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Association of Electrical Resistance of Vaginal Mucus (ERVM) with Follicle Diameter and Conception Rate in Crossbred Dairy Cows

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ABSTRACT

Background: Estrus detection by observing behavioral signs are time consuming and ineffective method that causes approximately 21 days loss with a significant financial consequence. This study highlights the value of electrical resistance of vaginal mucus (ERVM) during estrus and its association with genital changes, follicle diameter and first service conception rate (FSCR) in crossbred cows.

Methods: A total of 400 cows were selected from different states of North-East, India, out of which 200 numbers of cows were examined to determine the ERVM value prior to artificial insemination (AI) by using Draminski estrus detector.The remaining 200 number of animals were examined for studying the association of ERVM with genital changes, follicle diameter and FSCR.The ERVM value obtained prior to AI was divided into three groups such as 150-200 (Group I), 201-250(Group II) and 251-290 (Group III) ohm, comprising 50 animals in each group. A control groups comprising 50 animals were kept in the study.The genital changes were recorded by transrectal palpation and follicle diameters were recorded by using transrectal ultrasonographic (USG) probe.

Result: The mean ERVM value prior to AI was 196.26±1.25 ohm.The lowest ERVM value (150-200 and 201-250 ohm) was closely associated with free flowing vaginal discharge, open cervix, moderate tone of uterus and mature graffian follicle.The follicles diameters were differed significantly between groups. The FSCR was found to be higher when the cows were inseminated at ERVM value from 201-250 ohm. The result suggested that, ERVM value can be consider as a suitable tool for detection of proper time of AI in cows.

Key words: Crossbred cows, ERVM, First service conception rate, Ovarian follicle.

INTRODUCTION

Maintenance of sound reproductive health management is the foundation for a successful dairy production where detection of estrus is an essential attribute. Cattle breeding herds that utilize artificial insemination (AI) in their reproductive and genetic improvement can be ameliorated with accurate and practical methods of estrus detection (Heersche and Nebel, 1994). Because, estrus detection is one of the key component for improving reproductive efficiency, shortening calving interval and to increase conception rate among dairy animals. Each missed estrus represents the loss of a complete estrous cycle and potential production of approximately 21 days in a seasonally calving herd, with a significant financial consequence. The most common causes of poor estrus detection are shorter estrus duration, silent estrus, sudden change in feeding system, fluctuations in environmental temperatures and occurrence of onset of estrus during the late night to early morning. Visual observation based on various behavioral (urination, mucus discharge, sniffing, mounting, drop in milk yield, standing heat, *etc*.) and physical signs (swelling of vulva, congestion of vulvar mucus membrane) of estrus is a commonly used method of estrus detection which involves the trained personnel to recognize and record the signs of estrus in animals. This method is tedious, time-consuming and also high cost of more labor involvement. Hence, there is need of using other alternate and more efficient methods of estrus detection. Till today, there are several aids have been developed for detection of estrus in farm animals with some pros and cons.

The electronic resistance on vaginal mucus (ERVM) technology is one of the aids used for detection of estrus in farm animals. During estrus, the increasing level of estrogen may change the activity of metabolic pathway and produces adrenocorticotropic hormone and aldosterone (Noakes *et al*., 2019), which increases the level of sodium chloride (Nacl) in the vaginal mucus. As a result, NaCl decreases electronic resistance of vaginal mucus during estrus

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(Fehring, 1997). This low electrical resistance might be an indicator for detection of estrus and proper time of AI in cows. Several researches had been done throughout the globe using ERVM technology for detection of estrus in cows with varying degree of success. In cattle, electrical resistance of vaginal mucus at estrus has shown to vary within animals at successive estruses and between animals on the same day of the estrus cycle (Elving *et al.,* 1983).Therefore, the present work has been planned to find out the ERVM value prior to AI and its association with genital changes, follicle diameter and first service conception rate (FSCR) in crossbred dairy cows.

MATERIALS AND METHODS

This study was ethically approved by Institutional Animal ethical committee (IAEC) of Assam Agricultural University, Jorhat, India.

Selection of animals

A total of 400 healthy and reproductively sound crossbred cows were selected from individually owned farmers farm in different state of North-East, India. The study was conducted for a period of 17 months from October, 2019 to February, 2021. All the cows were in 2nd to 3rd lactation, body weight ranging from 200-250 kg, body condition score was 3.5 (Mishra *et al.*, 2016). Hand milking was performed twice in a day, producing 800-1200 kg of milk per cow per lactation. The data of animals were collected either by examination of animals or recorded by interviewing the farmers using questionnaire. All the animals were examined transrectally to detect any abnormalities in the genital tract, additionally white side test was also performed for screening out sub clinical endometritis. All the experimental animals were maintained in semi intensive system of rearing. All the animals were fed mostly on straw, green fodder and small amount of concentrate mixture comprising of wheat bran and rice polish. All the cows were vaccinated against Foot and mouth disease, Haemorrahagic septicemia and black quarter disease. Deworming was done at 6 month interval in all the animals.

Detection of estrus by visual observation

One trained observer monitored behavior signs of estrus every 6 hr interval for 30 min at 5:00 am, 11 am, 5:00 pm and 11:00 pm. Estrus was defined as a period at which a female animals shows sexual desire to male (Noakes *et al.*, 2019). Estrus efficiency was determined by calculating the heat score for each individual female by the method described by Roelofs *et al.* (2005). In this system, behavioral symptoms (urination, mucus discharge, sniffing, mounting, standing heat, *etc*.) of estrus were recorded and an individual score was assigned to each sign. If the sum of points during consecutive observation periods exceeded 100, the animal was considered to be in estrus. Onset of estrus was defined as the first observation period the animal showed estrous behavior minus 3.0 hr.

Recording of ERVM

A total of 200 numbers of cows were selected for recording ERVM value prior to AI. Estrus of cows was detected by the trained observer on the basis of visual observation of behavioral signs of estrus. After 14-16 hrs of onset of estrus or prior to insemination the value of ERVM was recorded by using Draminski estrus detector (Draminski Electronics in Agriculture, 17 Owocowa, 10-860 Olsztyn, Poland). After restraining the cows, the vulva of the cows were cleaned with mild antiseptic solutions and dried with clean cloth. The probe was inserted through vulva up to the cervix and value of ERVM was determined (Fig 1). The ERVM values were recorded as per method described by Islam *et al.* (2019). Three numbers of ERVM readings were taken at an interval of one minute prior to AI in each cow. A total of 600 numbers of observations were recorded and a mean resistance value obtained from analysis was used as standard ERVM value at the time of insemination in cows.

Correlation of ERVM with genital changes and ovarian follicle diameter

The remaining 200 numbers of selected animals were considered for studying the association of ERVM value with genital changes and ovarian follicle diameter at estrus.

Fig 1: Recording of ERVM value prior to artificial insemination.

The standard ERVM value obtained prior to AI on the first part of study were divided into three groups such as 150 to 200 ohm (Group I), 201 to 250 ohm (Group II) and 251 to 290 ohm (Group III), comprising 50 animals in each group. For studying the association of ERVM with genital changes transrectal palpation of genital organs were done. The ovarian follicle diameters were determined by using transrectal ultrasound probe and diameter of follicles were expressed in mm. Ultrasound scanning was done by using Fujifilm Sonosite M-Turbo™ portable Ultrasound Machine, Sonosite Inc. (USA) equipped with Sonosite L52X rectal Ultrasound probe. The genital changes were recorded for vaginal discharges, patency of cervix, uterine tonicity and presence of mature graffian follicle and corpus luteum in ovaries. Vaginal discharge was recorded as free flowing when distinct discharge was seen flowing from vulva, scanty when there was evidence of discharge sticking to the tail or perineal region of cow and absent when there was no evidence of discharge at all. Cervix was examined for patency and recorded as open and closed. Palpation of distinct depression on the external os was indicative of open cervix or otherwise closed. Both horns of uterus were palpated for uterine tonicity and recorded as good, moderate and mild, when the horn shows distinct turgidity and coiling, some degree of turgidity but no distinct coiling and little degree of turgidity respectively. Ovaries were examined for presence of mature graffian follicle, ovulatory depression and mature corpus luteum. The remaining 50 animals were kept as a control group (Group IV). Onset of estrus in Group IV animals were recorded based on behavioral signs of estrus. After 14-16 hours of onset of estrus or prior to AI transrectal palpation of genital organs and USG screening of ovarian follicle were done. Animals under Group I- III were inseminated based on the ERVM value and the control group animals were inseminated based on visual observation of behavioral signs of estrus. All the cows were bred artificially using quality frozen semen straw (0.25 ml) procured from Assam Livestock Development Agency (ALDA).

Pregnancy diagnosis

The cows after artificial insemination were further monitored for pregnancy diagnosis. The pregnancy diagnosis was confirmed by transrectal palpation of genital tract after 60 days of post AI.

Statistical analysis

Frequency of occurrence of genital changes at estrus was expressed in percentage. The ovarian follicle diameters in relation to ERVM during estrus were expressed as mean±SE (Standard Error). Means were analyzed using one-way analysis of variance followed by post hoc test to determine significance difference between ovarian follicle diameters in all the groups, using the SPSS (version 20.0, SPSS, Chicago, IL, USA). Correlation between ERVM and follicle diameter was determined by using Pearson correlation test. FSCR was expressed in percentage and significant differences were analyzed by using chi-square test. Difference with values of $p \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Electronic resistance of vaginal mucus (ERVM) prior to AI

The mean ERVM values prior to AI in 200 numbers of crossbred dairy cows were 196.26±1.25 ohms that ranged from 150 to 290 ohms was in agreement with the finding of Islam *et al.* (2019). The mean ERVM values on the day of estrus were 186.67±12.54 and 196.67±8.34 ohms in 20 and 21 days of estrus cycle length in crossbred cows by using of Draminski electronic estrus detector as reported by Islam *et al*. (2019). However, lower mean vaginal electrical resistance (VER) during estrus was recorded 82.2±2.2 ohms in dairy cattle of Ethiopia (Tadesse *et al.,* 2011) and 32.68±0.46 ohms in Rathi cows of India (Meena *et al.,* 2003)by using Ovatec device. These differences of ERVM values during estrus might be the result of changes in intravaginal probe design (Tadesse *et al.,* 2011), different stages of estrus cycle (Islam *et al.,* 2019), depth of the probe inserted into vagina (Aboul-Ela *et al.,* 1983), positioning of probe into the vagina (Foote *et al.,* 1979), pressure of the probe against the vaginal mucus and pathological condition of genital tract (Leidl and Stolla, 1976), limited area of genital tract and the electrical potential differences on its surface (Gupta and Purohit, 2001),the entrapment of air in the vagina during insertion of an electrode bearing probe or unequal hand pressure during taking resistance measure (Lehrer *et al.*, 1991) *etc*. However, necessary precaution was taken to eliminate aforesaid conditions for obtaining ERVM value in the present experiment.

Correlation between ERVM and genital changes prior to AI

The association between ERVM and frequency of occurrence of genital changes during estrus of crossbred cows are presented in Table 1. It was observed that greater proportion of animals showed free flowing vaginal discharge, moderate tone of uterus and presence of mature graffian follicle in the ovaries when the ERVM values ranging between 150 to 200 ohms (Group I) and 201 to 250 ohms (Group II). However, when the ERVM was between 251 to 290 ohms (Group III) most of the animals had scanty vaginal discharge, mild uterine tone and mature follicle which have ovulated. In control group cows 52, 52 and 58 % animals showed scanty vaginal discharge, moderate tone of uterus and mature graffian follicles in ovaries respectively. These findings clearly indicated that there was a close association between ERVM values and genital changes during estrus. The present finding was in agreement with the findings of Purohit and Gupta (2000) in cows and reported that lower VER ranging from 26 to 35 ohms showed vaginal congestion, good to fair vaginal discharge, uterine tone and a mature follicle. However, when VER was between 36 to 40 ohms the vaginal congestion, discharge and uterine tone were poor and ovulation had already taken place. The increasing levels of estrogen followed by production of corticotropic

Table 1: Frequency of occurrence (%) of genital changes at estrus in association with ERVM value.

Group	No of animals	Vaginal discharge			Cervix		Uterus			Ovaries		
		Free flowing	Scanty	Absent	Open	Closed	Good tone	Moderate tone	Mild tone	Mature graffian follicle	Ovulation depression	Mature corpus luteum
Group I (ERVM values 150 to 200 ohms)	50	42 (84.00)	7 (14.00)	1 (2.00)	50 (100.00)	0 (0.00)	15 (30.00)	32 (64.00)	3 (6.00)	47 (94.00)	3 (6.00)	0 (0.00)
Group II (ERVM values 201 to 250 ohms)	50	41 (82.00)	5 (10.00)	4 (8.00)	50 (100.00)	0 (0.00)	11 (22.00)	35 (70.00)	4 (8.00)	45 (90.00)	5 (10.00)	0 (0.00)
Group III (ERVM values 251 to 290 ohms)	50	12 (24.00)	31 (62.00)	7 (14.00)	46 (92.00)	4 (8.00)	6 (12.00)	19 (38.00)	25 (50.00)	16 (32.00)	34 (68.00)	0 (0.00)
Group IV (Visual observation)	50	19 (38.00)	26 (52.00)	5 (10.00)	48 (96.00)	2 (4.00)	10 (20.00)	26 (52.00)	14 (28.00)	29 (58.00)	21 (42.00)	0 (0.00)

Figure in the parenthesis indicate frequency of occurrence.

hormone and aldosterone during estrus resulting in increased level of NaCl in the vaginal mucosa. As a result, NaCl, decreases electrical resistance of vaginal mucosa during estrus than other time of estrous cycle (Fehring, 1997). In cattle decreased ERVM in the second half of estrus reported as the luteinizing hormone (LH) surge time and was most suitable time for insemination (Purohit and Gupta, 2000). Therefore, low ERVM value might be used as an indicator of diagnosis of estrus in cows in respect to genital changes. Moreover, in Group IV cows highest per cent of cows showed scanty vaginal discharge, moderate tone of uterus and 58% cows having mature graffian follicle in ovaries. This result might be indicated that estrus period of most of the cows were already completed. Therefore, there is a need of increasing the frequency of observations to detect the behavioral signs of estrus. Moreover, detection of estrus by observing behavioral sign is not only a superior method for artificial insemination programme.

Correlation between ERVM and ovarian follicle diameter prior to AI

The associations between ERVM values and ovarian follicle diameters have been presented in Table 2. The ovarian follicle diameters were differed significantly between groups. The mean highest diameters of follicles were recorded when ERVM value ranging between 201 to 250 ohms and lowest in 251 to 290 ohms. It was observed that, the follicle diameters of the cows were in between 13.5 to 15.5 mm (Fig 3, Fig 4); indicating presence of mature graffian follicle in the ovary (Fig 2A, 2B) when ERVM value ranged between 150 to 200 ohms and 201 to 250 ohms. However, when the ERVM was between 251 to 290 ohms and control groups the diameter of most of the follicles were between 4 to 6 mm (Fig 5, Fig 6) indicating that estrus was already completed and ovulation had taken place (Fig 2C). A negative correlation between ERVM value and ovarian follicle diameter was observed in the present study (Table 3). Similar observation was also recorded in dairy cowsby (Tadesse *et al.,* 2011). The authors recorded that the mean vaginal electrical resistance and diameter of largest follicle at estrus were 89.60±3.10 ohm and 14.30±0.10 mm and at diestrus were 106.8±1.4 ohm and 10.00±0.10 mm respectively. However, in Group III and IV most of the follicles diameters were in between 4 to 6 mm, indicating that presence of post ovulatory follicles. The ovarian follicle diameters were significantly differed between the groups. The mean lowest diameters of follicles were observed in Group III. The vaginal electrical resistance differed between follicular and luteal phase and the lowest vaginal electrical resistance correlated with the maximum follicular size and highest estrogen concentration during estrus (Islam *et al.*, 2019; Tadesse *et al.,* 2011). The present findings might be an indicated that low ERVM value resulting presence of largest diameters follicles in ovaries of cows. Moreover, in control group of presence of post ovulatory follicles might be due to inaccuracy of detection of estrus and estrus was already completed in most of the animals.

Table 2: Ovarian follicle diameter in relation to the electrical resistance of vaginal mucus (ERVM) in crossbred cows during estrus.

Group	No of animals	Ovarian follicle diameter (mm) by ultrasound scanning
Group I (ERVM values 150 to 200 ohms)	50	14.43^{c}±0.07
Group II (ERVM values 201 to 250 ohms)	50	14.55^{c}±0.07
Group III (ERVM values 251 to 290 ohms)	50	7.29^{a} ±0.57
Group IV (Control group)	50	10.44^{b} ±0.65

^{a,b,c} Means bearing different superscript in a column differ significantly (P<0.05).

Fig 2: Ultrasound scanning of ovarian follicle at estrus. Represents the presence of mature graffian follicle in the ovary.

Fig 2C: Ultrasound scanning of ovarian follicle at estrus. Represents that ovulation is already completed.

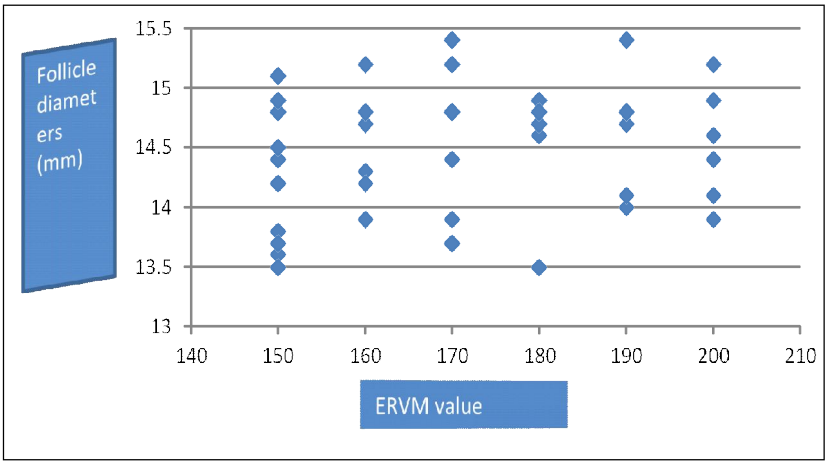

Fig 3: Correlation between ERVM values with follicle diameter at estrus of Group I (150 to 200 ohms) cows.

Correlation between ERVM and FSCR

The association between FSCR to the value of ERVM at the time of insemination is presented in Table 4. There was significant difference in FSCR percentage in all the groups. The highest FSCR was recorded when cows were inseminated at ERVM value ranging from 201 to 250 ohms followed by 150 to 200 ohms, control group and lowest in 251 to 290 ohms. The low conception rate in Group III and IV might be due to presence of cows with post ovulation and estrus was already completed. The present findings is

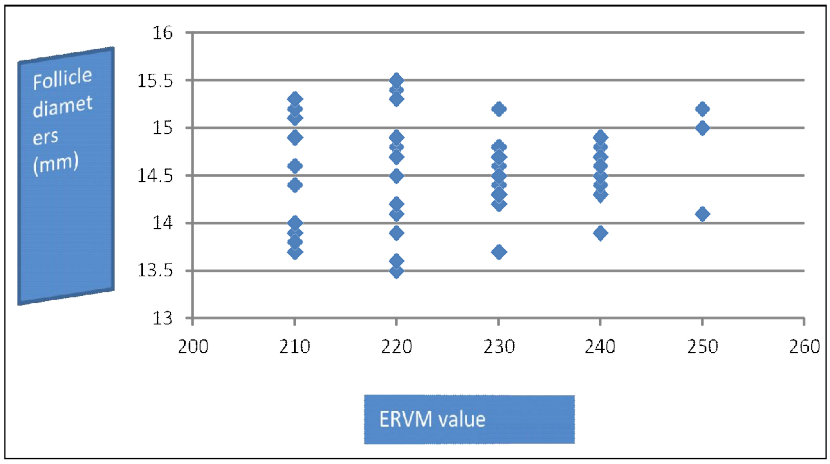

Fig 4: Correlation between ERVM values with follicle diameter at estrus of Group II (201 to 250 ohms) cows.

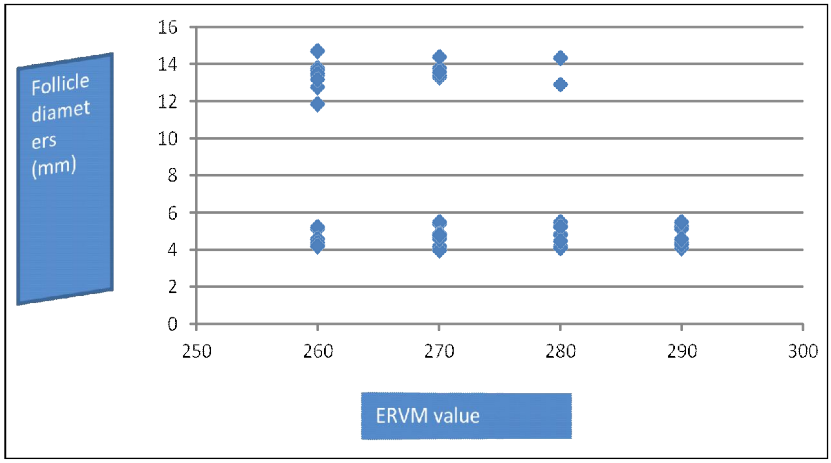

Fig 5: Correlation between ERVM values with follicle diameter at estrus of Group III (251 to 290 ohms) cows.

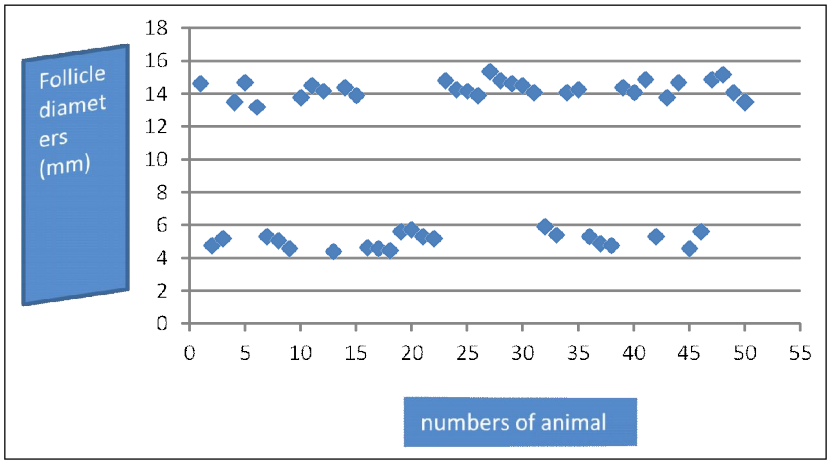

Fig 6: Diameters of follicles in control group cows.

Table 3: Correlations between electrical resistance of vaginal mucus (ERVM) value and follicle diameter.

		ERVM	Follicle
ERVM	Pearson correlation	1	-.680**
	Sig. (2-tailed)		0
	Sum of squares and cross-products	284536	-18537.44
	Covariance	1909.638	-124.412
	N	150	150
Follicle	Pearson correlation	-.680**	1
	Sig. (2-tailed)	0	
	Sum of squares and cross-products	-18537.44	2610.133
	Covariance	-124.412	17.518
	N	150	150

Table 4: First service conception rate (FSCR) in relation to the value of electronic resistance of vaginal mucus (ERVM) at the time of insemination.

Group	Number of cows inseminated	No of animals conceived	First service conception rate (FSCR %)	χ^2 value
Group I (ERVM values 150 to 200 ohms)	50	35	70	12.55*
Group II (ERVM values 201 to 250 ohms)	50	41	82	
Group III (ERVM values 251 to 290 ohms)	50	19	38	
Group IV (control)	50	22	44	

P≤0.05.

in agreement with earlier study where cows inseminated with low electrical resistance had higher pregnancy rate than those inseminated when electrical resistance was high (Ahmed *et al.,* 2017). Ahmed *et al.* (2017), reported that the ERVM values ranging 181 to 220 ohms had higher pregnancy rate (83.30%) as compared to cows inseminated with 221 to 280 ohms showing lower pregnancy rate (36.40%) by using Draminski estrus detector. A few studies also recorded that insemination of cows at low VER (26 to 30 ohms) resulting higher conception rates (84.00 to 92.50%) by using Ovotec device (Meena *et al.,* 2003; Purohit and Gupta, 2000). The present study indicated that ERVM value can be used for detection of time of AI in cows to obtain high FSCR. However, in previous study it was reported that ERVM was insufficiently reliable to predict time for AI in large farms conditions (Foote *et al.,* 1979) and ERVM value was not reliable method for predict the time of insemination after induction and synchronization of estrus in cows (Zuluaga *et al.,* 2017).

CONCLUSION

The present study can be concluded that the insemination of cows at ERVM value of 201 to 250 ohm is distinctly advantageous to obtain higher FSCR. However, further study should be implement to rule out the effectiveness of ERVM for detection of proper time of estrus in silent estrus cows and insemination of large numbers of cows should be conducted before application of the technology under field condition.

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