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Physico-Chemical Characterstics of Cervical Discharge in Endometritic Repeat Breeder Cow

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ABSTRACT

Cattle and buffalo encounter several reproductive problems during their productive life. Among various reproductive problems, Anoestrus and Repeat breeding are the two important problems which reduce fertility as well as productivity of the animals. A healthy uterus is the basis for high submission and conception rates. Vaginal discharge of animals may be turbid, thick and mucopurulent due to uterine and cervical infection. Cows with purulent, mucopurulent estrual discharge or containing white flakes and positive reaction to whiteside test is considered positive for endometritis. Forty (n=40) endometritic affected repeat breeder cows were selected which, were presented at veterinary hospital lying adjacent to Kumarganj area (Khandasha) and animal of villages in vicinity of university. The diseased cows were divided into five groups (G1, G2, G3, G4 and G5), each comprising of eight (n=8) animals. Overall recovery rate in the form of negative whiteside test was observed as 87.50% for levofloxacin, 75.00% for Giloy, 50.00 % for Ashwagandha & 75.00 % for Ashwagandha and Giloy combination and the conception rate was recorded as 75%, 50%, 37.50% & 50% for levofloxacin, Giloy, Ashwagandha & its combination. On the basis of present study, it is concluded that levofloxacin, Giloy, Ashwagandha and its combination have effective roll in the treatment of endometris in cows. Levofloxacin is more effective than ethno-veterinary medicine. Giloy, Ashwagandha and its combination having an immunomodulator, antibacterial, anti-inflammatory & anti-infective properties which can be utilize as alternative low cost medicine for treatment of endometritis in repeat breeder cows.

Key words: *levofloxacin, Tinospora cordifolia, Withania somnifera and its combination, Endometritic repeat breeder cows.*

INTRODUCTION

Livestock is an important source of income and employment in rural sector. Seventy per cent of livestock of country is owned by 67 % of small, marginal farmers and landless labour. Forty percent of the people living below poverty line are largely dependent on live stock for their precarious existence[33].

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Repeat breeders are animal cycling normally without any clinical abnormalities, but fail to conceive even after at least three successive inseminations. They have clinically normal reproductive tract, estrous cycles and estrous periods[37]. Repeat breeding is one of the important causes of infertility in cattle that results in delayed conception and increased calving interval, loss of milk production, reduction in calf crop, increased cost of treatment and culling of useful breeding animals leading to heavy economic losses to the dairy producers.

Incidence of repeat breeding in cows in various countries ranged from 10 to 18%[15]. The potential causes of the repeat breeding mainly include nutritional deficiency[28], anomalies of reproductive tract[2], hormonal asynchrony[3], delayed ovulation[14], inadequate luteal function[45] and other managemental factors[20]. The incidence of bovine endometritis varies from 15 to 57 %[38], and the variability mainly depends on sanitation practiced during peripartum and immediate postpartum period. The other important reasons are unhygienic A.I., service by an infected bull and systemic infections. The bacterial contamination of the uterus during and immediate to parturition virtually occurs in all the cows and if the contamination worsens due to bacterial colonization it leads to endometritic[43]. The uterus of cow also frequently becomes contaminated with specific and non – specific bacteria during pre and post-partum period, which may result in mucopurulent vaginal discharge. The uterine infection often resolved spontaneously. However, in some cow's discharge persists or become foul smelling and produces metritis or endometritic with a consequential effect on fertility. Endometritis, particularly subclinical endometritis has been the major cause of repeat breeding[1]. Furthermore, it is bacterial endometritis that constitutes major concern especially in cross bred cattle[21]. Subclinical endometritis alter the physico-chemical properties of cervical mucus and therefore, examination of cervical mucus for appearance, consistence and pH may be valuable in its diagnosis. As for as therapeutic approach to repeat breeding cow is concerned, it involves either antibiotics and antiseptics or hormonal therapy[13,54]. Periparturient complication like retained placenta, dystocia, twins, dead foetus, milk fever, etc., increases the risk of uterine infection because they delay involution and reduces the bovine ability to control uterine infections. Increases of pH 7.5-8.0 of vaginal discharges were in agreement with previous studies in cattle with endometritis[39,48]. This could be due to bacterial contamination of uterine fluids and the increased pH is not suitable for survival of spermatozoa and embryo in the uterus[36,44]. However, during infection bacterial toxin binds directly to uterine epithelial and stromal cell to stimulate the release of prostaglandins E2 which is luteotropic and may prevent luteolysis[5]. The bovine uterus is an anaerobic environment, thus antibiotics chosen for intrauterine use must be active in the absence of oxygen[10]. In addition, most antibiotics and chemicals depress the activity of uterine neutrophils and interfere with the uterine defence mechanism; therefore, the potential benefit of their use must be carefully weighed against their deleterious effect[52]. Organisms that cause postpartum uterine infections are usually sensitive to penicillin, but bacterial contamination during the first several weeks after calving produce penicillinase, which renders the drug ineffective if applied locally. By 30 days postpartum these organisms are usually eliminated, and intrauterine treatment with penicillin is more likely to be effective after that time[27]. Herbal preparation called as phytotherapy were used for treatment of infectious endometritis and repeat breeding condition in cow also.

MATERIALS AND METHODS

Preperation of experimental Animal:

Gynaecological cases were screened to identify repeat breeder cows. The cases of endometritic repeat breeder cow which, were presented at Veterinary hospitals lying adjacent to Kumarganj area (Khandasha) and Animal of villages in vicinity of university were used for the study. The cervical mucus from these cows were collected at pre and post-treatment estrus, visually examined and tested for change in colour, intensity to white side test and pH. The cases were also identified by using ultrasonography. In ultrasonography the endometrium is thick and copious mucous is present in uterus. The effect of different treatment on haematological & serum biochemical profile was also analized.

Selection of repeat breeder cows affected with endometritis:

Forty (n=40) repeat breeder cow were selected for the study. The repeat breeder animals were confirmed by history, thorough per rectal examination and physico-chemical characteristics of cervical mucus. The animals with purulent or mucopurulent estrual discharge or containing white flakes and positive reaction to white side test[29] were considered as positive for endometritis. Moreover, animals with history of repeat breeding and clear discharge but positive to white side test were also be included in the present study.

Collection of cervical mucus:

Estrual cervical mucus from all the animals was collected at 8 to 12 hr after the onset of behavioural estrus as described by Dhillon *et al*[8]. The vulvar and perineum region were washed with antiseptic solution and wiped properly with absorbable sterile cotton. The internal genitalia were massaged (per rectum) and mucus flown out was collected in sterilized disposable petridish.

White side test:

For conducting white side test, 1 ml of estrual cervical mucus was heated with equal volume of 5 to 10% sodium hydroxide (Merck, Mumbai, India) up to boiling point and after cooling the intensity of colour changes were studied and graded as normal (turbid or no colour), mild infection (light yellow colour), moderate infection (yellow colour) and severe infection (dark yellow colour).

pH:

The pH of the cervical mucus was assessed using pH indicator strips as described by Tsiligianni *et al*[51]. Each pH strip was inserted approximately 5 cm into each cow's vagina and pressed against the left lateral vaginal wall. Strips were maintained in the vagina for approximately ten seconds before removal. The nature of cervical mucus has pronounced influence on the fertilizing capacity of the spermatozoa in female reproductive tract and its physical properties have direct relationship with the fertility status of the animals[32]. Under the influence of gonadal hormones, the cervical mucus undergoes cyclic changes in physical and chemical properties[11]. At the time of estrus, because of hormonal changes, the physical properties of cervical mucus viz. colour, consistency, elasticity, fern pattern and pH gets altered in repeat breeding cows which ultimately results in lowered fertility[25]. Semen immediately on deposition comes in contact with estrual mucus, the phyio-chemical properties of which affect the spermatozoa viability and conception rate[24]. Clear cervical mucus is conducive for sperm penetration and conception, whereas sperm motility is arrested by turbidity of cervical mucus[7]. It has been found that thick cervical mucus is due to differences in arrangement of glycoprotein molecules of cervical mucus which significantly influence the sperm penetrability[7] thereby, preventing fertilization process. The pH of cervical mucus ranged from 6.5 to 9.0 with a mean of 7.95 ± 0.096 in repeat breeding cows[22].

Statistical analysis

The data obtained in this study were analyzed by using suitable statistical methods[49].

RESULTS AND DISCUSSION

The therapeutic study was carried out to evaluate therapeutic efficacy of antibiotic (Levofloxacin) and ethno-veterinary medicines (*Tinospora cordifolia*, *Withania somnifera* and its combination) on endometritic repeat breeder cow. The cervical mucus and blood sample were collected from affected animal at pre and post treatment estrus.

The blood sample was collected from affected animal at pre and post treatment estrus. Effect of antibiotic and herbal treatment on hematological profile and pregnancy rate were evaluated and data were analyzed experimently and results of different parameter are bellow;

Physico-chemical characteristic of cervical mucus:

Nature of cervical mucus:

Cervical mucus discharge observed in all affected animals of present study was Mucupurulent or hazy, which could be due to uterine and or cervical infection whereas, Mucupurulent or hazy discharge in G1, G2, G3, G4 & G5 group of endometritic affected repeat breeder cow were reported as 100%, 12.5%, 25%, 50% & 25% respectively in post treated animal. Untreated control animals were not expressed clear discharge, whereas all normal cyclic animals were shown clear discharge. In antibiotic treated group (G2) animals were expressed clean and mucupurulent or hazy mucus as 87.5% and 12.5% whereas, corresponding figures for Giloy treated group (G3) were 75% and 25%. In Ashwagandha treated group (G4) animal was expressed as 50% clear, 12.5% mucupurulent and 37.5% hazy discharge whereas, 75% clear, 12.5% mucupurulent and 12.5% hazy discharge obtained in (G5) group of post treated animal. Effect of Antibiotic (Levofloxacin) and Herbal drugs (*Tinospora cordifolia,Withania somnifera* and its combination) on physico-Chemical characterstics of cervical discharge in endometritic repeat breeder cow given in table -1.2. Our findings are in agreement with the observation of Kumar *et al*[17], whereas, Verma[53], reported lower recovery rate in treatment with levofloxacin. Our findings were self-explanatory that *Tinospora cordifolia*, *Withania somnifera* & its combination could be utilized for treatment of endometritis. This recovery might be due to antimicrobial properties of these herbal drugs. Direkbusarakom *et al*[9]., recorded direct antibacterial properties of *Tinospora cordifolia*. The reduction in the bacterial population was due to immunostimulatory activity of *Tinospora cordifolia*. Ashwagandha treated group of animals were expressed higher recovery rate than control. This recovery might be due to antibacterial & immunomodulatory properties of Ashwagandha. Our results are in agreement with the observation of Mishra *et al*[23]., and Rahi *et al*[31].

Mucupurulent & hazy discharge is due to uterine and cervical infection[39,46]. Similar finding was also recorded by Salphale *et al*[41]., Rangnekar *et al*[32]., and Modi *et al*[24]. Generally, clear cervical mucus has been reported to be a conductive factor for sperm penetration and conception whereas, turbidity in estrual mucus arrested sperm motility[12]. Uterine and cervical infection suggests that during parturition, the physical barrier of the cervix, vagina and vulva are compromised, providing the opportunity for bacteria to ascend in the genital tract. Normally, these infections are cured naturally by rapid involution of the uterus, discharge of the uterine contents and mobilization of the host defense including mucus, antibodies and phagocytes. Post parturient complication like retained placenta, dystocia, twins, dead foetus, milk fever etc. increases the risk of uterine infection because they delay the involution and reduces the bovine's ability to control uterine infections. Rearing in unhygienic condition

and insemination in non-sterile condition also cause uterine infection.

Colour reaction to white side test:

White side test was performed in the cervical mucus samples collected at pre-treatment and post treatment estrus. On the basis of visibility, it was divided in to dark yellow, moderate yellow and light yellow as shown in (Table - 1.1). The colour change of cervical mucus observed in white side test might be due to neutrophil infiltration and metrorrhagia which was normally seen during estrogen dominance[26]. Colour of cervical mucus was observed as 75% dark yellow and 25% moderate yellow in untreated control animals whereas, in antibiotic treated group 50% dark yellow, 25% moderate yellow and 25% light yellow colour recorded before treatment respectively. Whereas, only 12.5% animal expressed light yellow colour after treatment with levofloxacin. Animal exhibited better recovery rate after levofloxacin treatment than herbal therapeutics and control group. These finding are comparable with the observation of Bhattacharyya *et al*[4], Kumar *et al*[18]., and Verma[53]. On the basis of present findings, it can be concluded that in field condition, intrauterine infusion of fluoroquinolones (levofloxacin) are very much effective in treating clinical and sub clinical metritic cow. Levofloxacin third generations' fluoroquinolones have a potential of use for the treatment of uterine infection because of its spectrum cover over anaerobes. A *Tinospora cordifolia* herbal medicine less effective than levofloxacin but significantly higher recovery rate was observed for the treatment of endometritis than control. This recovery rate suggested the possible antimicrobial effect of *Tinospora cordifolia* and can be utilize as alternative of costly allopathic formulation for treatment of endometritis.

Tinospora cordifolia has been suggested to have direct antibacterial effect Besides causing nonspecific stimulation of immune response[35], it induces leukocytosis[50] and activates macrophages[30]. This may be the reason for white pus like mucoid discharge from the genital tract on the subsequent days following treatment. Rege and Dahanukar[34], reported that it increases the phagocytic and killing capacity of peritoneal macrophages in rats and man in a dose dependant manner. Further *Tinospora cordifolia* enhances antibody production by proliferation of B-cell[40]. The reduction in bacterial population in the present study may be due to this immunostimulatory activity of *Tinospora cordifolia*. The activated phagocytic cells are more competent in phagocytizing invading bacteria, while the raised antibody level help in better opsonization of bacteria for better phagocytosis or, cause direct lysis of bacteria. Similarly, Singh[47] reported reduction in bacterial load of milk sample in cases of mastitis in cow treated with intramammary infusion of aqueous extract of T. cordifolia. In Ashwagandha (G4) treated animals were expressed dark yellow, moderate yellow & light yellow colour of cervical mucus as 37.50%, 37.50% & 25%, respectively in pretreated animal and 12.5%, 25% and 12.5%, respectively in post treated observations. The results comprise that 50% animal recovered from infection after administration of *Withania somnifera*, but rate of recovery was lower than that recorded in antibiotic, Giloy and Giloy & Ashwagandha combination. Similar finding was also observed by Rahi *et al*[31]. Maximum cow shows thick mucus before treatment which is in agreement with the finding of Salvaraj *et al*[42], who also recorded a higher percentage (73.63%) of repeat breeder cow with thick mucus discharge.

In (G5) group, pretreated animals exhibited dark, moderate & light yellow colour mucus as

25%, 50% & 25%, respectively whereas, moderate and light yellow colour precipitation was observed as 12.5% each after treatment with *Withania somnifera & Tinospora cordifolia* combination. Post treated observation expressed that 75% animal recovered from infection after administration of *Withania somnifera & Tinospora cordifolia combination*. Our results agree with the finding of Bhoshey *et al*[6]. Present findings demonstrate the potential efficacy of Giloy sat & Ashwagandha powder as remarkable antimicrobial activity *in-vivo.*

pH:

The pH value of normal cyclic, repeat breeder and different treatment groups of cow were given in the (Table- 1.1 and Figure-1.1). Increase a pH (7.5-8.0) of vaginal discharge in repeat breeder was in agreement with previous studies[39,46]. This could be due to bacterial contamination of uterine fluids and increased pH is not suitable for survival of spermatozoa and embryo in the uterus[36]. The uterine infection also appears to disrupt the endocrine function of the endometrium and luteolysis of the ovarian corpus luteum. Luteolysis is induced by the release of PGF2α from the uterine endometriun, which passes to the ovary from the uterus *via* localized vascular pathways, to initiate the regression of the corpus luteum. However, during infection, bacterial toxin binds directly to uterine epithelial and stromal cell to stimulate the release of prostaglandin E2, which is luteotropic and may prevent luteolysis[5].

After treatment with levofloxacin, *Tinospora cordifolia*, *Withania somnifera* and combination of *Tinospora cordifolia & Withania somnifera*, cervical mucus pH was observed within the normal range. Similar, finding with levofloxacin treatment was also reported by Kumar *et al*[18]., and Verma[53]. Normal range pH in herbal treated animal might be due to this antimicrobial activity.

Conception rate:

The overall recovery rate in levofloxacin treated group was recorded as 87.50% and in Giloy, Ashwagandha and its combination was reported as 75%, 50% & 75%, respectively. The conception rate was also recorded as 75% for levofloxacin, 50% for Giloy, 37.50% for Ashwandha & 50% for Giloy and Ashwandha combination as presented in (Table- 1.2). The recovery rate was highest in levofloxacin treated group than Giloy, Ashwagandha and its combination, might be due to higher efficacy of levofloxacin in presence of debris.

Bhattacharyya *et al*[4]., and Markandeya *et al*[19]., have found higher conception rate with levofloxacin than the present finding. In herbal treated group, significantly higher conception rate was observed as compare to control, a self-explanatory that these ethno-veterinary medicine must had reduced bacterial population lead to reduced bacterial metabolites and also regulate hormonal control specially estrogen to reduce infection persist in uterus.

Krishna *et al*[16]., also reported that Giloy is widely used in veterinary folk, Ayurveda and other system of medicine for its general tonic, immunomodulatory, antioxidant, antibacterial, hepatoprotective and anti-inflammatory properties. The conception rate of *Tinospora cordifolia* had better result than control. It indicated a possible ameliorative effect of *Tinospora cordifolia* on uterine infection.

Ashwagandha was considered as adaptogenic which is a non-toxic herb that work on non-specific basis to normalize physiological function working on the hypothalamus pituitary axis and the neuroendocrine system.

Table 1.1: Effect of Antibiotic (Levofloxacin) and Herbal drugs (*Tinospora cordifolia,Withania somnifera* and its combination) on physico-Chemical characterstics of cervical discharge in endometritic repeat breeder cow

	parameters	Control		Treatment								
		G1		G2		G3		G4		G5		G○
1.	Nature of cervical mucus	Pre	post	pre	post	pre	post	pre	post	Pre	Post	Cyclic
	Mucupurulent	5(62.50%)	4(50.00%)	4(50.00%)	1(12.5%)	3(37.50%)	1(12.50%)	3(37.50%)	1(12.50%)	2(25.00%)	1(12.50%)	0(0.00%)
	Hazy	3(37.50%)	4(50.00%)	4(50.00%)	0(0.00%)	5(62.50%)	1(12.50%)	5(62.50%)	3(37.50%)	6(75.00%)	1(12.5%)	0(0.00%)
	Clear	0(0.00%)	0(0.00%)	0(0.00%)	7(87.50%)	0(0.00%)	6(75.00%)	0(0.00%)	4(50.00%)	0(0.00%)	6(75.00%)	8(100%)
2.	**White side test**											
	A . Positive	8(100%)	8(100%)	8(100%)	1(12.50%)	8(100%)	2(25.00%)	8(100%)	4(50.00%)	8(100%)	2(25.00%)	0(0.00%)
	Dark yellow	6(75%)	4(50%)	4(50.00%)	0(0.00%)	5(62.50%)	1(12.50%)	3(37.50%)	1(12.50%)	2(25.00%)	0(0.00%)	0(0.00%)
	Moderate yellow	2(25%)	1(12.50%)	2(25.00%)	0(0.00%)	2(25.00%)	0(0.00%)	3(37.50%)	2(25.00%)	4(50.00%)	1(12.50%)	0(0.00%)
	Light yellow	0(0.00%)	3(37.50%)	2(25.00%)	1(12.50%)	1(12.50%)	1(12.50%)	2(25.00%)	1(12.50%)	2(25.00%)	1(12.50%)	0(0.00%)
	B. Negative	0(0.00%)	0(0.00%)	0(0.00%)	7(87.50%)	0(0.00%)	6(75.00%)	0(0.00%)	4(50.00%)	0(0.00%)	6(75.00%)	8(100%)
3.	**pH of cervical mucus**	8.26±0.11	7.98±0.12	8.10±0.16	7.05±0.10	8.13±0.15	7.45±0.09	8.03±0.19	7.68±0.06	8.08±0.21	7.33±0.08	7.03±0.11

Table: 1.2: Effect of Antibiotic (Levofloxacin) and Herbal drugs (*Tinospora cordifolia, Withania somnifera* and its combination) on cervical mucus, recovery rate and conception rate in endometritis affected repeat breeder cows

S.N	Particulars	G0	G1	Treatment groups			
				G2	G3	G4	G5
1.	No of animal utilized for study	8(100%)	8 (100%)	8 (100%)	8(100%)	8(100%)	8(100%)
2.	No of animal positive white side test before treatment	0(0.00%)	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)
3.	Animal show white side test negative after treatment	8(100%)	0(0.00%)	7(87.50%)	6(75%)	4(50%)	6(75%)
4.	Animal inseminated	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)
5.	Animal pregnant	6(75%)	(0.00%)	6(75%)	4(50%)	3(37.50%)	4(50%)

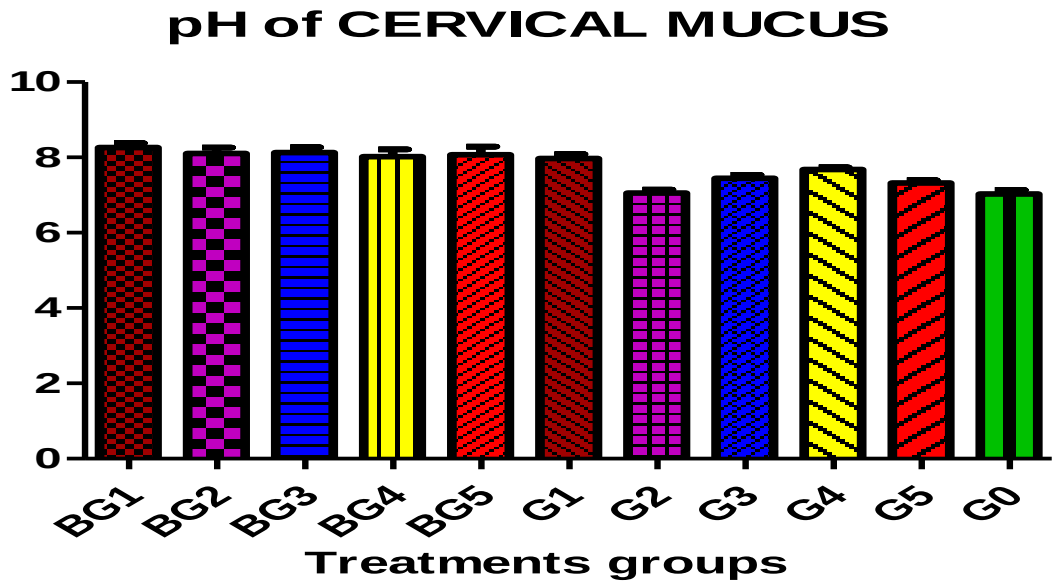

Fig. 1.1: Graphical representation of pH of cervical mucus values (Mean±S.E) in Endometritic repeat breeding, treated and cyclic cow with Levofloxacin, *Tinospora cordifolia, withania somnifera* and combination of both.

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