COMPARATIVE STUDY OF DIFFERENT ANTIBIOTICS AND POLYHERBAL INTRAUTERINE THERAPY IN CATTLE WITH ENDOMETRITIS

एंडोमेट्रिटिस के साथ गायों में विभिन्न एंटीबायोटिक दवाओं और पॉलीहेर्बल अंतर्गर्भाशयी थेरेपी का तुलनात्मक अध्ययन

SATISH KUMAR
B.V.Sc. & A.H.

THESIS

MASTER OF VETERINARY SCIENCE
(Veterinary Gynaecology and Obstetrics)

।पशुधनं नित्यं सर्वलोकोपकारकम्।

2018

Department of Veterinary Gynaecology and Obstetrics
College of Veterinary and Animal Science, Bikaner
Rajasthan University of Veterinary and Animal Sciences
Bikaner-334001

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THESIS

Submitted to the

Rajasthan University of Veterinary and Animal Sciences, Bikaner

In partial fulfilment of the requirements for the degree of

Master of Veterinary Science

FACULTY OF VETERINARY & ANIMAL SCIENCE
(Veterinary Gynaecology and Obstetrics)

By

Satish Kumar

2018

Rajasthan University of Veterinary and Animal Sciences, Bikaner

College of Veterinary and Animal Science, Bikaner

CERTIFICATE – I

Date…….

This is to certify that **Mr. Satish Kumar** has successfully completed the comprehensive examination held on ……. as required under the regulation for Master's degree**.**

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CERTIFICATE – II

Date……..…

This is to certify that the thesis entitled **"Comparative study of different antibiotics and polyherbal intrauterine therapy in cattle with endometritis"** submitted for the degree of Master of Veterinary Science in the subject of **Veterinary Gynaecology and Obstetrics** embodies bona fide research work carried out by **Mr. Satish Kumar** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the Advisory Committee on ……..

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This is to certify that the thesis entitled **"Comparative study of different antibiotics and polyherbal intrauterine therapy in cattle with endometritis"** submitted by **Mr. Satish Kumar** to Rajasthan University of Veterinary and Animal Sciences, Bikaner, in partial fulfilment of requirements for the degree of **Master of Veterinary Science** in the subject of **Veterinary Gynaecology and Obstetrics** after recommendation by the external examiner was defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory, we, therefore recommend that the thesis be approved.

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This is to certify that **Mr. Satish Kumar** of the **Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Bikaner** has made all corrections/modifications in the thesis entitled "Comparative study of different antibiotics and polyherbal intrauterine therapy in cattle with endometritis" which were suggested by the external examiner and the advisory committee in the oral examination held on…….. The final copies of the thesis duly bound and corrected were submitted on ……, are enclosed herewith for approval.

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one original and two copies of bound thesis. Forwarded to the Dean, Post Graduate Studies, RAJUVAS, Bikaner through the Dean, College of Veterinary and Animal Science, Bikaner

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(Satish Kumar)

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LIST OF ABBREVIATIONS

Abb.	EXPANDED FORM
AI	Artificial insemination
BCS	Body Condition Score
CVAS	College of Veterinary and Animal Science
D	Days
DIM	Day in milk
DLC	Differential leukocyte count
E	Eosinophils
EC	Endometrial cytology
etc.	Et cetera
Hb	Hemoglobin
h	Hour
IU	Intrauterine
ml	Milliliter
M	Monocytes
N	Neutrophils
ng	Nanogram
PI	Povidone iodine
PMNs	Polymorphonuclear cells
RAJUVAS	Rajasthan University of Veterinary and Animal Sciences
SE	Subclinical endometritis
TEC	Total erythrocyte count
TLC	Total leukocyte count
Viz.	Videlicet, namely
℃	degrees Celsius
%	Per cent
≥	Greater than or equal to
±	Plus minus
>	Greater than
<	Lesser than
/	Per
@	At the rate of

1. INTRODUCTION

Uterine infection is a major problem in reproductive management. A wide variety of genital tract diseases of female domestic animals are known to produce significant losses and responsible for poor fertility. However, cattle with 75% exotic blood have lower uterine immunity than natives and hence higher incidence (18-25%) of endometritis (Nanda and Singh 2008; Singh *et al.,* 2009a) that accounts for one of the major causes of repeat breeding syndrome. The endometritis may be subclinical (an inflammation of the endometrium in the absence of clinical signs) and clinical endometritis (the presence of a purulent/mucopurulent uterine discharge). One of the major key for excellent fertility in dairy herds is a healthy uterine environment that facilitates implantation and development of the embryo (Kaufmann *et al.*, 2009). The conception rates are lower in cattle with endometritis that result prolonged calving to conception interval and there are 3% more animal culled because of failure to conceive (Sheldon *et al.,* 2009).

The risk factors for endometritis include summer calving, parturient and postpartum complications such as retained placenta and metabolic disorders, (Gautam *et al*., 2010; Islam *et al*., 2014). Primiparous cows had 4.02 times higher frequency of clinical endometritis compared to second parity cows (Aghamiri *et al*., 2014). High plasma non-esterified fatty acids and high postpartum beta hydroxyl butyrate increase the risk for endometritis (Giuliodori *et al*., 2013). The rearing systems have a profound effect on the development of endometritis (Chunjie *et al*., 2013).

Subclinical endometritis (SE) is the most prevalent of all uterine diseases; it affects approximately 30% of lactating dairy cows, with prevalence ranging from 11 to >70% in some herds (Barlund *et al*., 2008; Galvao *et al*., 2009a; Gilbert *et al*., 2005; Hammon *et al*., 2006; Kasimanicham *et al*., 2004). The prevalence of subclinical endometritis

in repeat breeder cows was 12.7% (Pothmann *et al*., 2015) to 40.2% (Janowski *et al*., 2013).

Diagnosing endometritis at the earliest often reduces the chances of rising complications due to delayed interventions in affected animals. Routine methods for diagnosing endometritis involve uterine biopsies, lavage and swabs but these may cause irritation and distortion of cells. An inconsistent success following conventional therapies is achieved due to lack of diagnostic standards (Kasimanickam *et al.,* 2005a). Therefore, most of the recent studies have been focused on sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. A novel approach for uterine cytological examination is cytobrush technique which is considered a consistent and reliable method in dairy animals (Barlund *et al.,* 2008; Honparkhe *et al.,* 2014).

Following diagnostic accuracy, one has to use either appropriate anti- bioticthерapy or any other alternative therapy. The systemic and local antibiotic (intrauterine) therapies have been tried to combat with uterine infections in dairy animals (Kasimanickam *et al.,* 2005b; Kutty, 2005; Sandhu, 2006; Singh *et al.,* 2009b; Sharma *et al*., 2014) but that often requires compulsory milk disposal and frequent administration. Apart from high cost of the antibiotic therapy, it also results into development of microbial resistance and decrease in phagocytic activity of polymorphonuclear cells (PMNs) (Chastant-maillard, 2006).

Herbal plants have been used as a source of valuable medication in virtually all cultures worldwide due to presence of important antimicrobial principles, immunomodulatory activities, maintenance of general health, precious therapeutic properties and healing potentials; thus ensure prevention and cure for several diseases and disorders of humans and animals (Baquar, 1995; Rios and Receo, 2005; Mahima *et al*., 2012; Rahal *et al*., 2014)

Relationship between plants and animals has been time immemorial. They together flourished with the help and assistance of one another. This relationship was analyzed finely after the evolution of human civilization to a greater extent (Pieroni, 2010). The Rig-Veda describes a lot regarding the close association of human beings with methods, practices and beliefs about the care of their animals and to keep them healthy, which are acquired through practice (Tayang *et al*., 2007). Since centuries before the introduction of western and allopathic medicine, all livestock keepers relied on these traditional practices. The use of medicine in the treatment of disease as well as for increasing the productivity and reproductive potential of high yielding cattle has generated renewed interest in recent times as herbal preparations are increasingly being used in cattle health care practices (Rigat *et al*., 2009). It is significant to note that more than 90% of drugs used in traditional medicines practiced in India come from plants and 70% of modern medicines originate from natural resources and plants (Mukerjee and Wahile, 2006).

The present study was thus carried out with the following objectives.

I. To investigate the incidence of subclinical endometritis in repeat breeder cattle by cytobrush technique.
II. To study the comparative efficacy between different antibiotics and herbal intrauterine therapy in cattle with subclinical endometritis.

2. REVIEW OF LITERATURE

The reviews of literature of the present study are represented under the following sub-headings.

2.1. Incidence and diagnosis of subclinical endometritis

2.2. Hematological parameters

2.3. Conception rate

2.1. Incidence and diagnosis of subclinical endometritis

Kasimanickam *et al*. (2004) conducted a study to validate the use of endometrial cytology (EC) and ultrasonography (USG) to diagnose SE in clinically normal postpartum dairy cows, and to measure the impact of SE on reproductive performance. Holstein cows from two dairy farms were examined at Visit 1 (V1) at 20–33 days in milk (DIM), and clinically normal cows (n=228), based on the absence of abnormal discharge on external inspection and vaginoscopy, were selected. The reproductive tract of selected cows was evaluated by transrectal palpation, USG and EC. All cows in the study were re-examined at Visit 2 (V2) at 34–47 DIM (2 weeks after V1) and were subsequently followed for a minimum of 8 months (until pregnant or culled). Survival analysis was used to derive a case definition of SE, based on factors associated with decreased relative pregnancy rate. Positive EC at V1 (>18% polymorphonuclear leukocytes; PMN) or fluid in uterus at V1 (FIU1) were associated with a significant reduction in the relative pregnancy rate and identified cows with SE. Similarly, a positive EC (>10% PMN) at V2 or fluid in the uterus at V2 (FIU2), identified cows with SE. Cows with SE at V1 and at V2 had a relative pregnancy rate of 41% and 51% (hazard ratio for pregnancy of 0.59 and 0.49), respectively, compared to cows without SE. Given EC or US findings, no diagnostic criteria based on transrectal palpation of the uterus had predictive value for risk of pregnancy and concluded that

SE, diagnosed by EC or USG, was associated with reduced relative pregnancy rate.

Gilbert *et al*. (2005) examined five commercial dairy herds (Hostein cows, n=141) in central New York for endometritis by examination of endometrial aspirates for presence of inflammatory cells, principally neutrophils, using endometrial cytology at 40-60 days postpartum. The prevalence of cytologically-diagnosed endometritis was 53%; within herds the prevalence varied from 37 to 74% (P=0.02).

Lincke *et al*. (2007) reviewed the publications of SE in dairy cattle and its effect on fertility and stated that diagnosis of SE can be performed by ultrasonography or cytological examination of the uterus. The cytological examination is based on uterine lavage or the cytobrush-method. Prevalence of SE in different studies ranges from 16 to 90 % and depends on the diagnostic method and the time postpartum when the examination is performed. Affected cows showed significantly decreased conception rates, prolonged days to first service and days open as well as a reduced number of cows pregnant.

Barlund *et al*. (2008) examined eight commercial dairy herds of Holstein cows (n=221) for endometritis between 28 and 41 days postpartum using 5 diagnostic techniques: (1) vaginoscopy; (2) ultrasonographic assessment of uterine fluid volume; (3) ultrasonographic assessment of endometrial thickness; (4) EC collected by cytobrush; and (5) EC collected by uterine lavage. They observed that 4th technique was reliable amongst above 5 for diagnosis of endometritis. The risk of non-pregnancy at 150 days was 1.9 times higher in cows with more than 8% PMNs identified using cytobrush cytology than in cows with less than 8% PMNs (P=0.04). Twenty-one cows of 189 cows (11.1%) had >8% PMNs and were considered to be positive for endometritis. Cows with endometritis had a 17.9% lower first service conception rate (P=0.03) and a 24-day increase in median days open (P=0.04).

Galvão *et al*. (2009) conducted a study to determine the effects of $PGF_{2\alpha}$ treatment on the prevalence of SE and fertility of dairy cows. A total of 406 Holstein cows (167 primiparous and 239 multiparous) from 5 herds were used. Uterine lavage for diagnosis of SE, $PGF_{2\alpha}$ treatment, evaluation of body condition scores (BCS), and collection of blood samples for estrous cyclicity determination were performed at 21, 35, and 49 DIM. PMNs were quantified and thresholds for diagnosing SE were selected by receiver operating characteristics analysis. Cows classified as having SE at 35 DIM (>or=6.5% PMN) and 49 DIM (>or=4.0% PMN) had increased time to pregnancy; however, cows classified as having SE at 21 DIM (>or=8.5% PMN) did not. Median days to pregnancy were delayed by 30 (151 vs. 121 D) and 40 (169 vs. 129) D for cows classified as having SE at 35 and 49 DIM, respectively. Treatment with PGF (2alpha) did not affect the prevalence of SE either at 35 (37.9 vs. 38.4%) or at 49 DIM (34.0 vs. 40.4%). Treatment with $PGF_{2\alpha}$ did not affect time to first insemination (AI; median 76 DIM for cows treated with $PGF_{2\alpha}$; 79 DIM for control. Nonetheless $PGF_{2\alpha}$ treatment increased pregnancy to first AI in all the cows (35.5 vs. 24.1%) and hazard ratio (HR) of pregnancy in cows with BCS <or=2.5 when all of the cows were evaluated (HR = 1.5; 95% confidence interval; CI = 1.1 to 2.0) and when only cows without SE were evaluated (HR = 1.8; 95% CI = 1.2 to 2.7).

Kaufmann *et al*. (2009) conducted a study to investigate the prevalence of SE 4 hours after AI and its effect on first service conception rate (FSCR) in dairy cows. A total of 201 Holstein-Friesian cows with no signs of clinical endometritis were examined 4 hours after first AI for signs of SE. Endometrial samples were collected from the uterus using the cytobrush technique. The proportion of PMNs in the cytological sample was used to characterize an inflammation of the endometrium. Cows were categorized into three groups according to the proportion of PMNs in the sample. Cows with 0% PMN (n=115) were assigned to group Zero, cows with >0-15% PMNs (n=59) to group

Medium, and cows with >15% PMNs (n=27) to group High. Pregnancy diagnosis was performed between days 38-44 after AI by palpation of the uterus and its contents per rectum. The FSCR was significantly higher in group Medium than in groups Zero and High (57.6% vs. 39.1% and 29.6%).

Dubuc *et al*. (2010) conducted an observational study to determine and compare diagnostic criteria for postpartum endometritis in dairy cows. Data generated from 1,044 Holstein cows (6 herds) enrolled in a randomized clinical trial were used. Cows were examined for endometritis at 35±3 D (exam 1) and 56±3 D (exam 2) after parturition, using EC (cytobrush technique), vaginal discharge scoring (Metricheck device; Simcrotech, Hamilton, New Zealand), and cervical diameter measurement (transrectal palpation). Reproductive data were recorded until 200 D after parturition. Diagnostic criteria for cytological and clinical endometritis were determined based on detrimental effect on subsequent reproductive performance, using logistic regression and Cox proportional hazard models accounting for the effect of herd clustering. Comparison of diagnostic criteria was performed using EC as reference test or by quantifying the agreement between diagnostic approaches. At exam. 1, diagnostic criteria were ≥6% PMNs and mucopurulent or worse (purulent or foul) vaginal discharge for cytological and clinical endometritis, respectively. At exam. 2, diagnostic criteria were ≥4% PMNs and mucopurulent or worse vaginal discharge for cytological and clinical endometritis, respectively. Cows were classified as having cytological endometritis only, clinical endometritis only, or both cytological and clinical endometritis. Prevalence at exam 1 was 13.5, 9.4, and 5.8% for cytological endometritis only, clinical endometritis only, and both cytological and clinical endometritis, respectively. The detrimental effects of cytological and clinical endometritis on reproductive performance were additive. Among cows with clinical endometritis, only 38 and 36% had

cytological endometritis at exam 1 and exam 2, respectively. Combination of diagnostic criteria improved neither the accuracy for predicting cytological endometritis nor the agreement between cytological and clinical endometritis. Overall, these results suggested that cytological and clinical endometritis may represent different manifestations of reproductive tract disease. They also suggested that use of the terminology clinical endometritis may not be accurate and that purulent vaginal discharge may be more descriptive.

Plöntzke *et al*. (2010) conducted a study to determine the prevalence of SE and its impact on reproductive performance outcomes in clinically healthy postpartum dairy cows in a pasture-based extensive dairy farming system in Argentina. Lactating Holstein cows (n=201) at 18-38 days postpartum from three commercial dairy farms in Buenos Aires Province, Argentina, were examined for signs of clinical endometritis by external inspection and manual vaginal examination. Only cows without signs of clinical endometritis i.e. no vaginal discharge were enrolled in this study and examined for SE using the cytobrush technique. Cows with ≥5% PMNs in the cytological sample were regarded as affected by SE. All cows were reexamined 14 days later following the same examination protocol. Prevalence of SE 18-38 day post partum was 38% and decreased to 19% at re-examination. The proportion of cows pregnant at first service was 29% and proportion of cows pregnant at 360 post partum was 73% and 75% in cows with SE and those without, respectively. The probability of conception at first service, hazards of insemination and pregnancy, respectively, were not affected by SE. Primiparous cows had a greater chance for insemination (HR=0.66; 95% CI=0.47-0.92) and pregnancy (HR=0.63; 95% CI=0.45-0.90) than multiparous cows. In conclusion SE did not affect reproductive performance outcomes in a pasture-based, extensive dairy farming system in Argentina.

Barański *et al*. (2011) conducted a study for diagnosis of SE by uterine cytology (cytobrush technique) in two experimental herds. The incidence was 69.7% in one herd and 38.3% in the other herd during the fourth week post partum. Two weeks later, the incidence of this disease dropped to 43.3% and 17%, respectively. Only in 12.1% and 8.7% of cows after parturition the number of PMNs grew with the passage of time. They also suggested that more research is needed to evaluate the influence of cytological endometritis on reproductive performance and to examine the pathological and physiological nature of this disorder.

Cheong *et al*. (2011) conducted a study to obtain prevalence estimates for SE, determine cow- and herd-level risk factors, and evaluate the reproductive consequences of SE. A cross-sectional study was used to determine prevalence and risk factors with cows followed in a prospective study to determine reproductive outcomes. Lactating Holstein cows were sampled between 40 and 60 D in milk using low-volume uterine lavage, and cytology was evaluated to determine SE status. In total, 779 cows from 38 herds were used in the analysis. The cow-level prevalence of SE was 25.9%. Within-herd level prevalence ranged from 4.8 to 52.6% (median 26.3%, interquartile range 15.6 to 33.3%). Cow-level risk factors identified were ketosis [odds ratio (OR) 3.83; 95% confidence interval (CI) 1.82-8.07], acute metritis (OR 1.86; 95% CI 1.05-3.30], and the interaction between milk production and parity. Primiparous cows that produced more milk had increased odds of having SE, whereas multiparous cows that produced more milk had decreased odds of having SE. Herd-level risk factors identified were housing early postpartum cows on bedded packs (herd-level SE=36.1%), which increased herd prevalence of SE by 16.7% (SE 5.58) compared with early postpartum cows housed in freestalls (herd-level SE=19.4%), and straw bedding in the calving pen, which decreased herd prevalence of SE by 10.7% (SE 3.59) compared with

herds that used other bedding material. In this study, primiparous cows with and without SE had similar reproductive performance; however, multiparous cows with SE had median days open 44 D longer (159 D; 95% CI 126-186 D) compared with unaffected multiparous cows (115 D; 95% CI 106-132 D).

Barański *et al*. (2012) conducted a study to determine (1) how the prevalence of cytologically determined SE varies when using three different cytological threshold ratios to categorize cows as either with or without endometritis, (2) how the number of animals categorized as having endometritis changes from the fourth to the sixth week postpartum when using each threshold, (3) how SE influences the number of days open, and (4) how the results of cytological and bacterial examinations correlate. To answer these questions, 222 clinically healthy cows in two herds were examined in the fourth (Exam 1) and the sixth week (Exam 2) postpartum, when endometrial surface scrapings for bacteriologic and cytologic examination were collected by cytobrush from their uterine horns. After each examination, all cows were categorized using three different thresholds: (1) > 18% polymorphonuclear leucocytes in Exam 1 and > 10% in Exam 2, (2) > 8% in both exams, and (3) > 5% in both exams. It was found that: (1) The number of cows categorized as having endometritis increased as the threshold was lowered, and ranged from 18.9% to 75.4% according to herd, time of examination, and the threshold used; (2) with all three thresholds and in both herds, the number of cows categorized as having endometritis in Exam 1 was approximately double that in Exam 2; whereas depending on the herd and the threshold used, 6.1% to 17.0% of the cows that were negative in the first exam were positive in the second, and 7.4% to 33.3% were positive in both exams; (3) cows were open for a significantly greater number of days if categorized as having endometritis with the first threshold in Exam 1 (mean ± SEM 151.5 ± 9.5 vs. 115.9 ± 7.8; $P < 0.01$), or with either the first or the

second threshold in Exam 2 (mean ± SEM 155.0 ± 15.0 vs. 125.1 ± 6.6; P < 0.05); and (4). They opinioned SE seems to be associated more with the postpartum recovery of the endometrium than with bacterial infection.

Madoz *et al*. (2013) conducted an experiment in 4 commercial dairy farms in Buenos Aires province (Argentina), where lactating Holstein dairy cows (n=418) 21 to 62 DIM without clinical endometritis were studied. Samples of EC were collected with the cytobrush technique. Data were analyzed with receiver operator characteristic curves with Sigmaplot 10.0, and with PROC GLIMMIX, PROC PHREG, and PROC LIFETEST from SAS 9.1. Cutoff values for the diagnosis of SE in grazing dairy cows are 8% PMNs for 21 to 33 DIM, 6% PMNs for 34 to 47 DIM, 4% PMNs for 48 to 62 DIM, and overall 5% PMNs for 21 to 62 DIM; the prevalence of SE 21 to 62 DIM was 17%. They conducted that SE diagnosed at 21 to 62 DIM decreased the hazard for pregnancy (hazard ratio=0.668; 95% confidence interval=0.492-0.909) and increased the calving to conception interval by D 30 compared with normal cows (median 95% confidence interval=133 vs. 93, respectively)

Ribeiro *et al*. (2013) conducted a study to characterize the prevalence of periparturient diseases and their effects on reproductive performance of dairy cows in seasonal grazing farms. A total of 957 multiparous cows in 2 farms (555 in farm A and 402 in farm B) were evaluated. The prevalence of SE was 13.4% (≥ 5% PMNs) on the day of milk 46–52.

Sens and Heuwieser (2013) conducted a study to determine the relationship between bacterial findings in the uterus and PMNs in the early postpartum period and to compare 2 classification schemes for the diagnosis of SE. Cytological and bacteriological samples from the uterus were taken from 149 cows at 2 consecutive times postpartum

(10 ± 1 and 24 ± 1 DIM) and additionally, 131 cows were sampled at 21 to 27 DIM. For sample collection, the cytobrush technique was used and vaginal discharge was examined by vaginoscopy. PMNs cut points were set at 5, 10, and 18%. Cows positive with α-hemolytic streptococci at 10 ± 1 DIM had significant higher PMNs (%) at 21 to 27 DIM and greater median days to pregnancy (193.5 D) than cows not infected (123.0 D). Primiparous cows with more than 18% PMNs had greater median days to pregnancy (144.5 D) than cows with less than 5% (80.0 D) or cows between 5 and 18% PMNs (68.0 D). Cows between 5 and 18% PMNs or 10 and 18% PMNs showed the best reproductive performance. The results demonstrated that an early infection with α-hemolytic *Streptococci* increased the PMNs percentage 2 week later and that 18% PMNs at 21 to 27 DIM was a predictive cut point for primiparous cows to diagnose animals with or without SE.

Singh *et al*. (2016) examined 170 repeat breeding crossbred cattle at spontaneous estrus for the status of genitalia (through rectal palpation) and cervico-vaginal discharge. The cows with clear discharge were further subjected to uterine cytobrush technique for confirmation SE i.e. on the basis of presence of ≥ 4 % PMNs. The incidence of subclinical and clinical endometritis was recorded as 29.4 % (50/170) and 21.7 % (37/170), respectively.

Noori and Yimer (2017) conducted a study on 53 clinically healthy beef cows (28 Brangus and 25 Kedah-Kelantan breeds) 20-35 days postpartum from three beef farms were obtained. All cows were evaluated at 4 and 5 weeks postpartum, using cytobrush endometrial examination methods to diagnose cytological endometritis and results showed that 9.4% (5/53) of the cows exhibited cytological endometritis.

Sharma *et al*. (2017) conducted a study in which EC was performed in 18 cows, out of which 6 cows were diagnosed with SE. Reproductive performance of these cows was assessed via evaluation

of days to first artificial insemination, number of inseminations per conception and calving to conception interval.

2.2. Hematological parameters

2.2.1. Hemoglobin (Hb)

Kumar *et al*. (1986) revealed in their study that optimum level of Hb (11.71±0.12gm/dl) and PCV (33.40%) are required for sufficient transport of oxygen and they are essential for normal health and production in cows.

Awasthi and Kharche (1987) did not find any difference between Hb levels in fertile and non fertile estrus of repeater cows.

Kumar and Sharma (1991) studied the mean value of Hb and other constituents in the blood or serum during fertile and non fertile estrus and found that the Hb concentration was significantly low ($P<0.01$) in non fertile group, adversely affecting the normal reproduction.

Khan *et al*. (1995) reported, the Hb, TEC and TLC do not show any significant change in between the regular breeding, repeat breeding and anestrus cows. The values were within normal physiological range. However, the regular breeding cows show a slight non-significant increase in Hb and TLC as compared to repeat breeding and anestrus cows.

Ahmad *et al*. (2003) reported the various hematological values of cyclic, non cyclic and endometritic cross bred (Sahiwal×Friesian) cows. RBC was highest in endometritic cows and lowest in non cyclic ones and the difference was significant ($P<0.05$). Values of Hb concentration, erythrocyte sedimentation rate (ESR) and MCHC were highest in the cyclic cows and lowest in non cyclic cows. It was concluded that lower erythrocytic indices can be attributed to non cyclic condition in cross bred cows.

Kekan *et al*. (2005) conducted a study to evaluate and compare the hematological parameter in regular and repeat breeding cows on day of insemination, 7^{th} and 15^{th} day of estrus cycle and revealed that the mean Hb concentration and packed cell volume were significantly lower in repeat breeders than in regular breeders.

Kumar *et al*. (2006) revealed that Hb levels in normal cycling was higher as compared to repeat breeder cows, but there was non-significant difference on 4^{th} and 11^{th} day of estrus cycle and the mean Hb level was higher during estrus (0 D) in all the groups.

Mordak and Nicpon (2006) compared the values of blood Hb in thirty (30) pre and post partum cows which was found to be 9.56 and 9.54 gm/dl in both stages of parturition. Further, it was described that the knowledge regarding changes in blood parameters is an important sign to ascertain the healthy status of cow in farm conditions.

Pariza *et al*. (2009) in a study on subfertile cows with reproductive problems like anestrus, repeat breeding and uterine infection for various hematological parameters. That found the ESR was higher, whereas, Hb and packed cell volume (PCV) were lower in anestrus and repeat breeding cows than in control.

Mondal and Paul (2012) conducted a comparative study on hematological parameters in 18 lactating multiparous cows (4-6 years aged) from the out patients at the Addl. Block Animal Health Center, Matiali Block, Jalpaiguri, West Bengal, India. The cows were divided into three group, i.e normally cyclic, repeat breeder and post partum anoestrous. They observed that Hb and PCV values were low ($P<0.05$) in repeat breeder and anoestrous cows compared to cyclic ones.

Sahoo *et al*. (2014) conducted a study on the effect of uterine immunomodulation on hemato-biochemical parameters and total immunoglobulin concentration in cyclic non-breeding cows. They observed no significant difference in Hb concentration and TEC either between the experimental groups or between the days of sampling.

Sarkar *et al*. (2016) collected blood samples from 79 cows with different uterine infections (pyometra, endometritis, cervicitis and abortion) and 41 normal cows of non-descripts and crossbreds to study the prevalence of uterine infection in relation to hematological as well as biochemical changes in blood serum. They found a significant decrease in Hb and TEC in animals with uterine infection as compared to the control group.

Bhuyan *et al*. (2017) compared hematological metabolites of 18 normal cyclic with 54 metritic cross bred cows in their study. TEC and Hb recorded in the study were within the normal range in both normal and metritic cows. However, the values were significantly lower in metritic cows than in normal.

2.2.2. Total leucocyte count (TLC)

Boitor *et al*. (1976) detected that the number of leucocyte and bacteria increased from 12000 to 16000 per ml in animals with atony of uterus, retention of foetal membrane and puerperal endometritis. Further, they implied that the uterine secretion during normal puerperium showed a correlation of leucocyte number and bacterial content during involution of uterus. The number of leucocytes and bacteria increased in cows with necrotic metritis. They suggested that the number of leucocytes could be considering diagnosing uterine infection.

Hafez *et al*. (1983) working on buffaloes reported that WBC count fell during weeks preceding calving and return to normal 6 weeks after calving.

Kekan *et al*. (2005) evaluated the hematological parameters in regular and repeat breeding cows on the day of insemination, 7^{th} and 15^{th} day of the estrus cycle. The TLC was significantly greater in repeat breeding cows than in regular breeding cows. Slight variation in TLC was recorded for both the groups on day 0, 7^{th} and 15^{th} of the estrus cycle.

Kim *et al*. (2005) reported that dairy cow with post partum endometritis had higher total leucocyte count at all the stages i.e. one week prepartum parturition and one, two, three and four week post partum as compared to normal counterpart.

Pariza *et al*. (2009) studied on cows with reproductive problems like anestrus, repeat breeding and uterine infection where they found that total leucocyte count was significantly higher ($P<0.01$) in anestrus and repeat breeder cows than control group.

Mondal and Paul (2012) conducted a study to compare hematological parameters in which 18 lactating multiparous cows (4-6 years aged) were selected from the out patients at the Addl. Block Animal Health Center, Matiali Block, Jalpaiguri, West Bengal, India. The cows were divided into three group, i.e. normally cyclic , repeat breeder and post partum anoestrous and it was observed that TEC count was higher ($P<0.05$) in repeat breeder and anoestrous cows compared to cyclic ones.

Sahoo *et al*. (2014) conducted a study to observe the effect of uterine immunomodulation on hemato-biochemical parameters and total immunoglobulin concentration in cyclic non-breeding cows and revealed no significant difference in TLC between the experimental groups or between the days of sampling.

Sarkar *et al*. (2016) collected blood samples of 79 cows with different uterine infections (pyometra, endometritis, cervicitis and abortion) and 41 normal cows of non-descripts and crossbreds to study the prevalence of uterine infection in relation to hematological as well as biochemical changes in blood serum and revealed a significant increase in total leukocyte count in animals with uterine infection as compared to the control group.

Bhuyan *et al*. (2017) compared hematological metabolites of 18 normal cyclic with 54 metritic cross bred cows and observed that TLC in the metritic cows was higher than that in normal cows.

2.2.3. Differential leukocyte count (DLC)

Ahmad *et al*. (2003) reported various hematological value of cyclic, non cyclic and endometritic cross bred cows. DLC showed that neutrophils were significantly higher in endometritic cows than in cyclic and non cyclic cows. Eosinophil count was highest in non cyclic cows and lowest in endometritic cows and the difference was significant ($P<0.05$). The values of lymphocytes monocytes and basophils did not differ in the three groups.

Kekan *et al*. (2005) studied the hematological parameters in regular and repeat breeding cows on day of insemination, 7^{th} and 15^{th} day of the estrus cycle. Significant neutrophilia with relative lymphopenia was noted in repeat breeders. The eosinophil count was lower in repeat breeding group, whereas, monocyte and basophil counts were insignificantly different between the two groups. Significantly lower lymphocyte count and higher neutrophil count were observed on the day of estrus compared to that observed on the 7^{th} and 15^{th} days in both the groups.

Kim *et al*. (2005) stated that dairy cows with post partum endometritis had greater neutrophil, lymphocyte and monocyte count at all stages i.e. one week pre partum, parturition and one, two, three and four week post partum.

Heidarpoor *et al*. (2012) conducted a study to investigate the treatment-related changes in hematological parameters in dairy cows affected by clinical endometritis and SE. One hundred seventy postpartum Holstein dairy cows were selected from a large commercial dairy farm. They found significant increases in white blood cell (WBC) ($P < 0.05$), neutrophil ($P<0.001$), and lymphocyte ($P<0.001$) counts, in the clinical endometritis and SE groups when compared to healthy cows.

Sahoo *et al*. (2014) conducted a study on the effect of uterine immunomodulation on hematobiochemical parameters and total

immunoglobulin concentration in cyclic non-breeding cows and observed no significant difference in monocyte, basophil and eosinophil but significant difference in neutrophil between the experimental groups or between the days of sampling.

2.3. Treatment and conception rate of repeat breeding cows due to endometritis

Treatment of uterine infection is a challenging concern due to variable success following different therapeutics available. The type of drug, frequency and route of administartion, economicity and residual effects create perplexity among the practitioners/veterinarian about the use of any therapy. One has to be very much critical while treating uterine infection. The common intrauterine therapies used for treating uterine infections are as follows:

2.3.1. Iodine solutions

Dilute solution of lugol's iodine had been reported as an effective treatment of endometritis as it causes more or less intense irritation of endometrium, stimulates uterine tone and mobilizes neutrophils in to the uterine lumen (Roberts 1971; Watson 1979).

Koujan *et al*. (1996) examined the efficacy of either povidone-iodine (PI) or dichloroxylenol (Septocid) intrauterine infusions for the treatment of endometritis and/or cervicitis in cows. One hundred and twelve repeat-breeder Holstein cows (aged 3-7 years) were selected for this study. Rectal and vaginal examinations were applied to detect the signs of endometritis and/or cervicitis. Cows were assigned into two groups: the first group (n = 60) was treated with PI solution (0.5%) while the second group (n = 52) was given Septocid (0.1%) administered as intrauterine infusion (100-150 ml). Both treatments were repeated at least two times at 7-day intervals and the cows were rechecked. The responding animals were inseminated 10-12 hours after estrus detection using frozen semen from bulls of proven fertility. The recovery and conception rates obtained after PI treatment were better than those obtained after Septocid. Moreover, healthy cows (> or

= 500 kg body weight) and those inseminated before post-partum day 180, having not more than 4-7 previous services, responded well to either PI or Septocid treatment.

Torres *et al*. (2002) examined the effect of intrauterine infusion of 2% PI solution on the reproductive performance of repeat breeder cows. Sixty repeat breeder dairy cows were randomly divided into two groups: (a) the treatment group was given intrauterine infusion of 50 ml of 2% PI 24 hours after insemination, and (b) the control group was left untreated. Results showed that in the treated group, the average number of days open was 169.84 days; the number of services per conception was 1.54; conception rate at first treatment after service was 53.3%, and overall conception rate was 86.7%. The corresponding figures in the control group were 207.9 days, 2.05, 16% and 66.7%.

Honparkhe *et al.* (2005) found that single intrauterine infusion of lugol's iodine (0.5%) resulted in clear CVM at next estrus in 70 % cattle suffering from infection but the first service pregnancy rates was lower (42.8 %) than other treatment groups (antibiotics and LPS).

Polat *et al*. (2009) conducted a study to evaluate the reproductive indices in cows with retained placenta and endometritis in response to D-cloprostenol or povidone iodine (PI) foam treatment, as well as bacterial clearance and histopathological changes after PI foam intrauterine administration. The examinations were performed on a total of 113 Brown Swiss dairy cows. In experiment I, cows with retained placenta (n=42) and endometritis (n=50) were administered randomly with either PI (2%) foam (20-150 ml, intrauterine) or PGF2 α (150 µg, i.m.). The uterine microbial flora was also monitored in the cows before and after PI foam administration. In experiment II, 21 cows without reproductive diseases were administered with PI foam (20 ml, intrauterine) 10, 20, and 30 D before slaughtering to evaluate the histopathology of the uterine tissue. In the cows with retained placenta, there were no effects from the treatment on the number of days to 1st service (D), days open (D), 1st service conception rate (%), and

insemination index, which averaged 100, 124, 28.6, and 2.19, respectively. In the cows with endometritis, PI foam administration decreased the number of days to 1st service and days open - 13 and 17 D - as compared to $PGF_{2\alpha}$ administration, without altering the 1st service conception rate (26.4%) and insemination index (2.32). *E. coli* was the most common bacterium in these cows. On days 5 and 15, relative to PI foam administration, 56 and 100% microbial recovery were achieved. Furthermore, no degenerative changes in the luminal epithelium of uterus were detected upon PI foam administration. In conclusion on the basis of the microbial recovery and shortened days to 1st service and days open, they suggested that a low level of PI foam can be considered as a therapeutic agent against retained placenta and endometritis.

Singh *et al.* (2010) compared different concentrations of lugol's iodine intrauterine and observed that 0.5 % lugol's iodine was more effective as compared to 0.25% and 0.1 % concentration in clearing CVM (83 % vs 33 % and 0 %, respectively) and increasing conception rate (66.6 % vs 16.6 % and 0 %, respectively).

Farrag *et al*. (2012) studied on sensitivity of fungi isolated from the female genital organs with or without clinical signs in farm animals that failed to conceive after being bred with fertile males more than two times, to some. They found that povidone-iodine antiseptic solution was effective on all fungal isolates tested *in vitro* at 50 to 100 mg/ml, while it had no effect on some fungi at lower concentrations (12.5 and 25 mg/ml).

Ahmed and Elsheikh (2014) reported that intrauterine infusion of 1% Lugol's iodine can be used successfully for treating SE and to improve conception rate in repeat breeding Sudan dairy cattle.

Sago *et al*. (2016) conducted an experiment to find out the optimal concentration of PI for treating endometritis effectively in dairy cattle. 18 cows with clinical endometritis were treated with either 2.0% or 0.5% PI (n=9 in each group). Cytology samples and bacteria were

collected using a cytobrush on weeks 0 (W0), 1 (W1) and 2 (W2) after treatment. They found that intrauterine infusion of 2.0% PI was better than 0.5% in treating clinical endometritis in dairy cattle.

2.3.2. Antibiotics

The systemic and/ local antibiotics are most commonly used for treatment of endometritis in cattle but the success rate in terms of clearing infection and conception rate is not consistent. Moreover, use of antibiotic drugs in food producing animals is the matter of under critical debate.

The criteria for the selection of antibiotics and its route of administration have been well described by (Azawi, 2008). The antibiotic should be active against the main uterine pathogens and should maintain its activity in uterine environment. It should not inhibit the normal defense mechanisms and should be well tolerated and not induce irritation in the endometrium.

Awasthi and Tiwari (1999) achieved higher first service conception rate following post breeding intrauterine infusion of cephelexin in crossbred cattle with having sub clinical uterine infection.

Shams-Esfandabadi *et al.* (2004) used intra uterine antibiotics viz. oxytetracycline and procaine penicillin in cattle with mucopurulent discharge and concluded that the treatment with either antibiotic had no advantage relative to the control on first service conception rate.

Honparkhe *et al.* (2005) compared the efficacy of various intrauterine drugs through disappearance of turbidity in CVM in next estrus and first service pregnancy rate (FSPR). The CVM was found clear during next estrus in 70, 50, 80, 60, 90, 80 and 20 % cattle, whereas, FSCR was 42.8, 60, 50, 66.6, 55.5, 62.5, and 50 %, respectively, in Groups I (Lugol's iodine), II (Cephalexin + Metronidazole), III *(E. coli* LPS), IV (*E. coli* LPS + Lugol's iodine), V (Lugol's iodine + Gentamicin) VI (*E. coli* LPS + Gentamicin) and VII (control).

Sandhu (2006) revealed in his study that the combination of cephelexin (broad spectrum antibiotic) against aerobes and metronidazole against anaerobic bacteria has been shown to have good clinical efficacy against endometritis.

Singh *et al.* (2009) administered a combination of 2 gm of ciprofloxacin and 2.5 gm Metronidazole intravenously for 5 D and achieved 100% cure rate (in terms of clear discharge) and 40-50 % conceptions in chronic endometritis cases in buffaloes.

Bhattacharyya *et al*. (2011) conducted a study in which Levofloxacin and Ciprofloxacin were infused intrauterine to 44 cows suffering from different degree of sub-clinical metritis, diagnosed by White side test. Overall recovery and conception rate was recorded as 78.26 and 72.22% for Levofloxacin, and 85.71 and 72.22% for ciprofloxacin treated group, respectively. It was concluded that intrauterine fluorquinolones are very much useful for treating sub-clinical metritis in cows.

Bhat and Bhattacharyya (2012) conducted a study in which 36 cows suffering from metritis were divided into 3 groups i.e. group-IA (metritis without palpable CL), Group-IB (metritis without palpable CL) and group-II (metritis with palpable CL) keeping equal number of animals in each group. Animals of group-IA were treated with oxytetracycline 3.0 gm intrauterine daily for 2-4 days; whereas, group-IB animals were treated with Cephalexin 4 gm intrauterine daily for 2-4 days. Single intramuscular injection of Dinoprost ($PGF_{2\alpha}$) 25mg was given to animals of group-II. Recovery rate, on the basis of results of cultural examination, of 3 groups were found as 80.00, 71.43 and 91.67% respectively.

Kumar *et al*. (2014) conducted a study in which thirty four cross breed cows with the history of normal cycle without any clinical abnormality and returned to heat after three or more consecutive services were divided in to two group A and B. Group A was treated with Ciprofloxacin, where as Group B was treated with Levofloxacin

along with alpha tocopherol (vitamin E). All recovered animals were artificially inseminated at next estrus. Recovery and conception rate was recorded as 87.50 and 62.50% for Ciprofloxacin combination treated group and 83.34 and 77.78% for Levofloxacin combination treated group, respectively. Finally, they concluded that Levofloxacin, combination was more effective to control incidence of repeat breeding caused due to uterine infection in field conditions.

Resum and Singh (2016) conducted a study to evaluate the efficacy of different intrauterine agents on conception rates of repeat breeder cows. Forty repeat breeder cows having slightly turbid vaginal discharge were taken in the present investigation and were randomly divided into four treatment groups with 10 animals in each group. In group I, 50 ml of 1% Lugol's iodine, in group II, 8 ml Enrofloxacin diluted with 12 ml of normal saline solution, in group III, Cephalexin @ 4 gm diluted with 60 ml distilled water and in group IV, Levofloxacin + Ornidazole + α -Tocopherol combination @ 60 ml was infused intrauterine for 3 consecutive days. After treatment the cows were inseminated artificially using frozen thawed semen of acceptable quality. Pregnancy diagnosis was performed by per rectal palpation at 60 days post insemination. The conception rates were 30.00, 40.00, 60.00 and 60.00% for group I, II, III and IV, respectively.

Sharma *et al*. (2017) conducted a study to find out the bacteria from postpartum dairy cows, determination of their antimicrobial sensitivity, to diagnose SE via EC and evaluation of reproductive performance. Uterine discharge from normally parturated cows (n=23) were examined for aerobic bacterial identification and antimicrobial sensitivity at a weekly interval. Isolates include gram-positive bacteria (n=13), gram-negative bacteria (n=10) and mixed bacteria (n=7). The most frequently found isolates of bacteria were as follow: *Escherichia coli* (32.26%), *Bacillus cereus* (22.58%), *Staphylococcus aureus* (16.13%), *Bacillus cereus* + *Staphylococcus aureus* (9.68%) and *Escherichia coli* + *Proteus vulgaris* (3.23%). From all 13 antimicrobial

agents used in the culture sensitivity test, Levofloxacin and Ciprofloxacin were highly sensitive antibiotics and both gram-positive and gram-negative bacteria were found resistant against metronidazole. EC was performed in 18 cows, out of which 6 cows were diagnosed with SE. Reproductive performance of these cows was assessed via evaluation of days to first artificial insemination, number of inseminations per conception and calving to conception interval.

2.3.3. Herbal intrauterine

Herbal intrauterine drugs are considered as alternative approach to antibiotictherapy for treating uterine infections. The use of different herbal intrauterine has been well documented in dairy cattle suffering from endometritis.

Brindavan *et al.* (2002) used crude neem oil (40 ml thrice at 24 h interval) and recorded 88% recovery in endometritis cattle.

Barman *et al*. (2009) reported that neem oil showed antibacterial sensitivity against 95% bacterial isolates belonging to CVM of endometritic cattle and was comparable to antibiotics such as Enrofloxacin and Gentamicin.

Khillare *et al*. (2010) conducted a study to evaluate the efficacy of polyherbal intrauterine infusion AV/RMI/45 (M/s Ayurvet Limited Baddi, India) in treatment of endometritis, metritis and repeat breeding in cattle. Among the two experimental groups, one healthy control (I) (n=10) and other treatment (II) group (n=20). Group II animals were subjected to treatment with polyherbal uterine infusion AV/RMI/45@25ml once a day for five days. Treated animals recovered successfully exhibiting estrus with transparent discharge. Animals found in estrus were inseminated and conception rate was recorded to be significantly (P=0.05) higher (70%) in treatment than control group (40%).

Kumar *et al.* (2013) observed clear CVM with negative colour reaction to white side test in all animals treated with neem oil and reduction of bacterial load (96.02 ± 2.02%) at subsequent estrus and

maximum (70.73%) conception. They also reported recovery rate of 75% at subsequent estrus following treatment with garlic extract. The decline in bacterial load and conception rate was 91.80± 5.30% and 66.67%, respectively.

Walia *et al*. *(*2013) conducted a clinical study on 20 cows to evaluate the efficacy of herbal intrauterine infusion Uraksha liquid (a polyherbal intrauterine infusion) in treatment of uterine disorders. Cows on the basis of clinical history and turbid, off odour vaginal discharge were screened for prolonged anestrus, repeat breeding or endometritis. Confirmed cases were treated with 25 ml of Uraksha liquid administered as intra-uterine infusion once a day for three days or 3-5 days depending upon the severity of problem. The efficacy of treatment was judged by recording number of animals exhibiting signs of heat and nature of discharge in post-treatment estrus, number of treatments required for complete recovery and post-treatment conception rate. Out of total 20 animals treated with Uraksha liquid, 17 animals showed signs of estrus and 15 had clear vaginal discharge after 3-5 days treatment with Uraksha liquid. 75% of animals recovered from various uterine disorders and repeat breeding with a resultant conception rate of 72.6%.

Verma *et al*. (2016) revealed that 18 out of 20 repeat breeding animals (90%) showed signs of heat with clear discharge, recovered completely without causing any irritation, or severe irritation/sloughing of genital mucous membrane after Uterofix liquid (polyherbal intrauterine) treatment.

3 MATERIALS AND METHODS

3.1 Experimental location and animals

The present study was conducted at Veterinary Gynaecology and Obstetrics clinic, CVAS, RAJUVAS and private cattle dairy farms in Bikaner city. Repeat breeding cross bred cows with the history of regular inter-estrus interval (avg. 21-24 days) but failed to conceive after three or more consecutive breeding (natural/artificial) were used in the study. The animals between 4 to 9 years of age and more than 90 DIM with their body condition scores varying between 2.5-3.25 (Edmondson *et al*., 1989) were included in the study.

3.2 Experiment / methodology

The present experiment was carried out to diagnose SE in repeat breeding crossbred cows. The animals having clear cervico vaginal mucous (CVM) discharge were examined in their spontaneous estrus for uterine cytology (PMNs count). The cows diagnosed with SE were subjected to polyherbal and different antibiotic intrauterine infusions. In subsequent estrus uterine cytology was repeated to observe reduction in PMNs count. The cows were inseminated and later on their pregnancy status was examined by estimating serum progesterone concentration and by rectal palpation. Complete blood count of these animals was also done on the day of first uterine cytology and on the day of second uterine cytology.

3.2.1. Selection of subclinical endometritic cows

Repeat breeding cows were examined by rectal palpation for the status of genitalia and CVM discharge and by uterine cytology for PMNs count using cytobrush technique. The cows (n=60) having clear CVM and ≥ 4% PMNs (Singh *et al.,* 2016) were diagnosed with SE. These animals were randomly divided into 5 groups (4 treatment and 1 control) (table 1). The each group contained equal number of cows (n= 12).

Table 1: Experimental groups

Groups	Medicine used	Dose rate	Trade name	Manufacturer
Group 1	(Levofloxacin hemihydrate 20 mg, Ornidazole 25 mg and Alpha Tocopherol acetate 5 mg	30 ml/day for 3 days	Vodine-IU	Vetoquinol
Group 2	(Povidine iodine 5% and Metronidazole 1%)	30 ml/day for 3 days	Metricare-IU	Zydus
Group 3	IU (Cephalexin 1.5 g and Serratiopeptidase 10mg)	4 g dissolve in 60 ml of sterile water /day for 3 days	Utriguard-IU	Zydus
Group 4	(*Azadirachta indica* Lf. 0.040 g, *Aloe barbadensis* Lx. 0.010 g, *Acacia catechu* Ht. Wd. 0.10 g, *Bambusa arundinacea* Lf. 0.30 g, *Curcuma longa* Rz. 0.010 g, *Gossypium herbaceum* Rt. 0.30 g, *Plumbago zeylanica* Rt. 0.30 g, *Saraca indica* St. Bk. 0.40 g, Excipients q.s) /10 ml	25 ml/day for 3 days	Uraksha	Ayurvet
Control	No treatment was given			

Note:- Intrauterine medicines used in experiment are shown in figure 1.

Figure 1: Different intrauterine medicine used in the experiment.

3.2.2. Artificial insemination (AI)

All the animals (treatment + control) were inseminated with frozen semen in subsequent estrus (on the day of second uterine cytology). Animals those failed to conceive in first insemination were inseminated in next estrus.

3.2.3. Pregnancy diagnosis

The serum progesterone concentration was estimated by a solid-phase enzyme immunoassay (EIA) on day 24 post AI (excluding the animals which showed heat symptoms) for early pregnancy diagnosis. The minimum serum progesterone level set for the pregnancy diagnosis was 1.0 ng/ml as described by Adeyemo (1989). Rectal palpation was done for further confirmation of pregnancy on day 60 post AI.

Conception rate

The percentage of conception was calculated as follows:

$$\frac{\text{Number of cows diagnosed pregnant}}{\text{Number of cows inseminated}} \times 100$$

3.3.4. Blood collection and processing

Two blood samples were collected from each of the animal aseptically by jugular vein puncture. One in plain sterile glass tube and other in EDTA coated sterile glass tube. Serum was separated from plain tube by centrifuging the sample at 3000 rpm for 10 minutes and stored at -20℃ until conducting of progesterone assay. Blood sample containing blood anticoagulant was used for the estimation of haematological parameters.

3.3. Procedures/methods

3.3.1. Procedure of cytobrush technique

After proper restraining and following all hygiene measures, the vulvar lips were pulled apart by an assistant and the cytobrush assembly Figure 2 and 3) was introduced into vagina and then through the cervix to body of the uterus. After assuring its place, the stylette was pushed to expose cytobrush and then screwed gently in both directions (clockwise and anticlockwise). Gentle pressure was applied on its tip against the uterine body per rectum for proper contact of brush with endometrium. The inner stylette was then withdrawn into the outer catheter to its normal position and then the whole catheter was withdrawn from the reproductive tract.

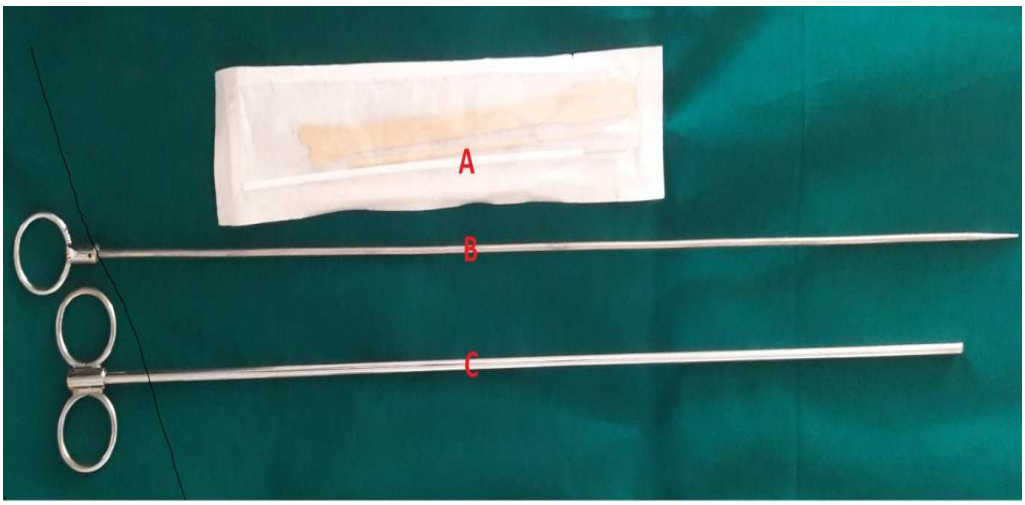

Figure 2: Cytobrush assembly: cytobrush (A), inner stylette (B) and outer catheter(C).

Figure 3: Taking of sample from uterus for cytology.

3.3.1.1. Staining method for endometrial cytology

Immediately after removal from reproductive tract, the cytobrush was rolled on clean sterlized glass slide and the slide was brought to the laboratory as soon as possible. The smear was stained with Geimsa stain. After drying the slide, 100 cells (endometrial cells + PMNs shown in Figure 4) were counted under microscope under oil immersion and percentage of PMNs was calculated.

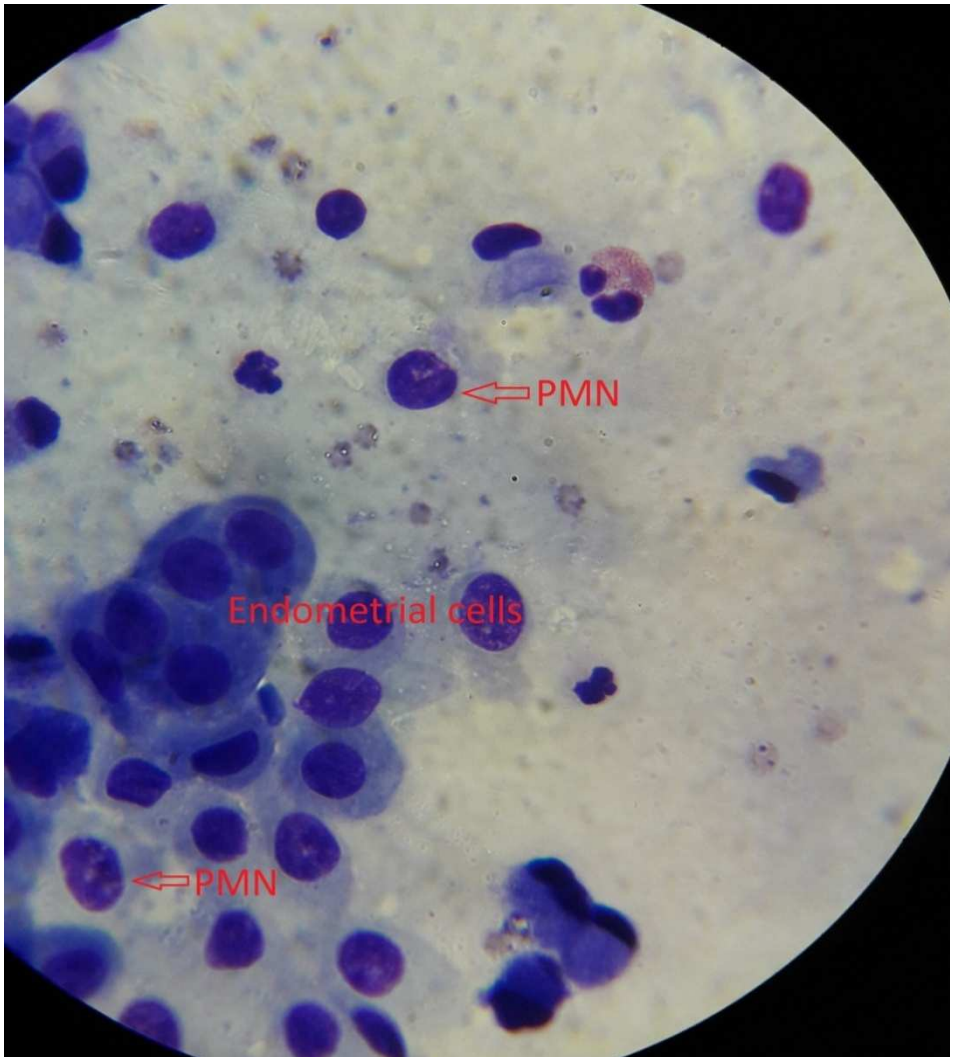

Figure 4: Endometrial cytology.

3.3.2. Estimation of haematological parameters

Haematological analysis was performed shortly after blood collection. Whole blood sample was used to measure haemoglobin concentration (Hb), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC). These parameters were analyzed as per the methods described by Jain (1986).

3.3.2.1. Haemoglobin

Haemoglobin was determined by Sahli-Hellige hemoglobinometer. Blood was drawn in Sahli's pipette up to 20 mm^3 mark. Then it was transferred to hemoglobinometer tube containing 4-5 drops of 0.1N hydrochloric acid and mixed well. The tube was then kept for 5 minutes for the Hb to change into acid haematin. The fluid was diluted with distilled water drop by drop and mixed after each drop until it matched to the colour of the standard comparison tubes. The hemoglobinometer tube was read to give the amount of Hb in g/dl of the blood.

3.3.2.2. Total erythrocyte count

The RBC pipette was filled up to 0.5 mark with the blood sample. The RBC diluting fluid (Hayem's fluid) was drawn up to 101 mark. After shaking the pipette gently for three minutes, the fluid in its stem was discarded. The counting chamber of the hemocytometer was carefully charged with the diluted blood after placing a cover slip. It was ensured that blood cells were evenly distributed over the counting chamber and overcharging was avoided. The red blood corpuscles present in the four corner small squares and one small central square of the large central square were counted under high power (40X) of the microscope.

Calculation

Numbers of red blood cells per cubic millimeter were calculated after multiplying the number of cells counted by 10,000 according to the following formula:

Total erythrocyte count (per mm^3) = Cells counted X 200 X 10 X 5

Where,

200 stands for dilution

10 stands for depth in mm

5 stands for the $1/5^{th}$ of square millimeter counted.

3.3.2.3. Total leucocyte count

The WBC pipette was filled up to 0.5 mark with blood sample and the WBC diluting fluid was drawn up to 11 mark. After shaking the pipette gently for three minutes, the fluid in its stem was discarded. The counting chamber of haemocytometer was carefully charged with the diluted blood after placing a cover slip. The cells were counted under low power (10X) objective of the microscope in the large four corner squares of the haemocytometer.

Calculation

The number of leucocytes in one cubic millimeter of blood was calculated by multiplying the total leucocytes counted by factor 50, according to the following formula:

$$\text{Total leucocyte count } (mm^3) = \frac{\text{Cells counted X 20 X 10}}{4}$$

Where,

20 stands for dilution

10 stands for depth in mm.

4 stands for the number of square millimeters counted.

3.3.2.4. Differential leukocyte count

A thin blood smear was drawn on a grease free slide. Blood smear was air dried and then stained by diluted Leishman's stain. Hundred (100) cells were counted, and percentages of different white blood cells were estimated as per method described by (Jain, 1986).

3.3.3. Progesterone (P_4) assay

Blood serum progesterone concentration was analyzed by ELISA reader (Tecan M Pro 200) as per the protocols given in the respective kits manufactured by XEMA Co. Ltd., Moscow, Russia (Figure 5 and 6).

Figure 5: Progesterone (P_4) assay kit with samples, micropipettes, microtips and tissue papers.

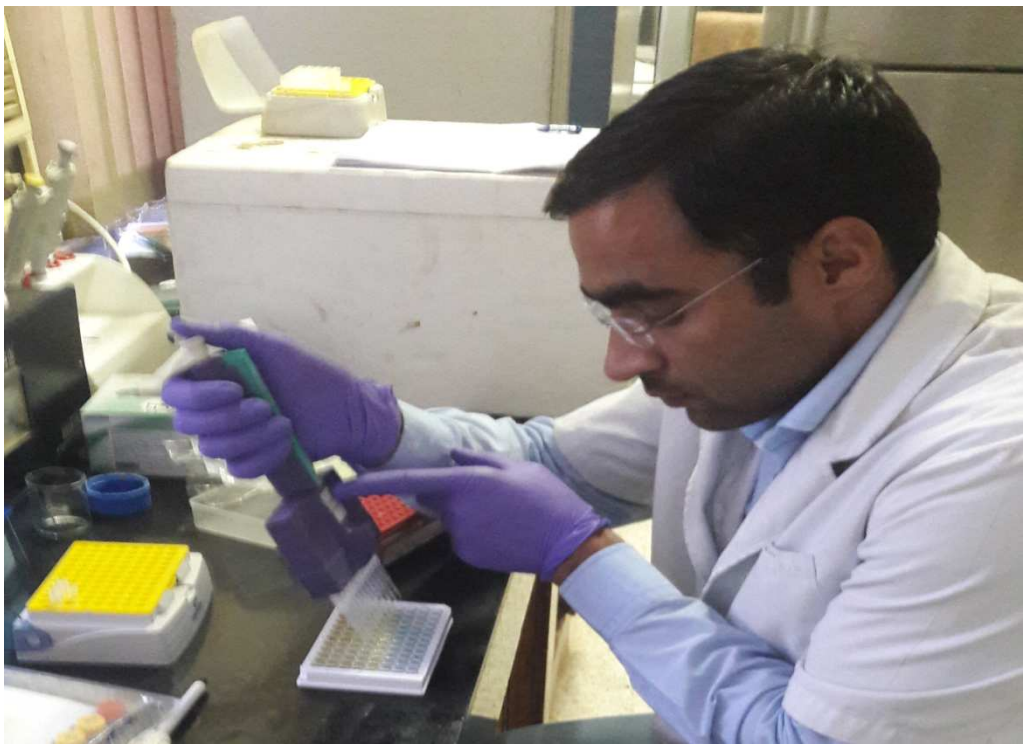

Figure 6: Handling of kit and ELISA plate.

3.3.3.1. Serum progesterone estimation

Assay for serum progesterone was done by solid phase enzyme immuno-assay using commercially available progesterone kits (XEMA Co. Ltd. Moscow, Russia). Each kit was having microplates for 96 tests.

3.3.3.2. Principle of assay

Solid phase enzyme immuno assay (EIA) is based on the competition principle. Tested specimen is placed into the microwells coated by specific murine monoclonal progesterone-antibodies simultaneously with conjugated progesterone-peroxidase. Progesterone from the specimen competes with the conjugated progesterone for coating antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen–substrate mixture, stop solution and photometry at 450nm. Optical density in microwell is

inversely related to the quantity of the measured analyte in the specimen.

3.3.3.3. Reagents

1. Calibrators (C_A, C_B, C_C, C_D, C_E, C_F, C_G): 7 x 0.8ml each vial. Before use, mixed for 5 minutes, with rotating mixer.

The calibrators have the following concentration of 17-α- CE OH progesterone.

Calibrators	C_A	C_B	C_C	C_D	C_E	C_F	C_G
nmol/l	0	1	3	10	30	100	300
ng/ml	0	0.318	0.954	3.18	9.54	31.8	95.4

2. Enzyme conjugate: The vial contains 22ml aqueous solution of progesterone coupled with horse radish peroxidase diluted on phosphate buffered solution preservative – 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride and red dye.

3. Micro well plate: The bag contains a microplate of 12 strips x 8 wells. Each well is coated with murine monoclonal to progesterone.

4. Substrate solution TMB: the vial contains 14 ml ready to use single component of TMB (3,3',5,5'-Tetramethylbenzidine) solution.

5. Stop solution: the vial contain 14 ml of 5.0% vol/vol solution of sulphuric acid.

3.3.3.4. Preparation of assay

1. All reagents were brought to room temperature.

2. Sufficient strips were left in the strip holder to enable the running of calibrators, controls, and samples in duplicate, plus one well for chromogen blank.

3. For a photometer blank 100 µl of substrate and 100 µl of stop solution were pipetted in one well.

4. The washing solution from the concentrate BUF WASH was diluted by 26 dilutions in distilled water.

3.3.3.5. Procedure of assay

1. Twenty five microliter of each Standard (calibrators) CAL 1-7, Control samples and unknown samples were pipetted into the respective wells of the Microtiter Plate.

2. Two hundred microliter of Enzyme Conjugate (HRP-Progesterone) was added into each well. The wells were covered by plate adhesive tape.

3. Plate was incubated for 120 minutes at 37℃.

4. Incubation solution was discarded. Plate was washed 5 times with 250µL of diluted Wash Buffer (BUF WASH 26X). The excess solution was removed by tapping the inverted plate on a paper towel.

5. Hundred microliter of Substrate Solution TMB (SUBS TMB) was dispensed into each well and incubated for 10-20 minutes at room temperature (18-25℃).

6. Substrate reaction was stopped by adding of hundred microliter Stop Solution into each well and contents were mixed by gently shaking the plate.

7. Optical density was measured with ELISA reader at 450 nm within 30 min after pipetting of the Stop Solution (Figure 7).

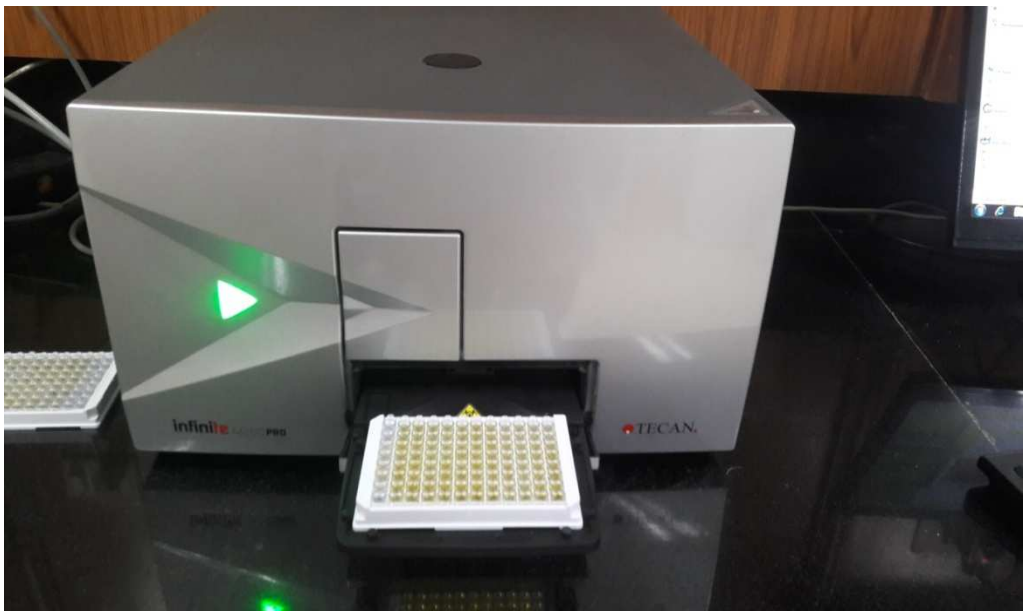

Figure 7: ELISA reader with microplate.

3.3.3.6. Calculation of results

The results were calculated by ELISA reader (Tecan M Pro 200) with normal programs for automatic data processing.

Statistical analysis

The data obtained in this research work were analyzed using conventional procedures as suggested by Snedecor and Cocharan (1994).

4. RESULTS

The results of the present study are represented under the following sub-headings.

4.1. Incidence and diagnosis of subclinical endometritis

4.2. Hematological parameters

4.3. Conception rate

4.1. Incidence and diagnosis of subclinical endometritis

Total 163 repeat breeding cows were screened, out of which 60 cows were diagnosed with SE. The incidence of SE was observed as 36.80% (60/163). The results of first and second uterine cytology are presented in Table 2 and Figure 8 in term of per cent PMNs. Significant (P<0.05) decline in mean PMNs (%) in subsequent estrus was observed in all the treatment groups after treatment.

Table 2: PMNs (%) (Mean+SE) in different groups in spontaneous and subsequent estrus.

Groups	**PMNs (%)**	
	In first uterine cytology (spontaneous estrus)	**In second uterine cytology (subsequent estrus)**
Group 1	5.66 ± 0.35^{aA}	2.41 ± 0.19^{cB}
Group 2	5.33 ± 0.28^{abA}	2.91 ± 0.14^{cB}
Group 3	6.08 ± 0.49^{aA}	2.25 ± 0.21^{cB}
Group 4	5.41 ± 0.31^{aA}	2.5 ± 0.15^{cB}
Control	5.33 ± 0.30^{abA}	4.58 ± 0.12^{bA}

Note:- The values with different superscripts in uppercase are significantly (P<0.05) differ within group.

The values with different superscripts in lowercase are significantly (P<0.05) differ between the groups.

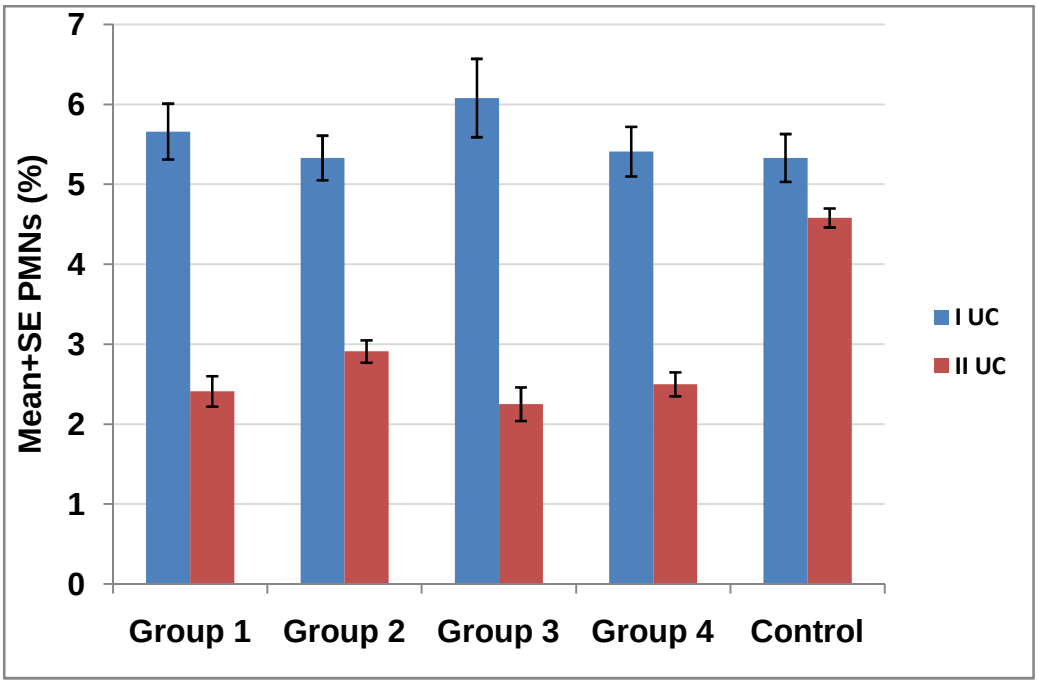

Figure 8: Graphical representation of mean+SE PMNs (%) in different groups in spontaneous and subsequent estrus.

I UC: First uterine cytology.

II UC: Second uterine cytology.

4.2. Hematological parameters

The blood parameters viz., Hb, TEC, TLC and DLC in all the groups at spontaneous and subsequent estrus are presented in Table 3. No significant (P>0.05) changes were observed for all the blood parameters between subsequent and spontaneous estrus in all the groups.

The range of Hb (g/dl) was recorded 8.96±0.21 to 9.6±0.64 and 8.85±0.29 to 10.26±0.47 in spontaneous and subsequent estrus, respectively.

The range of TEC (× 10^6µl) was recorded 5.68±0.21 to 6.59±0.45 and 6.01±0.27 to 6.78±0.36 in spontaneous and subsequent estrus, respectively.

The range of TLC (× 10^3µl) was recorded 8.32±1.04 to 11.95±0.38 and 8.4±0.75 to 11.7±0.40 in spontaneous and subsequent estrus, respectively.

The range of Lymphocytes (%) was recorded 55.5±1.84 to 60.83±2.90 and 54.08±1.72 to 58.25±2.76 in spontaneous and subsequent estrus, respectively.

The range of Neutrophils (%) was recorded 26.33±0.94 to 30.41±2.04 and 24.41±1.63 to 30.33±2.91 in spontaneous and subsequent estrus, respectively.

The range of Monocytes (%) was recorded 3.5±0.35 to 4.66±0.39 and 2.83±0.40 to 5.25±0.61 in spontaneous and subsequent estrus, respectively.

The range of Eosinophils (%) was recorded 4.16±0.86 to 7.83±1.05 and 3.08±0.64 to 7±0.92 in spontaneous and subsequent estrus, respectively.

4.3. Conception rate

The conception rates in different groups on day 24 (post AI) and on day 60 (post AI) are shown in Table 4. Highest and lowest 1st service conception rate was observed in group 4 (58.33%) and group 5 (8.33%), respectively (Table 5 and Figure 9). The serum progesterone (P4) concentration range (on day 24 post AI) was recorded 2.24 to 5.96 ng/dl and 2.49 to 2.51 ng/dl in pregnant and non pregnant cows, respectively.

Table 3: Different blood parameter values (mean+SE) in spontaneous and subsequent estrus

	Group 1		Group 2		Group 3		Group 4		Control	
Parameter	**SPE**	**SUE**	**SPE**	**SUE**	**SPE**	**SUE**	**SPE**	**SUE**	**SPE**	**SUE**
Hemoglobin g/dl	9.50±0.37	9.21±0.36	8.96±0.21	9.26±0.20	9.6±0.64	10 .26±0.47	9.13±0.43	9.91±0.31	9.38±0.36	8.85±0.29
TEC × 10^6µl	6.59±0.45	6.78±0.36	5.91±0.33	5.55±0.30	6.52±0.24	6 .61±0.33	6.12±0.28	6.47±0.27	5.68±0.21	6.01±0.27
TLC × 10^3µl	10.58±0.55	10.40±0.52	8.32±1.04	8.4±0.75	11.42±0.51	11.31±0.44	11.95±0.38	11.7±0.40	9.89±0.24	9.14±0.3 4
Lymphocytes %	55.91±2.30	54.08±1.72	55.5±1.84	54.91±1.40	60.75±2. 38	58.25±2.76	60.83±2.90	57.5±2.35	60.08±1.62	57.75 ±1.65
Neutrophils%	29±0.65	30.33±2.91	26.66±2.56	25.08±1.76	26.33±0.94	25.66±1.33	26.58±1.95	24.41±1.63	30.41±2.04	27.08± 1.13
Monocytes %	3.83±0.48	3.58±0.31	4.66±0.39	5.25±0.61	4±0.59	3.58 ±0.41	4.25±0.73	4.25±0.52	3.5±0.35	2.83±0.40
Eosinophils %	4.16±0.86[a]	3.08±0.64[a]	4.66±0.97[a]	3.91±0.58[a]	6.16±1.16[ab]	6.33±0.99[ab]	5.41±0.87[ab]	4.91±0.60[ab]	7.83±1.05[b]	7±0.92[b]

SPE: Spontaneous estrus; SUE: subsequent estrus after treatment.
Note:- The mean values with different superscripts differ significantly (P<0.05) between the groups.

Table 4: Conception rates in different groups

Groups	On day 24 post AI (%) (P_4 assay)	On day 60 post AI (%) (rectal palpation)	Accuracy (%)
Group 1	8/12 (66.66)	8/12 (66.66)	8/8 (100)
Group 2	7/12 (58.33)	6/12 (50)	6/7 (85.71)
Group 3	9/12 (75)	8/12 (66.66)	8/9 (88.88)
Group 4	10/12 (83.33)	9/12 (75)	9/10 (90)
Control	3/12 (25)	3/12 (25)	3/3 (100)
Overall	37/60 (61.66)	34/60 (56.66)	34/37 (91.89)

Table 5: Conception rates after 1st and 2nd AI in different groups

Groups	Number of cows pregnant after 1st AI	Number of cows pregnant after 2nd AI	Total number of pregnant cows (%)
Group 1	5	3	8/12 (66.66)
Group 2	3	3	6/12 (50)
Group 3	6	2	8/12 (66.66)
Group 4	7	2	9/12 (75)
Control	1	2	3/12 (25)

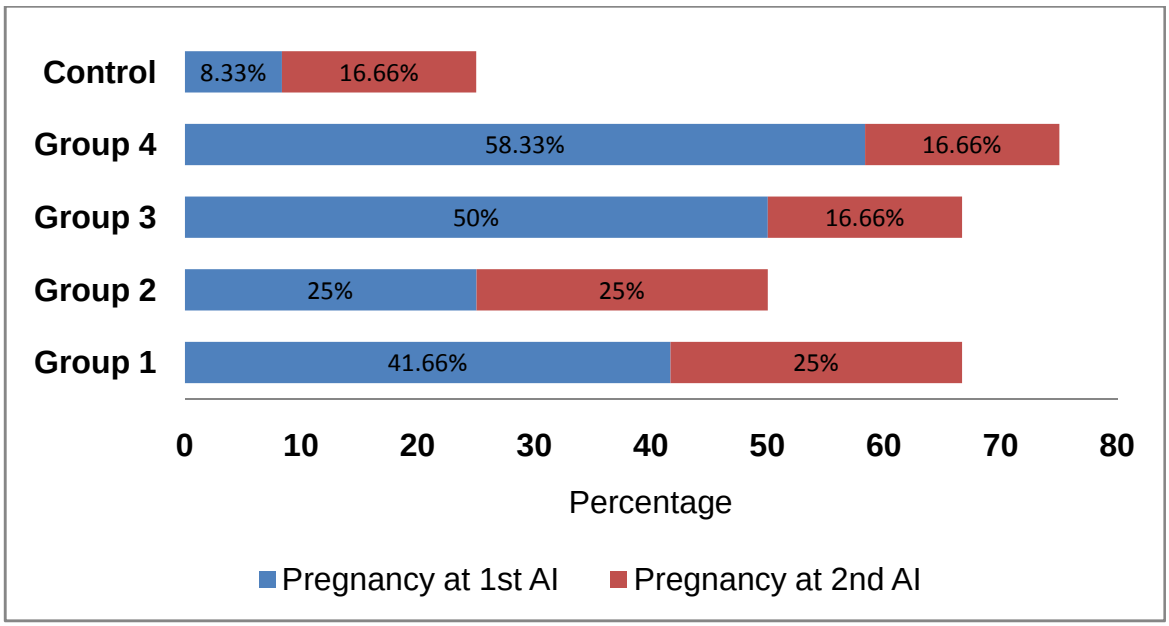

Figure 9: Graphical representation of conception rates after 1st and 2nd AI in different groups.

5. DISCUSSION

The results of the present study are discussed under following subheading

5.1. Incidence and diagnosis of subclinical endometritis

5.2. Hematological parameters

5.3. Conception rate

5.1. Incidence and diagnosis of subclinical endometritis

One of the major reproductive problems in dairy cattle prevalent at field level is existence of large number of repeat breeder cows. Under field conditions, repeat breeding is associated mainly with SE, delayed ovulation and corpus luteum deficiency, which cause fertilization failure or embryonic mortality (Stolla and de Kruif 1999, Parkinson 2009). In the present study, the prevalence of SE in repeat breeding cows was 36.80% (60/163). In the previous studies by Salasel *et al*. (2010) and Janowski *et al*. (2013), higher prevalence of SE in repeat breeding cows was observed which was 52.7% and 40.2%, respectively. In another studies, Pothmann *et al*. (2015) and Singh *et al*. (2016) observed lower percentage of cows with this disorder 12.7% and 29.4%, respectively. The difference in prevalence between the studies may be associated with different study populations, managemental practices and different environmental factors. The prevalence of SE depends on various factors viz., the time of examination in the postpartum period, the applied threshold for PMNs and herd specific factors (Wagener *et al*., 2017). Several factors have been suggested as risk factors for SE viz., acute metritis, ketosis, and high milk yield in the first lactation at cow-level, and housing early postpartum (bedded packs > free stalls), and bedding material (straw bedding < sand, sawdust, paper, or combination of any of these) at herd-level risk factors (Cheong *et al*., 2011). Animal farms in Bikaner city were unorganized and unhygienic except few, and different farms

have different feeding practices may also be the other factors involved in prevalence of SE. In many previous studies the threshold value of 4% to 18% PMNs was used as an indicative of SE at about 21-62 days (Kasimanickam *et al*., 2004; Gilbert *et al*., 2005; Lincke *et al*., 2007; Barlund *et al*., 2008; Santos *et al*., 2009; Fischer *et al*., 2010; Kaufmann *et al*., 2010; Plöntzke *et al*., 2010; Cheong *et al*., 2011; Baranski *et al*., 2012; Madoz *et al*., 2013; Ribeiro *et al*., 2013; Sens and Heuwieser, 2013; Singh *et al*., 2016). The applied threshold for PMN was ≥4% in the present study as the research was conducted long after parturition (>90 days postpartum).

The range of mean PMNs (%) was 5.33±0.28 to 6.08±o.49 on the day of 1st uterine cytology (spontaneous estrus) which was in accordance with the findings (4.4±0.27 to 5.5±0.78) of Singh (2016). In another study, the range was 5.60±3.82 to 12.50±2.96 on the day of selection (D=0) by Alagar *et al*. (2017). There was significant ($P< 0.05$) decline in PMNs (%) during subsequent estrus post treatment in all treatment groups which was indicative of recovery from infection. In control group no significant ($P>0.05$) change was observed as no treatment was given.

Cytobrush technique is an invasive technique to diagnose SE. The results of uterine cytology in the present study reflect that cytobrush technique is an effective tool to diagnose uterine infection in case of SE. This supports the earlier reports made by Barlund *et al.* (2008), Honparkhe *et al.* (2014) and Singh *et al*. (2016).

5.2. Hematological parameters

The range of Hb (g/dl) was recorded 8.96±0.21 to 9.6±0.64 and 8.85±0.29 to 10.26±0.47 in spontaneous and subsequent estrus, respectively and no significant change was observed in between, which indicates no adverse efffect of any treatment. The values of Hb reflect the general health of animals. The Hb values in blood in normal healthy

cows would be within the range of 8-15 g/dl (Blood *et al*., 1989). The present finding sustained with the observation made by Heidarpour *et al*. (2014) and Singh (2016) as they also did not find any significant chance in Hb values before and after treatment in the cows diagnosed with SE.

The range of TEC (× 10^6µl) was recorded 5.68±0.21 to 6.59±0.45 and 6.01±0.27 to 6.78±0.36 in spontaneous and subsequent estrus, respectively. The present finding was in accordance with the observation made by Heidarpour *et al*. (2014) as they also did not find any significant chances in the values before and after treatment.

The range of TLC (× 10^3µl) was recorded 8.32±1.04 to 11.95±0.38 and 8.4±0.75 to 11.7±0.40 in spontaneous and subsequent estrus, respectively. No significance difference was observed between the findings. The present results were in the range of previous finding made by Singh (2016). In contrast Heidarpour *et al*. (2014) observed significant decline in TLC values after treatment of SE.

Non-significant difference was observed in DLC values in all the groups before and after treatment. Similar observation was made by Singh (2016) in his study. Although, Heidarpour *et al*. (2012) observed a significant decrease in leucocytes ($P<0.05$), neutrophils ($P<0.001$) and lymphocyte ($P<0.001$) counts after treatment of SE.

5.3. Conception rate

In group 1st, 8 out of 12 repeat breeder cows conceived and conception rate was 66.66%. In a previous study, 60% conception rate was reported with Levofloxacin in combination with Ornidazole + *α* -Tocopherol by Resum and Singh (2016). However, Singh *et al*. (2014) reported higher conception rate (89.28%) with the similar combination. Kumar *et al*. (2013) also treated repeat breeder crossbred cows successfully using levofloxacin in combination with *α* -Tocopherol. High conception rate in this group might be due to presence of *α* -tocopherol which might have altered immune status of animals by enhancing host

defence against infections through improving phagocytic cell function and consequently improved the fertility. Uterine endometrial treatment with *α* -tocopherol might improve oxygen utilization, health of superficial layer of endometrium and provide assistance for metabolism as an antioxidant for effective innate defence (Kumar *et al*., 2014).

In group 2nd, 6 out of 12 repeat breeder cows conceived and the conception rate was 50%. Sago *et al*. (2016) observed 44.4% conception rate in endometritic cows using 2% PI intrauterine. Polat *et al*. (2009) observed 26.4% 1st service conception rate in endometritic cows with 2% PI foam. Bacterial exposure to 2.5 % PI for 2.15 seconds showed a sufficient antiseptic effect (Jose, 1986). Stephens and Slee (1987) reported conception rates 22/29 (75.86%) and 9/18 (50%) with Metronidazole + Ampicillin and Povidone iodine, respectively in cows with pyometra.

In group 3rd, 8 out of 12 repeat breeder cows conceived and conception rate was 66.66%. Cephalexin is 1st generation cephalosporin which acts by inhibiting synthesis of the peptidoglycan layer of the bacterial cell wall (Fisher *et al*., 2005) and also remains a drug of choice for intrauterine infusion as it works against gram positive as well as gram negative bacteria. Serratiopeptidase, a proteolytic enzyme from trypsin family, possesses tremendous scope in combating inflammation. Serine protease possesses a higher affinity for cyclooxygenase (COX-I and COX-II), a key enzyme associated with production of different inflammatory mediators including interleukins, prostaglandins and thromboxane etc. (Tiwari, 2017), and also contains fibrinolytic and caseinolytic properties (Bhagat *et al*., 2013). Intrauterine administration of cephalexin and serratopeptidase combination improved the conception rate in the present study could be due to its bactericidal effect and anti-inflammatory combination, thus eliminating SE. Similar results (60%) were reported by Resum and Singh (2016) with 4g cephalexin intrauterine. Better conception rate in repeat

breeding Gir cows (83%) and repeat breeding crossbred cows (76.92%) was reported by Parikh *et al*. (2017) and Singh *et al*. (2014), respectively, with the same treatment.

In group 4th, 9 out of 12 repeat breeder cows conceived and conception highest rate (75%) was observed in this group. Khillare *et al*. (2010) reported 70% (14/20) conception rate in endometritis, metritis and repeat breeding cows treated with AV/RMI/45 (polyherbal drug). Highest conception rate in this group may be due to the fact that different herbs contain different phytochemical properties. *Saraca asoca*, *Aloe barbadensis*, *Gossypium herbaceum*, *Plumbago zeylanica* and *Azadirachta indica* are scientifically proven for their antibacterial (Mukherjee *et al*., 1996), anti-inflammatory, antimicrobial activity (Valerie *et al*., 2003) and immunoregulatory activity (Qiu *et al*., 2000). *Acacia catechu* leaf extract act as antioxidant and free-radical scavenger (Devi *et al*., 2011; Saha *et al*., 2016) and also found to be nontoxic and safer through metabolic activity tetrazolium dye assay (MTT cell viability assay; Saha *et al*., 2016). *Bambusa arundinacea* : various parts of this plant such as leaf, root, shoot and seed possess anti-inflammatory, anti-oxidant, anthelmintic, astringent, emmengogue activity (Kumar, 2009; Rathod, 2011). *Curcuma longa* (Turmeric): curcumin is the main chemical compound of Turmeric and proven for its anti-inflammatory, antioxidant, antimutagenic, antibacterial, and pharmacological activities (Krup *et al*., 2013).

Lowest conception rate in the control group indicates that treating SE with antimicrobial or polyherbal drugs clears the infection and improves conception rate. Treatment also increased 1st AI pregnancy rate. Higher pregnancy rates in treatment groups (antimicrobial or polyherbal drugs) compared to the control (untreated) were also observed in the previous studies (Kasimanickam *et al*., 2005b; Denis-Robichaund and Dubuc, 2015; Khillare *et al*., 2010).

The conception rate on day 24 by P_4 assay was 66.66, 58.33, 75, 83.33 and 25% in group 1st, group 2nd, group 3rd, group 4th and control group, respectively. On day 60 by rectal palpation the same was 66.66, 50, 66.66, 75 and 25% in group 1st, group 2nd, group 3rd, group 4th and control group, respectively. The serum P_4 concentration range (on day 24 post AI) was recorded 2.24 to 5.96 ng/dl and 2.49 to 2.51 ng/dl in pregnant and non pregnant cows, respectively. The accuracy of P_4 assay for pregnancy diagnosis was 91.89% (34/37) in the present study. Muhammd *et al*. (2000) observed plasma P_4 level range between 2.3 to 3.8ng/ml and 0.1 to 2.6 ng/ml in pregnant and non pregnant Holstein Friesian cows, respectively and the accuracy was 83.3% on the day 25 post AI. Otava *et al*. (2007) observed 82% accuracy on the day 19 post AI and the minimum criteria for early pregnancy diagnosis of serum P_4 was 3ng/ml. Butterfield and Lishman (1988) observed milk P_4 values on the day 20 and 24 post AI and accuracy was 89.1 and 91.4%, respectively. The incorrect diagnosis of early pregnancy in the present study may be due to early embryonic loss. Early pregnancy losses are common upto D 55 in cows (Chaudhary and Purohit, 2012).

6. SUMMARY AND CONCLUSIONS

The objectives of the present study were to investigate the incidence of SE in repeat breeder cattle by cytobrush technique and study the comparative efficacy between different antibiotics and herbal intrauterine therapy in cattle with SE.

The present experiment was carried out at Veterinary Gynaecology and Obstetrics Clinic, CVAS, RAJUVAS and private cattle dairy farms in Bikaner. Repeat breeding crossbred cows (>90 days in milking) in their spontaneous estrus were examined for CVM by rectal palpation and uterine cytology by cytobrush technique. The cows (n=60) having clear CVM and ≥ 4% PMNs in uterine cytology were diagnosed with SE. These cows were randomly divided into 4 treatment groups (group 1, group 2, groupN 3 and group 4) and control group and each group contained 12 animals. The cows in group 1, group 2, group 3 and group 4 were treated with intrauterine medicine Vodine-IU 30 ml, Metricare-IU 30 ml, Utriguard-IU 4 g dissolve in 60 ml of sterile water and Uraksha 25 ml, respectively for consecutive 3 days. In subsequent estrus, uterine cytology was repeated to observe reduction in PMNs (%) and the cows were inseminated. Animals those failed to conceive in 1st insemination were subjected to 2nd insemination in the next estrus. The pregnancy status was examined by estimating serum P_4 concentration on day 24 and by rectal palpation on day 60 post AI. Complete blood count of these animals was also done on both the days of uterine cytology.

The incidence of SE was observed as 36.80% (60/163) in the present study. The mean PMNs (%) was 5.66±0.35, 5.33±0.28, 6.08±0.49, 5.41±0.31 and 5.33±0.30 in group1st, group 2nd, group 3rd, group 4th and control group, respectively on the day of 1st uterine cytology (spontaneous estrus); the same was 2.41±0.19, 2.91±0.14, 2.25±0.21, 2.5±0.15 and 4.58±0.12 on the day of 2nd uterine cytology

(subsequent estrus). Significant ($P<0.05$) decline in PMNs (%) was observed in all treatment groups.

No significant ($P>0.05$) changes were observed for all the blood parameters viz. Hb, TEC, TLC and DLC between subsequent and spontaneous estrus in all the groups.

The range of Hb (g/dl) was recorded 8.96±0.21 to 9.6±0.64 and 8.85±0.29 to 10.26±0.47 in spontaneous and subsequent estrus, respectively.

The range of TEC (× 10^6µl) was recorded 5.68±0.21 to 6.59±0.45 and 6.01±0.27 to 6.78±0.36 in spontaneous and subsequent estrus, respectively.

The range of TLC (× 10^3µl) was recorded 8.32±1.04 to 11.95±0.38 and 8.4±0.75 to 11.7±0.40 in spontaneous and subsequent estrus, respectively.

The range of Lymphocytes (%) was recorded 55.5±1.84 to 60.83±2.90 and 54.08±1.72 to 58.25±2.76 in spontaneous and subsequent estrus, respectively.

The range of Neutrophils (%) was recorded 26.33±0.94 to 30.41±2.04 and 24.41±1.63 to 30.33±2.91 in spontaneous and subsequent estrus, respectively.

The range of Monocytes (%) was recorded 3.5±0.35 to 4.66±0.39 and 2.83±0.40 to 5.25±0.61 in spontaneous and subsequent estrus, respectively.

The range of Eosinophils (%) was recorded 4.16±0.86 to 7.83±1.05 and 3.08±0.64 to 7±0.92 in spontaneous and subsequent estrus, respectively.

The conception rate on day 24 by P4 assay was 66.66%, 58.33%, 75%, 83.33% and 25% in group 1st, group 2nd, group 3rd, group 4th and control group, respectively. The serum P4 concentration

range (on day 24 post AI) was recorded 2.24 to 5.96 ng/dl and 2.49 to 2.51 ng/dl in pregnant and non pregnant cows, respectively.

The conception rate on day 60 by rectal palpation was 66.6, 50, 66.66, 75 and 25% in group 1st, group 2nd, group 3rd, group 4th and control group, respectively.

The following conclusions were drawn out of this study:-

1. Uterine cytobrush technique can be used efficiently for diagnosing sub-clinical endometritis in dairy cattle.
2. The polyherbal intrauterine drugs may be a good alternative of antimicrobial intrauterine therapy.

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Comparative study of different antibiotics and polyherbal intrauterine therapy in cattle with endometritis

M.V.Sc. Thesis
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ABSTRACT

The present study was conducted to investigate the incidence of subclinical endometritis (SE) in repeat breeder cattle by cytobrush technique and to study the comparative efficacy between different antibiotics and herbal intrauterine therapy in cattle with SE. The present experiment was carried out at Veterinary Gynaecology and Obstetrics Clinic, CVAS, RAJUVAS and private cattle dairy farms in Bikaner. Repeat breeding crossbred cows (>90 days in milking) in their spontaneous estrus were examined for cervico vaginal mucous (CVM) by rectal palpation and uterine cytology by cytobrush technique. The cows (n=60) having clear CVM and ≥ 4% polymorphonuclear cells (PMNs) in uterine cytology were diagnosed with SE. These cows were randomly divided into 4 treatment groups (group1, group2, group3 and group4) and control group and each group contained 12 animals. The cows in group1, group2, group3 and group4 were treated with intrauterine medicine Vodine-IU 30 ml, Metricare-IU 30 ml, Utriguard-IU 4 g dissolved in 60 ml of sterile water and Uraksha 25 ml, respectively for 3 consecutive days. In subsequent estrus, uterine cytology was repeated to observe reduction in PMNs (%) and the cows were inseminated. Animals those failed to conceive in first insemination were

subjected to second insemination in the next estrus. The pregnancy status was examined by estimating serum progesterone (P_4) concentration on day 24 and by rectal palpation on day 60 post AI. Complete blood count of these animals was also done on both the days of uterine cytology. The incidence of SE was observed as 36.80% (60/163) in the present study. Significant ($P<0.05$) decline in PMNs (%) was observed in all treatment groups. No significant ($P>0.05$) changes were observed for all the blood parameters viz. Hb, TEC, TLC and DLC between subsequent and spontaneous estrus in all the groups. The conception rate on day 24 by P_4 assay was 66.66, 58.33, 75, 83.33 and 25% in group 1, group 2, group 3, group 4 and control group, respectively. The serum P_4 concentration range was recorded 2.24 to 5.96 ng/dl and 2.49 to 2.51 ng/dl in pregnant and non pregnant cows, respectively. The conception rate on day 60 by rectal palpation was 66.66, 50, 66.66, 75 and 25% in group 1^{st}, group 2^{nd}, group 3^{rd}, group 4^{th} and control group, respectively. It was concluded that uterine cytobrush technique can be used efficiently for diagnosing SE in dairy cattle and the polyherbal intrauterine drugs may be a good alternative of antimicrobial intrauterine therapy.

Key words: Cytobrush, Endometritis, Levofloxacin, Ornidazole, Povidine iodine, Metronidazole, Cephalexin, Serratiopeptidase, Polyherbal, Repeat breeding.

एंडोमेट्रिटिस के साथ गायों में विभिन्न एंटीबायोटिक दवाओं और पॉलीहेर्बल अंतर्गर्भाशयी थेरेपी का तुलनात्मक अध्ययन

स्नातकोतर शोध ग्रंथ
पशु चिकित्सा एवं प्रसूति विभाग
पशु चिकित्सा एवं पशु विज्ञान महाविद्यालय, बीकानेर

उपस्थापन — सतीश कुमार
मुख्य उपादेष्टा — डॉ. संदीप धोलपुरीया

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वर्तमान अध्ययन फुराव करने वाले मवेशियों में साईटोब्रश तकनीक द्वारा सब क्लीनिकल एन्डोमेट्राईटिस की जांच एवं सब क्लीनिकल एंडोमेट्राईटिस वाले मवेशियों में विभिन्न एन्टीबायोटिक (रोगाणुरोधी) दवाओं एवं हर्बल अंतर्गर्भाशयी चिकित्सा के बीच तुलनात्मक प्रभावकारिका का अध्ययन करने के लिये आयोजित किया गया। वर्तमान प्रयोग पशु मादा रोग एवं प्रसूति क्लिनिक सी.वी.एस राजूवास एवं बीकानेर में निजी पशु डेयरियों में किया गया। फुराव करने वाली संकर गायों (90 दिनों से अधिक दूध में) का उनके स्वभाविक मदकाल में गुदा मार्ग जांच द्वारा मज्जा योनि श्लेष्म (सी.वी.ए.एम.) एवं साईटोब्रश तकनीकी द्वारा गर्भाशयी कोशिका जांच के लिये परीक्षण किया गया। स्पष्ट सी. वी.एम एवं गर्भाशयी कोशिका जांच में 4 प्रतिशत के बराबर या अधिक पॉली मॉफोन्यूक्लियर कोशिकाएं (पी.एम.एन.) रखने वाली गायों (एन-60) में सब क्लिनिकल एन्डोमेट्राईटिस निदान किया गया। इन गायों को बेतरतीब ढंग से 4 उपचार समूहों (समूह 1, समूह-2, समूह-3 और समूह-4) और नियंत्रण समूह में बांटा गया था। प्रत्येक समूह में 12 जानवर थे। समूह-1, समूह-2, समूह-3 और समूह-4 की गायों में अंतर्गर्भाशयी दवा क्रमशः वोडिन आईयू 30 मि.ली. मेट्रेकेयर 30 मि.ली., यूट्रीगार्ड आई यू 4 ग्राम 60 मिली जीवाणु रहित जल में घोलकर एवं यू रक्षा 25 मिली लगातार तीन दिन के लिये उपचारित की गई। आगामी मद में पी.एम.एन (प्रतिशत) में कमी की जांच के लिये गर्भाशयी कोशिका जांच को दोहराया गया एवं गायों को गर्भित किया गया। जो पशु प्रथम गर्भाधान में गर्भधारण होने में विफल रहे उनका अगले मद में पुनः गर्भाधान किया गया। औत्रिम गर्भाधान के चोबीसवें दिन सीरम प्रोजेस्ट्रोन (पी-4) सांद्रता के आकंलन द्वारा और 60 वें दिन गुदा मार्ग जांच द्वारा गर्भावस्था की जांच की गई। इन पशुओं की पूरी रक्त गणना भी गर्भाशयी कोशिका जांच वाले दोनों दिन की गई। वर्तमान अध्ययन में सब क्लीनिकल एन्डोमेट्राईटिस की घटना 36.80 प्रतिशत

(60/163) देखी गई। सभी उपचार समूहों में पी.एम.एन. में महत्वपूर्ण गिरावट (पी ढ0.05) देखी गई। स्वाभाविक एवं आगामी मदचक्र के बीच सभी समूहों में सभी रक्त मापदंडों का जैसे एच.बी., टी.ई.सी., टी.एल.सी. एवं डी.एल.सी. में कोई परिवर्तन (पीझ0.05) नहीं देखा गया। पी-4 परख द्वारा 24 वें दिन गर्भधारण दर समूह -1, समूह-2, समूह-3, समूह-4 एवं नियंत्रण समूह में क्रमशः 66.66, 58.33, 75, 83.33 एवं 25 प्रतिशत थी। सीरम पी-4 का विस्तार गर्भवती और गैर गर्भवती गायों में क्रमशः 2.24 से 5.96 एन.ग्राम / डी.एल. एवं 2.49 से 2.51 एन.ग्राम / डी.एल. पाया गया। 60 वें दिन गुदा मार्ग जांच द्वारा गर्भधारण दर समूह -1, समूह-2, समूह-3, समूह-4 एवं नियंत्रण समूह में क्रमशः 66.66, 50, 66.66, 75 एवं 25 प्रतिशत पाई गई। यह निष्कर्ष निकाला गया कि गर्भाशयी साईटोब्रश तकनीक डेयरी गायों में सब क्लीनिकल एन्डोमेट्राईटिस के निदान में फलोत्पादक हो सकती है और पोली हर्बल अंतर्गर्भाशयी दवाईयां रोगाणुरोधी अंतर्गर्भाशयी चिकित्सा का एक अच्छा विकल्प हो सकती है।