of herbal extract of garlic (crude) and ashwagandha (hydro-ethanolic) with antibiotic ciprofloxacin for the treatment of endometritis and repeat breeding condition in cross bred cows. They concluded that garlic + ashwagandha extract was found as the most effective treatment among all treated groups and, thus, can replace conventional antibiotic in future for bacterial endometritis leading to repeat breeding condition in cross bred cows.

2.8.2 Allopathic drugs

Singh et al. (2000) reported the effectiveness of lipopolysaccharide as an intrauterine immunomodulator curing bacterial endometritis in repeat breeder cattle. They concluded that administration of E. coli lipopolysaccharide (LPS) as single infusion in cows with bacterial
endometritis stimulated uterine defense mechanism and cleared the infection within one oestrous cycle, and improve fertility.

Subandrio et al. (2000) conducted their study on function of oyster glycogen in cattle. They reported that 500 mg of oyster glycogen led to marked improvement in the non-specific uterine defense and endometrial histopathological picture of cows with acute and chronic endometritis. They reported an increase in live phagocytosing PMNs in uterine fluid within 6 hrs., the bacterial load was almost cleared within 72 hrs. after treatment and conception rate improved significantly.

Drillich et al. (2005) found that PGF$_2$α is the treatment of choice for endometritis. The increase in oestrogen concentration and decrease in progesterone concentration is associated with follicular growth and luteolysis results in increased resistance of uterus to bacterial infection.

Azawi (2008) suggested that fertility should be improved when antibiotics is administered as a treatment of endometritis. Antibiotics reduce the multiplication of uterine pathogens and maintain its normal activity. It should not inhibit the normal uterine defense mechanism and should be well tolerated and not induce irritation in the endometrium.

Akhtar et al. (2009) observed higher curative and conception rate in cloprostenol treated than that in oestradiol treated animals. They concluded that cloprostenol is more effective than oestradiol for treatment of first degree endometritis in cow.

Singh et al. (2010) reported in their comparative study on different concentrations of Lugol’s iodine in treatment of endometritis and observed that 0.5 per cent Lugol’s iodine was more effective as compared to 0.25 per cent and 0.1 per cent concentration in clearing CVM and increasing conception rates.

Biswal et al. (2014) reported the effect of various immunomodulators (oyster glycogen and levamisol) on acute phase protein (APP) concentration and pregnancy rates in endometritic cattle and observed
decrease in APP following treatment. The overall conception rate was 60 per cent and 50 per cent in respective groups.

Honparkhe et al. (2014) administered proteolytic enzymes as intrauterine and achieved 86.7 per cent cure rate and 60 per cent pregnancy rate in sub-clinical endometritis.

3. MATERIAL AND METHODS

3.1 Locations of work

The present study was carried out at livestock farm (College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur), Adhartal, and at farmer's door in and around Jabalpur.

3.2 Animals used for study

Repeat breeder cattle which have normal or nearly normal oestrous cycles with apparently no palpable abnormalities of the reproductive tract but fails to conceive in three or more AI were selected for the present study.

3.3 General management of dairy cattle

The cattle were stall fed and kept in cemented shed with brick floor under intensive housing system. Seasonally available green fodder with wheat straw and concentrate mixture were fed to all cattle.

Supplement of mineral mixture was also provided. During morning and evening, clean drinking water was provided ad lib. The feeding system to all private farms in Jabalpur area were nearly same.

3.4 Preparation of extract

Neem Bark
Alcoholic extract of neem bark was prepared using soxhlet extraction assembly. Neem bark removed from plant, cleaned properly, dried in hot air oven and thoroughly crushed into powder form mechanically. The powdered neem bark was extracted with absolute alcohol in soxhlet apparatus, heated over the soxhlet extraction heater until the alcohol became dark coloured.

**Neem oil**

For preparation of methanolic fraction the neem oil obtained from local market was subjected to fractionation by mixing equal volume of methanol. This mixture was vigorously shaken for 10 minutes and poured in a separating funnel and allowed to stand for another 10 minutes. The uppermost, methanol miscible fraction was collected and stored till use.

### 3.5 Technical programme

The proposed study was conducted in three phases:

**Phase I** Study the incidence of repeat breeding.

**Phase II** Diagnosis of sub-clinical endometritis.

**Phase III** Therapeutic management of sub-clinical endometritis.

#### 3.5.1 Phase I: Study the incidence of repeat breeding

The incidence of repeat breeding in cattle was determined by using the formula

\[
\text{Incidence of repeat breeding (\%) = \frac{\text{No. of animals not conceived}}{\text{Total No. of animals inseminated}} \times 100}
\]

#### 3.5.2 Phase II: Diagnosis of sub-clinical endometritis

A total no. of 100 repeat breeder cattle (which not conceived after three or more AI) were screened, out of which 30 repeat breeder cattle were selected for the proposed work.

#### 3.5.2.1 Screening of animals
The cows were screened on the basis of history of conception failure since last three or more services, gynaecological examination for normal or apparently normal genitalia, nature of oestrul discharge, asymmetry of uterine horns, palpable ovarian structures and any other apparent anatomical abnormality. Cervico-vaginal mucus (CVM) was collected aseptically by syringe and pipette method (Reddy, 1973) and whiteside test was performed (Pateria and Rawal, 1990). The test was considered positive for endometritis if the colour turned yellow after boiling with 5 per cent sodium hydroxide solution. The samples with no colour reaction were considered negative for endometritis.

3.5.2.2 Experimental design

Out of 100 repeat breeder cattle, 30 cattle were selected for the experiment. They were divided into five groups to study the comparative diagnostic efficiency of sub-clinical endometritis in cattle by endometrial cytology and whiteside test.

3.5.2.3 Physical properties of cervico-vaginal mucus

The physical properties of CVM of each cow was examined immediately after collection for the purpose of-

1. Colour
2. pH
3. Whiteside test
4. Fern pattern

3.5.2.3.1 Colour

Colour of CVM was judged by direct examination of oestrual mucus immediately after collection and classified as per method described by Deo and Roy (1971). The classification consist of:
A) Clear with string
B) Turbid (cloudy in appearance)
C) Dirty colour (yellowish, grey red mucus)

3.5.2.3.2 pH
The pH of CVM was determined immediately after collection of sample using pH strip.

**3.5.2.3 Whiteside Test**

Oestrual CVM was subjected to whiteside test as described by Popov (1969) and if mucus turned yellow colour after boiling with equal volume of 5 per cent sodium hydroxide solution, then it was considered positive for endometritis.

The samples with no colour reaction were considered negative for endometritis.

**3.5.2.3.4 Fern pattern**

Few drops of freshly collected and well mixed cervical mucus were spread uniformly over grease free clean glass slide and air dried. The air dried slide was examined under microscope using low power objective (10x) for crystallization pattern of mucus known as fern pattern. The fern pattern of the observed mucus was grouped into three classes as described by Deo and Roy, (1971).

A. **Typical**: Fern pattern with primary, secondary and tertiary branches.

B. **Atypical**: Fern pattern with primary and secondary branches.

C. **Nil**: Fern pattern with no primary, secondary and tertiary branches.

**3.5.2.4 Endometrial cytology by cytobrush technique**

After proper restraining of the animal, evacuation of rectum through back racking is done. The vulva and perineal region were washed with clean water and sanitizer and after it, disinfected with spirit swab. With the help of an assistant, vulvar lips were pulled apart and the modified cytobrush assembly (Madoz et. al., 2014; Singh, 2014) was introduced in the vagina. The assembly consisted of stainless steel catheter and a stylette attached with cytobrush and covered with chemie. After reaching at external os of cervix, the outer sanitary sheath was perforated and the assembly was introduced into the cervix and then to the body of uterus. When assembly reached at body of uterus, then stylette was advanced to expose the
cytobrush into lumen of body of uterus, where it was gently rotated clock wise and anti clockwise. Gentle pressure was applied on uterine body to obtain cellular material from endometrium. The cytobrush and inner stylette were then retracted back into the outer catheter to its normal position and the whole assembly was withdrawn from the reproductive tract. The threshold cut off values for diagnosis of sub-clinical endometritis by endometrial cytology were more than 18 per cent PMNs between day 20-33 days postpartum and more than 10 per cent PMNs between day 34-47 days postpartum as described by kasimanickan et al. (2004).

Endometrial cytology samples were collected twice from group I to group V before and after treatment.

3.5.2.5 Staining method for endometrial cytology

After removal of assembly from reproductive tract, the cytobrush was rolled over on clean grease free glass slide to make a smear, air dried and transported to laboratory for staining and examination. The slide was stained with modified wright giemsa stain solution for 3 minute and then diluted with equal volume of triple glass distilled water and kept for 5 minute. The slide was then washed with triple glass distilled water and air dried. After drying slides, were screened for the presence of endometrial cells and polymorphonuclear cells (PMNs) or neutrophils. Cells were counted under the microscope at 400x and 1000x (oil immersion) and per cent PMN cells were calculated.

3.5.3 Phase III: Therapeutic management of sub-clinical endometritis

To study the therapeutic efficacy of different herbal plants and fertility response in cattle suffering from sub-clinical endometritis. Repeat breeder cattle (n=30) found positive for sub-clinical endometritis by endometrial cytology were randomly divided into five groups (n=06 per group) and were subjected to different treatment regimen as follows:

1. Treatment group I (n=06)

The cattle were administered intrauterine 25 ml normal saline at 24 hrs. interval for three days as placebo.
2. **Treatment group II (n=06)**

   These cattle were administered intrauterine 25 ml methanol fraction of neem oil at 24 hrs. interval for two days.

3. **Treatment group III (n=06)**

   These cattle were administered intrauterine 25 ml methanol fraction of neem oil at 48 hrs. interval for two days.

4. **Treatment group IV (n=06)**

   These cattle were administered intrauterine 25 ml extract of neem bark at 24 hrs. interval for three days.

5. **Treatment group V (n=06)**

   These cattle were administered intrauterine 25 ml extract of neem bark at 48 hrs. interval for three days.

3.6 **Statistical analysis**

   The results of incidence of repeat breeding, incidence of sub-clinical endometritis, whiteside test, fern pattern and conception rate were expressed in percentage. The data of pH and PMN per cent were analysed statistically by two way analysis of variance (ANOVA) as per the methods described by Snedecor and Cochran (1994). The means were compared using Duncan’s new multiple range test (DNMRT).
4. RESULTS

4.1 Incidence of repeat breeding

The incidence of repeat breeding in cattle was determined by the formula mentioned below.

\[
\text{Incidence of repeat breeding (\%)} = \frac{\text{No. of animals not conceived}}{\text{Total no. of animals inseminated}} \times 100
\]

Incidence of repeat breeding in cattle is presented in Table 01 and depicted in Figure 01. The overall incidence of repeat breeding was 30.47 per cent in total population of five villages, one private dairy farm and Livestock farm (LSF), Adhartal. Highest percentage of repeat breeding was recorded in the village Hinotia (35.08\%); followed by village Kudaria (32.35\%); Yadav dairy farm, Bilpura (31.81\%); Piperiya (30.95\%); LSF, Adhartal (29.33\%); Shalivada (29.16\%) and Jamtara (26.65\%).

Table 01: Incidence of repeat breeding in cattle

<table>
<thead>
<tr>
<th>Place of study</th>
<th>Animals presented for A.I.</th>
<th>No. of animals conceived</th>
<th>Repeat breeder cattle (%)</th>
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The incidence of sub-clinical endometritis was 33 per cent in total population of five villages, one private dairy farm and at LSF, Adhartal. Highest percentage of sub-clinical endometritis was recorded in the village Jamtara (38.46%), followed by village Piperiya (35.00%); Shalivada (33.33%); LSF, Adhartal (31.81%); Hinotia (30.76%); Kudaria (30.00%) and Yadav dairy farm, Bilpura (28.57%).

Table 02: Incidence of sub-clinical endometritis among repeat breeder cattle

<table>
<thead>
<tr>
<th>Place of study</th>
<th>No. of repeat breeder animals</th>
<th>Animals positive for sub-clinical endometritis</th>
<th>Sub-clinical endometritis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSF, Adhartal</td>
<td>22</td>
<td>7</td>
<td>31.81</td>
</tr>
<tr>
<td>Yadav dairy farm, Bilpura</td>
<td>7</td>
<td>2</td>
<td>28.57</td>
</tr>
<tr>
<td>Jamtara</td>
<td>13</td>
<td>5</td>
<td>38.46</td>
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<td>-------------------------</td>
<td>-------</td>
<td>---</td>
<td>------</td>
</tr>
<tr>
<td>Shalivada</td>
<td>15</td>
<td>5</td>
<td>33.33</td>
</tr>
<tr>
<td>Piperiya</td>
<td>20</td>
<td>7</td>
<td>35.00</td>
</tr>
<tr>
<td>Hinotia</td>
<td>13</td>
<td>4</td>
<td>30.76</td>
</tr>
<tr>
<td>Kudaria</td>
<td>10</td>
<td>3</td>
<td>30.00</td>
</tr>
<tr>
<td>Overall incidence</td>
<td>100</td>
<td>33</td>
<td>33.00</td>
</tr>
</tbody>
</table>

4.3 Physical properties of cervico-vaginal mucus

4.3.1 Colour

The colour of cervico-vaginal mucus of cattle is categorized as clear, turbid and dirty. Turbid and dirty colour of CVM is an indication of clinical endometritis, while clear CVM is the indication of healthy uterus or it may have sub-clinical endometritis. In the present study, only clear CVM was considered as a sample for whiteside test, pH and fern pattern.

4.3.2 Fern Pattern

Results of fern pattern before and after treatment are mentioned in table 03 and depicted in figure 03. The results revealed that typical fern pattern was observed in group II was maximum (83.33%) after treatment with neem oil for two days at 24 hrs. interval followed by neem oil administered for two days at 48 hrs. interval and neem bark administered for three days at 24 hrs. interval (66.67%).

The sub-clinical endometritic cows exhibited typical fern pattern as 16.67, 16.67, 0.00, 0.00 and 16.67 per cent, atypical fern pattern as 50.00, 66.67, 83.33, 66.67 and 66.67 per cent, nil fern pattern as 33.33, 16.67, 16.67, 33.33 and 16.67 per cent before treatment in group I, II, III, IV and V, respectively. After treatment the cows exhibited typical fern pattern as 33.33, 83.33, 66.67, 66.67 and 50.00 per cent, atypical fern pattern as 50.00, 16.67, 33.33, 16.67 and 33.33 per cent, nil fern pattern as 16.67, 0.00, 0.00, 16.67 and 16.67 per cent in group I, II, III, IV and V, respectively. Results revealed maximum number of animals exhibiting typical fern pattern in group II after treatment, followed by group III (66.67%), group IV (66.67%), group V (50.00%) and least number of animals exhibited typical fern pattern in group I (33.33%).
Table 03: Fern pattern of CVM (%) in sub-clinical endometritic repeat breeder cattle

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical</td>
<td>Atypical</td>
</tr>
<tr>
<td>Group I</td>
<td>16.67 (1)</td>
<td>50.00 (3)</td>
</tr>
<tr>
<td>Group II</td>
<td>16.67 (1)</td>
<td>66.67 (4)</td>
</tr>
<tr>
<td>Group III</td>
<td>0.00 (0)</td>
<td>83.33 (5)</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.00 (0)</td>
<td>66.67 (4)</td>
</tr>
<tr>
<td>Group V</td>
<td>16.67 (1)</td>
<td>66.67 (4)</td>
</tr>
</tbody>
</table>

Figures in parenthesis are the number of animals

4.3.3 Whiteside test

The selection of the animals for experiment was done on the basis of whiteside test. All the repeat breeder cattle positive for whiteside test, were selected for endometrial cytology.

Whiteside test was performed on CVM collected from the animals of all the groups before and after treatment. All the animals selected for the study were positive to whiteside test. The data presented in table 04 and figure 04 revealed that after treatment, at subsequent oestrus, 83.33 per cent cattle in group I, 0.00 per cent in group II, 33.33 per cent in group III & IV and 50 per cent in group V became positive for whiteside test. The analysis of data reveals that WST was negative in group II as compared to other treatment groups with treatment of neem oil administered at 24 hrs. interval for two days showing better effect of treatment.

Table 04: Whiteside test on CVM in sub-clinical endometritic repeat breeder cattle

<table>
<thead>
<tr>
<th>Groups</th>
<th>Positive before treatment</th>
<th>Positive after treatment</th>
</tr>
</thead>
</table>
4.3.4 pH

The mean pH values of oestral cervical mucus in sub-clinical endometritic repeat breeder cattle before and after treatment are presented in table 05 and depicted in figure 05.

The pH value of CVM before treatment from different groups varied from 7.40±0.05 to 7.67±0.05. The pH values before treatment varied significantly (p<0.05) between different groups. The difference was found non-significant (p>0.05) between treatment groups I and IV, II and III, II and V, III and V whereas, it was significant (p<0.05) between groups I and II, I and III, I and V.

The pH value of CVM after treatment in different groups varied from 7.00±0.05 to 7.50±0.04. The pH value after treatment was significantly decreased in all the groups (p<0.05). However, the post treatment difference was found statistically non-significant (p>0.05) between treatment groups II and III, II and IV, III and IV, whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV, I and V.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>7.67&lt;sup&gt;bp&lt;/sup&gt;±0.05</td>
<td>7.50&lt;sup&gt;aq&lt;/sup&gt;±0.04</td>
</tr>
<tr>
<td>Group II</td>
<td>7.40&lt;sup&gt;ap&lt;/sup&gt;±0.05</td>
<td>7.00&lt;sup&gt;cq&lt;/sup&gt;±0.05</td>
</tr>
<tr>
<td>Group III</td>
<td>7.50&lt;sup&gt;ap&lt;/sup&gt;±0.04</td>
<td>7.10&lt;sup&gt;cq&lt;/sup&gt;±0.04</td>
</tr>
</tbody>
</table>
### 4.4 Endometrial cytology

Polymorphonuclear cell (PMN) percentage in the endometrial cytology samples before and after treatment obtained in different treatment groups of sub-clinical endometritic repeat breeder cattle are presented in table 06, figures 06, 07 and plates 03 to 12.

Percentage of PMN in endometrial samples before treatment from different groups varied from 5.67±0.21 to 7.66±0.19. The PMN percentage before treatment varied significantly (p<0.05) between different groups. The difference was found statistically non-significant (p>0.05) between treatment groups I and V, II and III, whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV.

The PMN percentage in endometrial samples from different treatment groups after treatment varied from 2.61±0.34 to 6.00±0.44. The PMN percentage after treatment varied significantly (p<0.05) between different groups. The difference was found non-significant (p>0.05) between treatment groups II and III, II and V, IV and V, III and IV, whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV, I and V.

Significant decline (p<0.05) in the PMN percentage after treatment was observed in all the groups.

#### Table 06: Endometrial cytology in different groups of sub-clinical endometritic repeat breeder cattle

<table>
<thead>
<tr>
<th>Groups</th>
<th>Endometrial cytology</th>
<th>PMN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endometrial cells</td>
<td>PMN</td>
</tr>
<tr>
<td>Group I</td>
<td>Before treatment</td>
<td>268.50±1.34</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>272.67±1.12</td>
</tr>
</tbody>
</table>

Values with different superscripts (a,b,c) in column and (p,q) in row differ significantly (p<0.05)
4.5 Therapeutic efficacy of herbal extract in terms of conception rate

Fertility response was recorded in terms of conception rate in different treatment groups of sub-clinical endometritic repeat breeder cattle and has been depicted in table 07 and figure 09.

Table 07: Conception rate in different treatment groups of sub-clinical endometritic repeat breeder cattle

<table>
<thead>
<tr>
<th>Groups</th>
<th>I service conception rate</th>
<th>II service conception rate</th>
<th>Overall conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>00(0.00)</td>
<td>02(33.33)</td>
<td>02(33.33)</td>
</tr>
<tr>
<td>Group II</td>
<td>04(66.67)</td>
<td>01(16.67)</td>
<td>05(83.33)</td>
</tr>
<tr>
<td>Group III</td>
<td>02(33.33)</td>
<td>02(33.33)</td>
<td>04(66.67)</td>
</tr>
<tr>
<td>Group IV</td>
<td>01(16.67)</td>
<td>02(33.33)</td>
<td>03(50.00)</td>
</tr>
</tbody>
</table>

Values with different superscripts (a,b,c) between groups in column differ significantly (p<0.05)
Values with different superscripts (p,q) within groups in column differ significantly (p<0.05)
The first service conception rate in groups I, II, III, IV and V were 0.00, 66.67, 33.33, 16.67 and 16.67 per cent, respectively. The overall conception rate was seems to be higher (83.33%) in group II as compared to group III (66.67%), IV (50.00%), V (33.33%) and group I (33.33%).

5. DISCUSSION

The results of the present study are discussed in the light of the literature pertaining to this study.

5.1 Incidence of repeat breeding

In the present study, the overall incidence of repeat breeding in cattle was recorded as 30.47 per cent. However, highest incidence of repeat breeding was recorded in the village Hinotia (35.08%); followed by Kudaria (32.35%); Yadav dairy farm, Bilpura (31.81%); Piperiya (30.95%); LSF, Adhartal (29.33%); Shalivada (29.16%) and Jamtara (26.65%). Similar incidence of repeat breeding was also reported by Bhat et al. (2012) as 28.31 per cent, Gupta and Deopurkar (2005) as 5.5-33.30 per cent, Saxena (2004) as 33.33 per cent and Khan et al. (2016) as 21.67 per cent.
However, higher incidence was reported by Venkatasubramanian and Fulzele, (1996) as 68.75 per cent and lower (4.26%) by Naraladker et al. (1994).

The difference in the incidence of repeat breeding may be due to breed, season, parity, ovulatory disturbance, ovarian cyst and managemental practices.

5.2 Incidence of sub-clinical endometritis

In the present study, the overall incidence of sub-clinical endometritis among repeat breeder cattle was recorded as 33.00 per cent. However, highest incidence of sub-clinical endometritis among repeat breeder cattle was recorded in village Jamtara (38.46%); followed by village Piperiya (35.00%); Shalivada (33.33%); LSF, Adhartal (31.81%); Hinotia (30.76%); Kudaria (30.00%) and Yadav dairy farm, Bilpura (28.57%).

The above findings are in agreement with Pillai (2012), Plontzke et al. (2011), Singh et al. (2016) and Drillich et al. (2005) who recorded incidence of sub-clinical endometritis in cattle as 29.69 per cent, 38.00 per cent, 29.40 per cent and 37.00 per cent, respectively. Bajaj et al. (2016) also reported the incidence of clinical and sub-clinical endometritis as 24.67 and 26.00 per cent in buffalo.

However, higher incidence was reported by Lincke et al. (2007) as 16.00 to 90.00 per cent and lower (15.00%) by LeBlanc et al. (2012).

The difference in the incidence of sub-clinical endometritis may be due to excess uterine stretching due to dropsy of fetus and fetal membranes, rupture of genital tissue during dystocia, manipulation of genitalia by owners and quacks for stimulation of milk let down and unhygienic managemental practices.

5.3 Physical properties of cervico-vaginal mucus

5.3.1 Colour

The colour of cervico-vaginal mucus of cattle is categorized as clear, turbid and dirty. Turbid and dirty colour of CVM is indication of clinical endometritis, while clear CVM is the indication of healthy uterus or it may
have sub-clinical endometritis. In this study, only clear CVM was considered as a sample for whiteside test, determination of pH and fern pattern. The colour of CVM which was taken as a sample are in close accordance with Kasimanickam et al. (2004) who also included clear cervico-vaginal mucus for their study to diagnose sub-clinical endometritis.

5.3.2 Fern pattern

It is well known that absence of crystallization (arborization) pattern in the CVM is an indication of unfavourable uterine environment. The oestrual cervical mucus shows a typical arborization pattern of crystallization (Hafez, 1993). Typical fern pattern is said to be the indicative of ovulatory heat, whereas, in silent or weak oestrus, atypical fern pattern is observed.

In the present study, the sub-clinical endometritic cattle exhibited typical fern pattern as 16.67, 16.67, 0.00, 0.00 and 16.67 per cent, atypical fern pattern as 50.00, 66.67, 83.33, 66.67 and 66.67 per cent, nil fern pattern as 33.33, 16.67, 16.67, 33.33 and 16.67 per cent before treatment in group I, II, III, IV and V, respectively. The above findings are in accordance with the study of Modi et al. (2011) who concluded that the cattle with alkaline pH and showing typical fern pattern were normal breeder, whereas, those with acidic cervical mucus and showing atypical fern pattern were repeat breeder cattle.

In the present study, the cattle exhibited typical fern pattern as 33.33, 83.33, 66.67, 66.67 and 50.00 per cent, atypical fern pattern as 50.00, 16.67, 33.33, 16.67 and 33.33 per cent, nil fern pattern as 16.67, 0.00, 0.00, 16.67 and 16.67 per cent in group I, II, III, IV and V, respectively after treatment. The above findings are in accordance with the study of Rangnekar et al. (2002) who observed that out of 11 fertile oestrus cows, 9 (81.82%), 2 (18.18%) and 0 (0.00%) cows had typical, atypical and nil fern pattern, respectively.

Various research workers reported that the conception rate was higher with typical and atypical fern pattern, however, the conception rate was nil when the fern pattern was missing (Chhatry, 1998 and Nzar, 2004). The
results of present study are agreement with the above findings as conception rate was higher (83.33%) with higher typical fern pattern (83.33%) in group II.

Majority of fertile oestrus were with typical fern pattern of cervical mucus, while majority of non-fertile oestrus were associated with atypical fern pattern of cervical mucus. Better conception rate in cattle with typical fern pattern were observed, therefore, atypical/nil fern pattern signifies unfavourable uterine environment which leads to blocking/destruction of the sperm in the reproductive tract, thereby preventing fertilization process.

5.3.3 Whiteside test

All the repeat breeder cattle included in the present study were positive for whiteside test before treatment. After treatment, in group II (neem oil at 24 hrs. interval for two days), at subsequent oestrus, five (83.33%) cattle were declared positive for WST. None (0.00%) of the cattle were declared positive for WST in group II which indicated that administration of neem oil at 24 hrs. interval for two days was most effective treatment for sub-clinical endometritis in repeat breeder cattle.

In group III, IV and V at subsequent oestrus, 33.33, 33.33 and 50.00 per cent cattle were declared positive to WST in each group which also proves the efficacy of herbal drugs to cure the sub-clinical endometritis.

Above findings are in close accordance with Bhat et al. (2014) who reported that WST indicated positive correlation with bacterial culture. Positive reaction to WST could be explained on the basis of number of leukocytes present in uterine discharge. Gupta et al. (2011) stated that the RNA present inside the nucleus of WBC reacts with 5 per cent NaOH to produce the colour reaction. The normal discharge has less number of leukocytes to cause any change of colour, whereas, in sub-clinical endometritis, discharge contains increased number of leukocytes causing a colour reaction (Pateria and Rawal, 1990).

The absence of colour development to WST in higher number of cattle treated with herbal extract revealed their efficacy for combating infection. This result showed that neem oil and neem bark are
immunomodulatory and antibacterial in nature as they reduce bacterial load and subsequently inflammation process as reported earlier (Rahi et al., 2013; Kumar et al., 2013).

The conception rate in the present study was observed to be highest in group II (83.33 %) where the results of positive case of WST were 0.00 per cent and 33.33 per cent conception rate was observed in group I and V where the results of positive case of WST were 83.33 per cent and 50.00 per cent, respectively. This implies that the conception rate was higher when the results of WST were on negative side, this may be due to the reason that negative WST is indicative of reduced bacterial load which makes uterine environment favourable for conception.

5.3.4 pH

In the present study, the pH of oestrual cervical mucus was alkaline in all the groups before treatment (more than 7.4) indicating infection (Singh et al., 2004). The pH value of CVM from different treatment groups varied from 7.40±0.05 to 7.67±0.05 before treatment and from 7.00±0.05 to 7.50±0.04 after treatment. There was significant decline (p<0.05) in pH value after treatment in all the groups. A number of workers reported that higher pH towards alkaline side is associated with uterine infection (Gupta et al., 1989, Singla et al., 2004). This increase in pH may be caused due to metabolites of bacteria and inflammatory exudates in oestrual cervical mucus (Salphale et al., 1993).

In the present study, significant decline (p<0.05) in pH value after treatment was observed in all the groups. A significant decrease (p<0.05) in pH value of group II (7.00±0.05), group III (7.10±0.04), group IV (7.10±0.04) and group V (7.30±0.04) was observed as compare to group I (7.50±0.04). Similarly a significant decrease (p<0.05) in pH of group II (7.00±0.05), group III (7.10±0.04) and group IV (7.10±0.04) was observed as compared to group V (7.30±0.04). This reduction in pH may be due to decline in bacterial load and inflammatory process in uterus after treatment.

In the present study, the pH value of CVM was declined maximum in group II (neem oil at 24 hrs. interval for two days) after treatment as compared to other groups and also the conception rate was highest in
group II as compared to other groups. Thus, we can conclude that the reduced pH value towards neutral side is directly proportional to reduced bacterial load and because of this reason, the conception rate in this group was higher as compared to other groups.

Similarly, the pH value of CVM in group V was not considerably declined after treatment and thus the bacterial load was on higher side as compared to the other groups after treatment. Due to this reason, the conception rate was lower in group V as compared to other groups. Similar is the case in group I (control), where the pH value of CVM before and after treatment does not show considerable differences and thus the conception rate was lower.

The present study also revealed higher alkaline pH of CVM of repeat breeder cattle suffering from sub-clinical endometritis which is in accordance with Sahadev et al. (2007) who reported that the mean pH (8.12±0.04) of uterine flushings of endometritic cattle was significantly higher (7.34±0.09) as compared to that of healthy cattle. A number of workers have found that higher pH towards alkaline side is associated with uterine infection.

Kumar et al. (2007) reported that the mean Hydrogen ion concentration of uterine flushings was significantly (P<0.01) higher in endometritic cattle (7.88±0.11) than in healthy cattle (7.18±0.08).

5.3.5 Endometrial cytology by cytobrush technique

Polymorphonuclear cells (PMN) are the predominant inflammatory cells found in intrauterine fluid accumulations and the number of PMN in uterus determines the reproductive performance in postpartum cattle (Kasimanickam et al., 2005)

In the present study, the PMN per cent in endometrial samples from different groups varied from 5.67±0.21 to 7.66±0.19 before treatment. The PMN per cent before treatment varied significantly (p<0.05) between different groups. The difference was found non-significant (p>0.05) between treatment groups I and V, II and III, whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV. The cut off value of PMN per cent
before treatment in the present study are in accordance with Madoz et al. (2014) who reported PMN per cent more than or equal to 4 per cent in 48 days or more than 48 days postpartum cattle to declare sub-clinical endometritis.

The PMN per cent in endometrial samples from different groups varied from 2.61±0.34 to 6.00±0.44 after treatment and varied significantly (p<0.05) between different groups. The difference was found non-significant (p>0.05) between treatment groups II and III, II and V, IV and V, III and IV whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV, I and V. Significant decline (p<0.05) in PMN per cent after treatment was observed in all the groups. Moreover, literature is scant to compare the results of PMN per cent after treatment at subsequent oestrous cycle.

In the present study, the lowest PMN per cent was observed in group II (2.61±0.34) as compared to other groups after treatment. The lesser PMN per cent indicates reduced bacterial load which makes the uterine environment favourable for conception. Thus, the highest conception rate was observed in group II (83.33%) as compared to other groups. Group I (control) recorded highest PMN per cent (6.00±0.44) as compared to other groups after treatment, so the bacterial load was also high. Due to high bacterial load, the uterine environment was unfavourable for conception and because of this; the conception rate was lowest in group I (33.33%) as compared to other groups. Similar findings were also observed in group V.

Kumar et al. (2013) reported that there was significant rise in PMN when uterine flushings were collected after 24 hrs. of last treatment because herbal drugs stimulate uterine defense mechanism due to their immunomodulatory action. Neutrophils are known to play a primary role in the defense of the uterus against infection. Influx of neutrophils into the uterus is thought to be mediated by chemoattractants, chemokines and adhesion molecules, such as β2-integrin and I-selectin (Tizard, 2000). The increased uterine population and oxidative burst activity of neutrophils favours the spontaneous resolution of uterine infection (Mateus et al., 2002).

**5.3.6 Therapeutic efficacy of herbal extract in terms of conception rate**
In the present study, the first service conception rate was found to be highest (66.67%) in group II followed by III (33.33%), IV and V (16.67%) groups. Overall conception rate was higher (83.33%) in treatment group II as compared to treatment group III, IV (50.00%), I and V (33.33%).

In the present study, the overall conception (83.33%) rate with the use of neem oil intrauterine in group II administered at 24 hrs. interval for two days, followed by group III (66.67%) administered at 48 hrs. interval for two days. The above findings are in accordance with the study of Kumar et al. (2013) who reported 71.24 per cent conception rate with the administration of neem oil, intrauterine twice at 48 hrs. interval. However, the conception rate in present study was higher (83.33%) in group II as compared to the findings of Kumar et al. (2013). The higher conception rate in group II may be due to the reason that the interval of administration of neem oil in group II was reduced to 24 hrs. from 48 hrs.

In the present study, the overall conception rate with the use of neem bark extract intrauterine was higher (50.00%) in group IV administered at 24 hrs. interval for three days, followed by group V (33.33%) administered at 48 hrs. interval for three days. The conception rate observed in the present study was lower as compared to the findings of Kumar et al. (2013) who reported 75 per cent conception rate with the administration of neem bark extract intrauterine for seven days at 24 hrs. Interval. The lower conception rate in the present study may be attributed to lesser duration of treatment (3 days) as compared to the study of Kumar et al. (2013), where the duration of treatment was seven days.

6. SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

Postpartum reproductive disorders cause heavy economic losses in dairy sector. The annual postpartum uterine infection ranges from 10-50 per cent in cattle and 20-75 per cent in buffaloes. Uterine infections include pyometra, mucometra, endometritis, metritis and tumors. Among all of these, repeat breeding due to endometritis is one of the major gynaecological
problem affecting reproductive efficiency and economy of milk production in dairy animals. Endometritis in breeding cattle mostly occurs during the postpartum period which reduces fertility by extending the calving to conception interval and increasing the number of services per pregnancy. Under normal conditions, the uterine defense mechanism (UDM) prevents invading bacteria from colonizing in uterus but when this mechanism get impaired or weakened, bacteria may colonize in uterus and lead to endometritis. Endometritis alter the physico-chemical properties of cervical mucus. Therefore, examination of cervical mucus for appearance, consistency and pH may be valuable in its diagnosis.

The objectives of present study were: to study the incidence of repeat breeding in cattle; to study diagnostic efficacy of white side test and endometrial cytology in sub-clinical endometritis and to evaluate the therapeutic efficacy of herbal extract for management of sub-clinical endometritis.

A total of 100 repeat breeder cattle belonging to Livestock Farm, Adhartal, Jabalpur and different villages in and around Jabalpur were screened, out of which 30 cattle were selected for the study on the basis of history, gynaecological examination, nature of oestrual discharge and positive reaction to white side test. The cattle (n=30) positive for sub-clinical endometritis were divided in to five groups, having 6 animals in each group. For the examination of physical parameters of cervico-vaginal mucus, the samples were collected by aspiration through recto-vaginal technique at the time of oestrus from all the animals. The physical property of cervico-vaginal mucus of each cattle was examined immediately after collection for colour, pH, white side test and fern pattern.

The overall incidence of repeat breeding was 30.47 per cent. Highest incidence of repeat breeding was recorded in village Hinotia (35.08%), followed by village Kudaria (32.35%; Yadav dairy farm, Bilpura (31.81%); Piperiya (30.95%); LSF, Adhartal (29.33%); Shalivada (29.16%) and Jamtara (26.65%).
The overall incidence of sub-clinical endometritis among repeat breeder cattle was 33 per cent. Highest incidence of sub-clinical endometritis was recorded in village Jamtara (38.46%), followed by village Piperiya (35.00%); Shalivada (33.33%); LSF, Adhartal (31.81%); Hinotia (30.76%); Kudaria (30.00%) and Yadav dairy farm, Bilpura (28.57%).

The physical properties of cervical mucus observed were colour, pH, whiteside test and fern pattern in all groups before and after treatment. Only clear CVM was considered as a sample for observation of whiteside test, pH and fern pattern. The sub-clinical endometritic cows exhibited typical fern pattern as 16.67, 16.67, 0.00, 0.00 and 16.67 per cent, atypical fern pattern as 50.00, 66.67, 83.33, 66.67 and 66.67 per cent, nil fern pattern as 33.33, 16.67, 16.67, 33.33 and 16.67 per cent before treatment in group I, II, III, IV and V, respectively. After treatment the cows exhibited typical fern pattern as 33.33, 83.33, 66.67, 66.67 and 50.00 per cent, atypical fern pattern as 50.00, 16.67, 33.33, 16.67 and 33.33 per cent, nil fern pattern as 16.67, 0.00, 0.00, 16.67 and 16.67 per cent in group I, II, III, IV and V, respectively. All cattle were positive to white side test before the treatment but after treatment, 83.33 per cent cattle in group I, 0.00 per cent in group II, 33.33 per cent in group III, IV and 50 per cent in group V remained positive for whiteside test.

The pH value of CVM before treatment from different groups varied from 7.40±0.05 to 7.67±0.05. The pH value before treatment varied significantly (p<0.05) between different groups. The difference was found non-significant (p>0.05) between treatment groups I and V, II and III, II and V, III and V whereas, it was significant (p<0.05) between groups I and II, I and III, I and V. The pH value of CVM after treatment from different groups varied from 7.00±0.05 to 7.50±0.04. The pH value after treatment varied significantly (p<0.05) between different groups. The difference was found non-significant (p>0.05) between treatment groups II and III, II and V, III and IV whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV, I and V. Significant decline (p<0.05) in pH value after treatment was observed in all the groups.
The PMN per cent before treatment in endometrial samples from different groups varied from 5.67±0.21 to 7.66±0.19. The PMN per cent varied significantly (p<0.05) between groups before treatment. The difference was non-significant (p>0.05) between treatment groups I and V, II and III, whereas, it was significant (p<0.05) between groups I and II, I and III, I and V. The per cent PMN in after treatment endometrial samples from different groups varied from 2.61±0.34 to 6.00±0.44. The after treatment PMN per cent varied significantly (p<0.05) between groups. The difference was non-significant (p>0.05) between treatment groups II and III, II and V, IV and V, III and IV whereas, it was significant (p<0.05) between groups I and II, I and III, I and IV, I and V. Significant decline (p<0.05) in PMN per cent after treatment was observed in all the treatment groups.

The first service conception rate in groups I, II, III, IV and V were 0.00, 66.67, 33.33, 16.67 and 16.67 per cent, respectively. The overall conception rate was higher (83.33%) in treatment group II as compared to treatment group I (33.33%), III (66.67%), IV (50.00%) and V (33.33%).

6.2 Conclusion

1. Incidence of repeat breeding and sub-clinical endometritis among repeat breeder cattle were 30.47 and 33.00 per cent, respectively.

2. Endometrial cytology by cytobrush technique is found to be reliable and effective diagnostic technique for diagnosis of sub-clinical endometritis and proved to be better than whiteside test.
3. The therapeutic efficacy of methanol fractionated neem oil appeared superior over the neem bark in sub-clinical endometritic cattle.

6.3 Suggestions for further work

1. Studies on biochemical parameter of uterine fluid in endometritic cows, viz., levels of different amino acids, trace minerals, enzymes and TLC values may prove useful.
2. Endometrial cytological samples should be collected at 12 hrs. and 24 hrs. after treatment to evaluate the immunomodulatory effect of herbal drugs.

3. Combination of different types of herbal drugs should be tried to evaluate their therapeutic efficacy in sub-clinical endometritis.

4. Difference in spontaneously recovering cattle verses sub-clinical endometritic cattle with respect to the immunity status may be studied.

7. REFERENCES


Livestock Census (2012). Department of Animal Husbandry, Dairy and Fisheries, Ministry of Agriculture, Government of India. 19th livestock census online http://www.dahd.nic.in


