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Induction of ovarian activity in Bulgarian Murrah buffaloes by hormonal treatment in the early postpartum period

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ABSTRACT

Objective: To determine the possibilities for induction of ovarian activity in Bulgarian Murrah buffaloes by hormonal treatment in the early postpartum period. **Methods:** Twenty six clinically healthy animals, weighing 480–520 kg, 3–5 years of age, with normal parturition, without clinical signs of endometritis during the entire experimental period were divided into 2 groups: control (n=10) and experimental (n=16). Primiparous and multiparous buffaloes were proportionally allotted into both groups. By 5, 21, and 28 postpartum days, control animals were intramuscularly treated with 2 mL physiological saline, whereas experimental buffaloes received 500 mg PGF2α by the 5th postpartum day, 100 μg GnRH by the 21st postpartum day and a second prostaglandin dose 7 days later. All buffaloes were submitted to transrectal ultrasound examination on days 5, 21 and 28 after calving. Evaluation of ovarian activity was made on the basis of follicle type and detection of a newly formed corpus luteum. The percentage of animals with small, medium or dominant follicle and corpus luteum after either spontaneous or induced ovulation was determined. Insemination management included introduction of two proven fertile bulls into the herd at the start of the treatment. Pregnancy ultrasound checks were performed on postpartum days 58, 68, 120 and 150. The proportion of buffaloes with clinical signs of spontaneous or induced oestrus until the 35th postpartum day, pregnancy rates by the 35th and 90th postpartum days and service periods <60 days, 60–90 days and >90 days were registered. **Results:** On the 5th day after calving, small follicles were predominating in the ovaries of buffaloes from both groups. Similar findings were established on the 21st day in the control group, whereas the share of experimental buffaloes with small follicles was significantly (P<0.01) lower. By the 21st day, there were no medium follicles in untreated animals, while in hormonally treated ones they were found out in 25% of cases. The proportion of experimental buffaloes with dominant follicle (56.3%) by the 21st day was statistically significantly higher (P<0.01) compared to that of controls (10%). Cumulative percentage of animals with detected corpus luteum (66.8%) until the 28th postpartum day differed considerably (P<0.01) from that in controls (10.0%). There was a substantial between-group difference (P<0.05) between the total percentage of buffaloes with clinical signs of oestrus and pregnancy rates until the 35th day after calving. The share of buffaloes with service period <60 days was higher (P<0.05) compared to the untreated group. The analysis of results demonstrated that the PGF2α-GnRH-PGF2α treatment on 5, 21 and 28 postpartum days, was able to induce ovarian activity and oestrus in Bulgarian Murrah buffaloes. The total proportion of animals with signs of oestrus (both spontaneous and induced) and pregnancy rates by the 35th postpartum day were significantly higher (P<0.05) in the treated group compared to controls. **Conclusions:** The tested hormonal treatment protocol, combined with the presence of fertile bulls in the herd, could be successfully used for reduction of service period and calving interval in clinically healthy buffaloes.

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1. Introduction

The extended calving interval is the main reason for the low reproductive efficiency in buffaloes[1,2]. Numerous authors affirm that the first 90 days after calving are particularly important for optimization of reproductive process[3–5].

The high concentrations of placental and ovarian steroid during late gestational phase have a negative impact on hypothalamic–pituitary system and subsequently, on the recovery of ovarian activity [2,6,7]. The studies of Agrawal et al.[8] on ovaries obtained from slaughtered buffaloes on the day of calving showed the presence of a corpus luteum graviditatis 3.5 mm of size and insignificant follicular development. Similar findings are reported by Jainudeen et al.[9] which, using rectal examination, palpated a small rough structure prominating over the ovarian surface, <3 mm in diameter, by the 10th day. Usmani et al.[10] established a complete corpus luteum graviditatis regression within (21.8±1.1) days, whereas Momongan *et al*.[11] indicated an interval of (7±2) days, and Khasatiya *et al*.[4] (9.39±0.27) days. According to Georgiev et al.[12] the regression of corpus luteum graviditatis begins as early as the end of the pregnancy. Lohan et al.[13] reported that by the 6th day after calving, the average number of small and medium follicles was (8.10±5.67) and (1.00±0.00), respectively. All cycling animals between the 14th and the 20th postpartum day had a follicle (>8.5 mm) in diameter, that is interpreted as a parameter of ovarian activity resumption. In half of studied animals, spontaneous ovulation occurred after the 65th postpartum day. Having investigated the recovery of ovarian activity, Presicce et al.[3] demonstrated that the number of antral follicles ($\geqslant$2 mm) gradually decreased until the 60th day after calving. Follicles $\geqslant$3 mm of size increased during the first two postpartum weeks and were considerably more numerous in the ovary, contralateral to the corpus luteum graviditatis, and the balance was reinstated by the end of the first month.

The onset of the first ovulation and the first estrus in buffaloes after calving was extensively researched[14–18], but the subject is still not completely clarified. The pattern of restoration of cycling activity in buffaloes showed that during the formation of the corpus luteum after the first ovulation, 26%–86% of animals exhibited shortened luteal phase (6–13 days), occasionally accompanied by low progesterone levels[19,20]. According to Presicce *et al*.[3] 30% of primiparous and 10% of multiparous buffaloes remained anovulatory until the 60th day after calving.

The postpartum period in large ruminants is of primary importance for shortening of calving period[2,21]. Most investigations[4,16,18,19,22] on hormonally–induced ovarian activity in buffaloes have been conducted after the 30th postpartum day, and obtained results are often contradictory. The data regarding the hormonal induction of ovarian activity in buffaloes during the early postpartum period is limited.

The aim of the current study was to determine the possibilities for induction of ovarian activity in Bulgarian Murrah buffaloes by hormonal treatment during the first 30 days after calving.

2. Materials and methods

The trial was performed with 26 Bulgarian Murrah buffaloes, weighing 480–520 kg, at the age of 3–5 years. The average daily milk production was (5.6±0.6) kg with milking morning and evening. They were clinically healthy, with normal parturition, normally expelled placenta, without clinical signs of endometritis during the entire experimental period. Females were housed together with bulls over the entire period of the study and fed uniformly. The farm management included separation of calves from the dams immediately after the birth, feeding colostrum during the first 3 days, and a milk replacer thereafter. The daily ration comprised concentrate, rough feeds (straw, hay and 8–hour grazing on pasture); water was offered *ad libitum*. Buffaloes were reared in a farm located at latitude of 42.183 N, longitude 25.567 E. and experiment was conducted between April and August.

The buffaloes were divided into 2 groups: control (n=10) and experimental (n=16). Primiparous and multiparous buffaloes were proportionally allotted into both groups. By postpartum days 5, 21, and 28 control animals were intramuscularly treated with 2 mL physiological saline. Experimental buffaloes were hormonally treated with 500 mg PGF2α (2 mL PGF Veyx forte, Veyx–Pharma GmbH, Schwarzenborn, Germany) by the 5th postpartum day, 100 μg GnRH(Depherelin, Veyx–Pharma GmbH, Schwarzenborn, Germany) by the 21st postpartum day and a second prostaglandin dose 7 days after GnRH administration.

All buffaloes were submitted to transrectal ultrasound (SonoScape A5 Vet, SonoScape Co. LTD, Shenzhen, China) with multifrequency (7–12 MHz) linear transducer. Initial examinations were performed on days 5, 21 and 28 after calving.

The evaluation of ovarian status was based on the visualization of small (<6 mm diameter), medium (6–9 mm diameter) or dominant follicle (>10 mm diameter) with or without a corpus luteum into one of the both ovaries. The percentage of animals with the respective follicular structures during the different time intervals and with a corpus luteum after spontaneous, induced ovulation and cumulative values was determined.

The detection of estrous activity until the 35th postpartum day was performed by daily observation during the grazing period. By the beginning of the treatment, two proven fertile bulls were introduced into the groups to mount the cycling females. The percentage of animals exhibiting the standing estrus and mated by a bull was recorded. The percentage of animals with spontaneous oestrus, induced oestrus and the cumulative values were registered.

Pregnancy ultrasound checks were performed on postpartum days 58, 68, 120 and 150. Positive diagnosis was based on visualisation of enlarged uterine lumen filled with amniotic fluid and an embryo (foetus).

The pregnancy rates by the 35th and 90th postpartum days and the cumulative pregnancy rates were calculated after the last ultrasound examination. The percentage of buffaloes with service periods <60 days, 60–90 days and >90 days were registered in both groups.

Statistical analysis was performed with Stat–Soft 1984–2000 Inc. statistical software (Copyright©1990–1995 Microsoft. Corp.) by means of non–parametric analysis for comparison of two proportions, using Student's t–criterion. Differences were considered significant in P–values $<$ 0.05.

3. Results

The data for reproductive traits showed that by the 5^{th} day after calving, small follicles were predominant ovarian structures in both groups (Table 1). Almost the same finding (90% buffaloes with small follicles) was established by the 21^{st} postpartum day in control groups, whereas in the treated group, their number was substantially reduced (P<0.01) to 18.8%. The difference (P<0.05) was also present by the 28th postpartum day. Three buffaloes from the experimental group did not exhibit follicular activity. By the 21^{st} day, there were no medium follicles in control animals, but they were present in 25% of hormonally stimulated animals.

A similar trend was determined by the 28^{th} day, but the differences between groups was not significant (P>0.06).

A dominant follicle was observed in two untreated buffaloes (20%): one by the 21^{st} postpartum day and another one – by the 28^{th} day. In the experimental group, the number of animals with dominant follicle by the 21^{st} day (56.3%) was significantly higher (P<0.01) than that in controls (10%) and decreased to 31.3% by the 28^{th} day. Only one of untreated buffaloes had a corpus luteum after spontaneous ovulation by the 28^{th} day. In the experimental group, it was detected in 1 animal by the 21^{st} day, while the other 9 corpora lutea detected by the 28^{th} day were after GnRH-induced ovulation. Eight of ovulated animals had a follicle >10 mm on the day of gonadotropin administration, and in one buffaloes, the follicle diameter was 9 mm. The cumulative percentage of treated buffaloes with corpus luteum until the 28^{th} day (62.5%), was considerably higher (P<0.05) vs that in controls (10%).

Clinical signs of spontaneous oestrus within 28-35 postpartum days occurred in 20% of untreated animals. At the same time, 12.5% of experimental animals with medium-size follicles by the 21^{st} day exhibited estrous activity on the day of prostaglandin administration and were determined as buffaloes with spontaneous oestrus. Seven animals (43.8%) with corpus luteum and follicle (>9 mm) by the 28^{th} day were cycling 3 to 5 days after the prostaglandin treatment and were hence determined as subjects with hormonally induced oestrus. There was a significant difference (P<0.05) between the cumulative percentage of control and experimental animals with estrous activity until the 35^{th} day (20% and 56.3%, respectively).

The data from the first two ultrasound checks showed that until the 35^{th} day, 10% of control and 37.5% of buffaloes with PGF2$_{\alpha}$ induced oestrus have conceived (P<0.05). The ultrasound performed on postpartum days 120 and 150 did not exhibit significant between- group differences in pregnancy rates (20.0% and 18.8%) until the 90^{th} postpartum day. Nevertheless, the total relative share of pregnant buffaloes after hormonal treatment (43.8%), was higher than pregnancy rate of controls (30%).

The proportion of buffaloes with service period <60 days was higher in the experimental than in control group (P<0.05), whereas the number of animals from both groups with service periods of 60-90 days was similar. There was however a trend to reduction of the number of hormonally treated buffaloes with service period over 90 days.

Table 1

Ovarian structures and reproductive parameters in the control and experimental buffaloes during the postpartum period.

Parameters	Control group postpartum days (n=10)			Experimental group postpartum days (n=16)		
	5	21	28	5	21	28
Ovarian structures (%)						
Small follicles(< 6mm)	100.0 (10/10)	90.0 (9/10)^{a}	70.0 (7/10)^{c}	93.7 (15/16)	18.8 (3/16)^{b}	18.8 (3/16)^{d}
Medium follicles(6-9 mm)	0.0 (0/10)	0.0 (0/10)	20.0 (2/10)	6.3 (5/16)	25.0 (4/16)	50.0 (8/16)
Dominant follicle (> 10 mm)	0.0 (0/10)	10.0 (1/10)^{a}	10.0 (1/10)	0.0 (0/16)	53.6 (9/16)^{b}	31.3 (5/16)
Cumulative percentage of animals with corpus luteum until the 28^{th} day(%)		10.0 (1/10)^{c}			62.5 (10/16)^{b}	
Estrous activity until the 35^{th} day (%)						
Spontaneous		20.0 (2/10)			12.5 (2/16)	
Induced		-			43.8 (7/16)	
Cumulative		20.0 (2/10)^{c}			56.3 (9/16)^{d}	
Pregnancy rate (%)						
Until the 35th day		10.0 (1/10)^{c}			37.5 (6/16)^{d}	
Between 35th and 90th days		20.0 (2/10)			18.8 (3/16)	
Cumulative		30.0 (3/10)			56.3 (9/16)	
Service period (%)						
<60 days		10.0 (1/10)^{c}			37.5 (6/16)^{d}	
60-90 days		20.0 (2/10)			18.8 (3/16)	
>90 days		70.0 (7/10)			43.8 (7/16)	

Different superscripts indicate significant difference between control and experimental values form the same period, a,b P<0.01; c,d P<0.05.

4. Discussion

The presence of small follicles into the ovaries in most of buffaloes from both groups by the 5th day after calving, indicate lack of ovarian activity. These findings are comparable to the results of other researchers[8,9,13] and support the thesis for postpartum suppression of ovarian activity by high progesterone levels during the pregnancy.

The significantly higher number of experimental buffaloes with dominant follicle by the 21st postpartum day affirms enhance ovarian activity in treated animals. It could be attributed to lysis of the corpus luteum of pregnancy after the prostaglandin administration and lower progesterone concentrations. According to Zerbe *et al*.[23], the rapid drop of progesterone concentrations results a GnRH release, increase of LH and FSH pulsations and resumption of ovarian activity. Iqbal *et al*.[24] demonstrated that the application of PGF2α two hours after calving led to increase uterine involution, earlier resumption of follicular activity, expression of estrous activity and shorter calving interval. The data from the ultrasound examination of control buffaloes by the 28th day support this statement.

The presence of a dominant follicle in one animal only in control group, and especially the considerably higher percentage of buffaloes with small follicles (P<0.05) indicates a low level of ovarian activity. The inadequate LH pulses during that period could be a cause[25,26], for the decreased release of estrogens by smaller follicles[27], respectively inadequate hypothalamic stimulation. We assume that the inadequate ovarian activity is resulting from the maintenance of sufficiently high blood progesterone concentrations in untreated buffaloes over a prolonged time interval, but a categorical conclusion could not be made without blood progesterone assay. The absence of substantially increased number of animals with medium and dominant follicles in this group by the 28th day is also in support of the hypothesis. Palta and Madan[28] also report a strongly reduced pituitary sensitivity to gonadotropin-releasing hormone during the pregnancy and about its gradual restoration from the 2nd to the 35th day after calving. The lack of ovarian response in three of treated buffaloes could be explained by an inadequate response of the pituitary gland to gonadotropin stimulus in the early postpartum period[29], seasonal effect[30] or individual sensitivity of some animals to hormonal therapy[31].

The presence of a newly formed corpus luteum is indicating a spontaneous ovulation [13,32]. Its presence by the 21st postpartum day in the experimental and by the 28th day in control group is a sign of spontaneous ovulation of follicles occurring at an earlier stage. A similar phenomenon has been established by Presicce *et al*.[3] between the 15th and the 25th day, but in general, the newly formed luteal structure has a very short lifespan [19,20]. The presence of corpora lutea by the 28th days in the treated group showed a GnRH-induced ovulation of follicles with diameter > 9 mm. The results support the findings of Gimenes *et al*.[33] for ovulation of Murrah buffalo heifers with follicles from 8.5 to 10 mm induced by exogenous LH administration. Campanile et al.[34] have utilized GnRH agonists or human chorionic gonadotropin (hCG) in multiparous Italian Mediterranean buffalo cows and ovulation of follicles between 4.2 mm and 13.0 mm of size. On the other hand, the lack of corpus luteum in two of animals with follicles >8 mm and a third one with dominant follicle after the GnRH treatment indicated that successful ovulation is dependent not only on follicle size. One of possible reasons could be the inadequate production of gonadotropin hormone receptors, as shown by Sartori *et al*.[35] in investigations with dairy cows. Campanile *et al*.[36] supposed that the inadequate production of estradiol by follicle could result in the lack of preovulatory LH peak. In our opinion, these follicles have not ovulated or were not luteinized after the GnRH application[37,38], but continued their development until the preovulatory follicular stage. The hypothesis for the lack of sufficient number of receptors, LH surge and subsequent ovulation, was supported by the spontaneous estrus expression in 12.5% of buffaloes on the day of prostaglandin treatment. Probably, these cycles were anovulatory because the animals were not pregnant during the subsequent ultrasound examinations. The considerably higher (P<0.05) cumulative percentage of treated buffaloes with corpus luteum until the 28th day (62.5%), that in controls (10%) also state to benefits of PG-GnRH treatment on days postpartum 5 and 21, respectively.

The clinical estrus expression in 43.8% of buffaloes with corpus luteum until the 35th day shows a successful oestrus induction after PGF2α application. These results differ substantially from reported data about 100% expression of oestrus after treatment with 3 mL GnRH analogue by the 30th postpartum day, 2.5 mL PGF2α seven days latter[5]. Applying a GnRH-PG protocol in the same postpartum period, Usmani *et al*.[19] detected estrus only in 27.8% of treated buffaloes. The causes for discrepancies could be due to the time of treatment, the type and dose of used hormone or the methods for estrus detection and insemination. The small proportion of animals with estrous activity from the 35th to the 90th postpartum days in both groups despite the presence of the bull indicated a strong seasonal influence on the resumption of ovarian activity. Seasonal effects on ovarian functions are extensively studied and reviewed by El-Wishy[2] and Perera[39]. The lack of oestrus behaviour in experimental buffaloes with detected corpus luteum by the 28th day during the next 7 days could be attributed both to seasonal influence and insufficient functional activity of the corpus luteum, responsible for its inadequate sensitivity to prostaglandin injection. According to Cohan *et al*.[30] the efficacy of PGF2α treatment was considerably reduced out of the breeding season, and buffaloes with smaller corpus luteum and low blood progesterone concentrations exhibit a less reproductive response[40]. Regardless of that, the used hormonal treatment protocol resulted in cumulative estrous activity in significantly more buffaloes (56.3%) as compared to untreated animals (20%).

The results from the first two ultrasound pregnancy checks in the control group showed that the animals could conceive after the first spontaneous ovulation in normal uterine involution. This is in agreement with our previous findings in Bulgarian Murrah buffaloes, demonstrating complete uterine involution by the 34th day after calving [41] and spontaneous ovulation between days 19 and 34 (Yotov *et al*., unpublished data). Statistically differences (P<0.05) between pregnancy rates of control and treated buffaloes until the 35th day, showed that the hormonal treatment during the early postpartum period could induce ovulatory oestrus with subsequent fertilization. The cumulative percentage of pregnant animals until the 90th day corresponded to rate of 53.7% reported by Usmani *et al*.[19], but is in disagreement with the data of other researchers[5]. Unlike us, the last authors has studied swamp buffaloes reared under different climatic conditions, using higher doses of GnRH and PGF2α analogues and beginning of the treatment by the 30th postpartum day. According to Ziccarelli *et al*.[42], a large part (30%) of non-pregnant animals after prostaglandin-induced cyclic activity during the transition season, remain acyclic and are difficult to be fertilized. Irrespective of that, the results suggested a positive trend to more pregnancies after hormonal stimulation of ovarian function during the first 30 postpartum days. An additional argument in support of the benefits of such a treatment is the significantly higher proportion of buffaloes with service period <60 days in the experimental group and the relatively higher parts of animals with service period >90 days in the control group. The positive effect of hormonal treatment during in the latter period (between day 30th and 60th postpartum) for reduction of service period and calving interval is demonstrated in numerous investigations[4,18,24].

The analysis of results demonstrated that the PGF2α-GnRH-PGF2α treatment on postpartum days 5, 21 and 28, respectively, was able to induce ovarian activity and oestrus in Bulgarian Murrah buffaloes. The cumulative percentage of animals with estrous activity and pregnancy rates by the 35th postpartum day were significantly higher in the treated group compared to controls. The used hormonal treatment protocol, combined with the presence of fertile bulls in the herd, could be successfully used for reduction of service period and calving interval in clinically healthy buffaloes.

Confict of interest statement

We declare that we have no conflict of interest

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