

Monitoring vaginal electrical resistance, follicular waves, and hormonal profile during oestrous cycle in the transition period in Bangladeshi sheep

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Received: June 9, 2018

Accepted: December 12, 2018

Abstract

Introduction: The ovarian follicular dynamics, vaginal electrical resistance (VER), progesterone (P₄) and oestrogen (E₂) profiles were investigated during the oestrous cycle in four indigenous ewes. **Material and Methods:** Daily VER values were recorded with a heat detector. The follicles were observed and measured by trans-rectal ultrasonography. Blood was collected daily for hormonal profiles. **Results:** A significant variation in VER values ($P < 0.05$) in oestrus by ewes and position in the sequence of cycles was observed. Trans-rectal ultrasonography of ovaries revealed the presence of 2–4 waves of follicular growth. Study of hormonal profiles by ELISA revealed a positive correlation between E₂ concentration and development of follicles and a negative correlation between P₄ concentration and their development. The concentrations of oestradiol increased in oestrus and then decreased to a basal level. Follicular growth was accompanied by a rise in the concentration of serum oestradiol. Inversely, when follicles received the stimulation for ovulation, concentration of progesterone started to fall, but after ovulation, it climbed back to its peak and remained at this state until next ovulatory follicle reached its maximum diameter. **Conclusion:** This study could help to set up a manipulative reproductive technique for improving genetic values in indigenous sheep.

Keywords: ewes, vaginal electrical resistance, oestrus detector, ovarian follicle, progesterone, oestradiol.

Introduction

Broadly the oestrus cycle in ewes can be divided into follicular and luteal phases. In the follicular phase, the oestrogen level increases and progesterone level decreases to the basal level. In the luteal phase, the levels change in the reverse direction. When the progesterone level is low in blood, it indicates that the animal is in the follicular phase, that means that the animal may be in prooestrus or oestrus. Therefore, estimation of progesterone level is an important tool for detection of stages of the oestrous cycle. During oestrus, oestrogen levels are the highest; this causes NaCl levels to be high, resulting in a significantly lower level of electrical resistance than usual (12). Differences in vaginal electrical impedance (VEI) readings resulting from the oestrus cycle correspond to

its phases, providing vital information for efficient and effective breeding. Exploiting this phenomenon, an electrical device has been developed for efficient heat detection, termed a heat detector. Measurements are taken simply by properly inserting the probe in the vagina and taking the reading, and no prior technical knowledge on the part of the operator is required. The oestrus detector is responsive to the hormonal changes that coincide with oestrus and, by one report, its use is a much simpler and more efficient process than previous methods (27).

Manipulation of ovarian follicle(s) development, maturation, and ovulation, and timing of insemination is the key to assisted reproduction techniques used worldwide in animal reproduction to increase and improve animal origin food production. In Bangladesh, the use of assisted reproductive technologies (ARTs) is

absent in sheep at the field level, although some technologies such as artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) have been developed experimentally. Extension and expansion of the application of these techniques is essential for improvement of genetic characteristics in native sheep. For this, we must have a clear understanding of the follicular dynamics in our sheep; however, no study has yet been performed on this aspect in Bangladesh. Therefore, the present study was designed to monitor vaginal electrical resistance (VER) values, follicular waves, and hormonal profile during the oestrous cycle in indigenous ewes to determine the state of the cycle.

Material and Methods

Experimental site. The study was conducted at the Research Animal Farm, Department of Surgery and Obstetrics, Bangladesh Agricultural University, from February to May 2016.

Animals. Four indigenous ewes aged 1.5–2.0 years were randomly selected and reared under a semi-intensive system. They were given the designations HF-1, HF-3, HF-4, and HF-5. The animals were fed green grass and concentrate mixture (250 g of concentrate per day) according to their body weight. Vitamin premixes (Powder Megavit-DB, Novartis Animal Health, Bangladesh) were also supplied. All the selected ewes were dewormed using nitroxynil (Nitronex 34% Injection, Renata Animal Health, Bangladesh) and albendazole (Helmex Tablet, Renata Animal Health, Bangladesh) alternately at 3 month intervals. One vasectomised ram was reared with the ewes to detect behavioural oestrus in experimental ewes.

VER recording. Daily VER values of the ewes were recorded from the day of oestrus for two cycles (34–36 days) by using an electronic heat detector (Draminski, Poland). Heat was also detected by teaser ram. Physical characteristics such as swollen vulva and mucus secretion were also monitored to detect heat. A band was used to mark the length of the probe to be inserted into the vagina. The following steps were performed for VER recording in selected ewes using the heat detector:

- Prior to use, the probe of the heat detector was washed with tap water and then with 1% potassium permanganate (PPM) solution. No gel or oily substance was used as this could change the VER reading;
- The animal was restrained by an assistant and the vaginal opening was soaked with 1% PPM solution;
- The probe was inserted into the vagina. The probe

was placed against the right side to take a reading from the vaginal wall. Care was taken to insert the probe up to the marked length each time;

- The reading of VER was visible on the screen;
- The same procedure was repeated three times to obtain an average value;
- Then, the probe was removed from the vagina and washed with running tap water and 1% PPM solution for further use.

Use of ultrasonography to monitor ovarian follicular waves. Trans-rectal ultrasonography was performed in the ewes by ultrasound machine (Magic 5000, Eickemeyer, Germany) equipped with a 7.5 MHz linear probe to examine ovaries. All animals were examined by the same operator. Ultrasonography started on the day when oestrus was observed and continued for two cycles. All follicles ≥ 2 mm in diameter were recorded. After freezing, the image on the screen, the maximum internal diameter of each follicle was measured using the built-in electronic calliper. The diameter and relative position of the follicles was sketched in the daily record notebook. Also, the diameter and position of the corpus luteum (CL) were noted if observed in the same way. Each day, ovarian diagrams depicting the relative location of follicles were made to determine patterns of growth and regression of individual follicles to define a follicular wave. Firstly, the animal was restrained in dorsoventral position on a table by an assistant. Faeces were removed from the rectum using a gloved index finger lubricated with ultrasonographic gel. The ultrasound machine was prepared, and the probe was washed with running water and coated with ultrasonographic gel. The probe was inserted into the rectum, and ultrasonographic images of the ovarian follicles were observed, measured, and sketched on paper.

The following parameters of follicular waves were determined for each animal:

- i. The number of follicular waves,
- ii. Length of each follicular wave,
- iii. Average size of largest anovulatory follicle,
- iv. Average size of ovulatory follicle,
- v. Average number of ovulations in a cycle,
- vi. Day of appearance of dominant follicle with the largest diameter.

Measurement of serum progesterone and oestradiol values. A 3 mL blood sample was collected from the jugular vein daily with appropriate aseptic measures, without using any anticoagulant prior to ultrasonography. Then, serum was collected after centrifuging at 1,500 rpm for 15 min and stored at -80°C until determination by ELISA of the concentrations of progesterone and oestradiol hormone (17). The serum sample was equilibrated at room temperature for half an hour. The samples were assayed by ELISA technique using Progesterone and Estradiol ELISA kits (NovaTec Immundiagnostica GmbH, Germany). Concentrations were calculated using the

formula $y = -0.236 \ln(x) + 1.6163$ for oestradiol (Fig. 1a) and $y = -0.248 \ln(x) + 1.2527$ for progesterone (Fig. 1b) obtained from standard value curves.

Data analysis. VER values, follicular, data and hormonal (progesterone and estradiol) concentrations were analysed for their relationships. Descriptive analysis was performed to present the characteristics of follicular dynamics and VER during the oestrous cycle. The values of VER, follicular diameter, and hormonal profile were compared using a *t*-test (0.05%) by MSTAT-C software (Michigan State University, USA), and values for individual ewes were presented as a line graph.

Results

The VER values in oestrus are presented in Table 1. There was a significant variation ($P < 0.05$) in these

values in oestrus in the ewes. Significant variations ($P < 0.05$) were also observed in VER values in the same ewe at the same stage in different cycles. These phenomena indicated that the VER values should be recorded for individual ewes to detect the oestrus.

Trans-rectal ultrasonography was performed daily to monitor follicular waves in ewes. The follicles were detected as echo-free black circles and were measured if ≥ 2 mm. From emergence to disappearance of a follicle ≥ 5 mm was defined as the length of a wave. The emergence of follicular wave, maximum diameter of dominant follicles in a wave, and days when the dominant follicle reached maximum size are presented in Table 2. Results indicated the presence of two to four waves of follicular growth during the study period. The days taken for development of follicles to their maximum diameter were different.

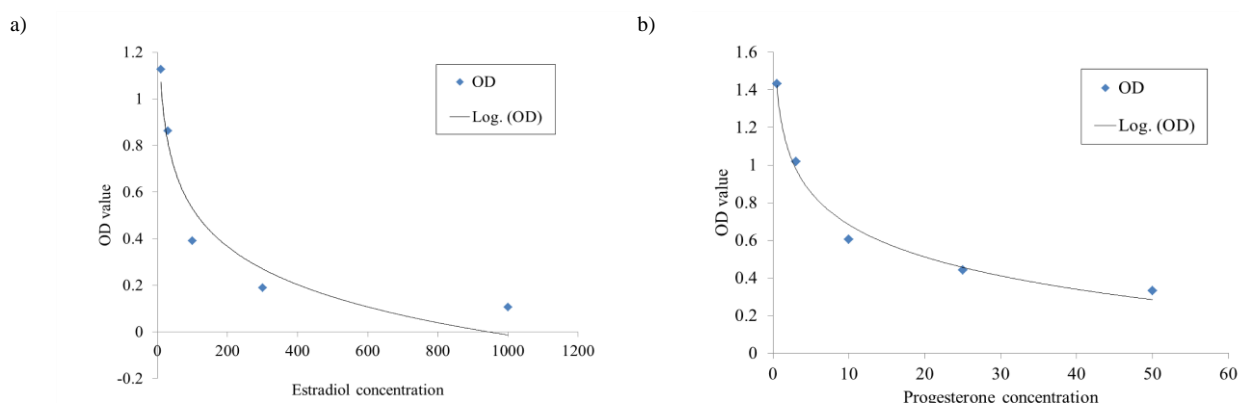


Fig. 1. Serum estradiol and progesterone values. (a) – standard curve for estradiol, (b) – standard curve for progesterone

Table 1. VER values on the day of oestrus

Ewes	VER value (in ohms, Ω) on the day of oestrus				
	1 st oestrus	2 nd oestrus	3 rd oestrus	Mean \pm SD	P-value
HF-1	360.0 ^a	460.0 ^b	430.0 ^{ab}	416.7 \pm 51.3 ^x	0.0343
HF-3	490.0 ^a	470.0 ^a	300.0 ^b	420.0 \pm 104.4 ^x	0.0382
HF-4	370.0	380.0	380.0	376.7 \pm 5.8 ^y	0.0530
HF-5	300.0 ^a	350.0 ^a	460.0 ^b	370.0 \pm 82.0 ^y	0.0219
P-value				0.0312	

a, b, c indicate significant variation in rows; x, y indicate significant variation in columns. Significant at 5% level of probability

Growth and regression of individual follicles, follicular waves, and VER values are shown in Figs 2, 3, 4, and 5. Some follicular waves were found to emerge in the presence of growing ovulation-sized follicles from a previous wave. We found that several follicles grew coincidentally during each follicular wave without affecting each other's growth. Results also showed that follicular diameter was negatively correlated with VER values. When follicular diameter increased, the VER value decreased correspondingly.

There was a decline in the concentration of progesterone before ovulation down to its lowest level of 1.18 ± 0.5 ng/mL with a range of 1.15–1.22 ng/mL. When ovulation completed, the concentration rose with

some minor fluctuations to its highest level of 1.42 ± 0.04 ng/mL with a range of 1.38–1.46 ng/mL. This might indicate the basal concentration was 1.18 ± 0.5 ng/mL during oestrus, and peak level concentration was 1.42 ± 0.04 ng/ml during dioestrus. Inversely, serum oestradiol concentration increased with the manifestation of behavioural oestrus to an uppermost concentration of 1.87 ± 0.3 pg/mL with the range of 1.71–2.27 pg/mL and after ovulation it started to decrease to a basal level of 1.59 ± 0.03 pg/mL with the range of 1.56–1.63 pg/mL. The relationship between follicular diameter and hormonal profile is shown in Figs 6 and 7.

Table 2. Presence of follicular waves and dominant follicles

Parameters	Wave 1	Wave 2	Wave 3	Wave 4
Days of emergence of wave				
4 waved cycle (n = 1)	1	5	10	13
3 waved cycle (n = 3)	0.7 ± 0.6	6.7 ± 1.5	13.3 ± 0.6	
2 waved cycle (n = 4)	1 ± 0	9.8 ± 2.2		
Day of maximum diameter of follicle in the wave (day)	5.25 ± 0.6	5.75 ± 1.0	4.83 ± 0.3	4.0 ± 0
Maximum diameter of follicle in the wave (mm)	5.22 ± 0.06	5.30 ± 0.06	5.41 ± 0.3	5.14 ± 0

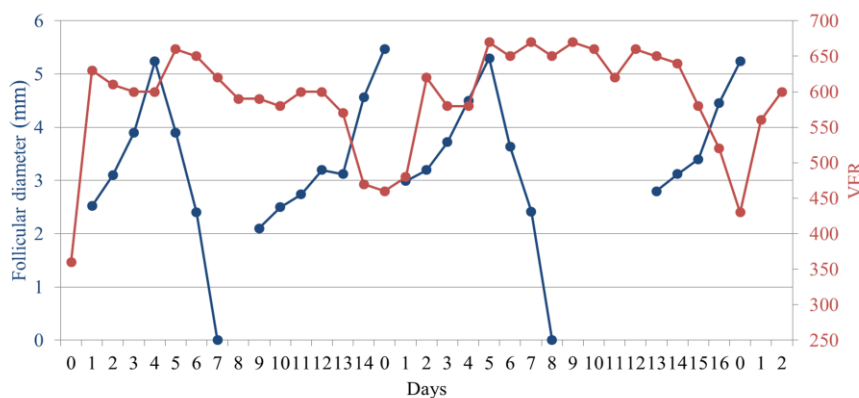


Fig. 2. Graphical presentation of follicular wave and VER values of ewe HF-1. Absence of ovulation-sized follicle observed on the previous day indicates ovulation. Follicular diameter 0 indicates regression of individual follicle during the oestrous cycles. Lengths of the first and second waves were four and seven days in cycle 1 and in cycle 2 lengths of the first and second waves were both five days

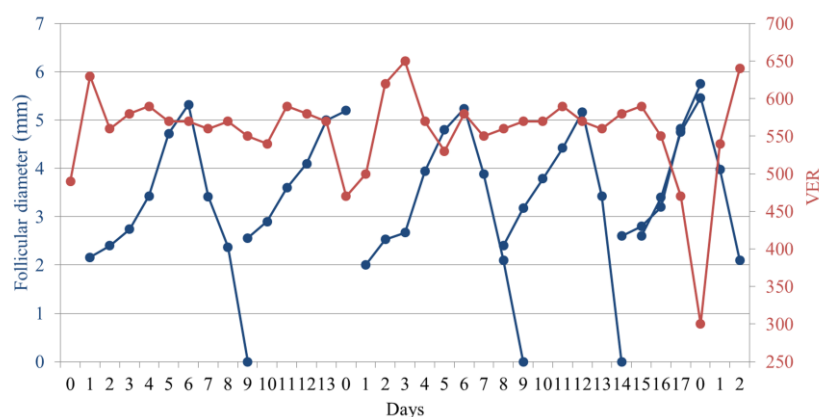


Fig. 3. Graphical presentation of follicular wave and VER values of ewe HF-3. Absence of ovulation-sized follicle observed on the previous day indicates ovulation. Follicular diameter 0 indicates regression of individual follicle during the oestrous cycles. Lengths of the first and second waves in cycle 1 were both six days. In cycle 2 the first wave was six days long and the second and third waves lasted five days

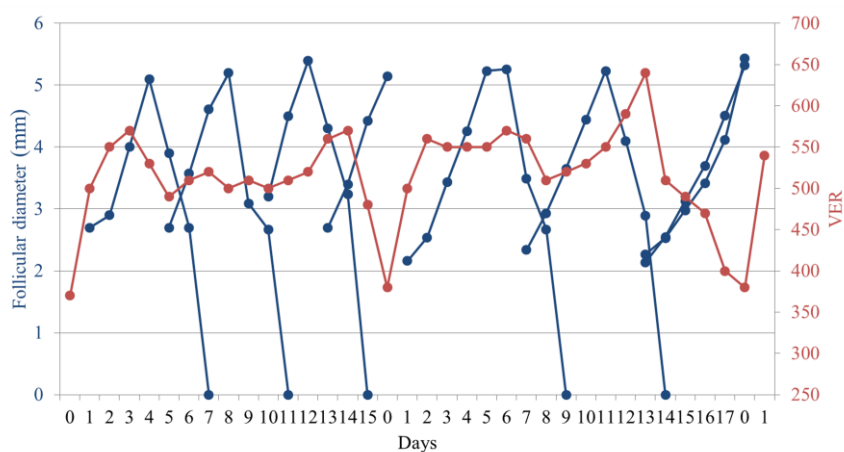


Fig. 4. Graphical presentation of follicular wave and VER values of ewe HF-4. Absence of ovulation-sized follicle observed on the previous day indicates ovulation. Follicular diameter 0 indicates regression of individual follicle during the oestrous cycles. Lengths of the first, second, and fourth waves were all four days and in the third wave was three days, in cycle 1. In cycle 2 the first and third waves were six days long and the second wave lasted five days

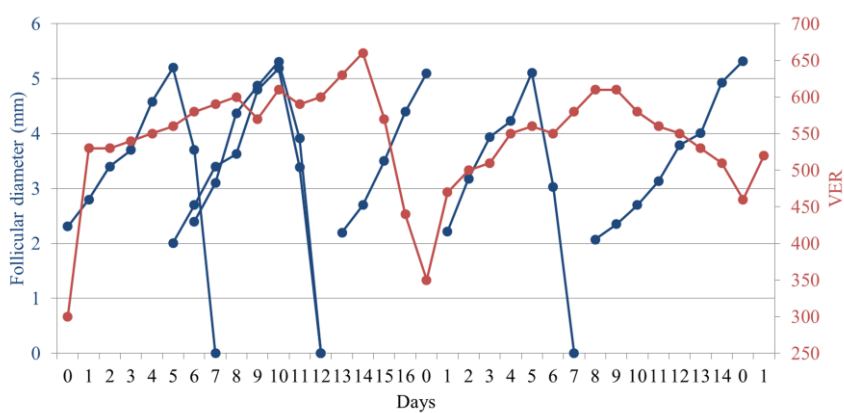


Fig. 5. Graphical presentation of follicular wave and VER values of ewe HF-5. Absence of ovulation-sized follicle observed on the previous day indicates ovulation. Follicular diameter 0 indicates regression of individual follicle during the oestrous cycles. Lengths of the first and second waves were both six days and in the third wave was five days, in cycle 1. In cycle 2 the first wave was five days long and the second wave lasted eight days

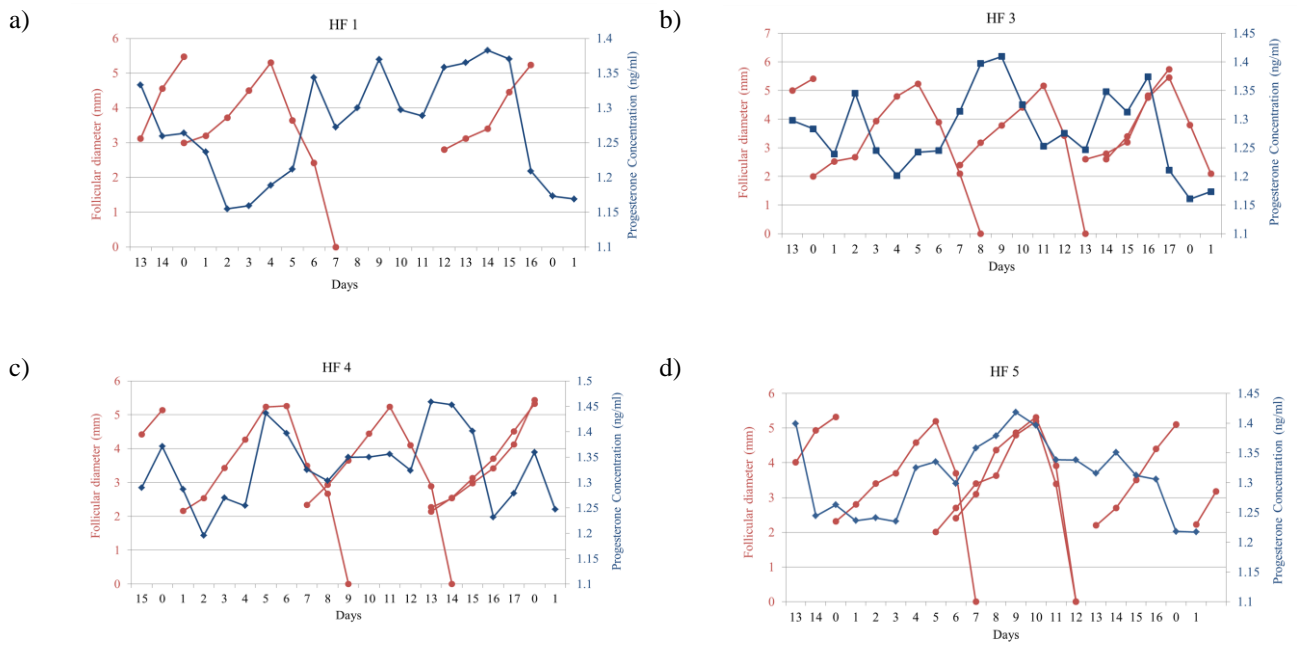


Fig. 6. Graphical presentation of progesterone concentration and follicular diameter of ewes HF-1 (a), HF-3 (b), HF-4 (c), and HF-5 (d). Near day 0 P₄ concentration declined which coincided with behavioural oestrus. After day 0 it increased to its highest level. Then, it started to decrease gradually to the low value of the next day 0. Follicular diameter reached its maximum, causing ovulation, when progesterone concentration was at its lowermost. So the graphs illustrate the negative correlation between progesterone concentration and development of follicles

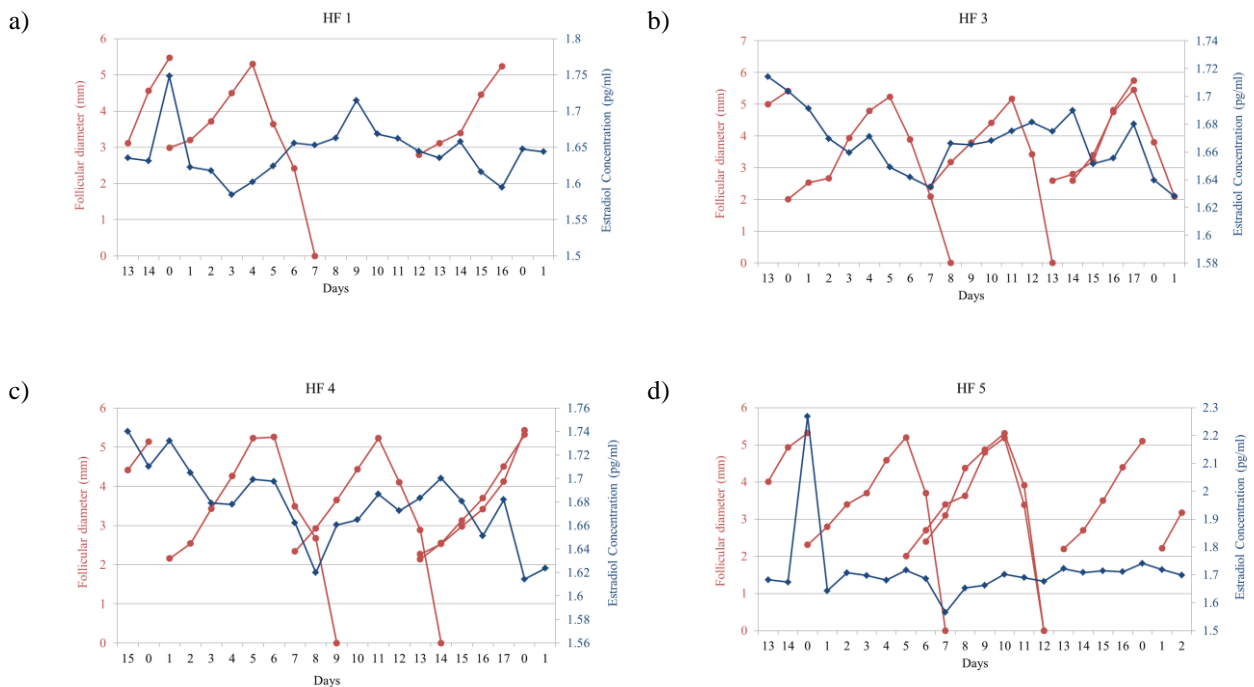


Fig. 7. Graphical presentation of oestradiol (E₂) concentration and follicular diameter of ewes HF-1 (a), HF-3 (b), HF-4 (c), and HF-5 (d). Near day 0 E₂ concentration increased which coincided with behavioural oestrus. After day 0 it decreased to its lowest level. Then, the concentration remained at basal level during luteal phase. Again before the time of ovulation it increased promptly to the highest level. Follicular diameter reached maximum size, causing ovulation, when E₂ concentration was at its peak. So the graphs show the positive correlation between E₂ concentration and development of follicles

Discussion

Understanding reproductive physiology is important to implement manipulative reproductive technologies in animals. Therefore, the study was conducted to monitor vaginal electrical resistance (VER), follicular dynamics, and P₄ and E₂ profiles in indigenous ewes. Daily VER values were recorded in four ewes during the oestrous cycle. Lower VER values were observed during oestrus and after oestrus, they increased to a high level. This finding supports Gupta and Purohit (16) who revealed that vaginal impedance is the lowest during the follicular phase and the highest during the oestrous luteal phase. The mean VER values for HF-1, HF-3, HF-4, and HF-5 were $416.7 \pm 51.3 \Omega$, $420.0 \pm 104.4 \Omega$, $376.7 \pm 5.8 \Omega$, and $370.0 \pm 82.0 \Omega$, respectively. Bartlewski *et al.* (5) studied the VER in two different breeds and concluded that the VER values $<40 \Omega$ indicated oestrus and greatly varied from the results of this experiment. This variation might be due to differences in instrument used, breeds, and climatic conditions. Significant variation ($P < 0.05$) was noted in VER values in oestrus in regard to individual ewes and cycles in this study. These phenomena indicated that the VER values should be recorded for one particular ewe to detect that specific animal's oestrus. The day difference between two successive lower peaks in VER waves ranged from 13 to 17 days, which might indicate the oestrous cycle in indigenous ewes. The length of oestrus cycle was 15.25 ± 0.5 days with the range of 13–17 days. This oestrus cycle length was similar to the findings of Zohara *et al.* (30), while the range was slightly lower than that of Kennedy (18) and Zieba *et al.* (29). The study of the relation between VER and follicular growth indicates that there is an inverse relationship between VER and follicular diameter. The plasma level of oestradiol increases during the follicular phase (19, 21). The largest non-atretic follicles are the main source of oestradiol (11), and this hormone's pertinent effect is to increase the level of NaCl in the vaginal mucous. NaCl itself decreases electrical resistance. During oestrus, oestrogen levels are at their highest levels and this causes NaCl level to be high, resulting in a significantly lower level of electrical resistance than usual (12). A higher electrical charge results in a higher detector reading value and a lower charge will give a lower value.

Understanding and investigating VER as a functional unit during the oestrus cycle could facilitate the development of an indicator value indicating oestrus and values pointing to the existence of an influence on ovarian function in both cyclic and non-cyclic animals. This could give a clear indication of the time of onset of oestrus, which is the most important detail to know for manipulation of ewe reproduction by administration of hormonal treatment for superovulation. There is no precise report available on VER of ewes in our country. This is the first study

on VER in ewes with a two-electrode technique in Bangladesh.

Trans-rectal ultrasonography of ovaries was done daily after VER recording to observe the follicular development in ewes during their natural oestrous cycle. It was found that a cohort of several follicles developed to ≥ 2 mm, of which only one or two developed to ≥ 5 mm, which indicated the wave-like pattern of follicular development in ewes. The observation of waves of follicular development in the sheep during the oestrous cycle is in agreement with previous reports using ultrasound to assess the follicular population (15, 25). The average number of follicular waves was 2.63 ± 0.6 per cycle, which was similar to that in the reports of Evans *et al.* (11) and Duggavathi *et al.* (10), but lower than that of Zieba *et al.* (29). Using ultrasonography, some lines of evidence have been cited for (11) and against (22, 23) the existence of a wave pattern of follicular development in sheep. Some authors noted that the numbers of follicles reaching a maximum diameter of 3 or 4 mm did not vary throughout the ovine oestrous cycle (15, 4).

Hormonal treatment acts on ≥ 2 mm follicles (antral follicle). Therefore, the developmental status of follicles needs to be known in order to set the time of FSH administration. The time of emergence of a follicular wave is crucial information. Through the present study, eight cycles of four ewes were observed and in these cycles, we found that waves emerged on the 1st, 5th, 10th, and 13th days after ovulation. The result is more or less similar to the reports of Ginther *et al.* (15), Bartlewski *et al.* (4), Zieba *et al.* (29) and Duggavathi *et al.* (10). In three-waved cycles, the average days of emergence of follicular waves were 0.7 ± 0.58 , 6.7 ± 1.53 , and 13.3 ± 0.58 . In two-waved cycles, 1 ± 0 and 9.8 ± 2.2 days were noted as averages when the waves emerged. The findings for the average day when the follicles were the largest in diameter were similar to the findings of Duggavathi *et al.* (10), but slightly higher than those of Bartlewski *et al.* (5). This data could facilitate assigning the proper time of hormonal treatment for superovulation in ewes after natural oestrus.

The average length of a follicular wave as observed here is lower than that reported by Seekallu *et al.* (24) but higher than that of McNeilly (20). The average size of an anovulatory follicle was 5.25 ± 0.058 mm and of an ovulatory follicle was 5.35 ± 0.16818 mm. Ovulatory follicles are the ones having achieved most development. Their average size was the average maximum size. The result corresponds well to the range noted by Ginther *et al.* (15), Bartlewski *et al.* (4), Evans *et al.* (11), Vinales *et al.* (28), and Ravindra *et al.* (22), who observed 5–7 mm ovulatory follicles in sheep. The findings are not much different from the values presented by Bartlewski *et al.* (5) and Souza *et al.* (25). The minor differences may reflect factors such as breed, climatic condition, environment, season,

location, management, and others. Ovulatory follicles appear to be more or less similar in maximum diameter to the largest follicles of anovulatory follicular waves of the cycle, granting a very slight difference found in the diameters which also agrees with the observations of Souza *et al.* (26) and Bartlewski *et al.* (4). The number of ovulations per cycle was 1.0, and this supports the findings of Bartlewski *et al.* (5). Cows only have one dominant follicle per wave (14), whereas sheep can have more than one, depending on the ovulation rate of the breed.

The pattern of oestradiol secretion in the ewe is pulsatile (3) and depends on the presence of a large oestrogenic follicle in the ovary. During the ovine oestrus cycle, there are periods of increased circulating concentrations of oestradiol (8). Serum oestradiol concentrations measured in this study show that the concentration increased with the manifestation of behavioural oestrus to an uppermost concentration of 1.87 ± 0.27 pg/mL with a range of 1.71–2.27 pg/mL and declined after ovulation to a basal level of 1.59 ± 0.03 pg/mL with a range of 1.56–1.63 pg/mL. Before, the time of ovulation when follicular size is maximal, the concentration of oestradiol increased to its highest level, and after ovulation, it decreased to its basal level. This concurs with the ovulation day oestradiol concentration fall to non-detectable levels observed by Findlay *et al.* (13) and Baird *et al.* (1) whose outcome was identical to that of the present study. The result is lower than the findings of Zieba *et al.* (29). A possible cause for this variation may be the seasonal effect. As sheep are shortday breeders, they express high oestrogenic behaviour in the winter season and serum oestradiol concentration remains higher in this period. Since the experiment was performed during spring and summer, the length of daylight might affect oestrus behaviour and oestradiol concentration as well. Serum oestradiol concentration changes coincide with the ovulation of the largest follicle of a follicular wave (4, 6, 26) which is exactly the results of the present study.

Progesterone secretion in the ewe is pulsatile during the luteal phase (2). Following ovulation, serum progesterone concentrations increase from day 0 to day 11 but then drop to the lowest content by day 15 after ovulation (4). In this study, progesterone concentration before ovulation declined to its lowest level of 1.18 ± 0.5 ng/mL with a range of 1.15–1.22 ng/mL. After ovulation it rose with some minor fluctuation to its highest level of 1.42 ± 0.04 ng/mL with a range of 1.38–1.46 ng/ml. We found the highest progesterone value of 1.42 ± 0.04 ng/ml during dioestrus and a lowest value of 1.18 ± 0.5 ng/mL in an indigenous Bangladeshi breed. The results of this experiment to a large extent confirm the range of progesterone concentration found in a previous experiment on a similar breed by Zohara *et al.* (30). However, Seekallu *et al.* (24) and Duggavathi *et al.* (10) stated a lower minimum concentration during oestrus. This variation might be connected with breed differences, as

variations of serum concentrations of progesterone between breeds have been evidenced (7).

FSH stimulates preantral follicular growth which increases the number of follicles reaching maturation and reduces the length of the follicular wave by hastening the growth phase of follicles. The hormone acts only on those follicles which are ≥ 2 mm (antral follicle) in diameter. For better understanding of this, study of follicular dynamics in coordination with hormonal measurement is necessary. The present study showed the relationship of follicular growth day to day with the fluctuation of progesterone and oestradiol.

It is concluded that that lower vaginal electrical resistance (VER) is indicative of the presence of oestrus. Detection of lower VER values would help farmers to detect oestrus. The differences between the days on which two successive lower extremes in VER values were recorded ranged from 13 to 17, which might help to indicate the oestrus cycle length in indigenous ewes. Progesterone concentration in serum exhibits negative correlation, and that of oestradiol exhibits positive correlation with follicular diameter. These results could help to determine the status of the oestrus cycle.

Conflict of Interests Statement: The authors declare there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The study was supported by the World Bank through HEQEP subproject no. CP 3015.

Animal Rights Statement: The study was designed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines and EU Directive 2010/63/EU for animal experiments and with permission from the Animal Experimentation Ethics Committee (AEEC), Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202 (Permission Number: 03/2016/AEEC).

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