Research Article

Open Access CrossMark

Oestrous cycle of cross-bred cows: Monitoring of VER, hormonal profiles and ovarian follicular dynamics

Abstract

Study of oestrous cycle of cows is prerequisite for reproductive manipulation aiming optimal genetic gain within existing reproductive and genetic potentialities of the cows. This experiment was carried out based on routine checkup of vaginal electrical resistance (VER), endocrinological assessment of hormonal profiles (progesterone and oestrogen) and ultrasound scanning to observe ovarian follicular dynamics during the course of oestrous cycles of the cross-bred cows. The animals were managed on a uniform field of nutrition, body condition score (BCS) varied from 3.0 to 3.5 (1-5 scale), careful attention was adopted for hygienic approach of experimented animals during reproductive records, instrumental preparation, precautions and application. VER values were registered by electronic oestrus detector (Draminski® Electronic Estrus Detector, Poland), blood was collected, processed to segregate serum by centrifugation (Universal 32R®, Hettich Zentrifugen, Germany), stored at -20^{0}C in a refrigerator followed by hormonal evaluation by ELISA test using commercial kits (Nova Tec Immunodiagnostica®, GmbH, Germany), and ultrasound scanning of ovaries to record follicular and luteal data by 7.5 MHz B Mode linear probe (Bionet®, Republic of Korea) daily at the scheduled time of a day. All the data originated during the routine observations of VER, hormonal profiles and ovaries were carefully noted, systematically organized and analysed statistically. The highest and lowest VER values, blood concentrations of progesterone and oestrogen were recorded as 350.56±7.96 and 186.67±12.54 ohms, 1.26±0.01 and 1.04±0.00 ng (nanogram)/ml, 3.09±0.02 and 2.94±0.01 pg (picogram)/ml, respectively. The patterns of VER and progesterone fluctuations were almost equally proportionate but inversely proportional relationship was also observed between VER values and oestrogen profiles. The wave number per cycle and the interovulatory interval (IOI) of the cycles varied from 2 to 3 and 20 to 21 days, respectively on ultrasonographical observations of ovaries of the experimented cows. The variations of VER, progesterone and oestrogen profiles at different stages of the oestrous cycles, and the correlation between VER and hormonal profiles were significantly reflected ($P<0.05$). Lowest VER values, maximum follicular size and serum concentration of excess oestrogen was indicative for oestrus (follicular phase), and opposite phenomena including hyper concentrations of serum progesterone was exclusively indicative for luteal phase of oestrous cycle.

Keywords: Oestrous cycle, vaginal electrical resistance (VER), hormones, follicular dynamics, cattle

Volume 8 Issue 2 - 2019

Islam MN,[1,2] Juyena NS,[2] Bhuiyan MMU,[2] Talukder MRI,[2,3] Bari FY[2]

[1]Department of Pathology and Parasitology (PPS), Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Bangladesh
[2]Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Bangladesh
[3]Department of Livestock Services, Bangladesh

Correspondence: MN Islam, Department of Pathology and Parasitology (PPS), Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh, Tel +8801716884939, Email islamvetpat@gmail.com

Received: November 06, 2019 | **Published:** December 31, 2019

Introduction

Reproductive efficiency is dependent upon optimization of management, health, and physiology of cows. Although many factors are interrelated with the reproductive efficiency of heifers such as body weight,[1] metabolic status,[2,3] heat stress,[4] and sufficient ovarian activity.[5] Reduced postpartum resumption of ovarian cyclicity is related to nutrition, body condition and body weight change[6–9] and the breed.[10–12] The negative energy balance due to pregnancy and lactation also diminish ovarian functions through suppressing pulsatile release of LH.[8,13] However, the interactions between nutrition, the hormonal systems, and altered reproduction in dairy cattle are increasingly being elucidated.[14–17] Therefore, the present research work was conducted to thorough investigation of oestrous cycle of the cross-bred cows following their VER patterns, hormonal status (progesterone and oestrogen) and ovarian follicular dynamics during the course of the cycle.

Methodology

Acclimatization of animals and experimental design

A total of 18 clinically healthy, reproductively sound, cross-bred cows, body weight ranging from 275-315 kilograms (kg), BCS varied from 3.0 to 3.5 were selected to detect their VER patterns, endocrinological profiles (oestrogen and progesterone) and ovarian follicular dynamics during oestrous cycles. All experimented cows were acclimatized in hygienically managed shed; routine coprological examination, anthelmintic dosing for controlling parasitisms, scheduled vaccination against the prevalent infectious diseases were strictly followed.

The routine experimental activities were started and concluded within a scheduled time in a day throughout the length of cycle. All tests belonging to the experiments were done in the Research Animal Farm (RAF), Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Recording of vaginal electrical resistance (VER)

VER of the experimented cows was recorded as per standard procedures.[18] Restraining of animals was done by an animal attendant followed by hygienic preparation, precautions both for animals and instrument prior to examine using appropriate ratios of disinfecting solution (Chlorhexidine, Savlon®, ACI Limited, Bangladesh) in water. The probe of electronic oestrus detector (Draminski® Electronic Estrus Detector, Poland) was scrubbed with clean paper towels before introducing into the vagina. Power supply of the heat detector was confirmed and then the probe was introduced into the vagina adjacent to cervix. The reading of the electronic heat detector was taken 3 times repeatedly. The data was registered systematically for individual animal.

Blood collection, serum preparation and storage

The blood (3-4 ml in volume) was directly collected in a vacuum tube (Clot Enhancer) from the jugular vein of the experimented cows following a standard procedures[19] and as per instructions of manufacturer of commercial ELISA kits for measuring progesterone and oestrogen (Nova Tec Immunodiagnostica®, GmbH, Germany). The tubes were kept in an undisturbed room for one hour at room temperature. Isolated sera were quickly centrifuged for 10 minutes at 1500rpm (Universal 32R™, Hettich Zentrifugen, Germany) to avoid haemolysis. The the sera were dispensed in eppendorfs, marked as per experimental animal and stored at -20°C in a refrigerator (Hitachi®, Japan).

Assessing of hormonal (oestrogen and progesterone) profiles

The sera were tested using commercial ELISA kits along with reagents as per instructions of the manufacturer (Nova Tec Immunodiagnostica®, GmbH, Germany).[20,21]

Reagent preparation and assay procedures

All reagents were brought to room temperature (18-22^{0}C), working solutions and buffer were prepared as per instructions with supplied reagents. The desired number of coated wells in the holder were secured and 25 microliter of sera were dispensed in each well. Allowing 100 microliter (µl) of working progesterone- HRP (for progesterone assay)/ Estradiol-HRP (for oestrogen assay) conjugates into each well was followed by thoroughly mixing for 30 seconds, incubation at room temperature for 90 minutes. The kits were rinsed and flicked 5 times with washing buffer.

Distributing 100 µl of TMB substrate into each well, gently mixed for 10 seconds, incubated for 20 minutes at room temperature, and then 100 µl of stop solution was added to each well, gently mixed for 30 seconds. Read absorbance at 450nm with ELISA reader (SPECTRAmax® 340PC384, USA) within 15 minutes was performed.

Calculation of the results

The mean absorbance value (A450) was calculated for each set of reference standards, controls end samples. A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against progesterone concentration in ng/ml or oestrogen concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis (Figure 1). The mean absorbance values for each specimen were used to determine the corresponding concentration of progesterone in ng/ml or corresponding concentration of oestrogen in pg/ml from the standard curve. Any values were obtained for diluted samples were further converted by applying the appropriate dilution factor in the calculations (Figure 1).

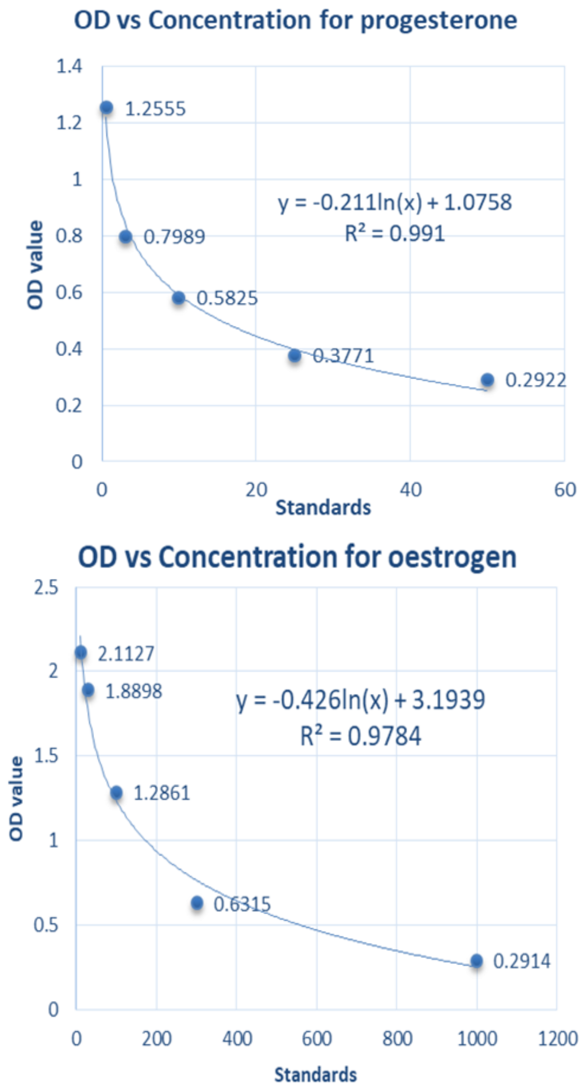


Figure 1 Construction of standard curves by plotting the mean absorbance obtained for each reference standard against progesterone (left) and oestrogen (right) concentrations.

Ultrasound scanning for ovarian follicular dynamics

Ultrasound scanning of ovaries was done using a high resolution real-time B Mode echocamera connected to a 7.5 MHz transducer (Bionet®, Republic of Korea).[22,23] Cattle was examined in standing position and restrained with a skilled animal attendant. The transducer was placed in appropriate position to visualize the ovaries. The image of ovaries were freezed on monitor, and the respective follicles and corpus luteum were measured by placing cursor on the structures (Figure 6).

The day to emerge of wave, number of waves, growing rate of the follicles, maximum diameter of dominant and ovulatory follicles, interovulatory intervals, and rate of follicular regression were observed and recorded.

Citation: Islam MN, Juyena NS, Bhuiyan MMU, et al. Oestrous cycle of cross-bred cows: Monitoring of VER, hormonal profiles and ovarian follicular dynamics. *MOJ Proteomics Bioinform.* 2019;8(2):54–64. DOI: 10.15406/mojpb.2019.08.00267

Statistical analysis

The data produced during routine examination of VER, hormonal profiles and ovaries were carefully noted in a record book, systematically organized in a Microsoft Excel data sheet and analyzed statistically using IBM® SPSS® version 22.

Capturing of images

The images of the different tests or experiments were captured either by cellphone (J 5, Samsung Galaxy, Korea) or directly from ultrasound machine, set in a computer for a simple modification for the better illustration of the results (Figure 2).

Figure 2 Multiple ways to confirm oestrus of the experimented cows (a. clear vaginal discharge, b. mounting, c. reduced VER, d. ultrasound scanning, e. larger follicle in ovary during the day of oestrus, f. hormonal analysis by ELISA test).

Results

VER patterns of the experimented cows

VER patterns were markedly varied from day to day and stage to stage of oestrous cycle of the experimented cows (Table 1, Figure 3). Cardinal features of VER of the cows were very closed each other in both 20 and 21 days length oestrous cycles (Figure 3–5).

The VER status was significantly lowered during the days of oestrus (D0) and subsequently started to rise with the advancement of days in metoestrus period. The maximal VER record was registered throughout the length of diestrus period and began to decline during the days of prooestrus stage of the cycles. The highest and lowest VER values were recorded as 350.56±7.96 and 186.67±12.54 ohms, respectively.

Endocrinology during oestrous cycle

Progesterone (P4) profiles

The blood concentrations of progesterone were remarkably fluctuated according to the days and stages of oestrous cycles of the cows under experimental observation (Table 1, Figure 4). The characteristic patterns of progesterone fluctuations were almost similar in both 20 and 21 day length of oestrous cycle (Figure 4).

The progesterone status was significantly lowered during the days of oestrus (D0) followed by rising of values during metoestrus periods. The greater blood concentrations of progesterone were exhibted throughout the length of diestrus periods and began to decline during the days of prooestrus stages. The maximum and minimum blood concentrations of progesterone were noted as 1.26±0.01 and 1.04±0.00 ng/ml, respectively.

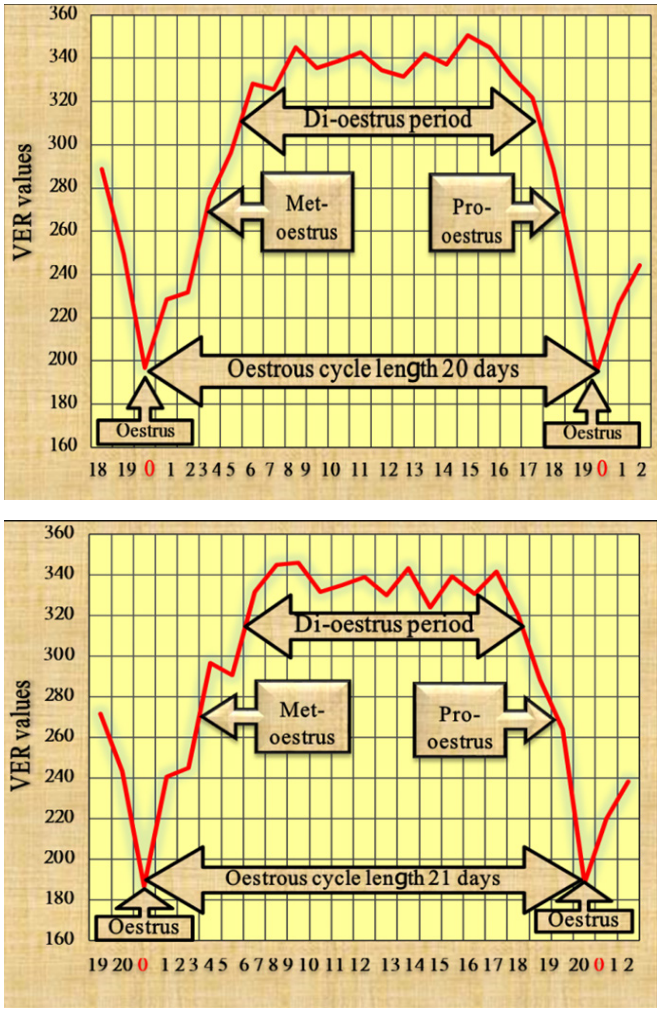
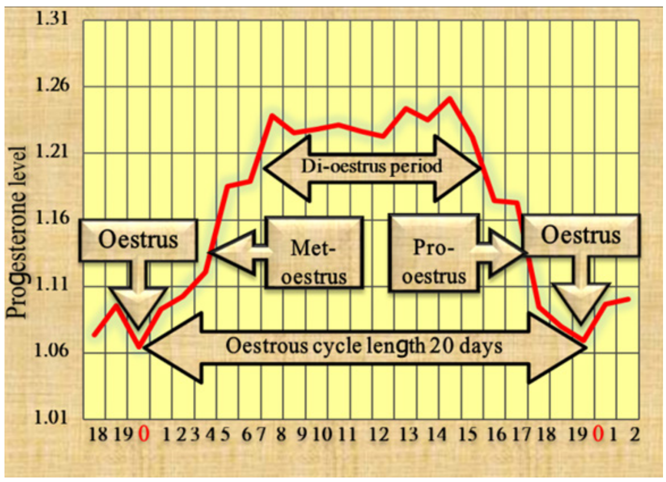


Figure 3 VER patterns during the course of oestrous cycle of the experimented cows (Left: Oestrous cycle with 20 days length, Right: Oestrous cycle with 21 days length) ('X' and 'Y' axis indicate the day of observation and VER values, respectively).



Citation: Islam MN, Juyena NS, Bhuiyan MMU, et al. Oestrous cycle of cross-bred cows: Monitoring of VER, hormonal profiles and ovarian follicular dynamics. *MOJ Proteomics Bioinform.* 2019;8(2):54–64. DOI: 10.15406/mojpb.2019.08.00267

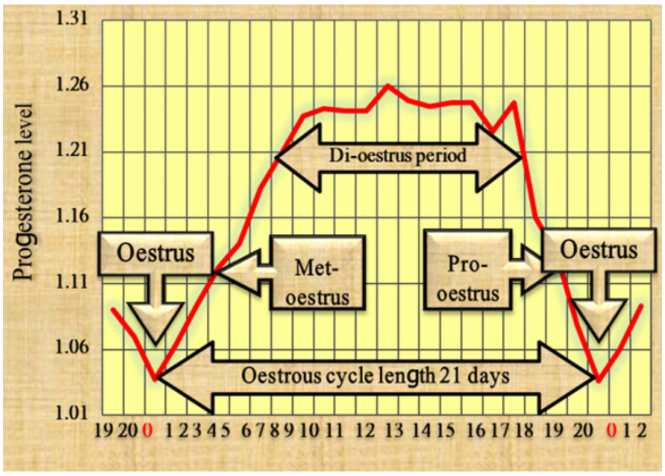


Figure 4 Progesterone profiles during the course of oestrous cycle of the experimented cows (Left: Oestrous cycle with 20 days length, Right: Oestrous cycle with 21 days length) ('X' and 'Y' axis indicate the day of observation and blood progesterone concentrations, respectively).

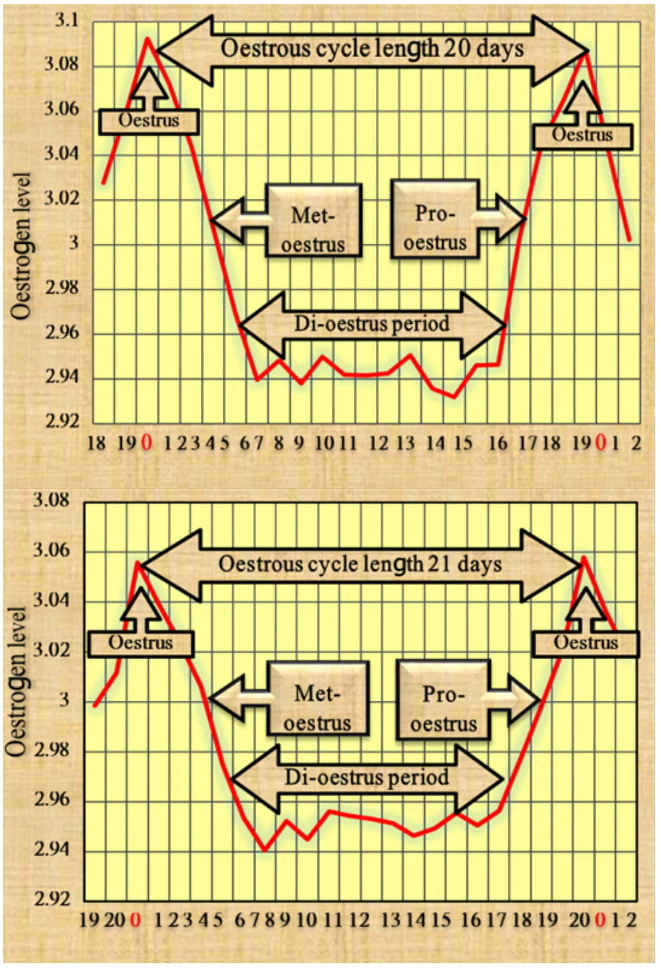


Figure 5 Oestrogen profiles during the course of oestrous cycle of the experimented cows (Left: Oestrous cycle with 20 days length, Right: Oestrous cycle with 21 days length) ('X' and 'Y' axis indicate the day of observation and blood oestrogen concentrations, respectively).

Oestrogen profiles

The blood concentrations of oestrogen were significantly differed as per days and stages of oestrous cycle of the cows (Table 1, Figure 5). The characteristic patterns of oestrogen fluctuations were almost similar both in 20 and 21 days pattern of oestrous cycles (Figure 5).

The oestrogen status was significantly increased during the days of oestrus (D0) and with the advancement of the days this oestrogen level started to fall in metoestrus period. The lowest blood concentration of oestrogen was exhibted throughout the length of dioestrus period and began to rise during the days of prooestrus stage. The maximal and minimal blood concentrations of oestrogen were recorded as 3.09±0.02 and 2.94±0.01 pg/ml, respectively.

Correlation between VER and hormonal (P4 and Oe2) profiles during oestrous cycles

There was a close relationship between VER values and hormonal profiles (positive with progesterone concentrations and negative with oestrogen concentrations) within the days and stages of the oestrous cycles of the experimental cows (Table 2) visualized during the course of observation (Table 1).

Day of observation 0: Day of oestrus (D_0). The superscripts indicate the level of significance each other.

There was an equal proportional relationship between the VER values and the progesterone profiles within the same days and stages of oestrous cycle of the cows but an inversely proportional relationship was also observed between VER status and oestrogen profiles in same days and stages of the oestrous cycle of the cows (Table 2).

Ovarian follicular dynamics

Ovarian follicular dynamics observed during oestrous cycle of the experimented cows were observed by ultrasound scanning of ovaries regularly in a specific time frame (Figure 6&7).

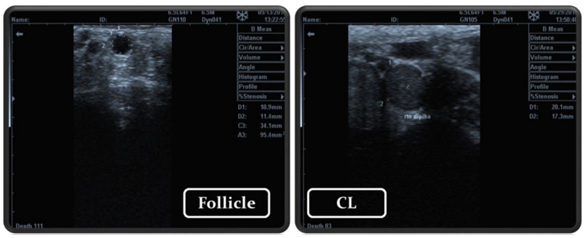


Figure 6 Measurement procedures of follicle and corpus luteum by ultrasound scanning of ovaries adopted in the experiment.

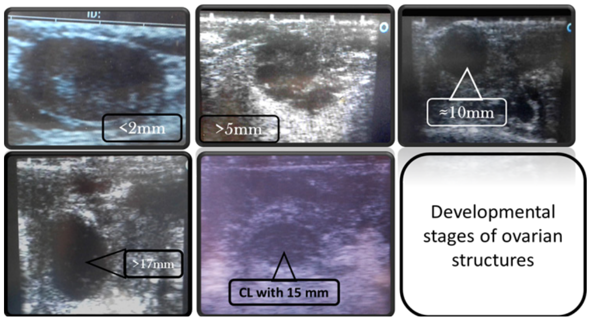


Figure 7 Ultrasound images of ovaries with different growth stages of follicles and corpus luteum (CL).

Table 1 Day-basis mean values of VER, blood concentrations of progesterone and oestrogen during oestrous cycle of the experimented cows

	Oestrous cycle length: 20 days				**Oestrous cycle length: 21 days**		
Day of observation	**VER (ohms) (Mean±SEM)**	**Progesterone (ng/ml) (Mean±SEM)**	**Oestroogen (pg/ml) (Mean±SEM)**	**Day of observation**	**VER (ohms) (Mean±SEM)**	**Progesterone (ng/ml) (Mean±SEM)**	**Oestroogen (pg/ml) (Mean±SEM)**
18	271.67±18.28^{cdef}	1.07±0.01^{a}	3.03±0.02^{cd}	**19**	288.89±9.10^{c}	1.09±0.02^{bcd}	3.00±0.01^{bc}
19	243.34±5.77^{bcd}	1.10±0.02^{a}	3.06±0.03^{de}	**20**	249.44±5.67^{b}	1.07±0.01^{ab}	3.01±0.01^{bc}
0	**186.67±12.54^{a}**	**1.06±0.02^{a}**	**3.09±0.02^{e}**	**0**	**196.67±8.34^{a}**	**1.04±0.00^{a}**	**3.06±0.01^{d}**
1	240.84±10.31^{bc}	1.09±0.03^{a}	3.07±0.02^{de}	**1**	228.33±7.49^{b}	1.06±0.01^{ab}	3.04±0.01^{cd}
2	245.00±12.87^{bcde}	1.10±0.02^{a}	3.04±0.02^{cd}	**2**	231.67±7.45^{b}	1.10±0.01^{bcd}	3.02±0.02^{bc}
3	296.67±8.50^{efgh}	1.12±0.02^{ab}	3.01±0.03^{bc}	**3**	275.17±8.17^{c}	1.12±0.01^{cdef}	3.01±0.00^{bc}
4	290.83±7.98^{efgh}	1.19±0.01^{cd}	2.97±0.01^{a}	**4**	296.28±10.81^{c}	1.14±0.02^{efg}	2.97±0.02^{ab}
5	331.67±12.36^{ghi}	1.19±0.03^{cd}	2.94±0.02^{a}	**5**	328.33±12.52^{d}	1.18±0.01^{gh}	2.95±0.01^{a}
6	345.00±10.67^{i}	1.24±0.02^{cd}	2.95±0.01^{a}	**6**	325.56±12.28^{d}	1.21±0.02^{hi}	2.94±0.01^{a}
7	345.84±11.50^{i}	1.23±0.01^{cd}	2.94±0.01^{a}	**7**	345.00±6.01^{d}	1.24±0.01^{i}	2.95±0.01^{a}
8	331.67±13.64^{ghi}	1.23±0.02^{cd}	2.95±0.00^{a}	**8**	335.56±7.68^{d}	1.24±0.02^{i}	2.95±0.01^{a}
9	335.00±23.19^{ghi}	1.23±0.02^{cd}	2.94±0.01^{a}	**9**	338.89±10.67^{d}	1.24±0.02^{i}	2.96±0.01^{a}
10	339.17±23.47^{hi}	1.23±0.01^{cd}	2.94±0.01^{a}	**10**	342.78±8.80^{d}	1.24±0.01^{i}	2.95±0.01^{a}
11	330.00±23.38^{ghi}	1.22±0.02^{cd}	2.94±0.01^{a}	**11**	334.45±12.84^{d}	1.26±0.01^{i}	2.95±0.01^{a}
12	343.34±22.69^{i}	1.24±0.01^{cd}	2.95±0.01^{a}	**12**	331.61±6.95^{d}	1.25±0.02^{i}	2.95±0.02^{a}
13	324.17±18.13^{ghi}	1.24±0.01^{cd}	2.94±0.00^{a}	**13**	342.22±5.49^{d}	1.24±0.01^{i}	2.95±0.03^{a}
14	339.17±13.63^{hi}	1.25±0.03^{d}	2.93±0.01^{a}	**14**	337.22±7.12^{d}	1.25±0.01^{i}	2.95±0.01^{a}
15	330.83±8.65^{ghi}	1.22±0.02^{cd}	2.95±0.01^{a}	**15**	350.56±7.96^{d}	1.25±0.01^{i}	2.96±0.01^{a}
16	341.67±12.58^{i}	1.17±0.01^{bc}	2.95±0.02^{a}	**16**	345.00±6.87^{d}	1.23±0.02^{i}	2.95±0.01^{a}
17	320.00±10.45^{ghi}	1.17±0.02^{bc}	3.00±0.01^{bc}	**17**	332.22±4.27^{d}	1.24±0.02^{i}	2.96±0.01^{a}
18	288.33±13.09^{defg}	1.09±0.03^{a}	3.05±0.02^{cd}	**18**	321.67±8.72^{d}	1.16±0.03^{fg}	2.98±0.01^{ab}
19	264.17±21.40^{bcd}	1.08±0.02^{a}	3.06±0.02^{de}	**19**	288.89±10.94^{c}	1.13±0.03^{def}	3.00±0.01^{bc}
0	**188.33±10.76^{a}**	**1.07±0.02^{a}**	**3.09±0.02^{e}**	**20**	241.11±5.75^{b}	1.08±0.02^{abc}	3.02±0.01^{bc}
1	220.00±9.91^{ab}	1.10±0.02^{a}	3.04±0.02^{cd}	**0**	**195.00±6.25^{a}**	**1.04±0.00^{a}**	**3.06±0.01^{d}**
2	238.34±3.47^{bc}	1.10±0.01^{a}	3.00±0.01^{bc}	**1**	226.11±7.52^{b}	1.06±0.01^{ab}	3.04±0.01^{cd}
P-value	0	0	0	**2**	240.00±8.98^{b}	1.09±0.01^{bcd}	3.02±0.01^{bc}
				P-value	0	0	0

Table 2 Statistical correlation between VER status and hormonal profiles of the cows during oestrous cycles

Parameters	20 days pattern of oestrous cycle		21 days pattern of oestrous cycle	
	Progesterone	**Oestrogen**	**Progesterone**	**Oestrogen**
VER	0.893**	-0.918**	0.953**	-0.980**
Progesterone		-0.942**		-0.955**
Level of significance	** Correlation is significant at the 0.01 level			

The interovulatory interval (IOI), diameter of the ovulatory follicle, day of follicular wave emergence, day at maximum diameter of the dominant follicle, maximum diameter of the dominant follicle, growth rate of the dominant follicle, day to onset of atresia or follicular regression following wave emergence and the number of waves in a cycle of the cows were determined and presented (Table 2, Figure 8). The ultrasound images of the ovaries, VER expressions and ELISA for hormonal evaluations in different period of oestrous cycle of the cows captured and presented herewith for better illustration of the results (Table 3, Figure 8).

Table 3 Characteristics of follicular waves and dominant follicles in two and three wave cycles in experimented cows

Parameter	Two wave cycle	Three wave cycle
	(Mean±SEM) (n=11)	**(Mean±SEM) (n=7)**
Occurrence of wave	61.11% (11/18)	38.89% (7/18)
Interovulatory interval (IOI) or oestrous cycle length (Days)	21	22
Diameter of the ovulatory follicle (mm)	19.76±0.41	18.86±0.40
Wave length in oestrous cycle		
1st wave length	13.00±0.43	12.86±0.55
2nd wave length	9.36±0.20	10.71±0.36
3rd wave length		8.14±0.26
Follicular wave emergence (Day)		
1st follicular wave emergence	1.82±0.23	1.57±0.30
2nd follicular wave emergence	8.73±0.27	5.43±0.20
3rd follicular wave emergence		11.43±0.20
Day at maximum diameter of the dominant follicle		
1st dominant follicle	5.27±0.24	6.43±0.30
2nd dominant follicle	4.72±0.14	5.43±0.20
3rd dominant follicle		4.57±0.20
Maximum diameter of the dominant follicle (mm)		
1st dominant follicle	10.73±0.25	11.65±0.24
2nd dominant follicle	11.80±0.16	10.03±0.13
3rd dominant follicle		10.11±0.11
Growth rate of the dominant follicle		
1st dominant follicle	1.24±0.04	1.33±0.03

Table continue

Parameter	Two wave cycle	Three wave cycle
	(Mean±SEM) (n=11)	(Mean±SEM) (n=7)
2nd dominant follicle	1.63±0.04	1.18±0.05
3rd dominant follicle		1.51±0.03
Onset of atresia following wave emergence (day)		
1st dominant follicle	6.64±0.20	7.43±0.30
2nd dominant follicle	7.27±0.24	6.43±0.20
3rd dominant follicle		5.57±0.20

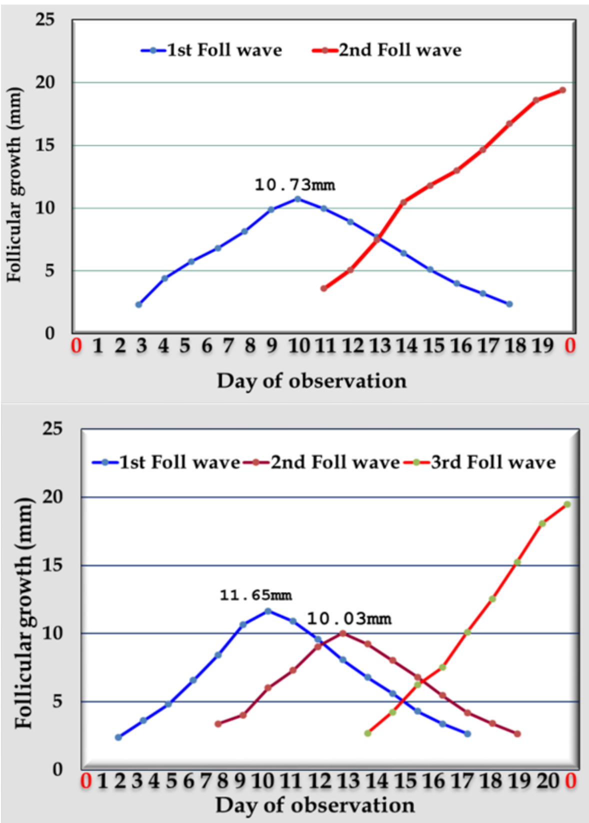


Figure 8 Ovarian follicular waves emerged during the course of oestrous cycles of the experimented cows (Left: Oestrous cycle with 2 follicular waves, Right: Oestrous cycle with 3 follicular waves).

Discussion

VER during oestrous cycle

VER of the experimented cows was daily monitored as per instructions provided by manufacturer of electronic oestrus detector (Manual Draminski® Electronic Estrus Detector, 2008). The data were recorded, organized and statistically analyzed. The VER patterns were significantly differed ($p<0.05$) from stage to stage of oestrous cycle of the cows in this study, and these variations might closely be related to physiological aspects like the presence of a mature follicle and a CL,[24] to the phases (follicular and luteal phases) of oestrous cycle,[18,24,25–27] to the serum concentrations of oestradiol during follicular phase. However, fluctuations of VER values are reflected due to cyclic stage of different species (Manual Draminski® Electronic Estrus Detector, 2008) such as lowest vaue exists during pro-oestrus period in pigs[28] and sheep;[29] during oestrus in goats,[30] horses,[31] buffaloes,[27] cattle.[25] Moreover, various factors may affect VER values such as depth of the probe inserted into vagina,[25] intravaginally position of the probe,[32,33] pressure against the vaginal mucosa and pathological conditions of genital tract,[34] limited area of genital tract and the electrical potential differences on its surface,[27] instability of the contact between the vaginal mucosa and electrodes due to entrapment of air, unequal hand,[35] etc., However, in above stated circumstances all necessary precautions under the limit were carefully approached to obtain VER records correctly in this study.

The rising of oestrogen during oestrus stimulates hyperproduction of adrenocorticotropic hormones and mineralocorticoid (aldosterone hormone) that increase the levels of NaCl in the vaginal mucosa resulting in a significantly reduction of electrical resistance than usual,[36] and thereby alterations of mucosal resistance due to existence of oestrogen and progesterone levels are clearly speculated.[37] In the present study the patterns of VER fluctuations of the cows were very closed each other during 20 and 21 days length of oestrous cycles. The degree of amplitudes of VER in different days and stages in this study were strongly related with predominant hormones of the oestrous cycle (progesterone and oestrogen) (Table 4).

Endocrinology (Hormonal profiles) during oestrous cycle

The blood was routinely collected from the experimented cows, processed for segregating sera to determine the amplitude of blood concentrations of progesterone and oestrogen during the course of oestrous cycle using commercial ELISA kits (Nova Tec Immunidiagnostica®GmbH, Germany).

Progesterone profiles

Serum progesterone concentrations in cows more than 1 ng/ml is considered to be cycling due to the presence of an active corpus luteum.[38,39] Blood concentrations of progesterone were maximum during the luteal phase of oestrous cycle and almost similarly documented by many researchers.[40,41] Rapid declination of progesterone concentrations occurred approximately during pro-oestrus period and remained at basal level during the onset of oestrus or on D0 (D0=Day of oestrus) as stated elsewhere.[42–44] The falling of progesterone concentration is associated with the secretory activity of the oestradiol from growing follicles followed by spontaneaous release of PGF2alpha from uterine endometrium[45] or the lifespan of CL or the concomitant regression of CL;[40,41] Elevation of progesterone level launched again 4-5 days after oestrus.[42–44]

The scenario of blood concentrations of progesterone variations was usually similar both in 20 and 21 days length of oestrous cycles. The highest and lowest blood concentrations of progesterone were noted respectively as 1.26±0.01 and 1.04±0.00 ng/ml during oestrous cycle in this study. Progesterone concentrations of the Holstein-Fresian cross-bred was measured in this study, although breed differences in amplitude and concentrations of progesterone have been reported.[46]

Table 4 Highest and lowest values of VER, blood progesterone and oestrogen in 20 and 21 days patterns of oestrous cycles of the cows

Level of the parameters	20 days pattern oestrous cycles			21 days pattern oestrous cycles		
	VER (ohm) (Mean±SEM)	Progesterone (ng/ml) (Mean±SEM)	Oestroogen (pg/ml) (Mean±SEM)	VER (ohm) (Mean±SEM)	Progesterone (ng/ml) (Mean±SEM)	Oestroogen (pg/ml) (Mean±SEM)
Highest level	345.00±10.67	1.25±0.03	3.09±0.02	350.56±7.96	1.26±0.01	3.06±0.01
Lowest level	186.67±12.54	1.06±0.02	2.94±0.01	195.00±6.25	1.04±0.00	2.94±0.01

Oestrogen profiles

Oestrogen is secreted from granulosa cells only or both theca interna and theca granulose cells,[47] and the level oestrogen in peripheral circulations were little bit fluctuated throughout the oestrous cycle of cows.[38,48]

Blood concentrations of oestrogen was started to rise during the initiation of pro-oestrus period and highest concentration was recorded during the day of oestrus in this study. The literatures show that the attempt to rise of oestrogen 4 days before oestrus, when the concomitant fall in progesterone allows the growing follicles to increase their secretions[49,50] and reaches the highest peak before 17-18 h of standing oestrus. Another small peak is displayed on D_{4-7} or D_{6-7} probably as a reflection of midcycle follicular growth.[41,48,51,52] However, the blood oestrogen level started to fall in metoestrus period and the lowest blood concentrations of oestrogen were exhibited throughout the length of di-oestrus period with tiny fluctuations in the present study.

Correlation between VER and hormonal profiles during oestrous cycle

VER is associated with various ovarian structures or oestrous cycle stages,[18,25,26,53] and daily impedance measurements are necessary to confirm the persistent stage.[54] A correlation (bivariate correlation) between VER and blood concentrations of either oestrogen or progesterone of the respective day of the cycle were attempted to statistically establish or to observe an outcome of their interrelationship in this study.

VER and blood progesterone concentrations were positively correlated (equally proportionate) but VER was inversely proportionate (negatively correlated) to blood oestrogen concentrations, where the patterns of hormonal concentrations in various cyclic stages was reflecting the opinions described in many published literatures.[40,44,48,51,52]

Ovarian follicular dynamics during oestrous cycle

A fixed number of primordial follicles categorized into gonadotropin independent and gonadotropin dependent stages is established during foetal development, and gonadotropin dependent follicular growth in cattle occurs in waves during oestrous cycle.[55,56] Ovarian follicular dynamics is concerned with ovarian physiology, and is characterized by the waves of follicular growth and regression during the oestrous cycle of cows and heifers.[59–59] Follicular dynamics is well documented in European breeds[22,56,57,59] and limitedly in zebu cattle.[61–63] After the initial growth period, normally a single follicle is selected to become larger (known as dominant follicle) and the subordinate follicles undergo regression.[64,65] Dominant follicle is the follicle that reaches the largest diameter, avoids its own regression by shifting its dependence from FSH to LH,[66,67] and becomes the ovulatory follicle during luteolysis.[68]

In this study the ovarian follicular dynamics was observed ultrasonographically using 7.5 MHtz B Mode linear transducer (Bionet®, Republic of Korea). The interovulatory intervals of the experimented cows were 20-21 d. However, bovine oestrous cycle length varies, normally ranging from 17 to 24 d (average 21 d)[69] and oestrus lasts 12 to 18h,[70,71] ovulation occurs 25 to 30 h after the onset of oestrus.[72,73] The wave number in a cycle of the cows in this study varied from 2[74,75] to 3. Oestrous cycle with 4 or 1 follicular wave was not found in this study, although oestrous cycle with 4 and even 1 (Savio et al., 1988) are also reported. In this study, the oestrous cycles evaluated evidenced a higher proportion of cycle (61.11%) with two follicular waves compared to three follicular waves (38.89%). The majority (greater than 95%) of bovine oestrous cycles are composed of either 2 or 3 follicular waves (Adams, 1999; Adams et al., 2008). In 2 and 3 wave patterns of oestrous cycle in this study, the wave length was minimally differed, the wave number was equally proportionate to the length of the cycle, and the length of oestrous cycle of the cows with 3 wave patterns was determined as 1 day longer (21 d) than in cycles with 2 wave patterns (20 d). The wave number can affect the oestrous cycle length in cows (Viana et al., 2000). The 3 wave pattern has an extra 2 days[57] or 3 days (Wael, 2003) oestrous cycle length compared to cows with 2 wave pattern. Oestrous cycle length in cows with 2 waves is on average 21 days, and 23 days in animals with 3 waves.[22,77] However, the overall length of the oestrous cycle as per wave numbers was somewhat shorter than that described.[22,77]

The day of follicular wave emergence, day at maximum diameter of the dominant follicle, maximum diameter of the dominant follicle (mm), growth rate of the dominant follicle and onset of atresia following wave emergence (day) both in 2 and 3 wave pattern oestrous cycle in this study strongly supported by researchers.[22,27,77–93]

Conclusion

Monitoring oestrous cycle of cows was observed with VER, endocrinological profiles and ovarian follicular dynamics, and the conclusions of the study were drawn as below:

a) Lowest VER, lowest blood concentrations of progesterone and highest level of blood oestrogen were noted during the day of oestrus but opposite reflections were found during dioestrus stage/luteal phase of oestrous cycle that were significantly differed each other (P<0.05).

b) VER is statistically equally proportionate to blood oestrogen concentrations but inversely proportionate to progesterone level.

c) Interovulatory interval of the experimented cows ranges from 20 to 21 days, follicular wave number per oestrous cycle varies from 2 to 3.

Acknowledgement

The author expressed his deepest sense of gratitude and profound respect to Dr. Maria Dattena, Responsible, Agricultural Research Agency of Sardinia (AGRIS), Italy for her valuable suggestions and instructions related to reproductive study during the course of oestrous cycle of ruminating animals in the period of training.

Conflicts of interest

Author declares that there are no conflicts of interest.

References

1. Chebel RC, Fernando FA, Dalton JC. Factors affecting reproductive performance of Holstein heifers. *Anim Reprod Sci*. 2007;101(3):208–224.
2. Bergfeld EGM, Kojima FN, Cupp AS, et al. Ovarian follicular development in prepubertal heifers is influenced by level of dietary energy intake. *Biologcal Reproduction*. 1994;51(5):1051–1057.
3. Ferguson JD. Nutrition and Reproduction in Dairy Herds. *Veterinary Clinics of North America Food Animal Practice*. 2005;21(2):325–347
4. Wilson SJ, Marion RS, Spain JN, et al. Effects of controlled heat stress on ovarian function of dairy cattle. *Journal of Dairy Science*. 1998;81:2124–2131.
5. Fortune JE. Follicular dynamics during the bovine oestrus cycle:A limiting factor in follicles during the development of follicular waves in cattle. *Theriogenology*. 1993;48(1):75–87.
6. Montgomery GW, Scott IC, Hudson N. An interaction between season of calving and nutrition on the resumption of ovarian cycles in post–partum beef cattle. *Journal of Reproduction and Fertility*. 1985;73(1):45–50.
7. Sasser RG, Williams RI, Bull RC, et al. Postpartum reproductive performance in crude protein restricted beef cows: Return to estrus and conception. *Journal of Animal Science*. 1988;66(12):3033–3039.
8. Butler WR, Smith RD. Inter–relationships between energy balance and postpartum reproductive function in dairy cattle. *J Dairy Sci*. 1989;72:767–783.
9. Galina CS, Arthur GH. Review of cattle reproduction in the tropics Part 2 Parturition and calving intervals. *Animal Breeding*. 1989;57(8):9–16.
10. Laster DB, Turman EJ, Stephens DF et al. Ovulation rates of beef cows and heifers treated with equine gonadotropin (PMS) and chorionic gonadotropin (HCG). *Journal of Animal Science*. 1973;33(2):443–449.
11. Thorpe W, Cruickshank DKR, Thompson. Genetic and environmental influences on beef cattle production in Zambia 1. Factors affecting weaner production from Angoni, Barotse and Boran dams. *Animal Production*. 1980;30(2):217–234.
12. Eduvie LO. Factors affecting postpartum ovarian activity and uterine involution in Zebu cattle indigenous to Nigeria. *Animal Reproduction Science*. 1985;8(1):123–128.
13. Dobson H, Alam MGS. Effect of beta–methasone treatment on the luteal lifespan and the LH response to GnRH in dairy cows. *Journal of Reproduction and Fertility*. 1987;80(1):25–30.
14. Lucy MC. ADSA Foundation Scholar Award – Reproductive loss in high producing dairy cattle:where will it end? *Journal Dairy Science*. 2001;84:1277–1293.
15. Wiltbank M, Lopez H, Sartori R, et al. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology*. 2006;65(1):17–29.
16. Chagas LM, Bass JJ, Blache D, et al. New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high–producing dairy cows. *J Dairy Sci*. 2007;90(9):4022–4032.
17. Sartori R, Bastos MR and Wiltbank MC. Factors affecting fertilization and early embryo quality in single– and superovulated dairy cattle. *Reproduction, Fertility and Development*. 2010;22(1):151–158.
18. Meena Rs, Sharma SS and Purohit GN. Efficiency of vaginal electrical resistance measurements for oestrous detection and insemination in Rathi cows. *Animal Science Journal*. 2003;76:433–437.
19. Henry JB. *Clinical diagnosis and management by laboratory methods*. Philadelphia: WB Saunders Company; 1979.
20. Shepard MK and Senturia YD. Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function. *Fertility and Sterility*. 1977;28(5):541–548.
21. Odell WD and Swerdloff RS. Abnormalities of gonadal function in men. *Clinical Endocrinology*. 1978;8:149–180.
22. Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real–time ultrasonography. *Biology of Reproduction*. 1988;39:308–317.
23. Adams GP, Jaiswal R, Singh J et al. Progress in understanding ovarian follicular dynamics in cattle. *Theriogenology*. 2008;69(1):72–80.
24. Tadesse M, Thiengtham J, Pinyopummin A, et al. The use of vaginal electrical resistance to diagnose estrus and early pregnancy and its relation with size of the dominant follicle in dairy cattle. *Kasetsart Journal of Natural Science*. 2011;45(3):435–443.
25. Aboul–Ela MB, MacDonald DC, Lindsay D, Topps JH and Mani R. The association between changes in the intra–vaginal electrical resistance and the in vitro measurements of vaginal mucus electrical receptivity in cattle. *Animal Reproduction Science*. 1983;5:323–328.
26. Canfield RW, Butler WR. Accuracy of predicting the LH surge and optimal insemination time in Holstein heifers using a vaginal resistance probe. *Theriogenology*. 1989;31(4):835–842.
27. Gupta KA, Purohit GN. Use of vaginal electrical resistance (VER) to predict oestrus and ovarian activity, its correlation with plasma progesterone and use for insemination in buffaloes. *Theriogenology*. 2001;56(2):235–245.
28. Rezac P, Kukla R, Poschl M. Effect of sow parity on vaginal electrical impedance. *Animal Reproduction Science*. 2002;72:223–234.

29. Bartlewski PM, Beard AP, Rawlings NC. The relation–ship between vaginal mucous impedance and serum concentrations of estradiol and progesterone throughout the sheep estrous cycle. *Theriogenology*. 1999;51(4):813–827.
30. Rezac P, Krivanek I, Poschl M. Changes of vaginal and vestibular impedance in dairy goats during the estrous cycle. *Small Ruminant Research*. 2001;42:183–188.
31. Brook D. An assessment of the efficiency of measuring the electrical resistance of vaginal mucus as a means of detecting ovulation in mares. *Veterinary Medicine, Small Animal Clinician*. 1982;77:1059–1067.
32. Foote R, Oltenacu E, Mellinger J, et al. Pregnancy rate in dairy cows inseminated on the basis of electronic probe measurements. *Journal of Dairy Science*. 1979;62(1):69–73.
33. Heckman G, Katz L, Foote R, et al. Estrous cycle patterns in cattle monitored by electrical resistance and milk progesterone. *Journal of Dairy Science*. 1979;62(1):64–68.
34. Leidl W, Stolla R. Measurement of electric resistance of the vaginal mucus as an aid for heat detection. *Animal Reproduction Science*. 1976;5:259–273.
35. Lehrer AR, Lewis GS, Aizinbud E. Electrical resistance of genital tissues during reproductive events in cows, and its possible on–farm applications:A review. *Wiener Tiera medical script*. 1991;78:317–322.
36. Fehring RJ. A comparison of the ovulation method with the CUE ovulation predictor in determining fertile period. *Journal of American Academy of Nurse Practitioners*. 1997;8(10):461–466.
37. Rezac P. Potential applications of electrical impedance techniques in female mammalian reproduction. Theriogenology. 2008;70(1):1–14.
38. Perry GA, Smith MF, Roberts AJ, et al. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J Anim Sci*. 2007;85(3):684–689.
39. Echternkamp SE, Thallman RM. Factors affecting pregnancy rate to oestrus synchronization and fixed–time artificial insemination in beef cattle. J Anim Sci. 2011;89(10):3060–3068.
40. Llewelyn CA, Munro CD, Luckins AG, et al. Behavioural and ovarian changes during the oestrous cycle in the boran (Bos indicus). *British Veterinary Journal*. 1987;143(1):75–82.
41. Alam MGS, Ghosh A. Reproductive performance in cows: its relation to parity and season. *Bangladesh Veterinary Journal*. 1989;22:51–61.
42. Donaldson LE, Bassett JM, Thorburn GD. Peripheral plasma progesterone concentration of cows during puberty, oestrous cycles, pregnancy and lactation and the effects of under nutrition or exogenous oxytocin on progesterone concentration. *Journal of Endocrinology*. 1970;48(4):599–614.
43. Kanchev LN, Dobson H, Ward WR, et al. Concentration of steroids in bovine peripheral plasma during the oestrous cycle and effects of betamethasone treatment. *Journal of Reproduction and Fertility*. 1976;48(2):341–345.
44. Lamming GE, Bulman DC. The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. *British Veterinary Journal*. 1976;132(5):507–517.
45. Villa Godoy, Ireland JJ, Wortman JA, et al. Effect of ovarian follicles on luteal regression in heifers. *Journal of Animal Science*. 1985;60:519–527.
46. Segerson EC, Hansen TR, Libby DW, et al. Ovarian and uterine morphology and function in Angus and Brahman cows. *Journal of Animal Science*. 1984;59:1026–1046.
47. Hay MF and Moor RM. Changes in the graafian follicle population during the follicular phase of the oestrous cycle. *In Control of Ovulation*. 1978;11:177–196.
48. Dobson H, Dean PDG. Radioimmuno assay of oestrone, oestradiol–17α and 17β in bovine plasma during the oestrous cycle and last stages of pregnancy. *Journal of Endocrinology*. 1974;61(3):479–486.
49. Dobson H. Plasma gonadotrophins and oestradiol during oestrus in the cow. *J Reprod Fertil*. 1978;52(1):51–53.
50. Glencross RG, Esslemont RJ, Bryant MJ et al. Relationships between the incidence of preovulatory behaviour and the concentrations of oestradiol–17β and progesterone in bovine plasma. *Applied Animal Ethology*. 1981;7:141–148.
51. Hansel W, Convey EM. Physiology of the estrous cycle. *J Anim Sci*. 1983;57(2):404.
52. Spicer LJ, Echternkamp SE. Ovarian Follicular Growth, Function and Turnover in Cattle: A Review. *Journal of Animal Science*. 1986;62(2):428–451.
53. Schams D, Schallenberger E, Hoffmann B, et al. The oestrous cycle of the cow:hormonal parameters & time relation¬ships concerning oestrus, ovulation, and electrical resistance of the vaginal mucus. *Acta Endocrinologica (Copenhagen)*. 1977;86(1):180–192.
54. Aizinbud E, H Schindler, L Adam. Impedometry of the bovine vagina and vulva for oestrus detection: present state of research and prospects. *Refuah Veterinarith*. 1980;37:156–162.
55. Rajakoski E. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left–right variations. *Acta Endocrinologica (Copenhagen)*. 1960;34(52):1–68.
56. Savio JD, Keenan L, Boland MP, et al. Pattern of growth of dominant follicles during the oestrous cycle in heifers. *J Reprod Fertil*. 1988;83(2):663–671.
57. Ginther OJ, Kastelic JP, Knopf L. Composition and characteristics of follicular waves during the bovine estrous cycle. *Animal Reproduction Science*. 1989;20(3):187–200.
58. Knopf L, Kastelic JP, Schallember E et al. Ovarian follicular dynamics in heifers:Test of two wave hypothesis by ultrasonically monitoring individual follicles. *Domest Anim Endocrinol*. 1989;6(2):111–119.
59. Taylor C, Rajamahendran R. Follicular dynamics, corpus luteum growth and regression in lactating dairy cattle. *Canadian Journal of Animal Science*. 1991;71(1):61–68.
60. Roche JF, Boland MP. Turnover of dominant follicles in cattle of different reproductive states. *Theriogenology*. 1991;35(1):81–90.
61. Figueiro RA, Barros CM, Pinheiro OL and Sole JMP. Ovarian follicular dynamics in Nelore breed (Bos indicus) cattle. *Theriogenology*. 1997;47(8):1489–1505.
62. Gambini ALG, Moreira MBP, Barros CM. Follicular development and timing synchronization in Gir cows. *Brasilera Magazine of Animal Reproduction*. 1998;19:9–22.
63. Viana JHM, A Ferreira, W Sá, et al. Follicular dynamics in zebu cattle. *Pesquisa Agropecuária Brasileira*. 2000;35(12):2501–2509.
64. Fortune JE. Ovarian follicular growth and development in mammals. *Biological Reproduction*. 1994;50:225–232.
65. Ginther OJ, Bergfelt DR, Kulick LJ et al. Selection of the dominant follicle in cattle:role of two–way functional coupling between follicle stimulating hormone and the follicles. *Biology of Reproduction*. 2000;62(4):920–927.
66. Campbell BK, Scaramuzzi RJ, Webb R. Control of antral follicle development and selection in sheep and cattle. *Journal of Reproduction and Fertility*. 1995;49:335–350.

67. Campbell BK, Baird DT, Webb R. Effects of dose of LH on androgen production and luteinization of ovine theca cells cultured in a serum–free system. *J Reprod Fertil*. 1998;112(1):269–277.

68. Kastelic JP, Ginther OJ. Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. *Animal Reproduction Science*. 1991;26(1):13–24.

69. Wishart DF. Observations on the oestrous cycle of the Friesian heifer. *The Veterinary Record*. 1972;90(21):595.

70. Hammond J. *The Oestrous Cycle*. In: The Physiology of Reproduction in the Cow. London: Cambridge University Press; 1927.

71. Nalbandov A, Casida LE. Ovulation and its relation to oestrus in cows. *Journal of Animal Science*. 1942;1(3):189–198.

72. Christenson RK, SE Echternkamp, Laster DB. Oestrus, LH, ovulation and fertility in beef heifers. *Journal of Reproduction and Fertility*. 1975;43(3):543–546.

73. Bernard C, Valet JP, Beland R et al. Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH. *Journal of reproduction and fertility*. 1983;68(2):425–430.

74. Ahmad N, Townsend EC, Dailey RA and Inskeep EK. Relationship of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. *Anim Reprod Sci*. 1997;49(1):13–28.

75. Bellmann A. *Follicular dynamics and corresponding hormone concentrations in cattle under the influence of a GnRH agonist in depot formulation (Decapeptyl®Depot)*. Germany: University of Leipzig; 2001.

76. Pierson RA, Ginther OJ. Follicular populations during the estrous cycle in heifers II Influence of Right and Left Sides and Intraovarian effect of the corpus luteum. *Animal Reproduction Science*. 1987;14(3):177–186.

77. Adams GP. Comparative patterns of follicle development and selection in ruminants. *J Reprod Fertil Suppl*. 1999;54:17–32.

78. Badinga L, Thatcher WW, Wilcox CJ, et al. Effect of season on follicular dynamics and plasma concentration of estradiol–17β, progesterone and luteinizing hormone in lactating Holstein cows. *Theriogenology*. 1994;42:1263–1274.

79. Carroll DJ, Barton BA, Anderson GW, et al. Influence of protein intake and feeding strategy on reproductive performance of dairy cows. *Journal of Dairy Science*. 1988;71(12):3470–3481.

80. Fogwell RL, Cowley JL, Wortman JA, et al. Luteal function in cows following destruction of ovarian follicles at mid–cycle. *Theriogenology*. 1985;23(2):389–398.

81. Foster JP, Lamming GE, Peters AR. Short–term relationships between plasma LH, FSH and progesterone concentrations in postpartum dairy cows and the effect of GNRH injection. *J Reprod Fertil*. 1980;59(2):321–327.

82. Friggens NC, Chagunda MG. Prediction of the reproductive status of cattle on the basis of milk progesterone measures: Model description. *Theriogenology*. 2005;64(1):155–190.

83. Hassan MM. *Prevalence and risk factors of repeat breeding in cows*. Bangladesh: Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University; 2017.

84. Henrieks DM, Dickey JF, Hill JR. Plasma estrogen and progesterone levels in cows prior to and during estrus. *Endocrinology*. 1971;89(6):1350.

85. Kindahl H, Basu S, Fredíksson G. Levels of prostaglandin F2α metabolites in blood and urine during early pregnancy. *Animal Reproduction Science*. 1981;7(1):133–148.

86. Knickerbocker JJ, Wiltbank MC, Niswender GD. Mechanisms of luteolysis in domestic livestock. *Domest Anim Endocrinol*. 1988;5(2):91–107.

87. Manual Draminski® Electronic Estrus Detector, Poland. 2008.

88. Rhodes FM, Death G, Entwistle KW. Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. *Animal Reproduction Science*. 1995;38(4):265–277.

89. Smith JF, Fairclough RJ, Payne E, et al. Plasma hormone levels in the cow. I. Changes in progesterone and oestrogen during the normal oestrous cycle. *New Zealand Journal of Agricultural Research*. 1975;18(2):123–129.

90. Van Eerdenburg FJCM, Loeffler HSH, Van Vliet JH. Detection of estrus in dairy cows:A new approach to an old problem. *The Veterinary Quarterly*. 1996;18(2):52–54.

91. Wael MBN. Ovarian follicular activity and hormonal profile during oestrus cycle in cows:The development of 2 versus 3 waves. *Reprod Biol Endocrinol*. 2003;1(50):1–6.

92. Walters DL, Schams D, Schallenberger E. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. *Journal of Reproduction and Fertility*. 1984;71(2):479–491.

93. Yamauchi S, Nakamura S, Yoshimoto T, et al. Prediction of estrous cycle and optimal insemination time by monitoring vaginal electrical resistance (VER) in order to improve the reproductive efficiency of the Okinawan native Agu pig. *Anim Reprod Sci*. 2009;113(1):311–316.