Research Article

Postpartum uterine involution and ovarian follicular dynamics in crossbreed Anatolian water buffalo (*Bubalus bubalis*) during summer season

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Abstract: It was aimed to monitor postpartum (pp) uterine involution and follicular wave pattern by transrectal ultrasonography and detection of concentrations of milk progesterone (P_4) in eleven water buffaloes during the summer season in Turkey. The data obtained from the first day of calving to positive pregnancy diagnosis were evaluated on a daily basis. Uterine position scores and nonechogenic material showed a decreasing grade pattern related to the time ($P < 0.0001$). A time effect was evident with uterine horn thickness ($P < 0.05$) or intercaruncular zone thickness ($P < 0.01$) and uterine horn lumen ($P < 0.01$). Uterine horn thickness showed a gravidity x time interaction ($P < 0.05$). Gravidity x time interaction was not evident in total uterine horn thickness but time effect was seen ($P < 0.0001$). Time effect was also evident for the thickness of uterine body ($P < 0.0001$) and cervix uteri ($P < 0.01$). The uterine body and horns were returned to previously nongravid state on day 26.00 ± 2.38 and the interval from calving to complete disappearance of uterine fluid was accomplished on day 31.44 ± 3.23 pp. The previously gravid and nongravid uterine horns, uterine body and cervix uteri involuted on day 31.42 ± 1.39, 28.37 ± 0.65, 29.37 ± 0.73 and 27.87 ± 0.47 pp, respectively. The interval from calving to first observation of dominant follicle of nonovulatory wave was 18.54 ± 6.91 days and on average 3.45 ± 1.91 nonovulatory waves were observed. Postpartum first ovulation occurred on day 64.45 ± 18.49. One and two wave models or combination of both waves were observed. The interval from calving to pregnancy was 72.36 ± 21.33 days. The maximum concentrations of milk P_4 during short or normal cycles and pregnancy were 0.62 ng/mL, 2.2 ng/mL, and 4.04 ng/mL, respectively. In conclusion, the presence of one or two waves should be considered when estrous synchronization programmes are planned in Anatolian water buffaloes during summer season.

Key words: Anatolian water buffalo, uterine involution, follicular dynamics, summer

1. Introduction

Buffalo have long been domesticated and bred in Asian, Mediterranean and Latin American countries for various purposes such as draught, capital, meat and milk [1,2]. Buffalo breeding is commercially very lucrative for the milk-cream industry in Turkey [3,4]. The water buffalo population of Turkey was more than 1 million in the early 1980s, but by 2010, there were less than 100,000 animals [4]. It has been reported that the rate of decline of Turkey's water buffalo population from 1980 to 2011, over a 30-year period, was 91.9% [5]. Since then, due to growing government support of the water buffalo breeding industry in Turkey, FAO data shows an increase in the water buffalo population of Turkey to 161,439 by the end of 2018.

As with other farm animals, buffalo production is highly dependent upon successful reproduction [6].

However, reproductive performance is negatively affected by late reproductive maturity, irregular estrous cycles, poor estrous activity, prolonged calving intervals and the effects of distinct seasonal reproductive patterns [7]. It is known that buffalo reproduction in both tropical and subtropical conditions is adversely influenced by day length, ambient temperature and relative humidity [7,8]. In addition, the estrous cycle patterns of buffalo in equatorial zones differ from those in nonequatorial zones despite nutritional conditions in both being equally adequate to maintain reproductive efficiency. Hence in areas further from the equator with milder summers, it is possible to observe both estrous and polyestrous patterns in buffalo throughout the year regardless of seasons [9]. Despite the presence of a polyestrous pattern in buffalo, better reproductive efficiency in Indian buffalo during winter compared to

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summer months, attributed to environmental factors, is evidenced by the exhibition of distinct seasonal variations in the display of estrus, conception rates and calving rates [10]. In Pakistani buffalo also, a higher breeding frequency is observed and reported during winter months as opposed to summer months [6]. In addition, in Italian buffalo seasonality of reproduction due to environmental factors has been observed and documented [9]. Along the same lines, it has already been postulated that calving and estrous activity occurs in a seasonal pattern in water buffalo in Turkey and that the highest estrous activity is observed between July and September with the densest annual calving rate between May and July [2]. Calving and estrous rates display seasonal patterns through the period April to August in another report in Turkey [3].

Prolonged calving intervals between two consecutive parturitions lead to poor reproductive efficiency and hence economic losses in the buffalo milk industry [11,12]. A rapid and complete uterine involution and resumption of cyclic ovarian activity is needed for an optimal intercalving period [11]. The improvement in fertility in female buffalo is closely related to the knowledge of follicular development. A large amount of information about ovarian follicular dynamics in domestic animals, intensively in cattle, has been provided by the ultrasound technology applied to animal reproduction [5]. The initial studies performed in buffalo for follicular growth were reported in Surti buffalo using rectal palpation by Singh et al. [13] and using histology of the ovaries by Danell [14]. Studies related to the follicular growth patterns and the reproductive properties of estrous cycle or genital tract organs in water buffalo in Turkey are very limited [5,15–17]. In addition and importantly, the transrectal ultrasonography of postpartum (pp) uterine involution and ovarian follicular dynamics in crossbreed Anatolian water buffalo in Turkey has not previously been investigated. Therefore, the objective of this present study was designed to monitor the pp uterine involution and follicular growth in crossbreed Anatolian water buffalo during summer season.

2. Materials and methods

2.1. Animals and feeding management
A total of eleven suckling crossbreed multiparous Anatolian water buffalo (Anatolian Water Buffalo X Murrah) (*Bubalus bubalis*) aged between 9 and 12, 7 days in milk (DIM) and housed in Afyon Kocatepe University Livestock Research and Practice Center (38°44′N, 30°34′E; altitude 1034 m) in Turkey were used in the study. All animals had normal calving with a single calf, spontaneous placental expulsion within the first 12 h after calving, without clinical signs of uterine infection and metabolic disease. They were milked twice daily- morning and evening. A Murrah breed buffalo

bull was kept in the same barn with the water buffalo cows for natural mating throughout the study. Water buffalo calves were separated from their dam 3 days after birth and they were allowed to stay with the dam to let the milk down before milking. The study was carried out during the warm season from May to September. All experimental procedures were approved by the Animal Ethics and Welfare Committee (AKUHADYEK/184).

All animals were housed in the same paddock (pen) and fed twice (8:00 and 20:00) a day with the same diet which was served ad libitum. The diet was prepared, due to 5%–7% refusals, to allow sufficient dry matter intake. Refusals were collected and weighed daily to accurately calibrate the daily feed intake per animal. The diet was formulated according to the nutrient composition of all ingredients. For this purpose, each foodstuff was analyzed for crude protein, crude ash, crude cellulose, ether extract, dry matter (according to Weende analysis methods), neutral detergent fiber (NDF) and acid detergent fiber (ADF) [18] before formulation. The NRC [19] was used as the primary source for the calculations in diet formulation. The ingredients and chemical composition of the feed ratio are shown in Tables 1 and 2, respectively.

2.2. Transrectal ultrasonography of postpartum uterus
Calving day was designated as the first day of the study. The transrectal ultrasonography (6.0 MHz, DP 20, Shenzen Mindray Bio-medical Electronics, Shenzen, China) of the pp uterus was revealed on a daily basis until 45 days pp. Nevertheless, the visualizing of the entire genital tract was not possible for six days due to the low depth rate of ultrasound waves or the location of ovaries and the size of the uterus. Therefore, the evaluation of images obtained from transrectal ultrasonography was initiated on seven days in milk (DIM). Real time transrectal ultrasonography images were recorded using a MP4 player device (Orite PMP500, Orite IT Solutions, Sydney, Australia) throughout each examination. The MP4 player device was connected to the ultrasound with a cable and the examination was recorded in video format. Video records were displayed on computer monitors using GOM Player (Gretech Corporation, Seoul, South Korea) and the video was frozen at the necessary points in time to measure each related parameter. The image was then transferred to an ImageJ program (National Health Institute, Bethesda, MD, USA). After the calibration of pixel value corresponding to 10 mm, the measurements were performed.

The parameters were measured on transrectal ultrasonography to evaluate the pp uterine involution of previously gravid and nongravid uteri. Briefly, the cranial part of the cervix uteri, at least 1.5 cm, was diagnosed as the uterine body [15], whereas the uterine horn was specified as the cranial part of the uterine body. The uterine horn thickness was measured between the external wall of the

Table 1. Dietary ingredients.

Feedstuffs	% Dry matter
	Forage
Alfalfa hay	21.55
Corn silage	20.87
Wheat straw	6.60
	Concentrate
Barley, ground	27.18
Concentrate mix	23.80
Total	100
	Concentrate mix
Feedstuffs	%
Sunflower meal (crude protein; 36%)	10
Cottonseed meal (crude protein; 26%)	10
Canola meal (crude protein; 32%)	5
Soft wheat bran	74
Premix (vitamins and minerals)*	1
Total	100

Premix composition in 1 kg; Vitamin A 200000 IU, Vitamin D 80000 IU, Vitamin E 2000 mg, Mn 300 mg, Fe 300 mg, Zn 1600 mg, Cu 300 mg, Co 17 mg, I 120 mg, Se 17 MG, Limestone 450 g, dicalcium phospate 190 mg, salt 90 g, magnesium oxyde 170 g.

Table 2. Chemical composition of the diet.Nutrients

Nutrients	% Dry matter
Metabolizable energy (Mcal/kg)	2.39
Crude protein	13.51
Ether extract	2.77
Crude ash	6.06
Neutral detergent fiber	39.48*
Acid detergent fiber	23.42**
Calcium	0.78
Phosphorus	0.45

Neutral detergent fiber content has been suggested around 36% of dry matter.
**Acid detergent fiber content has been suggested around 21% of dry matter.

uterine horn and the caruncle surface with the uterine lumen. The intercaruncular zone thickness was measured as the distance from the external wall of the uterine horn to the uterine lumen. The uterine horn lumen was defined as the image of nonechogenic material visualized by the ultrasonography and the transversal measurement of the lumen was the diameter of the uterine horn lumen. However, the measurement of thickness of the uterine horn and intercaruncular zone was ended on 14 DIM, due to difficulties in imaging the caruncle(s). Consequently, the distance between the dorsal and ventral external walls of the uterine horn was recorded as the total uterine horn thickness after 14 DIM (since both the dorsal and ventral external uterine walls of the uterine horn were not visualized in one image until 14 DIM).

The uterine body thickness was measured as the distance from the external wall of the uterine body to the lumen in which images of nonechogenic material were evident, whereas the total uterine body thickness was considered to be the distance between the dorsal and ventral external walls of the uterine body. Similarly, the cervix uteri thickness was measured as the distance from the external wall of cervix uteri (without crypt) to the lumen, where the image of nonechogenic material

was evident, whereas the total cervix uteri thickness was recorded as the distance between the dorsal and ventral external walls of the cervix uteri. The total thickness of the uterine body, uterine horns and cervix uteri were measured until 45 DIM.

Intrauterine fluid volume was evaluated by the method described by Scully et al. [20], which was based on the scoring of the amount of nonechogenic material using a scale of 0–3 (0 = no fluid; 1–3 = increasing amounts of fluid in the corpus or cornu uteri). Moreover, the position of the uterus relative to the pelvic brim was noted by the way of rectal palpation before each ultrasound examination, and the position was scored on a 0–3 scale (0 = when the uterus was easy to palpate within the pelvic canal and returned to previously nongravid state, 1–3 = when the uterine body and horns were falling further over the pelvic brim). Uterine involution was deemed complete from the occurrence of a minimal uterine body and horn diameters, with no change between two successive days [20]. The evaluation of intrauterine fluid volume and the position of the uterus were performed between 7 and 45 DIM. The involution criteria for nonechogenic material and uterine position were accepted, when the score reached 0 with no change between two successive days. Any vaginal discharge was noted for the assessment of the uterine health status as described in cows throughout the study [21].

2.3. Transrectal ultrasonography of ovaries
The detection of ovarian follicular dynamics was performed by daily transrectal ultrasonography until positive pregnancy diagnosis. Follicles or luteal tissue on left or right ovaries were separately recorded. Follicles ≥ 3 mm in diameter (starting of follicle wave) were monitored until the presence of dominant follicle (DF) that was ≥

10 mm and at least 2 mm larger than other follicles. The criterion of the number and diameter of DF and day of emergence of DF until first ovulation was used to describe the nonovulatory wave(s). The first ovulation after the postpartum nonovulatory wave and the subsequent follicular growth process were described as the ovulatory follicle wave. The time between ovulation (the absence of Graafian follicle and presence of luteal tissue at the same position of the Graafian follicle of the ovary) and the next ovulation was considered as the duration of estrous cycle. The optimum duration of estrous cycle was accepted as 19–22 days [22]. Moreover, other parameters such as follicle growth rate, duration of growth phase, duration of static phase, regression rate and duration of regression phase were evaluated. The positive diagnosis of pregnancy was provided by the imaging of embryo. The pregnancy was confirmed by the daily reexamination of the animal for 10 days.

2.4. Detection of milk progesterone concentration

Milk samples were collected daily into potassium dichromate added sterile tubes until positive pregnancy diagnosis and stored at 4 ℃ further analysis of the concentrations of milk progesterone (P_4). Samples were centrifuged at 4 ℃, 3000 rpm for 30 min and the milk fat was discarded in order to avoid misinterpretation of the values due to progesterone variations in the fat content. Then, the samples were transferred to Eppendorf tubes and centrifuged at 15,000 rpm for 30 min to separate the milk protein and milk fat. The concentrations of milk P_4 were measured by ELISA with commercial test kits (P_4, EIA – 1561, DRG, USA). The concentrations of P_4 above 1 ng/mL for at least five successive days were regarded the occurrence of ovulation and hence the presence of corpora lutea which was confirmed by the transrectal ultrasonography.

2.5. Statistics

Data of thickness of uterine horn and intercaruncular zone, uterine horn lumen and total uterine horn were evaluated using PROC MIXED of SAS (SAS Institute, Cary, NC, USA, 2019) as repeated measures. Fixed effects were determined as the previous gravidity status (gravid or nongravid), examination time point and their interaction. Each examination was evaluated as a random effect in the model. The REPEATED statement was used for time-dependent evaluation. Degrees of freedom were calculated by using Kenward–Roger option in the model, and the most appropriate covariance structure was chosen for each of the parameters by the Bayesian information criterion (BIC) among the selected covariance structure with TYPE statement in the REPEATED statement (heterogenous or nonheterogenous autoregressive, spatial power, Toeplitz, heterogenous or nonheterogenous compound symmetry, variance components, and unstructured) [23]. Studentized

residuals were determined using the MIXED statement of SAS with all fixed effects and interactions. The results with studentized residuals of <–4 or >4 were considered outliers and removed from the model. The PDIFF statement was used for a two-side comparison of the observed parameters. The aforementioned values were represented as least square means with LSMEANS statement of SAS in the graphs. In addition, SPSS software (16.0, SPSS Inc., Chicago, IL, USA, 2007) was used for the analyses of data including uterine position score, nonechogenic material score, uterine body, uterine body lumen, cervix uteri, total uterine body and total cervix uteri. The distribution of normality of data were analysed by Shapiro–Wilk normality test. It was found that all data had the normal distribution. The parameters of thickness of uterine body lumen, uterine body, cervix uteri, total cervix uteri, nonechogenic material score and uterine position score regarding the time were analysed by repeated measures ANOVA with Bonferroni adjustment. Differences were considered significant at a P value of less than 0.05.

3. Results

3.1. Postpartum uterine involution

Uterine position scores and nonechogenic material showed a decreasing grade pattern related to the time (P < 0.0001) as the DIM increased. The uterine body and horns were easy to palpate within the pelvic cavity and returned to previously nongravid state on day 26.00 ± 2.38 (Figure 1a), whereas the interval from calving to complete disappearance of uterine fluid was accomplished on day 31.44 ± 3.23 pp (Figure 1b). Moreover, no vaginal discharge related to metritis was observed.

The data obtained on day 8 and 10 showed significant difference (P < 0.05) for the measurement of uterine horn thickness (Figure 2a), whereas no significant difference was observed between uterine horns for the measurements of intercaruncular zone (Figure 2b) and uterine horn lumen (Figure 2c). However, a time effect was evident with the decreasing uterine horn thickness (P < 0.05) or intercaruncular zone thickness (P < 0.01) as well as the uterine horn lumen (P < 0.01). Uterine horn thickness (Figure 2a) showed a gravidity x time interaction (P < 0.05), while there was no interaction with the data of gravid or nongravid uterus of intercaruncular zone thickness (Figure 2b) and uterine horn lumen (Figure 2c). A decreasing thickness pattern was observed for the uterine body (Figure 2d), uterine body lumen (Figure 2e) and cervix uteri (Figure 2f), nevertheless the time effect was not significant.

No significant difference was evident between uterine horns for the measurement of total uterine horn thickness and gravidity x time interaction, whereas a time effect was seen (P < 0.0001) (Figure 3a). Time effect was also evident

Time < 0.0001

(a)

Time < 0.0001

(b)

Postpartum Days
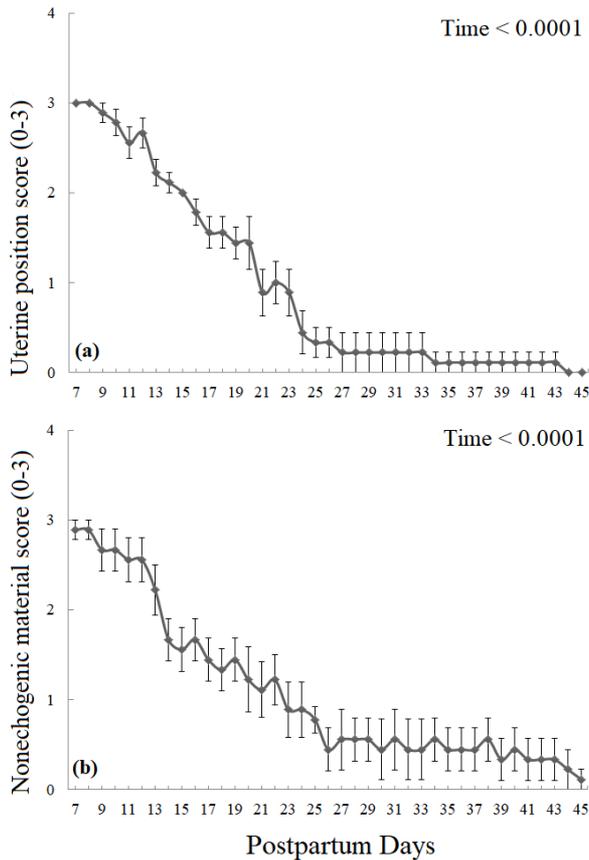
Figure 1. The scoring of the position of uterus relative to the pelvic brim by rectal palpation (a; 0 = when the uterus was easy to palpate within the pelvic canal and returned to previously nongravid state, 1–3 = when the uterine body and horns were falling further over the pelvic brim) and the amount of nonechogenic material by transrectal ultrasonography (b; 0 = no fluid; 1–3 = increasing amounts of fluid in the corpus or cornu uteri) between 7 and 45 days in milk.

for the thickness (P < 0.0001) of total uterine body (Figure 3b) and thickness (P < 0.01) of total cervix uteri (Figure 3c). Moreover, the evaluation process of the occurrence of the minimal diameters of different parts of the genital tract with no change between two successive days, showed that the gravid and nongravid uterine horns, uterine body and cervix uteri involuted on 31.42 ± 1.39, 28.37 ± 0.65, 29.37 ± 0.73 and 27.87 ± 0.47 DIM, respectively.

3.2. Ovarian follicular dynamics
Postpartum nonovulatory wave pattern was evaluated. The interval from calving to first observation of dominant follicle of nonovulatory wave was 18.54 ± 6.91 days. Accordingly, it was found that 3.45 ± 1.91 nonovulatory wave was observed until postpartum first ovulation (Table 3). The growth phase of nonovulatory dominant follicles was 8.27 ± 3.39 days (1.08 ± 0.40 mm/day). They had static

phase for 4.27 ± 2.86 days. Similarly, the regression phase was 7.34 ± 2.34 days (1.20 ± 0.44 mm/day). It was seen that the growth and regression phases of last dominant follicles before the first ovulation were 9.12 ± 1.55 and 8.75 ± 2.65 days, whereas static phase was 4.00 ± 1.06 days. The growth phase of first, second and third ovulatory follicles were 14.33 ± 3.50, 9.75 ± 2.98 and 12 days, respectively, whereas growth rate of overall ovulatory follicles displayed 12.85 ± 3.75 days. The maximum diameter of last dominant follicles before the first ovulation was 13.21 ± 1.24 mm. However, the maximum diameter of first, second, third and overall ovulatory follicles were 14.32 ± 1.94 mm, 13.75 ± 2.98 mm, 16.01 mm and 14.28 ± 1.73, respectively (Table 3).

The evaluation of follicle wave patterns showed that three or more wave model was not evident in Anatolian water buffalo. Accordingly, three buffalo cows displayed one wave (Figure 4a); two wave follicle patterns were only observed in one buffalo cow (Figure 4b) and both one and two wave follicle growth (Figure 4c) again was seen in just one buffalo cow. Moreover, it was detected that pregnancy was achieved in six buffalo cows at pp first ovulation (Figure 4d) (between 31 and 83 days), whereas overall calving pregnancy interval was 72.36 ± 21.33 days. The duration of estrous cycles between ovulations showed short (11.2 ± 0.95 days) or normal (19 days) patterns, while there was no long cycle(s) (Table 4). Accordingly, it was seen that the buffalo cows which showed short cycle were pregnant at second ovulation. The diameter of the ovulatory follicle of first ovulation was 14.32 ± 1.94 mm. However, the diameter of the second ovulatory follicle in short cycle was 13.75 ± 2.98 mm, whereas normal cycle showed the ovulatory follicle at 14.41 mm. During the third ovulation, the diameter of the ovulatory follicle was 16.01 mm. The diameter of the corpus luteum during pregnancy (21.86 ± 2.60 mm) was slightly larger than those detected at first (16.71 ± 1.71 mm) and second ovulation (13.81 mm). The concentrations of milk progesterone until first ovulation were ranged with a maximum 0.42 ng/mL. The maximum concentrations of milk progesterone during short or normal cycles and pregnancy were 0.62 ng/mL, 2.2 ng/mL and 4.04 ng/mL, respectively.

4. Discussion
The data obtained in this study support previous in vitro and in vivo reports [5,13,14,24–26] and provide new evidence that wave patterns of follicular growth play an important role in fertility in water buffalo. Moreover, the present study elucidates the summer anestrous status of water buffalo in Turkey by demonstration of pp uterine involution process and ovarian follicular dynamics. It has been previously reviewed that the gravid horn becomes located on the pelvic brim by 14 DIM, then in the pelvic cavity by 21–25
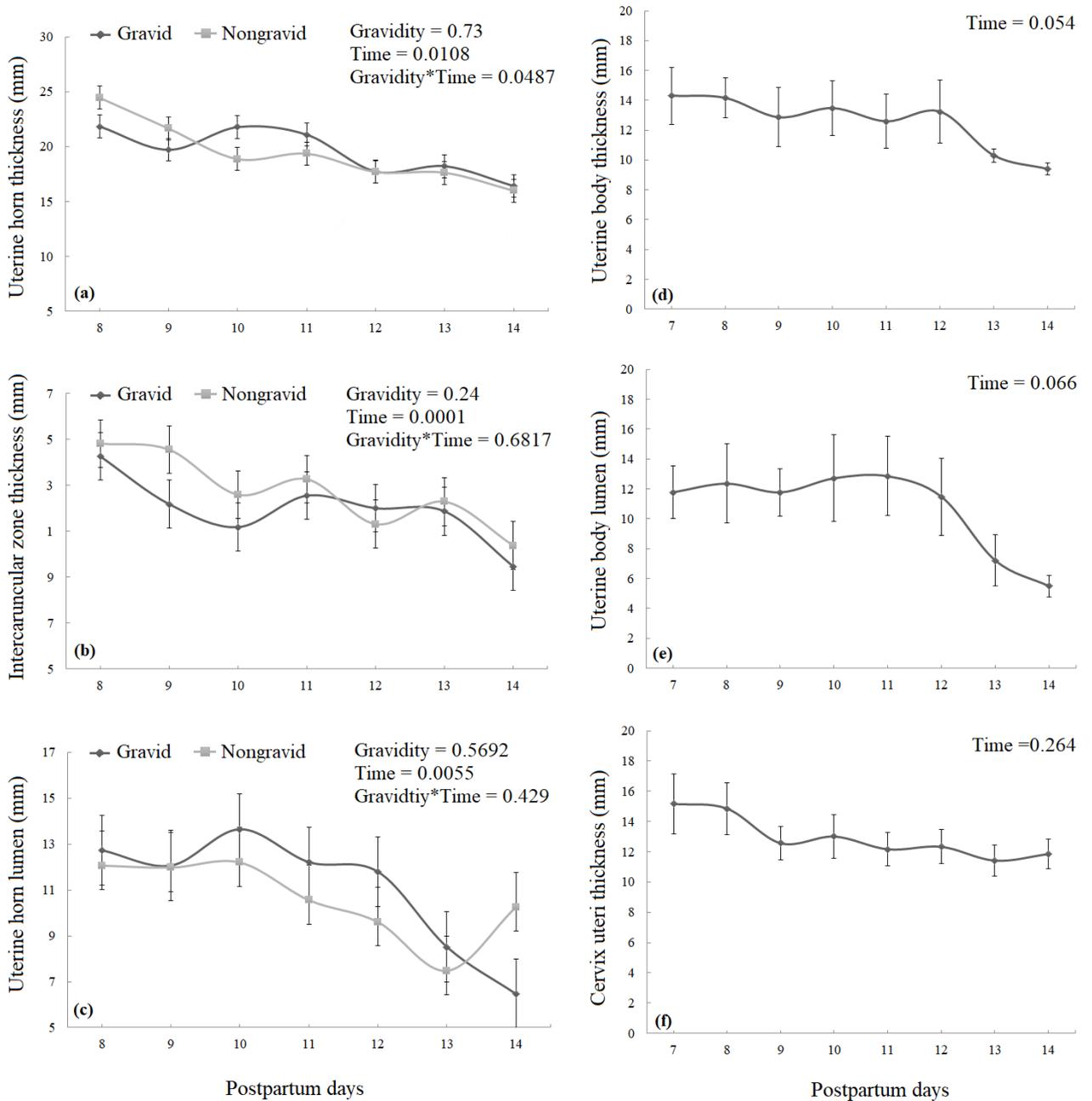
Figure 2. The changes of thickness of uterine horn (a) or intercaruncular zone (b), uterine horn lumen (c), uterine body (d), uterine body lumen (e), and cervix uteri (f) throughout the postpartum days in Anatolian water buffaloes.

DIM, whereas 24% of dairy buffaloes clinically completed the involution of the uterus on pp fourth week and 36% on fifth week [27]. In the present study, a consistent result was found that the uterus returned to its previously nongravid state on day 26.00 ± 2.38. However, longer durations for involution day were also reported [28]. The discrepancies might be related to factors such as feeding, seasonal and management conditions or the criteria used for intervals between examinations, as well as the subjective nature of rectal palpation. The scoring of the amount of uterine fluid is another method to evaluate the involution process in lactating or nonlactating cows in which the complete disappearance of nonechogenic material occurred at approximately 40 days [20]. The present study demonstrated that the interval from calving to complete disappearance of pp uterine fluid was accomplished on day 31.44 ± 3.23, indicating that there was no persistent uterine inflammation indicative of uterine infection.
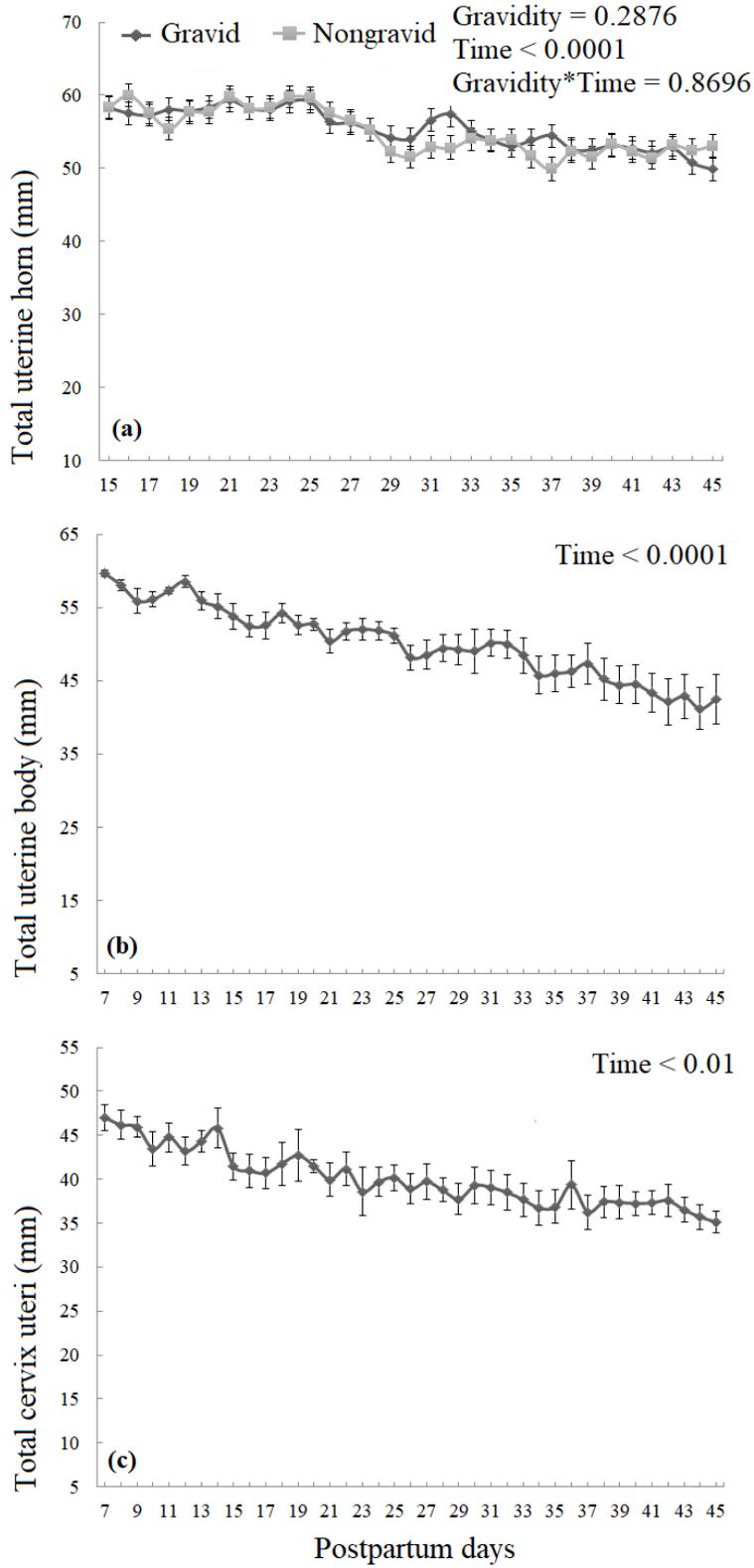
Figure 3. The changes of thickness of total uterine horn (a), total uterine body (b), and total cervix uteri (c) throughout the postpartum days in Anatolian water buffaloes.

Table 3. The number, growth phase (day), growth rate (mm/day), static phase (day), regression phase (day) and regression rate (mm/day) of the nonovulatory wave pattern and the characteristics of last dominant follicle (DF) before postpartum first ovulation and first, second or third ovulatory follicle (OF) and total OFs (mean ± SD).

Parameter	Non-ovulatory	Last DF	First OF	Second OF	Third OF	Total OF
Number	3.45 ± 1.91	10	11	5	1	17
Growth phase (day)	8.27 ± 3.39	9.12 ± 1.55	14.33 ± 3.50	9.75 ± 2.98	12	12.85 ± 3.75
Growth rate (mm/day)	1.08 ± 0.40	0.89 ± 0.32	0.77 ± 0.37	1.31 ± 0.67	1.18	0.95 ± 0.51
Static phase (day)	4.27 ± 2.86	4.00 ± 1.06				
Regression phase (day)	7.34 ± 2.34	8.75 ± 2.65				
Regression rate (mm/day)	1.20 ± 0.44	1.03 ± 0.30				
Max. diameter (mm)	12.88 ± 1.66	13.21 ± 1.24	14.32 ± 1.94	13.75 ± 2.98	16.01	14.57 ± 1.74

Moreover, this finding is in line with the uterus position scores indicating both scores might be used to assess the uterine health status of the water buffalo cow.

The more accurate means of examination of the reproductive system to detect dynamic changes in the reproductive tract after parturition is ultrasonography [22]. The methods for measuring the different parts of the uterus, the variety of examination days and the criteria for the end of involution day display various results in reports. Therefore, this study focused on the measuring of the thickness of intercaruncular zone of the uterus, as well as the uterine horn thickness by ultrasonography. The present study stated that the thickness of gravid or nongravid uterine horn or intercaruncular zone and uterine horn lumen decreased in a time-dependent manner between days 7 and 14, as expected. Moreover, the imaging of caruncles was not possible on day 15 in all examined water buffalo cows. This observation was consistent with El-Wishy [27] who reported the complete degeneration of caruncles by the 15th day postpartum in the uteri of slaughtered buffalo. Sloughing of necrotic caruncular material and reepithelisation of caruncle that is slowly covered with the growth of the surface epithelium are needed for the involution process of caruncles [29]. The presence of no difference in gravid or nongravid intercaruncular zone thickness in the study revealed that involution of intercaruncular zones might not be associated with gravidity status of the uterus. Nevertheless, higher uterine horn thickness on day 8 and lower thickness on day 10 in nongravid uterine horn showed significant difference as compared to gravid one. It is suggested that involution of caruncular zone of nongravid uterus might be faster than those of gravid uterus due to less damage during calving.

The present study displayed that second week of postpartum period did not cause changes for the measurements of uterine body, uterine body lumen and cervix uteri, while the decreasing thickness of gravid and nongravid total uterine horns, uterine body or cervix uteri was stabilized on days 31.42 ± 1.39, 28.37 ± 0.65, 29.37 ± 0.73, and 27.87 ± 0.47 pp, respectively. Observations in dairy buffaloes have indicated that the clinically completed uterine involution of the uterus ranged with a maximum of 74 days [28]. Accordingly, in Pakistani buffaloes, 55% of them were assumed to complete uterine involution by 35 days pp and 85% by 50 days, whilst the average rate was 32.5 days [30]. However, in another study, it has been reported that the uterine involution of swamp buffalo ranges between 22 and 41 days, and is 28.74 days on average [31]. In contrast, Presicce et al. [32], reported that the uterine involution of primiparous buffalo was 31 days, while 33 days was observed in multiparous Mediterranean buffalo. The longest duration required for uterine involution in Indian and Egyptian buffalo has been indicated as 45 and 40.3 days, respectively [30]. However, it has been demonstrated that the involution of gravid and nongravid uterine horn is completed on days 25 and 22 postpartum by oxytocin treatment in Bulgarian Murrah buffaloes, while cervix uteri involutes on day 25 [33]. Uterine and cervical involutions were noted as 29.3 and 37 days in another study performed in Murrah buffaloes [34]. Although the present results were consistent with the previous data, discrepancies in the above mentioned observations indicated that factors such as parity [32], suckling [35], barn management [30], cyclicity [36,37] and season [37] might all affect the involution process. Furthermore, it is suggested that nongravid uterine horn, body, and cervical involution completed simultaneously, and earlier than previously gravid uterine horn.

The criteria used for the diagnosis of a nonovulatory wave, which was the presence of nonovulatory dominant follicles (DF) ≥ 10 mm and at least 2 mm larger than other follicles, revealed that the interval from calving to first observation of dominant follicle of the nonovulatory
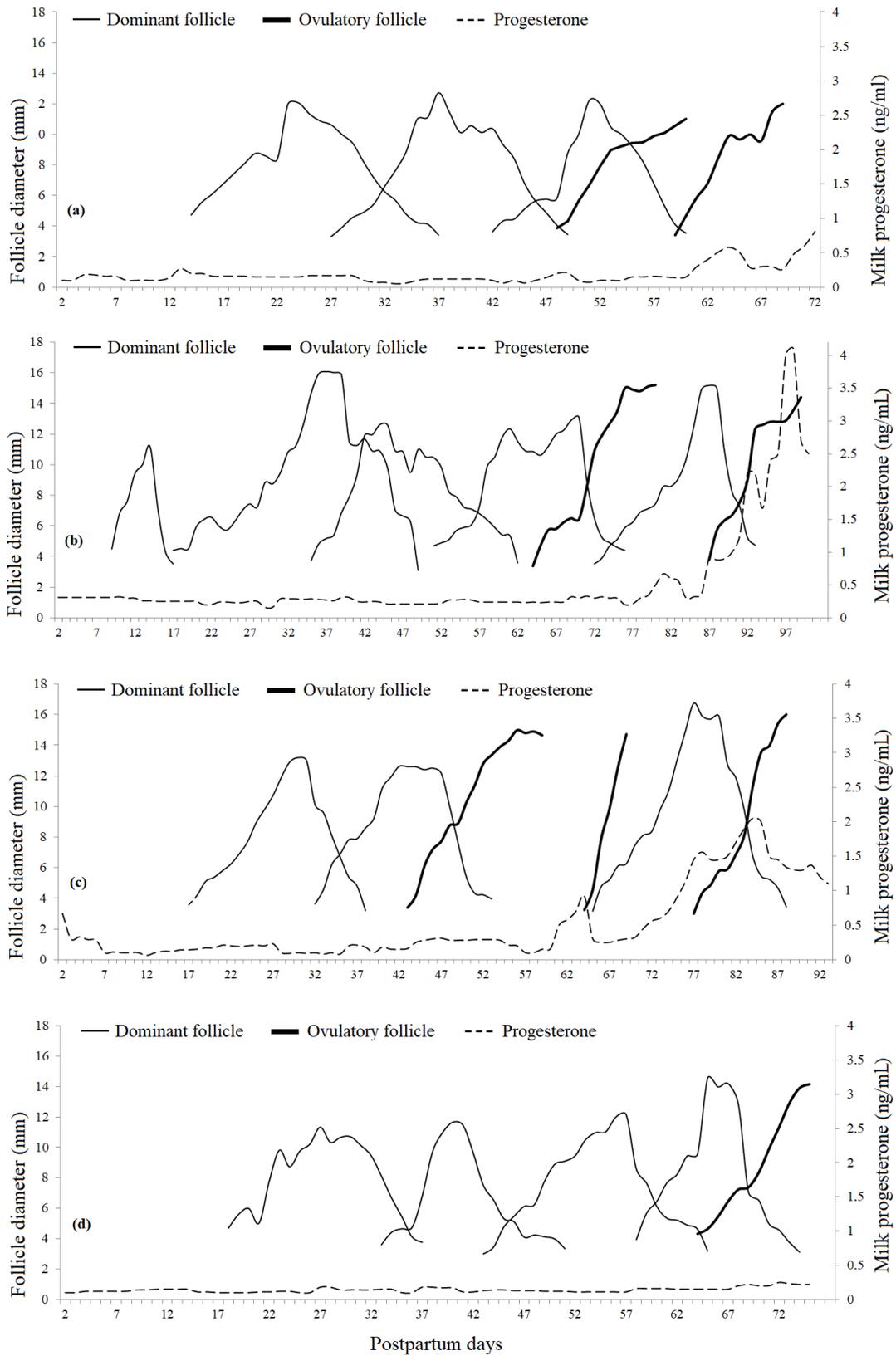
Figure 4. Representative graphics of milk progesterone concentrations (ng/mL) related to one wave (a), two wave (b), both one and two wave (c) patterns and pregnancy detected after postpartum first ovulation (d).

Table 4. The calving-first ovulation interval (day), duration of estrous cycle (short, normal), ovulatory follicle (OF) diameter (mm), first-second ovulation interval (day), second-third ovulation interval (day), number and diameter of corpus luteum (CL) (mean ± SD).

Parameter	Estrous cycle	Number of follicles	Diameter of OF	Ovulation intervals	Number of CL	Diameter of CL
Calving-first ovulation	---	11	14.32 ± 1.94	64.45 ± 18.49		
First*-second ovulation	Short	4	13.75 ± 2.98	11.20 ± 0.95	5	16.71 ± 1.71
	Normal	1	14.41	19		
Second*-third ovulation	Normal	1	16.01	19	1	13.81
Pregnancy					11	21.86 ± 2.60

* The diameter (mm) of corpus luteum of nonpregnant Anatolian water buffalo.

wave was 18.54 ± 6.91 days. It has been reported that the largest follicle on any pp day does not grow more than 8 mm up to day 21 pp, whereas the appearance of large sized follicles (>8.5 mm) in cyclic buffalo is evident between 10 to 62 days pp and 15 to 47 days in acyclic animals [36]. The first detection day of large follicles in multiparous Bulgarian Murrah buffalo was estimated at 16.33 ± 4.09 days and at 19.0 ± 4.74 days in primiparous animals [38]. The present study displayed compatible results with the abovementioned reports. Moreover, it is suggested that the follicular growth pattern is observed in waves during early pp periods, from the end of first week pp onwards. The growth rate and the largest size of the last dominant follicle before the first ovulation, as well as the parameters of ovulatory follicles, were similar to those detected by Yindee et al. [22]. Furthermore, the previous observation of Presicce et al. [32], who found the time (7–14 days) needed for the first pp dominant follicle (14.1 ± 0.4 mm) to grow and ovulate, was consistent with the findings of the present study. However, the duration of pp first ovulation, found to be 64.45 ± 18.49 days in this study, was longer than those detected in Mediterranean Italian buffaloes (15.5 ± 1.3 days, [32]), Bulgarian Murrah buffaloes (38.6 ± 12.46 days, [38]), Thai swamp buffaloes (39.81 ± 3.38 day, [22]) but similar to limited suckled Nili-Ravi buffaloes (60.7 ± 3.3 day, [39]) and suckled buffaloes (88.0 ± 4.7 day, [40]). Observed discrepancies could be a consequence of differences in climate and barn management strategies. Moreover, inappropriate feeding conditions such as using poor quality fodder or straw as the main roughage ingredients of the diet [41], low energy intake [42] or unbalanced protein/energy ratios [43], might have adversely affected the reproductive efficacy in buffalo. Since the animals in the present study were fed with low quality roughage, concentrate mix was used to compensate, and indeed it is possible that the low digestibility and high ADF content of the diet could have been a major dietary factor leading to the observed delay in pp first ovulation. This would be consistent with the findings of Qureshi et al. [41] who reported that feeding buffaloes with low quality crop residues led to a decrease

in reproductive performance. However, in the present study it was expected that the calving pregnancy interval might be extended due to the prolonged pp first ovulation. Interestingly, the calving pregnancy interval was 72.36 ± 21.33 days (between 31 and 100 days) due to the presence of pregnancy in six buffalo cows at pp first ovulation, and in four animals at pp second ovulation following short luteal phase, and in one buffalo cow at pp third ovulation. Increasing the daily feed consumption and energy intake by expanded rumen volume allowing animals to consume more feed could be the reason for the improvement in calving pregnancy interval. It should be noted that among the economic factors of water buffalo production in Turkey, the cost of prolonged calving interval per day (6.49 USD), and the bull cost per pregnancy (6.07 USD), have been indicated totally at 12.56 USD [44]. Moreover, because of the tradition of keeping the water buffalo bull with the dam in rural areas of Turkey, it is suggested that the interval to first ovulation might be included among the traits for selection of service period of water buffalo, for improving pregnancy rates and hence enhancing economic returns.

Several authors have reported the predominance of two wave pattern in buffalo [5,25,45–47]. Besides the absence of three or more wave model was evident in this study, one or two wave follicle patterns have been observed. The common observation of short estrous cycles between first and second ovulations in those wave models was consistent with other reports [22,32,35]. It has been indicated that pluriparous buffaloes have more short luteal phases with lower progesterone concentration from the developing corpus luteum following first postpartum ovulation, as compared to primiparous animals [32]. This may explain the lower progesterone concentrations of short cycles obtained in this study and why the water buffalo cows with short cycles were pregnant after their second ovulation.

In conclusion, the present study firstly demonstrates in vivo pp uterine involution and follicular dynamics in crossbreed Anatolian water buffaloes. Results of the present study suggest the presence of one or two wave

patterns in pp period of Anatolian water buffaloes. Therefore, these suggestions should be considered when estrous synchronization programmes are planned in Anatolian water buffaloes during summer season.

Acknowledgment

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