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# Effect of Ovarian Structures upon the Clinical Signs of Estrus and Conception Rates in Bulgarian Murrah Buffaloes after Synchronization of Estrus and Ovulation

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## ABSTRACT
The purpose of the present study was to investigate the clinical signs of estrus and conception rates after synchronization of ovulation in Bulgarian Murrah buffaloes depending on ovarian structures. Sixty buffaloes age between 4-8 years, weighing 550-650 kg with body condition score 3.5 were included in the study. Depending on ovarian structures, two experimental groups were formed. All animals had their estrus synchronized according to the GnRH-$PGF_{2\alpha}$-GnRH protocol. The ease of cervical passage, uterine tone and presence of cervical mucus were determined. The conception rates and the signs of spontaneous oestrus in non-fertilized animals were also registered. An increased uterine tone, good ease of cervical passage and cervical mucus were observed in 100, 77.4 and 35.5% of buffaloes from group I, respectively and in 100, 62.1 and 41.4% of animals from group II. Spontaneous estrus in non-fertilized animals until the 20th day after the artificial insemination was detected in 52.2 and 25% of animals from group I and II, respectively ($p<0.05$). The ultrasound identification of ovarian structures in buffaloes could be recommended in decision making when selecting candidates for synchronization schedules. The presence of cervical mucus at the time of artificial insemination indicated a better conception rate. The application of schedules for estrus and ovulation synchronization with fixed-time insemination could be successfully used in Bulgarian Murrah buffaloes.

**Key words:** Buffaloes, estrus induction, conception rate, cervical mucus

## INTRODUCTION
The reproductive function is a major factor determining the economic importance of buffalo cows (Barile, 2005). The reproductive status is mainly influenced by the age of sexual maturity onset (Jainudeen and Hafez, 2000; Mondal and Prakash, 2004), the prolonged interval between calvings and the weak ovarian activity during the hot months of the year (Singh *et al.*, 2000; Bughio *et al.*, 2000; De Rensis and Lopez-Gatius, 2007; Dimitrov *et al.*, 2009). Improvement of reproductive ability is possible with reduction in the age of first calving by use of artificial insemination and other advanced reproductive technologies (Ali *et al.*, 1999; Sejian *et al.*, 2010).

Artificial insemination is with limited application in female buffaloes (Barile, 2005). This is determined by the difficult detection of animals in estrus due to the lack or weak oestrus behavioural signs in most cases. The different duration of estrus (from 4-64 h) also impedes the exact determination of the proper time for insemination (Neglia *et al.*, 2003).

The utilisation of schedules for estrus and ovulation synchronization could partly eliminate the aforementioned problems and contribute to the broader use of artificial insemination for improving reproductive and production traits in this animal species (Presicce *et al.*, 2005; Baruselli *et al.*, 2007). Pursley *et al.* (1995) were the first to use a protocol for synchronization of the oestrus and the ovulation in cows with fixed-time insemination. Then the model was adopted and tested in buffaloes during different seasons (Berber *et al.*, 2002; Baruselli *et al.*, 2003; Neglia *et al.*, 2003; De Rensis *et al.*, 2005; Paul and Prakash, 2005; Carvalho *et al.*, 2007; Warriach *et al.*, 2008).

The application of schedules for synchronization of the estrus and the ovulation with fixed-time insemination in Bulgarian Murrah buffaloes is not yet fully explored. The purpose of the present study was therefore, to investigate the opportunities for induction of oestrus, clinical signs and conception rates after synchronization of ovulation with fixed-time insemination in this buffalo breed depending on ovarian structures.

## MATERIAL AND METHODS

**Animals:** The experiment was conducted with 60 Bulgarian Murrah buffaloes reared in a buffalo farm in central North Bulgaria (geographic coordinates 42.95 N, 25.13 I). The study was performed between July 2010 and January 2011.

The buffaloes were 4-8 years of age, live body weight between 550 and 650 kg and body condition score of 3-4 on a five-point scale 1 = emaciated, 5 = fat (Edmonson *et al.*, 1989). All of them were lactating and were reared in the same housing system.

An initial transrectal ultrasonography of the uterus and ovaries was carried out with ultrasound Aloka SSD 500 Micrus (Aloka Co. Ltd, Tokyo, Japan), supplied with a 5 MHz linear probe. The findings were documented on a thermal video printer Mitsubishi P91 E (Tokyo, Japan). Two experimental groups were formed depending on ovarian structures: group I (n = 31) with follicles >5 mm in diameter and a corpus luteum (Fig. 1) and group II (n = 29) with follicles >8 mm in diameter without a corpus luteum (Fig. 2).

**Treatment:** The synchronization of estrus and ovulation was performed by intramuscular administration of 10 μg GnRH analogue (Buserelin acetate, Receptal®, Intervet/Schering-Plough Animal Health) on days 0 and 9 and intramuscular injection of 500 μg $PGF_{2\alpha}$ analogue (Ñloprostenol Sodium, Estrumate®, Intervet/Schering-Plough Animal Health) on the 7th day after the first GnRH dose.

All buffaloes were inseminated artificially on the 16 and 25th h after the second GnRH injection. To this end, plastic straws with frozen semen obtained from a certified buffalo bull were used.

The clinical signs of estrus-ease of cervical passage, uterine tone and presence of cervical mucous (Stevenson *et al.*, 1983; Loeffler *et al.*, 1999) were detected. The reproductive traits conception rate and spontaneous oestrus manifestation in non-fertilized animals were recorded.

On the 40th day after the fixed-time insemination, ultrasonography was performed to detect pregnancy. The tentative diagnosis of pregnancy was based on visualization of enlarged uterine lumen filled with amniotic fluid and presence of an embryo.

Data were statistically processed by StatSoft (Microsoft corp. Ink) software. Results were considered statistically significant at $p<0.05$.


Fig. 1: Ovary with follicle 6 mm and corpus luteum

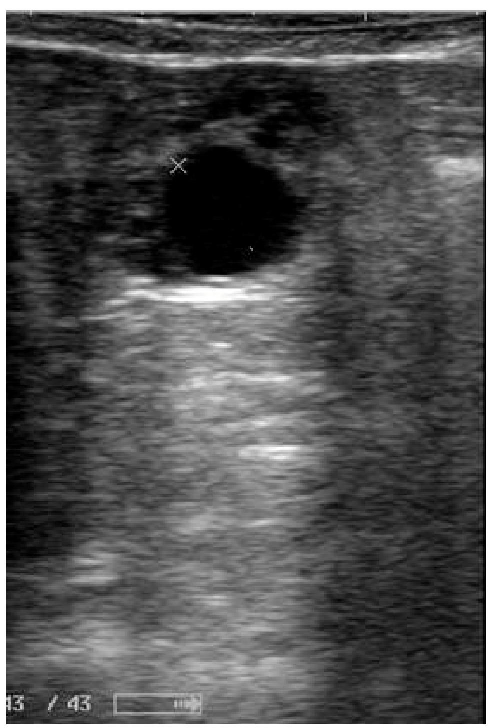

Fig. 2: Ovary with follicle 11 mm without corpus luteum

## RESULTS AND DISCUSSION

The results are presented in Table 1. At the day of artificial insemination, increased uterine tone was detected in 100% of animals in both groups. Easy cervical passage was present in 77.4% of buffaloes from group I and 62% in group II, the average percentage for all animals was 70%.

Presence of cervical mucus was observed in 35.5% of animals with corpus luteum and in 41.4% of cases where it was absent. For both experimental groups, oestral secretion was observed in 38.3% of buffaloes. The presence of cervical mucus always correlated to easy cervical passage.

The relative share of fertilized animals in the second experimental group (44.8%) was considerably higher ($p<0.05$) than that of group I (25.8%). The total conception rate was 35%. The simultaneous presence of easy cervical passage and cervical mucus in fertilized animals was 75% for group I, 61.5% for group II and 66.7% for all animals. Independent occurrence of either ease of cervical passage or uterine discharge was registered in 19 and 4.8% of cases, whereas 9.5% of fertilized buffaloes exhibited neither oestrus sign.

Spontaneous oestrus in non-fertilized buffaloes until the 20th day after the artificial insemination was manifested in 52.2% of animals from group I and 25% from group II ($p<0.05$).

Estrus synchronization in buffaloes is a powerful tool for optimisation of reproduction in this species (Berber *et al*., 2002; Baruselli *et al*., 2003). The treatment is aimed at attaining synchronous ovulation in a group of animals within a short time after the second GnRH injection. It allows for fixed-time artificial insemination and elimination of the need for identification of receptive buffaloes (Barile, 2005).

Uterine muscle tone increases during the periovulatory period and gradually decreases after ovulation (Bonafos *et al*., 1995). It is directly influenced by estrogens released by preovulatory follicles in ovaries (Rodriguez-Martinez *et al*., 1987).

The observed raised uterine tone in all experimental animals (100%) indicates the presence of oestrogen-producing tertiary follicles. This finding is consistent with data by Neglia *et al*. (2003), reporting rigid uterus in 88% of synchronised buffaloes with tertiary follicle present in one ovary. Bostedt *et al*. (1976) and Gordon (1996) affirm that the presence of increased uterine tone in cows detected by rectal palpation during spontaneous oestrus, is a prerequisite for successful fertilization.

On the contrary, Berber *et al*. (2002) did not show a relationship between this parameter and conception rates in buffaloes. We suggest that uterine rigidity could indicate oestrus but could not be a predictor of fertility.

Table 1: Clinical signs of oestrus at the time of insemination and conception rates in Bulgarian Murrah buffaloes after application of the estrus and ovulation synchronization scheme

| | | Clinical signs of oestrus % (n) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Good uterine tone | | Ease of cervical passage | | Uterine discharges | | Conception rates | | Signs of spontaneous oestrus after the last insemination | |
| No. of animals | Ovarian strnctures | No. | % | No. | % | No. | % | No. | % | No. | % |
| Group I (n = 31) | Follicles>5 mm in diameter with corpus luteum | 31/31 | 100 | 24/31 | 77.4 | 11/31 | 35.5 | 8/31 | 25.8[a] | 12/23 | 52.2[a] |
| Group II (n = 29) | Follicles>8 mm in diameter without corpus luteum | 29/29 | 100 | 18/29 | 62.1 | 12/29 | 41.4 | 13/29 | 44.8[b] | 4/16 | 25[b] |
| Total (n = 60) | | 60/60 | 100 | 42/60 | 70.0 | 23/60 | 38.3 | 21/60 | 35.0 | 16/60 | 41 |

Values within a column with different letters designate statistically significant difference at $p<0.05$

Clinical signs of oestrus and presence of cervical mucus are reported very rarely in buffaloes in spontaneous (Jainudeen and Hafez, 2000) or synchronised oestrus (Neglia *et al*., 2003). In our study, they were registered in 38.3% of all experimental animals. A similar relationship was reported by Berber *et al.* (2002) in 31.5% of buffaloes submitted to ovulation synchronisation protocol. The insufficient percentage of animals with clinical oestrus signs could be attributed to the fact that the time needed for follicles to reach the ovulation stage when the oestrus is induced is substantially shorter than that in spontaneous oestrus. In such instances, the uterus exposure to oestrogens is shorter, the amount of secretion is significantly lower and its consistency-thicker (Tsiligianni *et al*., 2003). Unlike, Loeffler *et al.* (1999), we believe that uterine discharges at the time of artificial insemination are an indicator for full oestrus and better conception rates. This is supported by the relatively high number of pregnant animals (71.4%) with uterine discharges at the time of insemination 16 h after the second GnRH application. A similar relationship is reported by Stevenson *et al.* (1983) in cows.

The insignificant differences between groups with regard to the ease of cervical passage indicated that this parameter was not relevant to conception after artificial insemination as also reported by Stevenson *et al.* (1983).

The total conception rate obtained in this study-35%, was similar to that, reported by Neglia *et al.* (2003) and Paul and Prakash (2005), 36 and 33.3%, respectively.

Warriach *et al.* (2008) also outlined a similar trend, accounting for the effect of the season and established a conception rate of 36.3% during the oestral and 30.4% during the anoestral season. In contrast to our findings, Berber *et al.* (2002), Baruselli *et al.* (2003) and Carvalho *et al.* (2007) reported considerably higher conception rates-56.5, 48.8 and 46.8%, respectively.

The difference in conception rates of the both groups, 25.8% for group I and 44.8% for group II, deserves attention. The echography of ovaries has shown that the majority of animals in group II were in the beginning of the reproduction season, determined by environmental factors and by the photoperiod in particular (Zicarelli, 1997).

Most probably, synchronous ovulation has occurred in most animals after the first GnRH injection. This is essential for the start of a new follicular wave, formation of $PGF_{2\alpha}$ sensitive corpus luteum and presence of preovulatory follicle at the time of the second GnRH dose (Baruselli *et al.*, 2003; De Rensis *et al*., 2005). A similar development of follicles was not observed when the treatment occurred between post ovulation days 2-5 of the preceding oestral cycle. At that time, a corpus luteum is developing in ovaries but a dominant follicle is absent (Baruselli *et al*., 1997; Vasconcelos *et al*., 1999). Previous studies of ours in buffaloes from the same breed have shown that the follicle size was important for the efficient synchronisation (Atanasov *et al*., 2011). A similar result could be anticipated when the first GnRH treatment is performed between the 13th and the 15th day after the preceding ovulation when progesterone levels are still elevated and the dominant follicle undergoes atresia (Moreira *et al*., 2005). The start of the synchronisation protocol during these two oestrus intervals, results very often in preliminary spontaneous ovulation or follicle atresia before the second application of GnRH (Baruselli *et al*., 2003). In our view, these were the reasons for the lower conception rates in buffaloes from group I. The hypothesis is supported also by the relatively high proportion (52.2%) of animals from group I which exhibited spontaneous oestrus within 20 days after the treatment.

## CONCLUSION

It could be affirmed that the ultrasound identification of ovarian structures in buffaloes at the start of the breeding season could be recommended as a criterion for selection of candidates for synchronisation schedules. Presence of the cervical mucus at the time of artificial insemination is indicator for better conception rates. The application of estrus and ovulation synchronization scheme with fixed-time insemination could be successfully used in Bulgarian Murrah buffaloes.

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