International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; SP-9(1): 395-401

www.veterinarypaper.com
Received: 03-12-2023
Accepted: 07-01-2024

Harshad Chaudhary
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Keshav
Department of Veterinary Gynaecology and Obstetrics, M. B. Veterinary College, Dungarpur, Rajasthan, India

Ravindra Jadav
Department of Veterinary Gynaecology and Obstetrics, M. B. Veterinary College, Dungarpur, Rajasthan, India

Sachin Kalaswa
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Vipul Solanki
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Vinod Kumar Sharma
Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Corresponding Author:
Keshav
Department of Veterinary Gynaecology and Obstetrics, M. B. Veterinary College, Dungarpur, Rajasthan, India

Gyneco-clinical and microbiological characteristics of cervical mucus in repeat breeder crossbred cows following ceftiofur therapy

Harshad Chaudhary, Keshav, Ravindra Jadav, Sachin Kalaswa, Vipul Solanki and Vinod Kumar Sharma

DOI: https://doi.org/10.22271/veterinary.2024.v9.i1Sf.1014

Abstract
The present study was carried out to investigate gyneco-clinical and microbiological 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 that the oestrus duration, tonicity of uterus and number of artificial insemination were derailed from normalcy but post-therapeutic characteristics of all these traits improved in repeat breeder cows and resembled to normal cyclic cows. The bacterial load of cervical mucus of repeat breeder cows ($3.1\times10^8\pm0.65\times10^8$, Group-I and $3.6\times10^8\pm0.72\times10^8$, Group-II) was significantly higher than normal cows (2.1×10^3, Group-III) which, however, decreased significantly at post-treatment oestrus in both groups of repeat breeders. *E. coli*, *Staphylococcus*, *Klebsiella*, *Anthracoid* and *Streptococcus* spp. were isolated from cervical mucus of pre-treatment groups of repeat breeders. Although, prevalence of microorganism varied but *E. coli* was predominant followed by *Staphylococcus* spp. However, all of them disappeared from post-treatment cervical mucus of repeater cows.
It is concluded from the finding that ceftiofur therapy of repeat breeder cows was beneficial to restore normalcy of oestrus duration, doughy tonicity of uterus and reduction in total bacterial load in uterus.

Keywords: Cervical mucus, ceftiofur, repeat breeder, oestrus, bacterial load

1. Introduction
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) [27]. 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) [3].
The repeat breeding occurs due to defects in gametes, endocrine dysfunctions, infections, nutritional deficiency etc. which culminate to either the fertilization failure or early embryonic death as evidenced from earlier reports that these two factors account for 10-20 and 45 percent, respectively in overall conception failure in cattle (Diskin *et al.*, 2008 and Inskeep *et al.*, 2005) [9, 13]. During the oestrus apart from other changes, cervical mucus is produced by mucus secretary cells that line the cervical epithelium (Cortes, 2014) [6]. 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.

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 gyneco-clinical and microbiological characteristics of cervical mucus of repeat breeder cows.

2. Materials 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 Palanpur were 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) [32].

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: Six normal 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) [22].

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) [11]. Therefore, samples were diluted with triple glass distilled water and thoroughly mixed by means of magnetic stirrer (Reddy, 1974) [25].

2.4.1 Gyneco-Clinical Attributes

The oestrous cycle length, oestrus duration (Ahmed *et al.*, 2016) [1] and number of AI/services were studied from the records and uterine health by per-rectal examination of genitalia whereas the post treatment oestrus duration was recorded by observing external sings of oestrus at six hrs interval.

2.4.2 Microbiology of Cervical Mucus

2.4.2.1 Bacterial Isolation

Cervical mucus samples obtained from treated and non-treated repeat breeder cows were transferred aseptically immediately to laboratory into the transport media subsequently, the streaking of the each mucus sample was done primarily for bacterial growth on Brain Heart Infusion Agar (BHI Agar, HiMedia) and left for incubation at 37 ºC temperature for 24-48 hrs.

The identification of gram positive (+ve) and gram negative (+ve) bacteria was done by gram staining and biochemical test of 3% KOH.

For further identification of bacteria colonies were transferred on MacConkey agar and Eosin Methylene Blue Agar (EMB Agar). Then used IMViC biochemical tests for confirmation of organisms (Gani *et al.*, 2008) [10].

IMViC test

The IMViC tests represented a group of individual tests used in microbiology lab testing to identify an organism in the coliform group. A coliform is a gram negative, aerobic or facultative anaerobic rod which produces gas from lactose within 48 hours.

The term "IMViC" is an acronym for each of these tests. "I" is for indole test, "M" is for Methyl Red test, "V" is for Voges-Proskauer test, and "C" is for Citrate Test. The lower case "i" is merely for "in", as the Citrate test requires coliform samples to be placed "in Citrate".

Indole test

In this test, the organisms under consideration were grown in peptone water broth. It contained tryptophan, which under the action of enzyme tryptophanase converted to an Indole molecule, pyruvate and ammonium. The indole was then extracted from the broth by means of xylene. To test the broth for indole production, Kovac's reagent was added after incubation. A pink/red layer formation on top of the liquid was identification of positive results.

Methyl Red and Voges–Proskauer test

The MRVP broth was used in these tests for bacterial growth. After growth, the broth was separated into two different tubes, one for the Methyl Red (MR) test and another for the Voges-Proskauer (VP) test.

The Methyl Red test detects production of acids formed during metabolism through mixed acid fermentation pathway using pyruvate as a substrate. The pH indicator Methyl Red was added to one tube and a red color appeared at pH's lower than 4.2, indicating a positive test. The solution remaining yellow at pH 6.2 or above indicated a negative test which means the butanediol fermentation pathway was used.

The VP test uses alpha-naphthol and potassium hydroxide to test the presence of acetylmethylcarbinol (Acetoin), an

intermediate of the 2, 3-butanediol fermentation pathway. After adding both reagents, the tube was shaken vigorously then allowed to stand for 5-10 minutes. The development of pinkish-red color indicated a positive test which means the 2, 3-butanediol fermentation pathway was used.

Citrate test

This test uses Simmon's citrate agar to determine the ability of a microorganism to use citrate as its sole carbon source. The agar contains citrate and ammonium ions (nitrogen source) and bromothymol blue as an indicator. The citrate agar is green before inoculation, and turns blue as a positive test indicator which reflected the utilization of citrate.

Result based on following table

Spp.	Indole	Methyl Red	Voges -Proskauer	Citrate
E. coli	+ ve	+ ve	- ve	- ve
Shigella spp.	- ve	+ ve	- ve	- ve
Salmonella spp.	- ve	+ ve	- ve	+ ve
Klebsiella spp.	- ve	- ve	+ ve	+ ve
Proteus vulgaris	+ ve	+ ve	- ve	- ve
Proteus mirabills	- ve	+ ve	- ve	+ ve
Citrobacter freundii	- ve	+ ve	- ve	+ ve
Enterobacter aerogenes	- ve	- ve	+ ve	+ ve

2.4.2.2 Bacterial Count

The bacterial count (cfu/ml) in the cervical mucus of repeat breeder cows was carried out the serial dilutions of sample by making as per standard technique of Diliello (1979) [8].

The 5 fold (10^5) dilutions of samples were made and plated in replicates of 3.

After dilution of the sample, 0.1 ml of diluted sample was taken and spreaded plate across the surface of a Brain Heart Infusion Agar (BHI Agar). The plates were allowed to dry before incubation at 37 ºC temperature for 24 hrs. Subsequently, colonies counted manually.

In each of the plates which possessed no. of colonies between 30 and 300. The plates which did not have standard number of colonies on surface of BHI agar were discarded.

The cfu/plate was read from a plate in the linear range, and then cfu/ml of the original calculated mathematically in consideration to dilution factor and no. of colonies grew on BHI agar by following formula:

B = N×D
B = number of bacteria
N = number of colonies counted on a plate
D = dilution factor

2.5 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) [30].

3. Results and Discussions

3.1 Gyneco-clinical attributes

3.1.1 Uterine Tonicity

The percentage of doughy uterus at pre-treatment oestrus was observed in 80.00 and 86.67 repeat breeder cows of Group-I and II, respectively whereas it was 16.67 per cent in normal cyclic cows. Following treatment of repeat breeders, the number of cows which had doughy uterus decreased and it was found in 13.33 and 6.67 per cent cows (Table 3.1).

On the other hand, a reverse trend was observed for tonicity of uterus at pre- and post-treatment oestrus. The percentage of tonic uterus in repeat breeders was 20.00 and 13.33 in Group-I and II, respectively which increased drastically to 86.67 and 93.33 at post treatment oestrus in both groups. The regaining of uterine tonicity in both treatment groups was almost similar to normal cyclic cows (86.33%).

The results pertained to uterine tone, length of oestrus cycle and duration of oestrus are depicted in Table 3.1. The repeat breeder cows had uterus of less tonicity in comparison to normal cyclic cows which, however, was improved at post treatment oestrus in repeat breeder cows of Group-I (86.67%) and II (93.33%). These findings are at par with normal cyclic cows of present study. The tonicity of uterus classified as pronounced, moderate/mild has been studied by various workers (Mehta, 1986) [18]. Duration to which the uterus remains tonic has also been reported in relation to status of ovary and uterus (Bhat and Bhattacharya, 2012) [4]. These findings indicated that duration of uterine tonicity decreased significantly when animal had normal ovulation but possesses infection in uterus. The uterus remains maximum tonic when oestrus is intense (Mehta, 1986) [18]. The post-treatment findings of present study are indicative of elimination of infection from uterus and thereby return of uterine musculature to normal texture which might be deviated in repeat breeders by bacterial invasion as reported earlier (Azawi *et al*., 2008) [2].

3.1.2 Number of Artificial Insemination

Table 3.1 showed the average number of artificial insemination required for conception in different groups of cows. The repeat breeder cows of Group-I and II were found to have more number of artificial insemination (4.73±0.50 and 4.20±0.28). The difference between groups of repeat breeders was non-significant whereas at post treatment oestrus, the number of artificial insemination observed to decrease (1.78±0.22 and 1.50±0.18) which did not differ than that of artificial insemination in normal cyclic cows (1.00±0.00) but the difference between pre- and post-treatment average of artificial insemination was significant in Group-I (4.73±0.50 and 1.78±0.22) and Group-II (4.20±0.28 and 1.50±0.18).

3.1.3 Oestrus Cycle Length

The results pertained to length of oestrus cycle in repeat breeder cows have been presented in Table 3.1. The average length of cycle was found to be 21.53±0.95 and 21.27±0.68 days in repeat breeder cows of Group-I and II, respectively. Following administration of ceftiofur crystalline free acid through intramuscular (Group-I) and subcutaneous (Group-II) routes, the length of oestrus cycle reduced to 20.93±0.34 days in Group-II whereas it was observed more or less similar to duration of pre-treatment length in Group-II. The differences between groups of treatment were non-significant.

3.1.4 Duration of Oestrus

The average duration of oestrus in repeat breeder crossbred cows was longer in Group-I (44.40±2.86 hrs) and Group-II (48.00±3.31hrs), which reduced to 34.00±2.08 and 35.20±2.33 hrs following treatment in both groups (Table 3.1). The difference was significant ($p<0.05$) between pre- and post-treatment duration of oestrus in Group-I and II but succeeding oestrus was found non-significant in two groups of treatment (34.00±2.08 hrs and 35.20±2.33 hrs).

Table 1: Gyneco-clinical attributes of cervical mucus in treatment and control groups.

Treatment groups		No. of animal	Gyneco-clinical Attributes				
			Uterine tonicity (%)		No. of AI	Length of oestrus cycle (days)	Duration of oestrus (hrs)
			Doughy	Tonic			
Group-I	Pre-treatment oestrus	15	80.00	20.00	4.73±0.50^{a} (3.0-10.0)	21.53±0.95^{a} (16.0-27.0)	44.40±2.86^{a} (36.0-72.0)
	Post-treatment oestrus		13.33	86.67	1.78±0.22^{b} (1-3)	21.67±0.29^{a} (20.0-24.0)	34.00±2.08^{b} (24.0-48.0)
Group-II	Pre-treatment oestrus	15	86.67	13.33	4.20±0.28^{a} (3.0-10.0)	21.27±0.68^{b} (16.0-25.0)	48.00±3.31^{a} (24.0-72.0)
	Post-treatment oestrus		6.67	93.33	1.5±0.18^{b} (1-3)	20.93±0.34^{ab} (19.0-23.0)	35.20±2.33^{b} (24.0-48.0)
Group-III	Normal cyclic (control)	6	16.67	86.33	1.0±0.00^{b}		32.00±3.69^{b} (24.0-48.0)

Mean ± SE bearing different superscripts differed significantly (p<0.05).
Figures in parenthesis represent range values.

Table 2: Analysis of variance for gynecol-clinical attributes of cervical mucus in treatment and control groups

Character	Sources of variation	d.f.	Sum of Squares	Mean Square	Cal F
Length of oestrus cycle	Between Groups	3	54.717	18.239	3.086*
	Within Groups	56	330.933	5.910	
	Total	59	385.650		
Duration of oestrus	Between Groups	4	2511.273	627.818	5.910*
	Within Groups	61	6480.000	106.230	
	Total	65	8991.273		

*Significant at 5 percent.

In normal cyclic cows, the oestrus duration of 32.00±3.69 hrs was observed which did not reveal any significant difference than that of post treatment oestrus duration of both groups (34.00±2.08 and 35.20±2.33 hrs).

Longer duration of oestrus in repeat breeder cows than normal cyclic animals is apparent from the results presented in Table 3.1. The difference was significant (p<0.05). The oestrus duration in repeat breeder cows of Group-I and II was shortened following treatment which also differed significantly but was not significant from the normal cyclic groups. The repeat breeder crossbred cows have been reported to show prolonged oestrus extended beyond 36-48 hrs (Singh, 1997 and Dadarwal *et al.*, 2005) [28, 7] which corroborate the present findings at pre-treatment oestrus whereas oestrus duration of 34.0±0.85 hrs is reported in normal crossbred cows (Qureshi *et al.*, 2000) [23] which is agreeable to present observations too in normal cows. Normal duration of oestrus in various breeds of cattle had been reported as 2-30 hrs (Noakes, 2001) [21] and 12-28 hrs in crossbred cows (Roberts, 1986) [26].

The prolonged duration of oestrus is postulated to be caused by hormonal imbalance, though, the exact etiology and pathogenesis remains elusive. Dadarwal *et al.* (2005) [7] reported that supra-basal progesterone levels during oestrus might be an important factor in causation of prolonged oestrus which in turn results in repeat breeding syndrome. However, it remains indescribable in the present study as the efforts are not made to investigate hormonal profiles of repeat breeder animals.

3.2 Microbiological Investigation

3.2.1 Bacterial Load and Isolation

Total bacterial load and types of bacteria in cervical mucus of treatment and control groups are presented in Table 3.3. The total bacterial load of $3.1 \times 10^8 \pm 0.65 \times 10^8$ with range a range of $0.72\text{-}7.6 \times 10^8$ was found in cervical mucus of repeat breeder cows of Group-I while it was $3.6 \times 10^8 \pm 0.72 \times 10^8$ with a range of $0.67\text{-}8.3 \times 10^8$ in repeaters of Group-II. The difference between two groups was non-significant but it was significantly higher (p<0.05) than that of normal cyclic cows ($2.1 \times 10^3 \pm 2.1 \times 10^3$). In comparison to higher bacterial load in pre-treatment cervical mucus, none of the post-treatment mucus samples showed bacterial growth in both treatment groups.

The pre-treatment cultural isolation from cervical mucus of repeat breeder cows showed that *E. coli*, *Staphylococcus* spp., *Klebsiella* spp., *Anthracoid* spp. *and Streptococcus* spp. were among the types of organism whose percentage was 53.33, 33.33, 6.67 and 6.67, respectively in Group-I whereas corresponding figures were 40.00, 20.00, 13.33, 6.67 and 20.00 per cent in Group-II. In normal cyclic cows, only *Streptococcus* spp. was identified (16.67%) while none of the cervical mucus sample showed bacterial growth in mucus obtained during post-treatment oestrus in both groups.

Bacterial isolation of the cervical mucus from repeat breeder crossbreds and normal cyclic cows yielded a wide range of isolates as depicted in Table 3.3. It is discernible from the results that *E. coli* was found in majority of mucus samples of repeat breeders followed by *Staphylococcus* spp. among the pathogenic groups of organism. The presence of *Klebsiella* spp., *Anthracoid* spp. and *Streptococcus* spp. was also identified in pre-treatment samples of cervical mucus in repeat breeder cows of Group-I and II whereas none of the mucus samples revealed presence of organism at post-treatment oestrus in these groups.

The uterine microbiology of healthy cows and that developed uterine diseases has been studied by various workers (Joshi *et al.*, 2013 and Zaman *et al.*, 2015) [14, 31]. The findings of these workers corroborate the isolation spectra of most organisms as discerned in present study. However, the present findings showed variation with respect to prevalence of different microorganisms than those reported earlier which might be attributable to various sites of genital organs to collect the samples (Azawi *et al.*, 2008) [2]. Histo-pathological changes in uterus to extent of mild, moderate and severe depend upon type of bacterial. *E. coli* was reported abundantly to cause mild to moderate type changes in uterus whereas severe uterine changes by *Arcanobacterium pyogenes* (Azawi *et al.*, 2008) [2]. Since *E. coli* was predominant over other bacterial isolates in repeat breeders prior to treatment, it might have alter uterine environment probably hostile to spermatozoa and thus resulting into conception failure and poor physical properties of cervical mucus. On the other hand reverse might be true at post-treatment oestrus in repeat breeder cow where none of mucus sample revealed the presence of organisms.

The bacterial load recorded in the present study was higher in repeater breeder cows than the normal cyclic animals (Table 3.3). It decreased significantly at post-treatment oestrus as discerned in repeat breeders of Group-I and II. Similar findings of decreased bacterial load at subsequent oestrus after treatment have also been reported (Kumar *et al.,* 2013 and Rahi *et al.,* 2013) [16, 24]. However a wide variation was evident in decreased load of bacteria from different studies conducted earlier which is agreeable to the action of various antibiotics and their potency used to inhibit the bacterial organism in uterus. Further, such a decrease in bacterial load after treatment with ceftiofur might be attributable to migration of leucocytes in to uterine lumen which have a phagocytic action on micro-organisms.

Table 3: Microbiological investigation of cervical mucus in treatment and control groups

Treatments Groups		No. of animals	Microbiology		
			Bacterial spp. (%)		Bacterial load (cfu/ml) (mean± SE)
Group-I	Pre-treatment oestrus	15	*E. coli*	53.33	$3.1\times10^{8} \pm 0.65\times10^{8a}$ (7.2×10^{7}-7.6×10^{8})
			Staphylococcus spp.	33.33	
			Klebsiella spp.	6.67	
			Anthracoid spp.	6.67	
	Post-treatment oestrus		0.00		0.00^{b}
Group-II	Pre-treatment oestrus	15	*E. coli*	40.00	$3.6\times10^{8}\pm0.72\times10^{8a}$ (6.7×10^{7}-8.3×10^{8})
			Staphylococcus spp.	20.0.0	
			Klebsiella spp.	13.33	
			Anthracoid spp.	6.67	
			Streptococcus spp.	20.00	
	Post-treatment oestrus		*0.00*		0.00^{b}
Group-III	Normal cyclic (control)	6	*Streptococcus* spp.	16.67	$2.1\times10^{3} \pm 2.1\times10^{3b}$

Mean ± SE bearing different superscripts differed significantly ($p<0.05$).
Figures in parenthesis represent range value.

Table 4: Analysis of variance for Microbiological (Bacterial load) of cervical mucus in treatment and control groups

Sources of variation	d.f.	Sum of squares	Mean square	Cal F
Between group	4	1.81×10^{18}	4.5×10^{17}	13.974*
Within group	61	1.97×10^{18}	3.24×10^{16}	
Total	65	3.78×10^{18}		

*****Significant at 5 percent.

3.3 Number of artificial insemination and conception rate

The overall conception rate was observed to 60.00 per cent in treatment Group-I whereas it was 73.33 per cent in Group-II. Out of 15 cows in Group-I, the first, second and third service per conception rate was 26.67, 20.00 and 13.33 per cent whereas corresponding figures for cows of Group-II were 40.00, 26.67 and 6.67 per cent, respectively. The maximum number of cows (4/15) and (6/15) were observed to conceived at first service in Group-I and II, respectively. Whereas the first service conception rate was 100 per cent in Group-III normal cyclic cows.

The findings of overall conception rate in repeat breeder and normal cyclic cows (Table 3.5) revealed that all cyclic cows conceived to first service and resulted into 100 per cent conception. In repeat breeder cows also, the conception rate of 60.00 and 73.33 per cent was discerned in Group-I and II, respectively which is quite comparable to normal cyclic group. Besides, the first service conception was also higher in repeaters in Group-II than Group-I. The higher conception rate is documented to be associated with physical properties of cervical mucus as it has been reported to improve when cervical mucus is clear, stringy and possesses typical fern pattern (Lim *et al.,* 2014 and Negash *et al.,* 2014) [17, 20]. Further, the present findings are agreeable to earlier reports of intrauterine antibiotic infusion of repeat breeder cows advocated following *in vitro* sensitivity test (Bhat *et al.,* 2013 and Singh *et al.,* 2015) [5, 29]. However, a wide variation in conception was revealed from findings of these authors which might be attributable to difference in the pharmacokinetic characteristics of different antibiotics which have been reported to vary from one to another formulation: Gentamycin (Haddad *et al.,* 1987) [12], Enrofloxacin (Kaartinen *et al.,* 1995) [15], Oxytetracyclin (Morro, 1980) [19].

The higher conception rate in repeat breeder cows of Group-I is a fair indication of better bioavailability of drug used in the present study which in turn might have attained maximum concentration to eliminate/or kill microorganisms of uterus and thereby production of clear colored and thin consistency of cervical mucus to enable the spermatozoa for maintaining better viability and motility required for conception.

Table 5: Conception rate in following treatment in different groups of animals

Groups	No. of animals	No. of service	No. of animals conceived	Conception (%)	Over all conception (%)
Group-I	15	First	4	26.67	60.00(9)
		Second	3	20.00	
		Third	2	13.33	
Group-I	15	First	6	40	73.33 (11)
		Second	4	26.67	
		Third	1	6.7	
Group-III	6	First	6	100	100 (6)

Figures in parenthesis denote number of animal conceived.

Table 6: Number of services per conception in treatment and control groups

Groups	No. of animals	No. of service/conception		
		First	Second	Third
Group-I	15	4 (26.67)	3 (20.00)	2 (13.33)
Group-II	15	6 (40.00)	4 (26.67)	1 (6.67)
Group-III	6	6 (100)	-	-

Figures in parenthesis denote conception rate.

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.

5. Acknowledgment

The authors thank the Dean, College of Veterinary Science and Animal Husbandry for providing the necessary facilities for this research.

6. Funding Information

This research was funded by College of Veterinary Science and Animal Husbandry Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar - 385506, India.

7. Conflict of interest statement

The authors declare no conflict of interest with respect to this research.

8. Data availability statement

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

9. References

1. Ahmed N, Kathiresan D, Ahmed FA, Lalrintluanga K, Mayengbam P, Gali JM. Pattern of induced *estrus* and conception rate following *ovsynch* and *ovsynch* based *gonadotropin-releasing hormone* treatment initiated on day 6 *estrus* cycle in repeat breeding crossbred cows. Vet World. 2016;9(1):342-245.
2. Azawi OI, Omran SN, Hadad JJ. A study on repeat breeding of Iraqi buffalo cows. Buffalo Bull. 2008;27(4):274-283.
3. Bartlett PC, Krik JH, Mather EC. Repeated insemination in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. Theriogenology. 1986;26:309-322.
4. Bhat FA, Bhattacharyya HK. *Oestrus* duration and status of reproductive organs in repeat breeding cows. Iran J Appl. Anim. Sci. 2012;2(3):295-299.
5. Bhat FA, Bhattacharya HK, Hussain A, Nadeem M, Wani AR. Microbial profile, antibiogram and conception rate following treatment in repeat breeder cows. Intas Polivet. 2013;14(1):42-48.
6. Cortes ME. Morphological and ultrastructural characterization of different types of bovine cervical mucus using light and scanning electron microscopy [doctoral thesis]. Santiago de Chile: Pontificia Universidad Catolica de Chile; c2014.
7. Dadarwal D, Singh J, Honparkhe M, Cheede GS, Kang RS. Investigation on repeat breeding crossbred cattle with a history of prolonged *estrus*. Indian J Anim. Sci. 2005;75(8):922-924.
8. Diliello LR. Clinical microbiology. Westport, CT: Publishing Co. Inc.; c1979.
9. Diskin MG, Morris DG. Embryonic and early foetal losses in cattle and other ruminants. Reprod Domest Anim. 2008;43:260-276.
10. Gani MO, Amin MM, Alam MGS, Kayesh MEH, Karim MR, Samad MA, *et al*. Bacterial flora associated with repeat breeding and uterine infection in dairy cows. Bangla J Vet. Med. 2008;6(1):79-86.
11. Gibbons RA, Boyed LJ, Dixon SN, Parker J, Sellwood R, Jasker JB. Chemical and physical characteristics of the macromolecular components of the cervical mucus from cows after synchronization of *oestrus* with melengestrol acetate. J Reprod. Fertil. 1973;35:469.
12. Haddad NS, Ravis WR, Pedersoli WM, Carson RLJR. Pharmacokinetics and residues of gentamycin in lactating cows after multiple intramuscular doses are administered. Am. J Vet. Res. 1987;48:21-27.
13. Inskeep EK, Daily RA. Embryonic death in cattle. Vet Clin. North. Am Food Anim. Pract. 2005;21:437-461.
14. Joshi R, Mudasir M, Sharma D, Saraswat N, Singh R. Bacterial microflora with repeat breeding in crossbred dairy cattle. Indian Vet J. 2013;90(6):52-54.
15. Kaatinen L, Salonen M, Alli L, Pyosala S. Pharmacokinetics of enrofloxacin after single intravenous, intramuscular and subcutaneous injection in cows. J Vet Pharmacol Ther. 1995;18:357-362.
16. Kumar A, Gupta HP, Prasad S. Studies on the immunomodulatory and therapeutic efficacy of neem (*Azadirachta indica*) in endometritis-affected repeat breeding crossbred cows. Indian J Anim. Reprod. 2013;34(2):1-5.
17. Lim HJ, Son JK, Yoon HB, Beak KS, Kim YS. Physical properties of *estrus* mucus in relation to conception rates in dairy cattle. J Embryotransfer. 2014;29(2):157-161.
18. Mehta GB. Studies on repeat breeding condition in crossbred (K x J and K x HF) cattle with special reference to cervical mucus. MVSc thesis. Anand: GAU; c1986.
19. Morrow DA. Current Therapy in Thetiogenology. Philadelphia: W.B. Saunders Company; c1980. p. 210.
20. Negash G, Gebrekidan B, Ashebir G, Weldemarian H. Effect of non-hormonal interventions in improving conception rate in repeat breeder dairy cows. Eur J Appl Sci. 2014;6(4):78-82.
21. Noakes DE. Endogenous and exogenous control of ovarian cyclicity. In: Noakes DE, Parkinson TJ, England GCW, editors. Arthur's Veterinary Reproduction and Obstetrics. New Delhi: Harcourt (India); c2001. p. 19.
22. Panangala VS, Fish NA, Barnum DA. Microflora of the cervico-vaginal mucus of repeat breeder cows. Can Vet J. 1978;19:83-89.
23. Qureshi MS, Khan A, Mirbahar KB, Samo MU. Productive and reproductive performance and their interaction in crossbred cattle under field conditions in district Bannu. Pak Vet J. 2000;20(1):31-33.

24. Rahi S, Gupta HP, Prasad S, Baithalu RK. Phytotherapy for endometritis and subsequent conception rate in repeat breeding crossbred cows. Indian J Anim. Reprod. 2013;34(1):9-12.
25. Reddy VNV. Studies on certain physical and biochemical properties of cervical mucus of bovine in oestrus. Indian Vet J. 1974;60:731-734.
26. Roberts SJ. Physiology of female reproduction. In: Roberts SJ, editor. Veterinary Obstetrics and Genital Diseases. Theriogenology. New Delhi: CBS Publishers; 1986. p. 13.
27. Saxena MS. Repeat breeding in cows and buffaloes. In: Proceedings of the National Symposium on Conservation and Propagation of Indigenous Breeds of Cattle and Buffaloes. Panthnagar, Uttaranchal; c2004. pp. 26-28.
28. Singh N. Effect of GnRH treatment on pregnancy in repeat breeding dairy cattle. M.V.Sc. Thesis. Ludhiana, India: Punjab Agricultural University; c1997.
29. Singh NJ, Singh A, Patel AK. Employing the effect of gentamycin and enrofloxacin treatment on pregnancy rate of repeat breeder dairy crossbred cows. World J Pharm Res. 2015;4(8):1144-1148.
30. Snedecor GW, Cochran WG. Statistical Methods. 8th ed. New Delhi: Oxford and IBH Publishing Co.; c1994.
31. Zaman MI, Sharma U, Taku AK, Kumar S, Kumar S. Therapeutic studies and microbial profile in repeat breeding cows. Indian Vet J. 2015;92(8):56-58.
32. Bhat S, Acharya UR, Adeli H, Bairy GM, Adeli A. Autism: cause factors, early diagnosis and therapies. Reviews in the Neurosciences. 2014 Dec 1;25(6):841-50.