Original Research Article

PHYSICO-CHEMICAL CHARACTERISTICS OF CERVICAL MUCUS IN REPEAT BREEDER CROSSBRED COWS FOLLOWING CEFTIOFUR THERAPY

Abstract

The present study was carried out to investigate physio-biochemical attributes of cervical mucus following treatment of repeat breeder crossbred cows (HF×K) with ceftiofur crystalline free acid @ 6.6 mg/kg body weight administer separately in two groups of cows through intramuscular (Group-I) and subcutaneous route (Group-II). Each group included 15 repeat breeder cows whose selection was random and based on history of conception failure, gyneco-clinical observation of genitalia and number of artificial insemination per conception.The findings envisaged higher percentage of thick consistency (53.33%) and cloudy colored cervical mucus (93.33%) which returned to their normal characteristics of thin and clear type at post-treatment oestrus in both groups (60.00 and 80.00 %, Group-I; 66.67 and 93.33%, Group-II).The calcium and inorganic phosphorus contents of cervical mucus were significantly higher ($p<0.05$) at post-treatment oestrus in repeat breeders of Group-I and II whereas total cholesterol declined following treatment (24.05±1.85 and 23.45±1.91 mg/dl) then pre-treatment values (31.12±2.68 and 35.40±4.74 mg/dl) in two groups of repeaters but difference was significant only in Group-I. On the other hand, cervical mucus concentration of sodium, potassium and acid phosphates decreased while total lipids and alkaline phosphatase increased non-significantly at post-treatment oestrus in repeat breeder cows of both groups.

It is concluded from the finding that ceftiofur therapy of repeat breeder cows was beneficial to restore normalcy of physio-biochemical properties of cervical mucus in both groups.

Keywords: cervical mucus, ceftiofur, repeat breeder, conception rate

1. INTRODUCTION:

The profitable rearing of dairy cattle lies in ensuring proper and optimal reproductive rhythm of each individual female within the normal physiological limits. Any deviation in breeding rhythm results in

progressive economic losses due to widening of the dry period, the calving interval as well lactation during the life time of the animals. Among the deviations of the reproductive origin, repeat breeding is one of the important and commonly encountered disorders in cross-bred cows, whose incidence has been reported from 5.5 to 33.33 percent in our country (Saxena, 2004). The affected animals have a regular oestrus cycle and appear normal on superficial clinical examination but fail to become pregnant following three or more breeding to artificial insemination /or natural service (Bartlett *et al.,* 1986).

The diagnosis and therapy of repeat breeder are of the major concern in order to maintain efficient dairying and normal breeding rhythm that animal give birth to a healthy calf every 12-13 months and be in milk to at-least 300 days in lactation. However, the diagnosis of repeat breeder is difficult due to multi-factorial etiology and routine investigation of affected females is restricted to clinical examination of the genitalia to draw inferences into the exact cause involved in conception failure. Therefore, it should infact also include a careful investigation of the physical, physiological and biochemical status of the factors involved in the overall reproductive process besides important cyclic secretary changes during the oestrus period which may have profound effect on the fertility of female.

During the oestrus apart from other changes, cervical mucus is produced by mucus secretary cells that line the cervical epithelium (Cortes, 2014). The consistency of mucus is visco-elastic and varies with the stages of oestrous cycle. It has profound effect on fertilizing capability of the spermatozoa. The cervical mucus at the time of ovulation provides a suitable environment for the maintenance of metabolic activity of spermatozoa and helps in its penetrability, migration and livability. Deviation in oestrual mucus properties from the normal may thus affect the spermatozoa adversely disrupting the fertilizing process and culminate

into infertility. Therefore, physical and biochemical properties of oestrual mucus need to be investigated in repeat breeders.

Intrauterine therapy of antibiotics has been used widely over the years to combat the uterine infection in-spite of their restriction in food producing animal. However, the success of such therapies varies from beneficial to no effect for recommendation due to the fact that many of drugs like penicillin is inactivated through bacterial production of enzyme penicillinase while tetracyclines are required in large dose to get tissue concentration which could be toxic to the animal (Olson *et al.,* 1985). Besides, anaerobic environment of uterus makes aminoglycoside group of antibiotics: gentamicin, kanamicin, streptomicin and neomycin ineffective because of non-availability oxygen for their activity. Bacterial activity of sulfonamides, aminoglycosides and nitrofurazones is also reduce in environment containing blood, pus, necrotic tissue, products of leukocytes and tissue damage. Further the poor response intrauterine treatment is also likely to manipulation which traumatizes the genitalia thereby inhibiting the phagocytosis essential for clearance of infection (Paisley, 1986).

The disadvantages associated with intrauterine application of antibiotics hypothesized to advocate systemic treatment of diseased uterus which might be efficacious and does not result in antibiotic residue in milk. In the recent past, systemic treatment of clinical endometritis 21 days to 27 days post-partum with ceftiofur on three consecutive day has been reported to improve clinical cure rate and conception rate compared with a protocol consisted of 2 treatment with PGF2alfa analogue (cloprostenol) at 14 days interval (Risco *et al.,* 2003). The present investigation was, therefore, under taken on systemic administration of ceftiofur crystalline free acid to determine its efficacy for the treatment of repeat breeder cross-bred cow with the objective to study the pre-and post therapeutic physio-chemical characteristics of cervical mucus of repeat breeder cows.

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL PROTOCOL

2.1.1 Selection of Experimental Animals

A total of 36 repeat breeders cross bred cows from Sherpura village of Banas Dairy Palanpurwere selected randomly on the basis of gyneco-clinical history of regular oestrus and appeared normal on clinical examination but failed to conceive following three or more breeding to artificial insemination with semen of good quality. The selection was restricted further to those animals which did not have any disorder of ovulation but showed abnormal oestrual cervical mucus detected by white side test (Bhat *et al.,* 2014).

2.1.2 Groups of Animals

The selected animals were divided in the three groups. The each treatment group was consisted of 15 repeat breeder cows while six normal cyclic cows were assigned to control group.

Group-I The animals of this group received single dose of Ceftiofur Crystalline Free Acid (Excede, Zoetis Inc. 2605 East Kilgore Road, Kalamazoo, Michigan 49001, USA) @ 6.6 mg/kg body weight through intramuscular route.

Group-II A total of 15 cows were categorized in this group to receive a single injection of similar preparation at the same dose rate by subcutaneous route.

Group-III Sixnormal cyclic animals which conceived within three breeding were kept as control. No medication was given to these cows.

2.2 OESTRUS DETECTION

The oestrus was detected by observing external signs of heat such as bellowing, frequent urination, presence of cervical mucus, edema of vulva and congestion of vulvar mucus membrane. Each cow identified

oestrus was bred by artificial insemination using frozen-thawed semen of good quality.

2.3 COLLECTION OF CERVICAL MUCUS

Oestrus cervical mucus was collected aseptically by recto-vaginal technique from each oestrus cow before breeding. The external genitalia was cleaned with mild antiseptic solution and dried with cotton. The mucus samples were obtained by aspiration using a sterilized plastic sheath connected to a disposable syringe. The plastic sheath was followed per-rectally to confirm its position in the cervical canal (Panangala*et al.,* 1978).

The first sample of cervical mucus from the repeat breeder cows was collected at the time of oestrus prior to commencement of treatment whereas subsequent sample was followed at the time of post therapeutic oestrus. The samples thus obtained were transferred to suitable containers for transportation in the wide mouth thermos containing ice crystals to the laboratory for physical and various biochemical constituents.

2.4 PARAMETERS TO BE STUDIED

The bovine cervical mucus is extremely inconvenient to handle with conventional laboratory apparatus (Gibbons and Sellwood, 1973). Therefore, samples were diluted with triple glass distilled water and thoroughly mixed by means of magnetic stirrer (Reddy, 1974)

2.5.2 Physical Properties of Cervical Mucus

2.5.2.1 Color

The color of cervical mucus was classified as clear or cloudy (milky white or whitish flacks) as per the method described by Sukhdeo and Roy (1971).

2.5.2.2 Consistency

The mucus consistency was classified as thin, medium or thick (Reddy, 1974). The mucus which flowed freely and rapidly on a tilted glass slide was graded as thin and sticky mucus slowly moved on a tilted glass slide was graded as thick.

2.5.2.3 Hydrogen Ion Concentration (pH)

The pH of cervical mucus was observed using wide range pH strips, 1-14 (Merck Pvt. Ltd, Mumbai) immediately after collection of samples (Rangnekar *et al*., 2002).

2.5.3 Biochemical Profiles of Cervical Mucus

Total protein, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, alkaline phosphatase, acid phosphats were estimated in cervical mucus of repeat breeder and normal cyclic cows using standard kits (AGAPPE Diagnostic Ltd., Agappe Hill's, Dist.-Ernakulam, India) whereas total lipid was estimated from cervical mucus using standard kit (CHEMELEX, S. A., Pol. Ind. Can Castells. Industria 113 Nau J08420 Canovells, Barcelona, Spain).

2.6 STATISTICAL ANALYSIS

The data were analyzed statistically to know the changes in cervical mucus of normal cycling and repeat breeding crossbred cows by using Post-hoc test of mean by Duncan's test (Snedecor and Cochran, 1994).

3. RESULT AND DISCUSSION

3.1 PHYSICAL CHARACTERISTICS OF CERVICAL MUCUS

3.1.1 Color

The cervical mucus from all normal and repeat breeder crossbred cows was examined by visual examination to determination the color variation which was classified as clean and cloudy. The cervical mucus of 6.67 and 13.33 per cent cows in Group-I and II of repeat breeders showed clear color during oestrus prior to administration of ceftiofur whereas in Group-III, which included normal cyclic cows, all the animals showed clear

color mucus. The percentage of clear color mucus following treatment increased to 80.00 and 33.33 per cent in Group-I and II, respectively (Table 1). On the other hand, the cervical mucus of 93.33 and 86.67 cows in Group-I and II of repeat breeder showed cloudy color mucus during oestrus whereas none of the normal cyclic cows in Group-III exhibited cloudy color mucus. Following treatment, the percentage of cows showed cloudy mucus at post treatment oestrus 77decreased to 20.00 and 6.67 in Group-I and II, respectively (Fig..1).

The data pertained to color of cervical mucus of normal and repeat breeder crossbred cows (Table 1) showed that majority of repeat breeders secreted cloudy color mucus prior to treatment with ceftiofur (93.33%, Group-I and 86.67%, Group-I) whereas none of the normal cyclic cows secreted cloudy color mucus. A high percentage of cloudy color mucus in repeat breeder cows was also reported by earlier author (Satheskumar and Punniamurthy, 2007 and Modi *et al.*, 2011) whereas Methai*et al.,* (2005) reported the purulent discharge in oestrus mucus of repeat breeder cows. The transparent, opaque, milky and yellowish colored cervical mucus in repeat breeder cows were reported by Bhat *et al.* (2015). The author further reported higher percentage of ovulation in cows which showed opaque colored mucus which indicated the presence of infection. The color of mucus may be affected with contamination, uterine secretion as well as local infiltration of leukocyte. During mid cycle, uterine glands are more active and produce uterine milk which is thick yellowish fluid resembling pus and contains flat globules in heavy concentration.

Following treatment of repeat breeder cows, the color of cervical mucus returned to clear in majority of cows (80.00%, Group-I and 93.33% in Group-II). Shehata *et al.* (1978) reported that cervical mucus secretion in cows and buffalos played active role in the process of reproduction and any physical alteration in secretion affects the process of fertilization. Clear cervical mucus is conducive for sperm penetration and thereby gives better conception rate Hamana*et al.* (1971) whereas turbidity

arrests sperm motility in oestrual mucus (Dev *et al.,* 1997) and thus lowered the conception. Shehata *et al.* (1978) further reported the presence of plentiful, fairly adhesive cervical mucus containing no cellular debris at oestrus. The present finding of clear colored cervical mucus in repeat breeder crossbred cows following treatment and turgidity resembled to cervical mucus of normal cyclic groups wherein no cellular debris was found, seem to in agreement with finding of above workers because low conception rate and more cloudy color mucus was exhibited by repeat breeder crossbred cows in this investigation.

3.1.2 Consistency

The consistency of cervical mucus was classified as thin, medium and thick. The data pertained to three types of consistency has been presented in Table1. The cervical mucus of repeat breeder crossbred cows prior to treatment had a thick consistency in both treatment Groups (53.33%, Group-I and 40.00%, Group-II) than that of normal cyclic cows (6.67%, Group-III). The thick consistency of mucus declined to 6.67 per cent at post treatment oestrus each in treatment Group-I and II.

In normal cyclic group, the thin, medium and thick consistency of oestrus mucus was observed in 66.67 and 33.33 per cent cows, respectively whereas none of the normal cyclic cows had thick consistency of mucus. The percentage of thin and medium consistency mucus was observed to 60.00 and33.33; 66.67 and 26.67 per cent during post treatment oestrus in Group-I and II, respectively.

The cervical mucus of thin and medium consistency (66.67 and 33.33%) was secreted by normal crossbred cows. However, repeat breeder cows secreted mucus of all three categorized but difference in cervical mucus of normal and repeat breeder cows was notable in respect to medium and thick consistency. The percentage of thin consistency of cervical mucus was higher in normal cows whereas thick mucus secreted by repeat breeder prior to initiation of their treatment. In earlier studies

also repeat breeder cows have been reported to secret cervical mucus of thick consistency (Sharma and Tripathi, 1987; Dev *et al*., 1997 and Modi *et al.,* 2011).

Agraval and Dutta (1977) reported that during most of the ovulatory phase, the cervical mucus was scanty, thick, viscous and also resistant to sperm penetration. Immediately prior to ovulation under estrogenic influence, the mucus became thin and its penetrability reaches maximum. Thus, the conception most likely occurs when cervical mucus is most fluid. The findings of Kumar *et al*. (2010) and Sharma *et al.* (2011) also indicated higher conception in cows having clear and stringy oestrus mucus than those with thick consistency mucus. The consistency of cervical mucus influences the spermatozoa penetration (El-Nagar and Bakshi Horvath, 1971). It was also reported that cervical mucus becomes minimum, cohesive and contained little debris during luteal phase wherein spermatozoa transport is inhibited causing lower conception leading to poor infertility in repeat breeder cows. Following treatment of repeat breeder cows, the consistency of cervical mucus returned to thin type in 60 and 67 per cent cows in Group-I and II, respectively which was at par to normal cyclic cows. These findings revealed that consistency of cervical mucus could be a fair indication to assess the effect of ceftiofur treatment on breeding behavior of cows that showed very negligible thick consistency of mucus vis-a-vis better conception in both treatment groups. The present findings are agreeable to Kumar *et al.* (2010) who also reported a significant decrease in thick consistency of cervical mucus coupled with higher conception.

3.1.3 Hydrogen Ion Concentration (pH)

Table1 showed the pH of cervical mucus in treatment and control groups. The average values of 8.13±0.13 and 8.40±0.11 was observed in repeat breeder cows prior to initiation of treatment with ceftiofur which were higher than the normal cyclic cows (7.75±0.21). The differences

were significant ($p<0.05$) between values of normal cyclic cows and repeat breeders of only Group-II (Table 2).

Following treatment, the pH of cervical mucus during oestrus decreased to averaged of 7.87±0.13 than that of preceding values of 8.13±0.13 in Group-I whereas corresponding values in Group-II were 7.20±0.18 and 8.40±0.11, which did not differ significantly.

The pH of cervical mucus in repeat breeder crossbred cows of both treatment groups tend to be alkaline in nature whereas it was towards isotonic side in normal cyclic cows (Table.1). Alkaline pH of cervical mucus in repeat breeder crossbred cows than the normal cyclic cows has also been reported in cross bred (Wani *et al.*, 1982) Kankrej (Modi *et al*., 2011) and Deoni cows (Dodamani*et al.,* 2010). A significant decline in cervical mucus pH of repeat breeder cows after the treatment with various antibiotics, lugol's and alcoholic extract of neem bark is evident from the earlier studies (Kumar *et al.,* 2011; Kumar *et al.,* 2013 and Palanisami *et al.,* 2014). However, a non significant decline was observed in repeat breeder cross bred cows following their treatment with ceftiofur.

The alkaline pH of oestrual cervical mucus in repeat breeders in comparison to normal cyclic cows was reported to be associated with bacterial infection (Bhat *et al.*, 2015). Verma *et al*. (2014) reported that conception rate decreased in either side of pH between 7.5-8.0, and it resulted maximum pregnancies (42.0%) in comparison to pH more than 8.0 and 7-7.5. The findings of alkaline pH in present study are in agreement to these authors and are further supported with better conception at average pH of 7.75±0.21 in normal cyclic cows and also in cows of treatment Group-I and II where post-treatment pH of cervical mucus turned to slightly alkaline. The improved conception rate following treatment might be attributed to better progressive sperm motility and suitable and congenial uterine environment in alkaline pH of cervical mucus as reported earlier Rangnekar *et al.* (2002).

3.2 BIOCHEMICAL PROFILE OF CERVICAL MUCUS

3.2.1 Macro-Minerals

3.2.1.1 Calcium

The mean values along with corresponding standard errors of calcium content in cervical mucus of repeat breeder crossbred cows were 8.53±0.64 with a range of 4.8 to 13.20 mg/ml in Group-I whereas the corresponding values for Group-II repeat breeder cows were 10.16±0.55 with a range of 7.2 to 13.8 mg/ml. The differences were non-significant. On the other hand, the post-treatment average concentration of calcium increased and was found to be 11.12±0.86 with a range of 6.0 to 17.4 mg/ml in Group-I and 13.92±0.77 with a range of 9.0 to 18.6 mg/ml in Group-II. The differences between pre- and post-treatment values were significant ($p<0.05$) in both treatment groups (Table 4).

In case of normal cyclic crossbred cows, the average values of calcium was found to be 12.17±0.52 with a range of 10.2 – 13.8 mg/ml which were almost in close proximity of post-treatment values in two groups of cows, which did not differ significantly.

It is apparent from Table 3 that a level of calcium was lower in cervical mucus of repeat breeders than the normal cyclic cows. The present findings does not corroborate the earlier observations of higher calcium in repeat breeders (Umashankar *et al*., 1984and Soylu *et al*., 1999) but are in agreement to those reported by Sood *et al*. (2013). Further, the calcium level discerned in normal cows (12.17±0.52 mg/ml) is at par with earlier findings (11.04±2.62 mg/ml) reported by Umashankar *et al*. (1984). The author further reported a wide range of calcium values between normal and repeater cows (11.04±2.62 v/s 23.32±0.25 mg/ml).

The post-treatment calcium levels in Group-I and II increased significantly than the pre-treatment levels. However, the calcium level at this juncture was almost similar to that observed in normal cyclic cows. The calcium has been reported as one of the major cations in the cervical mucus. Its optimum level maintains selective permeability of cell

membrane (Banerjee, 2008), stimulateglycolysis thereby sustain the viability, motility and metabolism of sperm (Sidhu and Guraya, 1985) whereas excessive levels inhibit sperm motility (Vadodariya and Prabhu, 1990). Although, the higher calcium was discernible at post-treatment oestrus in repeat breeders of Group-I and II but these levels were at par with normal cyclic cows who revealed 100 per cent conception in the present study, therefore, the post-therapeutic calcium levels in cervical mucus of repeat breeders might be attributable for glycolysis to produce energy require for maintaining viability and metabolism of spermatozoa and thereby resulting into better conception.

3.2.1.2 Inorganic phosphorus

Prior to treatment, the values of inorganic phosphorus ranged between 5.4 to 14.4 and 5.4 to 11.4 mg/dl in repeat breeder crossbreds of Group-I and II, respectively. The mean ± SE were 7.64±0.56 and 8.20±0.46 mg/dl in these two groups of cows. The difference between two mean was non-significant (Table.3). The post-treatment inorganic phosphorus increased to 9.92±0.65 and 10.04±0.79 mg/dl in Group-I and II, respectively. The values were significantly higher ($p<0.05$) than that of pre-treatment values in cows of two groups but were non-significant when compared with normal cyclic cows (10.70±0.36 mg/dl).

The mean values of inorganic phosphorus in cervical mucus of normal cyclic and repeat breeding cows duringpre- and post-treatment oestrus havebeen depicted inTable 3. In general, the values were higher in post-treatment and normal cyclic groups of cows.

Perusal of table 3 revealed the level of inorganic phosphorus in repeat breeder crossbred cows at pre- and post-treatment oestrus in two groups and also in normal cyclic cows. Significant difference was discernible in the levels of inorganic phosphorus in cows of Group-I whereas in Group-II, the post-treatment levels also increased than the preceding levels (10.04±0.79 v/s 8.20±0.46 mg/dl) but difference was not

significant. The post-treatment increasedlevels of phosphorus in both groups of cows are in close proximity to phosphorus levels of normal cyclic cows. The findings also revealed significantly higher inorganic phosphorus in normal cyclic cows than those of repeat breeders, which are corroboratedwith earlier findings of Shukla and Sharma (2006) and Sood *et al*. (2013). The role of inorganic phosphorus in cellular metabolism is well established. Lardy and Phillips (1943) and White (1953) have reported that presence of at least a minimum concentration of inorganic phosphate is essential for energy transportation at cellular level. It has also been associated with sperm glycolysis and respiration. Thus, levels of inorganic phosphorus in normal cows and repeat breeders too at post-treatment osetrus might enhance spermatozoal motility and transport thereby resulting into better conception in the present study. Further, the endometrial glands and cervical crypts in the repeat breeder cows may not be capable of providing inorganic phosphorus to the extend it is available in cervical mucus of normal cows as well as repeat breeders following their treatment in both groups.

3.2.1.3 Total cholesterol

The range of this attributes varied from 16.20 - 46.8 and 12.0 – 37.20 mg/dl in cervical mucus of repeat breeder crossbred cows of Group-I and II, respectively. The Mean ± SE values of total cholesterol in cervical mucus of these two groups were 31.12±2.68 and 35.40±4.74 mg/dl before administration of ceftiofur, respectively. Whereas pre-treatment values of cholesterol were higher in two groups of treatment, the values were declined at post-treatment oestrus in Group-I (24.05±1.85 mg/dl) and Group-II (23.45 ± 1.91 mg/dl) which, however, differedsignificant ($p<0.05$) than those of preceding values. In normal cyclic cows, the mean values of total cholesterol ranged between24.6 - 33.6 with a Mean ± SE of 29.20 ± 1.32 mg/dl (Table 3). These values were marginally higherin comparison topost-treatment average of total

cholesterol in Group-I and II. However,differences were non-significant statistically.

Results depicted in Table.3 revealed that total cholesterol was higher in repeat breeder cows than the normal cyclic animal. However, the difference was not significant. The levels at post-treatment oestrus decreased than preceding values in repeat breeder cows of Group-I and II. Non-significant difference was discernible in calcium content at pre- and post-treatment oestrus in Group-I while it was significant in Group-II. Cholesterol concentration in repeat breeder observed as observed in present study is at par with earlier reports of Manjunath *et al.* (2001) who quantified it to average of 34.24±2.60 mg/dl. The higher cholesterol in non-conceived in comparison to conceived buffaloes has been reported by Vadodaria (1987). This clue seems to be agreeable to support the present findings of post-treatment and normal oestrus where conception rate was better to first service than the repeat breeders who required more number of artificial inseminations but failed to conceive.

3.2.1.4 Total lipid

Total lipid in cervical mucus of normal cyclic and repeat breeder crossbred cows of treatment groups are depicted in Table.3. This content varied from 2.89 – 4.96 and 1.07 mg/dl in repeat breeder cows of Group-I and II, respectively whereas in normal cyclic groups, it ranged from 3.51-5.49 mg/dl. The values for Mean ± SE were 3.79±0.17 (Group-I) and 3.99±0.31 mg/dl (Group-II) in repeat breeder cows prior to initiation of treatment which, however, elevated to 4.58±0.40 and 4.28±0.27 mg/dl at post-treatment oestrus in two groups, respectively but the values did not differ from the pre-treatment and also with that finding qualified in normal cyclic cows.

It is apparent from the results (Table.3) that total lipids are lower in cervical mucus of repeat breeder crossbred cows (Group-I and II) than normal cyclic animals (Group-II). However, significant differences were not discernible. Further, the average mean values at post-treatment

oestrus were elevated in both treatment groups and tend at par with normal cyclic cows. The results of present study are in agreement with those reported earlier (Sharma and Tripathi, 1987 and Umashankar *et al.* 1984). Lipids perse have decisive role to play as faras the protective action and consequently motility of spermatozoa are concerned. It has also been reported by Hartree and Srivastava (1965) andMann (1981) that lipids are metabolized by spermatozoa in the absence of reducingsugars and thus the energy is made available to the spermatozoa thoughthe breakdown of lipids. The high total lipids in normal cows and at post-treatment oestrus in both groups of repeaters might gave better environment for spermatozoal metabolism and thus, may also give a conducive environment for the transport of spermatozoa whereas reverse might be true for repeater cows prior to treatment with ceftiofur who are not able to provide congenial vehicular atmosphere for spermatozoa to achieve their normal transport resulting into more number of services for conception as observed in present study (Table.1).

3.2.1.5 Total protein

The contents of total protein in cervical mucus of repeat breeder crossbred cows ranged from 0.66 – 2.28 and 1.2 -3.42 g/dl in Group- I and II, respectively while itsrange in normal cyclic cows (Group-I) was observed to be 1.8 – 2.64 g/dl. The average values normal cyclic cow was 2.14±0.13 g/dl. The corresponding values in repeat breeding crossbred cows were 1.39±0.12 and 1.94±0.18 g/dl in Group-I and II, respectively. These values were observed to increased in cervical mucus of repeat breeder cows of treatment groups, 1.88±0.21 g/dl; Group-I and 3.00±0.25 g/dl; Group-II. The difference of pre- and post-treatment mean was significant ($p<0.05$) in Group-II only. The post-treatment rise in total protein concentration in Group-II was also found significant than that of normal cyclic values. On the other hand, the difference was non-significant in cervical mucus of cows in Group-I.

It is discerned from Table 3 that total protein was significantly higher in normal cyclic cows than the pre-treatment average values of repeat breeders in Group-I and II. It also remained higher than preceding concentration at post-treatment oestrus in Group-II whereas in Group-I, the difference was not significant. Higher total protein in cervical mucus of normal cyclic cows than those of repeat breeder have also been reported by earlier workers (Gohel *et al.,* 2012a and Sood *et al.,* 2013). It has also been found to be associated with conception (Pankaj *et al.,* 2000) and endometritis (Rao and Seshagiri, 1998).

Total protein in uterine flushingof repeat breeder crossbred cows following treatment with antibiotics and plant based immunomodulators has been reported by Kumar *et al.* (2011). These findings revealed a non-significant rise in protein values following treatment except antibiotics wherein the protein level after treatment decreased significantly than the pre-treatment values. In the present study, higher pre-treatment protein values in repeat breeding cows of Group-II revealed the killing of bacteria effectively as a result inflammatory response in uterus might have reducedwhich intern resulted to elevated protein influx towards uterus/or cervical mucus.

The higher concentration of protein observed in present study following treatment in the both groups of cows as well as better physical properties (Table 1) might provide better trend for spermatozoal transport in the cervical mucus and thereby might raise the conception rate in normal and repeat breeder cows following treatment.

3.3.2 Ions

3.3.2.1 Sodium

Sodium content varied from 163.8-328.2 and 148.8-328.8 MEq/L of cervical mucus in repeat breeder crossbred cows designated to treatment Group-I and II, respectively (Table 5). Themean values ± SEwere found to be 217.88±15.77 and 251.27±14.21 MEq/L in pre-treatment oestrual mucus of both treatment groups which did not vary significantly. Post-

treatment sodium content of oestrual cervical mucus was observed to decline in both treatment groups (187.20±11.11 MEq/L, Group-I and 217.±12.74 MEq/L, Group-II) than that of preceding mean ± SE at pre-treatment osetrus (217.88±15.55 and 251.27±14.21 MEq/L) but differences were not significant statistically. In normal cyclic cows, the mean ± SE was 202.40±13.93 with a range of 165.6-264.6 MEq/L of cervical mucus. Although, these values were observed at par with post-treatment mean values but statistically the difference were non-significant.

Data presented in Table 5 revealed higher sodium content in cervical mucus of repeat breeder cows of treatment Group-I and II which decreased at post-treatment oestrus in cows of both groups. However, the difference was not significant. The values of sodium in cervical mucus at post-treatment oestrus were slightly elevated than the normal cyclic cows. The higher sodium content in cervical mucus of repeat breeder crossbred cows than the normal cyclic in the present study are corroborated with earlier study of Siddiquee*et al.* (2006). However, Sood *et al*. (2013) reported higher sodium in cervical mucus of normal cows than the repeaters. Levels of sodium have been reported to change due to inflammatory condition of genital organs as evident from findings of higher sodium in first degree uterine infection (Nandi *et al.*, 2002).

3.3.2.2 Potassium

The values of potassium content in cervical mucus of normal cyclic and treatment groups are shown in Table 5. The content ranged between 6.6 – 18.6 and 7.8-19.2 MEq/L in cervical mucus of repeat breeder crossbred cows of Group-I and II, respectively whereas the mean ± SE in these cows prior to administration of ceftiofur was found to be 11.68±0.75 and 12.04±0.90 MEq/L respectively. The difference was not significant. The post treatment oestrual mucus potassium averaged to 9.64±0.17 and 9.76±0.65 with a range of 4.8 -15.6 and 6.0-15.0 MEq/L in repeat breeders of Group-I and II respectively. Although, these values were

observed to decrease in comparison to their preceding values at pre-treatment oestrus which, however, were found non-significant statistically.

In normal cyclic cows, the potassium averaged to 10.30±0.29 with a range of 9.6 – 11.4 MEq/L at oestrus. Apparently, mean values of normal cyclic group and post-treatment oestrus in Group-I and II showed variations but when compared statistically, none of the mean values reveal significant difference.

The results pertained to potassium (Table 5) revealed its higher average values in cervical mucus of repeat breeder crossbred cows prior to treatment in Group-I and II while the values declined at post-treatment oestrus in animals of both groups, which fall in close proximity of potassium concentration in normal cyclic cows. However, the differences between groups of animals were not significant.

The present finding of potassium in cervical mucus of normal cows is corroborated by earlier study of Schultz *et al.* (1971). A marginal variation in potassium content of normal and repeat breeder cows has also been reported by Siddiquee (2006). Potassium is the major intracellular cation and perse have vital role in the crystallization pattern of genital discharge (Roland, 1962). It has also been reported increased significantly in genital infection irrespective of degree of inflammation.

3.2.3 Enzymes

3.2.3.1 Acid phosphatase

Acid phosphatase content ranged from 4.91 – 7.40 with average value of 5.91±0.31 U/L in cervical mucus of normal crossbred cows whereas it averaged between 6.82±0.40 and 5.84±0.37 U/L with a ranged of 4.40 - 9.38 and 3.85 – 7.70 U/L in repeat breeder crossbred cows of treatment Group-I and II, respectively (Table 7). The difference of mean values was not significant between repeat breeder and normal cows, although, values were slightly higher in repeat breeders.

Following treatment, the mean values of acid phosphatase declined to 5.84±0.37 and 5.41±0.29 U/L in Group-I and II, respectively which also did not differ significantly from those of their preceding values and the normal cyclic group (Table.8).

The increased levels of acid phosphatase in repeat breeder cows of Group-I and II are seen from Table.7 which, however, declined at post-treatment oestrus in both groups. Much difference was not discernible in enzyme activity between repeat breeder and normal cyclic cows which are corroborated by findings of Sharma and Tripathi (1986) and Bugalia and Sharma (1988). However, significantly higher acid phosphatase in normal than repeat breeder cows was reported by Selvaraj *et al*. (2002).

3.2.3.2 Alkaline phosphatase

Enzyme alkaline phosphatase varied from 168.56 – 253.78 with an average of 204.57±14.86 U/L in cervical mucus of normal cyclic cows (Group-III) whereas it varied from 80.57 – 352.92 and 96.68 – 338.39 with mean values of 192.47±21.35 and 184.69±19.87 U/L in repeat breeder cows of Group-I and II, respectively (Table 7). Pre-treatment mean values of repeat breeder cows (Group-I and II) did not differ significantly than the values observed in normal cyclic cows (Group-III).

Following treatment of repeat breeder cows, the mean values were found to elevate in both treatment groups (242.07±25.49 U/L, Group-I; 224.33±21.27 U/L, Group-II). Although, these values were higher than their preceding average and were also in close proximity to that of normal cyclic group but statistically differences were not significantly (Table.8).

Perusal of Table 7 reveals that there is an increasing trend in the alkaline phosphatase of repeat breeder cows at post-treatment oestrus in Group-I and II. However, the difference in enzyme activity in pre-and post-treatment oestrus was not significant. These values also differed non-significantly than the normal cyclic cows. Similar finding of alkaline phosphatase in cervical mucus of normal and repeat breeder animals have

been reported by earlier workers (Sharma and Tripathi 1986 and Bugalia and Sharma 1988) while Nandi *et al*., (2002) and Selvaraj *et al.* (2002) reported significant variation, enzyme being higher in normal cows than repeaters.

As such the alkaline phosphatase activity observed in cervical mucus of normal animals might help to produce enhanced energy required for spermatozoa transport and thus help in settling the cows in a better way than the repeaters. These clues seem to be agreeable to support the findings of increased alkaline phosphatase following treatment of repeat breeder cows in the present study.

4. CONCLUSION

It can be concluded that the abnormal color and consistency of cervical mucus in repeat breeder crossbred cows returned towards their normalcy following ceftiofur administration through intramuscular and subcutaneous routes.The concentration of calcium and inorganic phosphorus in cervical mucus of repeat breeder cows after treatment increased with both routes

8. DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1: Physical characteristics of cervical mucus in treatment and control groups.

Treatments Groups		No. of animal	Physical Characteristics					
			pH (Mean ± SE)	Color (%)		Consistency (%)		
				Clear	Cloudy	Thin	Medium	Thick
Group-I	**Pre-treatment oestrus**	15	8.13±0.13^{ab} (7.0 -9.0)	6.67	93.33	6.67	40.00	53.33
	Post-treatment oestrus		7.87±0.13^{b} (7.0-8.5)	80.00	20.00	60.00	33.33	6.67
	Pre-treatment oestrus		8.40±0.11^{a} (8.0-9.5)	13.33	86.67	6.67	53.33	40.00

Group-II	**Post-treatment oestrus**	15	7.20±0.18^{ab} (7.5-10.5)	93.33	6.67	66.67	26.67	6.67
Group-III	**Normal cyclic (control)**	6	7.75±0.21^{b} (7.0-8.5)	100.00	0.00	66.67	33.33	0.00

Mean ± SE bearing different superscripts differed significantly ($p<0.05$).

Figures in parenthesis represent range values.

Table.2: Analysis of variance for pH of cervical mucus in treatment and control groups.

Sources of variation	**d.f.**	**Sum of squares**	**Mean Square**	**Cal F**
Between Groups	4	3.056	.764	2.541*
Within Groups	61	18.342	.301	
Total	65	21.398		

*Significant at 5 percent.

Table 3: Macro-mineral profile of cervical mucus in treatment and control groups.

Treatments Groups		**No. of animals**	**Macro-minerals (mean±SE)**				
			Calcium (mg/ml)	**Inorganic phosphorus (mg/dl)**	**Total cholesterol (mg/dl)**	**Total lipid (mg/dl)**	**Total protein (g/dl)**
Group-I	**Pre-treatment oestrus**	15	8.53±0.64^{c} (4.8 – 13.2)	7.64±0.56^{c} (5.4 – 14.4)	31.12±2.68^{ab} (16.2 –	3.79±0.17^{a} (2.89 – 4.96)	1.39±0.12^{c} (0.66 – 2.28)

					46.8)		
	Post-treatment oestrus		11.12±0.86 [b] (6.0 – 17.4)	9.92±0.65 [a b] (6.0 -16.2)	24.05±1.85 [b] (12.0 – 37.2)	4.58±0.40 [a] 1.07 – 6.60	1.88±0.21 [bc] (0.78 – 3.12)
Group-II	**Pre-treatment oestrus**	15	10.16±0.55 [bc] (7.2 – 13.8)	8.20±0.46 [b c] (5.4 – 11.4)	35.40±4.74 [a] (15.0 – 79.2)	3.99±0.31 [a] (1.8 – 6.49)	1.94±0.18 [bc] (1.2 – 3.42)
	Post-treatment oestrus		13.92±0.77 [a] (9.0 – 18.6)	10.04±0.79 [ab] (6.6 – 17.4)	23.45±1.91 [b] (13.2 – 39.2)	4.28±0.27 [a] (2.03 – 5.76)	3.00±0.25 [a] (1.68 – 4.92)
Group-III	**Normal cyclic (conrol)**	6	12.17±0.52 [ab] (10.2 – 13.8)	10.70±0.36 [a] (9.6 – 11.4)	29.20±1.32 [ab] (24.6 – 33.6)	4.60±0.32 [a] (3.51 – 5.49)	2.14±0.13 [b] (1.8 – 2.64)

Mean ± SE bearing different superscripts differed significantly ($p<0.05$).

Figures in parenthesis represent range values.

Table.4: Analysis of variance for macro-mineral characters of cervical mucus in treatment and control groups.

Macro-minerals					
Character	**Source of variation**	**d.f.**	**Sum of Squares**	**Mean Square**	**Cal F**
Calcium	Between Groups	4	237.993	59.498	8.648*
	Within Groups	61	419.671	6.880	
	Total	65	657.664		
Inorganic phosphorus	Between Groups	4	82.819	20.705	3.773*
	Within Groups	61	334.716	5.487	
	Total	65	417.535		
Total cholesterol	Between Groups	4	1498.361	374.590	2.943*
	Within Groups	61	7765.339	127.301	
	Total	65	9263.699		
Total lipid	Between Groups	4	6.347	1.587	1.236[NS]
	Within Groups	61	78.289	1.283	

	Total	64	84.636		
Total protein	Between Groups	4	20.773	5.193	9.563*
	Within Groups	61	33.125	.543	
	Total	65	53.898		

*****Significant at 5 percent.

NS= Nonsignificant at 5 percent.

Table 5: Sodium and potassium ions of cervical mucus in treatment and control groups.

Treatments Groups		**No. of animal**	**Ions (mean±SE)**	
			Sodium (MEq/L)	**Potassium (MEq/L)**
Group- I	**Pre-treatment oestrus**	15	217.88±15.77^{ab} (163.8 – 328.2)	11.68±0.75^{a} (6.6 – 18.6)
	Post-treatment oestrus		187.20±11.11^{b} (132.0 – 298.8)	9.64±0.71^{a} (4.8 – 15.6)
Group-II	**Pre-treatment oestrus**	15	251.27±14.21^{a} (148.8 – 328.8)	12.04±0.90^{a} (7.8 – 19.2)
	Post-treamentoestrus		217.11±12.74^{ab} (124.2 – 310.8)	9.76±0.65^{a} (6.0 – 15.0)
Group-III	**Normal cyclic (control)**	6	202.40±13.93^{b} (165.6 – 264.6)	10.30±0.29^{a} (9.6 – 11.4)

Mean ± SE bearing different superscripts differed significantly ($p<0.05$).

Figures in parenthesis represent range values.

Table 6:Analysis of variance for ions characters of cervical mucus in treatment and control groups.

Ions					
Character	**Source of variation**	**d.f.**	**Sum of Squares**	**Mean Square**	**F**
Sodium	Between Groups	4	32223.943	8055.986	3.063*
	Within Groups	61	160435.375	2630.088	
	Total	65	192659.318		
Potassium	Between Groups	4	72.321	18.080	2.270*
	Within Groups	61	485.772	7.963	

	Total	65	558.093		

*****Significant at 5 percent.

Table 7: Enzyme activity of cervical mucus in treatment and control groups.

Treatments Groups		No. of animal	Enzymes (mean±SE)	
			Acid phosphatase (U/L)	Alkaline phosphatase (U/L)
Group-I	**Pre-treatment oestrus**	15	6.82±0.40[a] (4.40 – 9.38)	192.47±21.35[a] (80.57 – 352.92)
	Post-treatment oestrus		5.84.±0.37[ab] (3.85 – 7.70)	242.07±25.49[a] (99.70 – 378.67)
Group-II	**Pre-treatment oestrus**	15	6.07±0.48[ab] (4.05 – 7.57)	184.69±19.87[a] (96.68 -338.39)
	Post-treatment oestrus		5.47±0.29[b] (4.34 - 11.62)	224.33±21.27[a] (77.55 – 346.14)
Group III	**Normal cyclic (control)**	6	5.91±0.37[ab] (4.92 – 7.40)	204.57±14.86[a] (168.56 – 253.78)

Mean ± SE bearing different superscripts differed significantly ($p<0.05$).

Figures in parenthesis represent range values.

Table 8: Analysis of variance for enzymes of cervical mucus in treatment and control groups.

Enzymes					
Character	**Source of variation**	**d.f.**	**Sum of Squares**	**Mean Square**	**F**
Acid phosphatase	Between Groups	4	15.552	3.970	1.831[NS]
	Within Groups	61	132.298	2.169	
	Total	65	148.180		
Alkaline phosphatase	Between Groups	4	32661.516	8165.379	1.194[NS]
	Within Groups	61	417051.516	6836.916	
	Total	65	449713		

NS=Non-significant at 5 percent.