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Original Contribution

CONCEPTION RATE IN BULGARIAN MURRAH BUFFALOES AFTER A PRESYNCH/OVSYNCH PROTOCOL APPLIED IN NON-BREEDING AND BREEDING SEASON

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ABSTRACT

The aim of the presented study was to establish the conception rate in Bulgarian Murrah buffaloes submitted to estrus synchronization via Presynch/Ovsynch protocol applied in non-breeding and breeding season. The experiment was conducted in 24 buffaloes with at least 40 days postpartum, allotted into two groups according to the treatment season: Group I (n=8) – non-breeding season, Group II (n=16) – breeding season. On day 0 and day 35 after artificial insemination, the buffaloes were examined by transrectal ultrasound to identify ovarian structures and prove pregnancy, respectively. The initial ultrasound examination revealed the presence of a corpus luteum and a follicle with a diameter ≥10 mm in 33.33% (8/24) of all cases, solely a follicle ≥10 mm in 45.84% (11/24) and follicles with a diameter <10 mm in 20.83 % (5/24). The total conception rates between Group I (37.50%, 3/8) and Group II (43.75%, 7/16) did not differ significantly (P = 0.39), and the overall conception rate after estrus synchronization (41.67%, 10/24) was within the normal range for the species. The applied protocol leads to successful conceptions of buffaloes in both seasons. The high percentage of conception is registered in the hormonal treatment of buffaloes with follicle > 10 mm.

Key words: Buffaloes, Presynch, Ovsynch, conception rate

INTRODUCTION

Water buffaloes (*Bubalus bubalis*) are polycyclic animals that are prone to seasonality in their reproductive functionality in dependence on the daylight duration (1).

As in the other livestock, herd replacement management in buffalo husbandry is based on successful reproduction. Furthermore, the profitability of a buffalo farm is essentially dependent on the age of first calving and calving interval, rather than on milk yield, as their economic weights indicate (2). The breeding efficiency of buffaloes is affected by late maturity, seasonality of calving, prolonged anestrus after calving (especially out of season), weak signs of estrus (silent estrus), low conception rates, early embryonic mortality, and long calving interval (3). In this respect, different protocols for estrus synchronization have been developed to make a great portion of a group of animals come in heat at a fixed moment. The efficiency of protocols for estrus and ovulation synchronization increases the percentage of pregnant animals (4, 5).

Combined administration of gonadotropins and prostaglandins during the breeding season or out of season has the purpose to optimize the effect of the applied protocols for synchronization of estrus and ovulation (6, 7). On this basis different protocols have been developed, affording fixed-time artificial insemination of buffaloes (8-10).

The objective of the presented research work was to establish the conception (pregnancy) rate in

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Bulgarian Murrah buffalo cows are subjected to a Presynch/Ovsynch-based protocol for estrus synchronization during the breeding season and out of the season.

MATERIALS AND METHODS

Animals and housing technology

The experiment was carried out during March-November, 2019. The buffaloes were bred on the farm of Agricultural Institute – Shumen (North-East of Bulgaria, latitude: 43.28N, longitude: 26.93E). The farming system is intensive – tie stalls with exercise yard.

The experiment was conducted in 24 pluriparous Bulgarian Murrah buffaloes at least 40 days postpartum. The buffaloes' live weight was from 550 to 650 kg, and the normal lactation milk yield – from 1,800 to 2,100 kg at two-time daily milking basis.

Experimental design

The animals were allotted into two groups corresponding to the season of planned synchronization: Group I (n=8) – non-breeding season, Group II (n=16) – in the typical breeding season. The applied hormonal treatment was as follow: Day 0 – 500 UI Synchrostim (Equine Serum Gonadotropin, Ceva Sante Animale, France) i.m. + 25 mg Enzaprost (Dinoprost Trometamol, Ceva Sante Animale, France) i.m., Day 3 and Day 10 – 100 μg Ovarelin (Gonadorelin, Ceva Sante Animale, France) i.m., Day 17 – 25 mg Enzaprost (Dinoprost Trometamol, Ceva Sante Animale, France) i.m., Day 19 – 100 μg Ovarelin (Gonadorelin, Ceva Sante Animale, France) i.m. and artificial insemination 16 hours after the last GnRH treatment **(Figure 1).** In Group I the protocol was applied in March, and for Group II – in November. All treatments were performed at 8.00 am on the respective day. Artificial insemination was applied by one and the same technician, using cryopreserved semen from tested buffalo bulls.

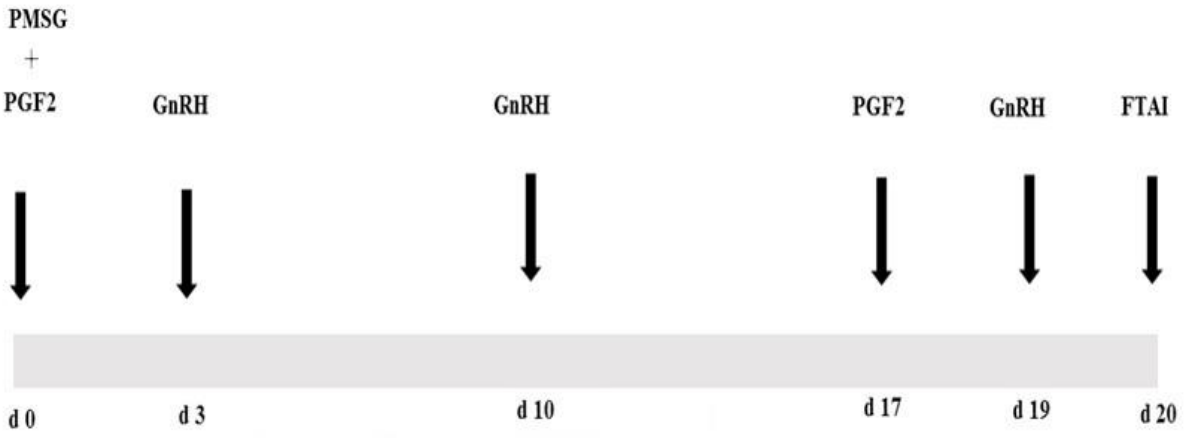

Figure 1. Treatment protocol used in the study.

Ultrasound examination

The ultrasound examination was performed with a SonoScape S2 Vet (SonoScape Co. LTD, Shenzhen, China), a multi-frequency (7-12 MHz) linear probe and a transrectal approach. On Day 0, the buffaloes were examined by transrectal ultrasound to determine the status of ovarian structures, as well as 35 days after artificial insemination, as a fixed time for early pregnancy diagnosis.

The criteria for early pregnancy were visualization of an enlarged uterine lumen, anechoic amniotic fluid and an echogenic embryo.

Statistical analysis

The records from the examinations and observations for each group and for the overall set of animals were processed under the computer statistical program Statistica 7 and compared using a non-parametric method for comparison of proportions. The differences were considered significant at $P<0.05$.

RESULTS

At the preliminary ultrasound exam of the animals in Group I on Day 0, corpus luteum (CL) together with a follicle of ≥10 mm in diameter was established in 37.5% (3/8), solely a follicle (F) of ≥10 mm – in 50.0% (4/8), and a follicle of <10 mm – in 12.5% (1/8). The ultrasonography in Group II revealed corpus luteum with a follicle of ≥10 mm in 31.25% (5/16), solely a follicle of ≥10 mm in 43.75% (7/16), and a follicle of ≤10 mm in 25.0% (4/16) of the buffaloes **(Table 1).** It is noteworthy that in our study the proportion of animals with follicle size ≥10 mm (with or without CL) is relatively high – 87.50% out of season and 75.0% in the breeding season. The percentage of non-cycling buffaloes during the non-breeding season is 62.5% (5/8) and in the typical breeding season – 68.75 (11/16).

Table 1. *Proportion of ovarian structures in buffaloes on Day 0 of Presynch/Ovsynch treatment and pregnancy rates 35 days post fixed-time AI*

Ultrasound findings in ovaries	Day 0		Pregnancies after FTAI	
	n	% of total n	n	% of total n
Group I – non-breeding-season				
CL + follicle ≥10 mm	3	37.50	1	12.50
Solely follicle ≥10 mm	4	50.00	2	25.00
Follicle <10 mm	1	12.50	0	0.00
Total	8	100.00	3	37.50
Group II – breeding season				
CL + follicle ≥10 mm	5	31.25	2	12.50
Solely follicle ≥10 mm	7	43.75	2	12.50
Follicle <10 mm	4	25.00	3	18.75
Total	16	100.00	7	43.75
Overall				
CL + follicle ≥10 mm	8	33.33	3	12.50
Solely follicle ≥10 mm	11	45.84	4	16.67
Follicle <10 mm	5	20.83	3	12.50
Total	24	100.00	10	41.67

* The difference between Group I and Group II was not statistically significant (P = 0.39)

On day 35 after AI, in Group I pregnancy was diagnosed in 12.5% (1/8) of the cases with CL and F > 10 mm, in 50.00% (4/8) of those with solely F > 10 mm, and in 0% (0/8) of the F < 10 mm cases; and in Group II respectively in 12.5% (2/16) of CL and F > 10 mm or a solely F > 10 mm and in 18.75% (3/16) of F< 10 mm.)

The achieved conception rates in the overall number of buffaloes as a result of the applied Presynch/Ovsynch protocol and fixed-time artificial insemination in the different categories of ovarian structures are similar – 16.67% (4/24) in the cases of solely a follicle of ≥10 mm and 12.5% (3/24) in those with corpus luteum and a follicle of ≥10 mm and in those with a follicle of ≤10 mm.

The total pregnancy rate for Group I was established to be 37.5% (3/8), for Group II – 43.5% (7/16), and the overall pregnancy rate for all animals in the experiment was 41.7% (10/24) **(Table 1).** The difference between Group I and Group II was not substantial and not statistically significant (P = 0.39).

DISCUSSION

The application of estrus synchronization protocols without previously identifying the formations present in the ovaries, the results in terms of conception rate usually vary in a wide range from 0% to 50% for non-cycling animals (8, 11). For the achievement of good results after using of synchronization protocols, there are a number of important factors, such as the size of the follicle during GnRH treatment, synchronization of the new follicle wave, hormone levels, and duration of follicular dominance (12). The presence of a follicle in diameter > 9 or 10 mm during GnRH treatment is

a prerequisite for the success of the synchronization scheme in ancestral dairy buffaloes (11).

Sharma *et al.* (13) developed a protocol named Ovsynch-Plus involving injection of eCG (500 IU) three days before starting Ovsynch. The administration of eCG increases the size of the dominant follicle and synchronizes the follicular development in the ovaries of all animals at the time of the initial GnRH treatment. The authors report 30% conception rates in non-cycling buffaloes.

In our study, the buffaloes without detected corpus luteum were 66.67% (16/24), but after using the current protocol was obtained conception rate of 43.50% (7/16). Neglia *et al.* (14) reported a conception rate of 36% - 57.0% in buffaloes treated with different protocols for estrus synchronization. After the introduction of Ovsynch protocol with a fixed time of artificial insemination, Rabida and Gofur (15) recorded 56,70% conception rate. Studies by Berber *et al.* (16) in crossbreds (Murrah x Medetterian buffaloes) and Bartolomeu *et al.* (17) in Brazilian buffaloes registered a conception rate in programmed artificial insemination 55.6% and 64.2%, respectively. These results are better than obtained one in our experiment. The reasons for different conception rate can be attributed to the differences in breed of animals, management and environment.

The conception rate registered during the non-breeding season 37.5% and 43.75% during the breeding season after application of the Presynch/Ovsynch protocol to buffaloes of the Bulgarian Murrah breed are close to the reported 41.7% and 45% by Souza *et al.* (18) and Oropeza *et al.* (19) using presynchronization protocols and higher than the result (30.7%) reported by Cordoba and Fricke (20). The season also has an influence on the conception rate. According to Baruselli *et al.* (21) and Warriach *et al.* (10), the reproductive performance of buffaloes during the breeding season (autumn and winter) is better compared to non-breeding season. Serena (22) obtained higher conception rate in autumn than in spring after using of Ovsynch protocol and programmed artificial insemination.

In this study high percentage of conception was registered in the hormonal treatment of buffaloes with follicle > 10 mm observed on Day 0. Estrus synchronization protocols in large ruminant allow the use of artificial insemination in fixed time with no estrus detection. For the achievement of a positive result importance has the follicular size during the first treatment and the synchronization of the ovulation from the second treatment with GnRH (23-26).

The results of the experiment show that the application of a protocol based on gonadotropins and prostaglandins in Bulgarian Murrah buffalo cows can be used to manage the breeding season, in order to make the production of milk more regular throughout the year, but mostly in order to overcome the longer anestral period during the non-breeding season, and hence to improve the genetic progress and profitability of buffalo farming.

CONCLUSION

The applied Presynch/Ovsynch protocol in the Bulgarian Murrah buffaloes leads to synchronized estrus and successful conceptions in non-breeding and breeding season. High percentage of conception is registered in hormonal treatment of buffaloes with follicle > 10 mm.

Conflict of Interest

The authors have no conflicts of interest to declare.

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