INFLUENCE OF POSTPARTUM LUTEAL ACTIVITY ON THE REPRODUCTIVE PERFORMANCE IN BULGARIAN MURRAH BUFFALOES

Stanimir Yotov[1], Anatoli Atanasov[1], Julieta Dineva[2]
[1]Trakia University, Faculty of Veterinary Medicine, Department of Obstetrics
Reproduction and Reproductive Disorders
Malka Verea, Student campus, 6000 Stara Zagora, Bulgaria
[2]Institute of Biology and Immunology of Reproduction, Department of Immunobiology of Reproduction
Tzarigradsko Shose 73, 1113, Sofia, Bulgaria

Corresponding author: Stanimir Yotov
E-mail: stanrad@abv.bg; Phone: + 359 42 699 513

Abstract. The aim of the present study was to investigate the pattern of luteal activity in Bulgarian Murrah buffaloes during the first 50 postpartum days by blood progesterone analysis and ultrasonographic corpus luteum detection and its effect on reproductive performance of animals.

The study was conducted with 15 clinically healthy Bulgarian Murrah buffaloes after normal delivery of a single foetus, reared and fed uniformly in the presence of a breeding bull.

Luteal activity was detected through enzymatic immunoassay of blood progesterone concentrations at 3-day intervals between postpartum days 1 and 50, and ultrasonographic detection of corpus luteum in one ovary. Pregnancy status was used as a criterion for reproductive performance evaluation. After birth records exploration and ultrasound pregnancy check on the 11th month after the beginning of the experiment, two groups of buffaloes were formed: group I (n=5) consisting of non-pregnant buffaloes and group II (n=10) – pregnant animals. Mean blood progesterone concentrations and the cumulative percentage of animals with corpus luteum different than the gestational one, in both groups were determined.

According to the results of the study, pregnant buffaloes showed basal blood progesterone levels by the 7th postpartum day and exhibited high luteal activity between postpartum days 19–34, characterised by ultrasonographic detection of a corpus luteum different from the gestational one and blood progesterone concentrations > 0.5 ng/ml after day 34 postpartum. Buffaloes with blood progesterone concentrations < 0.25 ng/ml until the 50th postpartum day and inadequate functional activity of the corpus luteum could remain non-pregnant for a long time after parturition.

In conclusion, the pattern of luteal activity in Bulgarian Murrah buffaloes during the first 50 postpartum days had an effect on their reproductive status. The received data could be used for improvement of the protocols for oestrus synchronization and postpartum anoestrus reduction.

Keywords: buffaloes, postpartum, luteal activity, ultrasonography.

GELTONKŪNIO AKTYVUMO PO JAUNIKLIO ATSIVEDIMO LAIKOTARPIU ĮTAKA MURRAH VEISLĖS BUIVOLIŲ REPRODUKCINĖMS SAVYBĖMS

Stanimir Yotov[1], Anatoli Atanasov[1], Julieta Dineva[2]
[1]Akušerijos, reprodukcijos ir reprodukcinių sutrikimų katedra, Veterinarinės medicinos fakultetas
Trakijos universitetas
Studentų miestelis „Malka Verea“, 6000 Stara Zagora, Bulgarija
[2]Reprodukcijos imunologijos katedra, Biologijos ir reprodukcinės imunologijos institutas
Tzarigradsko Shose 73, 1113 Sofija, Bulgarija

Santrauka. Šio darbo tikslas – ištirti geltonojo kūno aktyvumą Bulgarijos Murrah veislės buivolių organizme pirmosiomis 50 dienų po jauniklio atvedimo ir jo įtaką reprodukcinėms savybėms. Ultragarsiniu sensoriumi nustatytas geltonasis kūnas ir progesterono koncentracija kraujyje.

Bandymui panaudota 15 kliniškai sveikų po vieną jauniklį atsivedusių Murrah veislės buivolių, vienodai prižiūrimų ir šeriamų šalia reproduktoriaus.

Ultragarsiniu sensoriumi nustačius geltonąjį kūną vienoje kiaušidėje, jo aktyvumas toliau buvo tikrinamas kas tris dienas (nuo pirmos iki penkiasdešimtos dienos po atsivedimo) imunologiškai tiriant progesterono koncentraciją kraujyje. Pagrindinis kriterijus vertinant reprodukcines savybes – progesterono koncentracija veršingų buivolių kraujyje. Vienuoliktą mėnesį nuo eksperimento pradžios, tada, kai buvo išanalizuoti gimdymo parametrai ir atliktas ultragarsinis tyrimas, buvo sudarytos dvi buivolių grupės. Pirmą grupę (n=5) sudarė neveršingos buivolės, o antrąją (n=10) – veršingos. Abiejų grupių buivolams nustatyta progesterono koncentracija ir bendras procentas gyvulių, kuriems geltonasis kūnas nebuvo būdingas vaikingumo laikotarpiu.

Pagal tyrimo rezultatus apvaisintų buivolių progesterono lygis kraujyje septintą dieną po veršiavimosi buvo bazinis, o geltonojo kūno aktyvumas nuo 19 iki 34 dienos po veršiavimosi buvo didelis. Praėjus 34 dienoms po jauniklio atsivedimo, progesterono koncentracija apvaisintų buivolių kraujyje buvo > 0,5 ng/ml. Buivolės, kurių kraujyje progesterono koncentracija iki penkiasdešimtos dienos po veršiavimosi buvo > 0,25 ng/ml ir kurių geltonojo kūno funkcija buvo netinkama, ilgą laiką gali likti neveršingos.

Galima daryti išvadą, kad Murrah veislės buivolių geltonojo kūno aktyvumas per pirmąsias 50 dienų po veršiavimosi turėjo įtakos jų reprodukcinėms savybėms. Tyrimo duomenys galėtų būti naudingi sinchronizuojant rujas ir trumpinant laikotarpį tarp rujų.

Raktažodžiai: buivolės, laikotarpis po jauniklio atsivedimo, geltonojo kūno funkcija, ultragarsinis tyrimas.

Introduction. One of the precise indicators to optimal reproductive performance in large ruminants is the pregnancy (Abdalla, 2002; Perea and Inskeep, 2008). The resumption of ovarian activity in early puerperium is an important factor to high conception rate achievement in buffaloes (Yendraliza et al., 2011). An evidence for postpartum ovarian activity and successful ovulation is the detection of a functional corpus luteum, different from the gestational one (Barkawi, 1993; Martin et al., 2010). The *in vivo* determination of luteal activity in buffaloes is most commonly done via rectal palpation (El-Wishy, 2007[2]), analysis of blood and milk progesterone (Perera et al., 1987; Jazayeri et al., 2010) and ultrasonography (Honparkhe et al., 2004; Terzano, 2005). The diagnostic value of the different methods is however variable (El-Wishy, 2007[2]).

Rectal palpation is a routine technique for diagnostics of ovarian structures, but it does not provide adequate information about their function (Younis et al., 1994). A primary technique for evaluation of the functional status of the corpus luteum is the analysis of progesterone concentrations (Abdalla, 2002; Campanile et al., 2010). However, progesterone levels are influenced by a number of factors as the season (Qureshi et al., 1999), presence of herd bull (Gokuldas et al., 2010), level of nutrition (Wongsrikeao, 1990) etc. The introduction of transrectal ultrasonography in large ruminant reproduction (Ginther, 1995; Terzano, 2005), increased the potential for real-time detection of changes occurring in ovaries and provides a substantial support in diagnosing the postpartum ovarian function in buffaloes (Presicce et al., 2005; Yindee et al., 2011).

Having determined puerperal ovarian activity, Jainudeen et al. (1983) established a very rapid regression of the gestational corpus luteum after the parturition and by the 10th day, palpated a small rough structure prominent over the ovarian surface with a diameter of < 3 mm. According to Momongan et al. (1990) the complete luteal regression occurs within 7 ± 2 days. Numerous researchers (Prakash and Madan, 1986; Eissa et al., 1995; Tiwari et al., 1995) linked this event with attaining basal progesterone concentrations at parturition, varying from 0.1 to 0.6 ng/ml. Bahga and Gangwar (1988) however, observed the lowest progesterone levels by the 6th postpartum hour, whereas El-Belely et al. (1988) demonstrated a gradual decrease in progesterone up to basal concentrations by the 15th day, when the residual gestational corpus luteum has completely disappeared.

The diagnostics of postpartum ovarian activity in swamp buffaloes has shown that in 67% of cases, progesterone concentrations over 0.2 ng/ml confirmed the palpable corpus luteum in ovaries, while lower levels did not indicate a functioning luteal structure (Jainudeen et al., 1981).

Bahga and Gangwar (1988) determined the progesterone profile of Indian Murrah buffaloes between postpartum days 0–57. They observed increased progesterone concentrations by days 8, 29 and 43 during the spring and by days 15, 36 and after the 50th day during the summer and reported seasonal differences in oestrus onset and conception rates. Investigating the issue in local buffaloes in Sri Lanka, Perera et al. (1987) proved that 75% were in oestrus and were fertilised before a corpus luteum could be identified by rectal palpation and milk progesterone concentrations were over 0.5 ng/ml. In the belief of Ullah et al. (2006) buffaloes could be described as acyclic at blood progesterone levels < 0.25 ng/ml and cyclic (suboestral) when blood progesterone was > 1 ng/ml, whereas Honparkhe et al. (2009) used 0.5 ng/ml as threshold value. Martin et al. (2010) recommends assaying blood progesterone during the post calving period as an indicator of initial luteal activity and for detection of impaired luteal function

There are single reports (Presicce et al., 2005; Yindee et al., 2011) about the combined use of rectal palpation, progesterone assay and transrectal ultrasonography for determination of puerperal ovarian function in buffaloes and its relationship with reproductive status. Presicce et al. (2005) established the first ovulation on postpartum days 25.5±6.9 and 15.5±1.3 in primiparous and multiparous Mediterranean Italian buffaloes, respectively. In 66% of ovulated buffaloes, the new corpus luteum was in the ovary contralateral to the one bearing the gravidic corpus luteum. Yindee et al. (2011) indicated an average period from parturition to first postpartum ovulation of 39.8 ± 3.38 days in swamp buffaloes with blood progesterone < 0.7 ng/ml.

Most of the cited studies were based upon the independent use of one of the three mentioned techniques and with emphasis on the diagnostics of postpartum anoestrus. The information about the puerperal corpus luteum regression and the onset of luteal activity in the different buffalo breeds is contradictory. No data are available for the time course of luteal activity of Bulgarian Murrah buffaloes and its relation with the reproductive performance of animals.

The aim of the present study was to investigate the model of luteal activity in Bulgarian Murrah buffaloes during the first 50 postpartum days and its effect on reproductive performance by blood progesterone analysis and ultrasonographic corpus luteum detection.

Material and methods. The study was conducted with 15 Bulgarian Murrah buffaloes, weighing 500–550 kg, aged 3–6 years, with body condition scores 3.5–4 by the scale of Edmonson et al. (1989). The animals were clinically healthy, given birth to single calf, with normal parturition, without signs of endometritis during the experimental period and submitted to the same antiparasitic and immunoprophylaxis treatments.

Calves were separated from dams immediately after the parturition and fed a milk replacer. Female and male animals were reared together in a private farm located at latitude of 42.183 N, longitude 25.567 E.

All buffaloes were fed uniformly. The daily ration included bioconcentrate, hay, straw, 7 hours controlled grazing and water ad libitum. The study was carried out from April to May.

Changes in serum progesterone concentration and ultrasound identification of corpus luteum different than corpus luteum graviditas were used to assess luteal activity. Blood samples were collected by jugular venipuncture at 3-day intervals between the 1st and 50th days postpartum, early in the morning, immediately after milking and before pasture. After collection, blood serum was separated from coagulated blood samples by centrifugation of 3000 g for 15 minutes and stored in a sterile tube at – 20^0C until analysis. Serum progesterone levels were measured by an enzyme immunoassay (EIA) using progesterone kit (Human, PROG ELISA, GmBh, Germany). The analytic sensitivity of progesterone ELISA test was 0.03-0.07 ng/ml (range of 0 - 40 ng/ml) with an intra- inter assay coefficient of variation < 10%.

The scanning of ovaries and the uterus was performed via transrectal ultrasonography by the same operator. An ultrasound Aloka SSD 500 Micrus (Aloka Co. Ltd, Tokyo, Japan) and a 5 MHz linear transducer were used; findings were documented on a thermal video printer Mitsubishi P91 E (Tokyo, Japan). According to owner's will to minimise stress, when a non-gestational corpus luteum was detected, ultrasound examination was no more performed and only blood samples were collected. Due to the fact that no pregnant animals were detected until postpartum day 50 regardless of the presence of herd bull, the number of calved buffaloes and the time of calving were recorded 11 months after the beginning of the experiment, while those having not given birth were examined ultrasonographically for pregnancy. Pregnancy status was used as a criterion for reproductive performance evaluation. On this base, two groups were formed: group I (n=5) consisting of non-pregnant buffaloes and group II (n=10) – pregnant animals. Mean blood progesterone concentrations were calculated for the different periods as well as the cumulative percentages of animals with newly formed corpus luteum for each group. The production technology did not allow exact record keeping of data related to the onset and duration of oestrus and time of mating, therefore they are not provided.

The statistical analysis of blood progesterone levels and ultrasound findings was performed by one-way analysis of variance (ANOVA) and a non-parametric analysis for comparison of proportions, using the Student's t-criterion using statistical software (StatSoft 1984-2000 Inc. Copyright © 1990-1995 Microsoft Corp.). Differences were considered significant at the $P<0.05$ level.

Results. The birth records and ultrasound checks performed 11 months after the beginning of the experiment showed that 2 buffaloes have calved, 7 were pregnant in the last stage of gestation, one has aborted about the 5th gestation month and 5 animals were not pregnant.

Immediately after the parturition, mean blood progesterone concentrations between the groups differed insignificantly (Table 1). They dropped sharply between postpartum days 1 and 4 and attained 0.09 ± 0.00 ng/ml and 0.16 ± 0.051 ng/ml in groups I and II, respectively. Low concentrations persisted until the 7th day, when the basal progesterone level was 0.12 ± 0.05 ng/ml.

Table. 1 **Mean values of progesterone in non-pregnant** (I group) **and pregnant** (II group) **buffaloes**

Postpartum days	Progesterone concentration (ng/ml)	
	I group (n= 5)	II group (n= 10)
	Mean ±SD	Mean ±SD
1	0,49±0,21	0,62±0,59
4	0,09±0,00	0,16±0,05
7	0,10±0,01	0,12±0,05
10	0,16±0,00	0,47±0,33*
13	0,11±0,08	0,31±0,25
16	0,15±0,01	0,17±0,22
19	0,06±0,01	0,80±0,43*
22	0,25±0,16	0,22±0,22
25	0,17±0,24	0,31±0,11
28	0,10±0,04	0,32±0,16*
31	0,06±0,08	0,38±0,28*
34	0,15±0,01	1,72±1,42**
37	0,12±0,07	0,65±0,60
40	0,19±0,16	0,93±1,19
43	0,13±0,03	0,64±0,27**
46	0,23±0,20	1,86±2,18*
50	0,15±0,07	4,20±3,62*

*Values within a column are significantly different at $P<0.05$

**Values within a column are significantly different at $P<0.01$

The average concentration on postpartum day 10 (0.47 ± 0.33 ng/ml) in the pregnant animals was significantly higher ($P<0.05$) compared to the non-pregnant animals (0.16 ± 0.00 ng/ml). Between days 13–16, blood progesterone was low in both groups, varying within 0.11 ± 0.08 ng/ml and 0.31 ± 0.25 ng/ml.

By the 19th day, a substantial increase ($P<0.05$) in blood progesterone occurred in buffaloes from the second group (0.80 ± 0.43 ng/ml). In the first group, the lowest mean progesterone concentration for the entire period was detected (0.06 ± 0.01 ng/ml).

Between postpartum days 22 to 25, the differences between progesterone levels of both groups were insignificant again with values not higher than 0.31 ± 0.11

ng/ml. After that time, buffaloes from the second group showed higher hormonal activity ($P<0.05$ and $P<0.01$) compared to group one. Average progesterone levels ranged within 0.10±0.04 ng/ml (postpartum day 28) and 0.15 ± 0.01 ng/ml (postpartum day 34) in group I, whereas on the same days, the respective concentrations in group II were 0.32 ± 0.16 ng/ml and 1.72 ± 1.42 ng/ml.

The differences in progesterone levels between days 37 and 40 were insignificant, despite the fact that the lowest concentration in pregnant buffaloes was 0.65 ± 0.60 ng/ml, and the highest one in non-pregnant buffaloes – 0.15 ± 0.01 ng/ml. In the last mentioned group, a slight increase in blood progesterone from 0.23 ± 0.20 ng/ml by day 46 was established. In group II, average progesterone levels increased sharply between postpartum days 43 and 50 and were considerably higher than those in group I, attaining a mean value 4.20 ± 3.62 ng/ml on day 50 after parturition.

By ultrasonography, a corpus luteum other than the gestational one was observed in one animal (10%) in the second group as early as the 13th postpartum day (Fig. 1). It appeared as an echogenic oval structure with the central cavity distinguished from the ovarian stroma (Fig. 2A). At the same time small and medium follicles were observed in the contralateral ovarium (Fig. 2B). Then the cumulative percentage of animals with corpus luteum increased from 30% by the 19th day to 60% by the 28th day. Substantial differences ($P<0.05$) were present on postpartum days 13 and 28.

The highest percentage (90%) was observed by the 34th day, statistically significantly different ($P<0.05$) from those in the previous time intervals.

In group I, the first corpus luteum was observed in 20% of buffaloes by the 40th day and in 60% by the 46th day. The differences between cumulative percentages in group II by the 34th day and group I by the 40th day were statistically significant ($P<0.01$).

The retrospective analysis of data from the final examination on postpartum month 11 demonstrated that the animals which have given birth were fertilised between the 28th and the 40th postpartum days, provided that the gestation period in Bulgarian Murrah buffaloes was 312-315 days.

Discussion. The postpartum cyclic activity depends on the synchronous activity of hypothalamic-pituitary-ovarian axis (Perea and Inskeep, 2008). The concentrations of placental and ovarian steroids during the late gestation and the puerperium have a significant effect on hypothalamic-pituitary system, which reflects on ovarian function (El-Wishy, 2007[1]).

Blood progesterone concentrations on the first postpartum day (0.49 ± 0.21 ng/ml and 0.62 ± 0.59 ng/ml) were identical in both groups of buffaloes. They were close to values reported by Bahga and Gangwar (1988) during the summer (0.62 ± 0.11 ng/ml), but different from spring values (1.41 ± 0.19 ng/ml). We assume that the discrepancy between blood hormone levels could be related to the breed, climatic conditions of the rearing location, or to differences in the level of nutrition as suggested by others (Wongsrikeao, 1990, Qureshi et al., 1999).

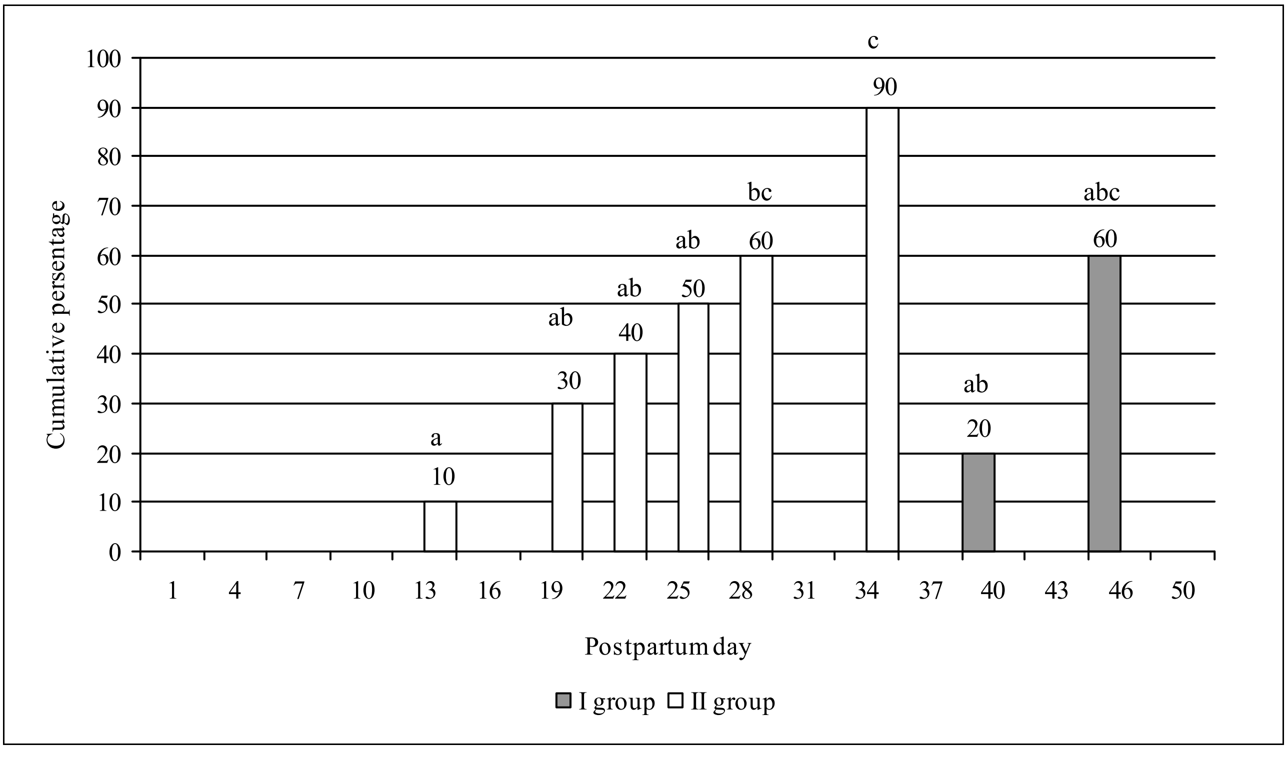

Fig 1. **Cumulative percentage of buffaloes with a corpus luteum that was registered at the first time**

Values bearing different superscripts differ significantly at $P<0.05$

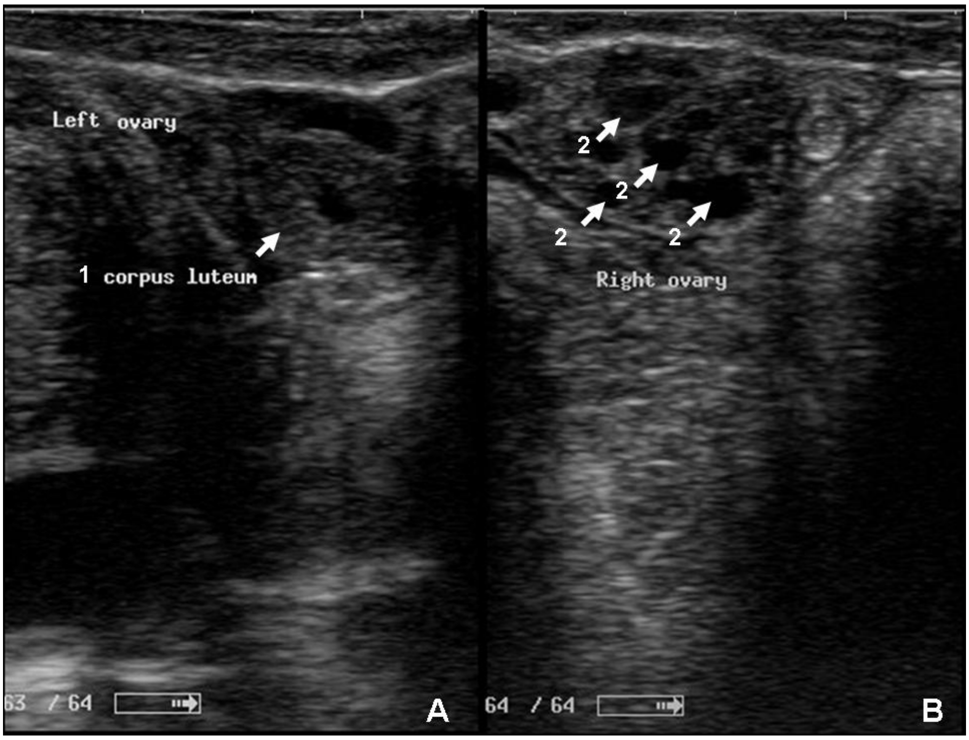

Fig 2. **Echographic picture of the ovaries in buffalo on day 13th postpartum**

A – Left ovarium; B – Right ovarium (1 – corpus luteum; 2 – follicles)

The rapid decline in progesterone from the 1st to the 7th postpartum day in both groups is corresponding to the results of Eissa et al. (1995) and supports data from earlier investigations (Jainudeen et al., 1983; Momongan et al., 1990) showing a rapid regression of the gestational corpus luteum during the first 7–14 days after calving. The attained basal progesterone concentration by the 7th postpartum day in group II indicated a complete regression of the gravidic corpus luteum, although luteal remnants were still visible by ultrasonography in the ovaries of some buffaloes. Comparable data about basal progesterone concentrations about the 7th postpartum day were also reported by Khasatiya et al. (2006). Statistically significantly different ($P<0.05$) blood progesterone concentrations and the cumulative percentages of buffaloes with corpus luteum after the first postpartum week showed differences in the puerperal luteal activity pattern in animals that were conceived latter, compared to non-pregnant ones, as well as its effect on reproductive performance. Blood progesterone concentrations < 0.25 ng/ml in group I between postpartum days 0 and 40 indicated absence of luteal activity and acyclicity (Bahga and Gangwar, 1988; Ullah et al., 2006).

The identification of a corpus luteum in three of animals within postpartum days 43–46 could be accepted as a criterion for occurring ovulation.

The low blood progesterone by the 50th day (0.15 ± 0.01 ng/ml) however indicated an inadequate functional activity of these corpora lutea and short life-span. Perera et al. (1987) also provided evidence for corpus luteum without increase in progesterone concentrations or high progesterone without palpable ovarian corpus luteum. Moreover the buffaloes from the above group were non-pregnant despite the bull presence in the herd. Usmani et al. (1990) reported that after a shortened oestrus cycle, the short luteal phase entailed a long anovulatory anoestrus. Probable reason to the low progesterone and lack of ovulation and pregnancy in the animals, respectively, could be the seasonal effects. In our latitude the period from May to August is defined as a hot season with meagre pasture. Similar influence of the agro-ecological zones on the hormonal levels in buffaloes was reported by Ali et al. (2009).

Until the 19th postpartum day, there were no buffaloes from group II with blood progesterone higher than 0.5 ng/ml, pointed out as ovulation indicator by Honparkhe et al. (2009), but as early as the 13th day, one of animals showed a corpus luteum. Although the first postpartum ovulation was reported to occur between the 37th and the 48th days (Usmani et al., 2001; Yindee et al., 2011) this finding indicated a rather early ovulation corresponding to the progesterone rise by the 10th postpartum day. Our statement is supported by the data of Presicce et al. (2005) about the occurrence of first ovulation by the 8th postpartum day in multiparous Mediterranean Italian buffaloes. The statistically significant difference ($P<0.05$) in blood progesterone between groups, the average value (0.80 ± 0.43 ng/ml) and the cumulative percentage of animals with corpus luteum between postpartum days 19 and 28 were signs of first postpartum ovulation and high luteal activity in group II. In experiments with Indian Murrah (Gokuldas et al., 2010) and swamp buffaloes (Wongsrikeo et al. 1990) the first ovulation was detected significantly later, respectively on postpartum days 56 ± 4

and 67 ± 7. The differences could be related to the breed and the season (El-Wishy, 2007[2]), but in our belief, the diagnostic technique also is important for the exact detection of the first postpartum ovulation.

The results obtained in the Bulgarian Murrah breed with respect to the interval between calving and first ovulation were similar to those reported by El-Shafie et al. (1983) – 28 ± 1 days, and by Presicce et al. (2005) – 25.5 ± 6.9 days. The decline in blood progesterone between postpartum days 13-16 and 22-25 could be attributed to the shortened luteal phase after the first ovulation, demonstrated in 26-86% of animals in different research works (Sharma and Kaker, 1990; Usmani et al., 2001; Yindee et al., 2011).

High blood progesterone (1.72 ± 1.42 ng/ml) by the 34th postpartum day together with the highest cumulative percentage of buffaloes with evidence of corpus luteum (90%) indicated that after that day, the animals with high luteal activity could exhibit oestrus with normal luteal phase duration. This is confirmed by the fact that progesterone levels persisted over 0.5 ng/ml until the 50th day, which, according to Jainudeen et al. (1983) and Honparkhe et al. (2009) indicates cyclicity. This luteal activity pattern is comparable to that reported by Bahga and Gangvar (1988) in Indian Murrah buffaloes calved during the summer. However, the mentioned authors did not establish blood progesterone concentrations over 0.97 ± 0.25 ng/ml until the 57th postpartum day.

Increased blood progesterone by the 50th day (4.20 ± 3.62 ng/ml) could be attributed to a corpus luteum in buffaloes ovulated for the second time. The primary reason however was the presence of pregnant animals in the group, conformed later by retrospective analysis of birth records. Earlier restoration of ovarian activity and the onset of oestrus in buffaloes reared with a bull or an early fertilisation are reported by other researchers (El-Shafie et al., 1983; Khattab et al., 1995; Gokuldas et al., 2010).

The analysis of results showed that the model of luteal activity in the early puerperium was closely related to the reproductive performance in Bulgarian Murrah buffaloes. The animals with basal progesterone concentrations by the 7th postpartum day exhibited a high luteal activity between days 19 and 34, characterized by ultrasonographic visualization of a non-gestational corpus luteum and blood progesterone over 0.5 ng/ml after day 34 postpartum. These animals showed successful ovulation followed by a luteal phase of normal duration and could be served by the heard bull. Buffaloes with blood progesterone concentrations < 0.25 ng/ml until the 50th postpartum day had an inadequate luteal activity between postpartum days 40-46 with short life-span of the corpus luteum and could remain non-pregnant for a long period postpartum if the animals entered on a non-breeding season. Zicarelli (2010) reported that ovarian activity stops if conception does not occur within 3 to 5 ovarian cycles.

In conclusion, the pattern of luteal activity in Bulgarian Murrah buffaloes during the first 50 postpartum days influenced their reproductive performance. The luteal activity evaluation by the progesterone level determination and ultrasound corpus luteum detection could be a criterion for selecting candidates for the different oestrus synchronization protocols. The present data could be successfully used in the development of methods for reduction of postpartum anoestrus and infertility in the buffaloes.

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