Bulgarian Journal of Veterinary Medicine (2011), **14**, No 3, 165–170

INDUCTION OF OESTRUS AND CONCEPTION RATES IN BULGARIAN MURRAH BUFFALOES AFTER FIXED-TIME ARTIFICIAL INSEMINATION (A PRELIMINARY STUDY)

A. ATANASOV[1], S. YOTOV[1], A. ANTONOV[1] & P. KOLEV[2]

[1]Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Stara Zagora, [2]Private veterinarian, Sevlievo; Bulgaria

Summary

Atanasov, A., S. Yotov, A. Antonov & P. Kolev, 2011. Induction of oestrus and conception rates in Bulgarian Murrah buffaloes after fixed-time artificial insemination (a preliminary study). *Bulg. J. Vet. Med.*, **14**, No 3, 165–170.

The aim of the present study was to investigate the effect of oestrus induction and timed artificial insemination on conception rates in Bulgarian Murrah buffaloes. Fifteen lactating non-primiparous buffaloes weighing 550–650 kg, fed and reared uniformly, were included in the survey. The animals were submitted to transrectal ultrasonography and divided into two groups depending on the ovarian follicle size: Group I (n=8) with follicle size <10 mm and group II (n=7) with follicle size >10 mm. The Ovsynch protocol for oestrus synchronization and programmed insemination was used. The clinical signs of oestrus (uterine tone, oestral discharges, cervical passage), conception rates and oestrus signs in non-fertilized animals were determined. The results showed that follicle size could be used as a criterion in selecting candidates for oestrus and ovulation synchronization in buffaloes from the studied breed. Oestrus-related discharges were observed in considerably higher number of animals from group II (71.4%; P<0.05) as compared to group I (37.5 %). This oestrus synchronization and scheduled insemination protocols could be successfully used in Bulgarian Murrah buffaloes.

Key words: buffaloes, conception rate, oestrus induction, Ovsynch

INTRODUCTION

The reproductive function of buffaloes is a major factor determining the economic significance of this animal species (Barile, 2005). It is influenced by: the late onset of sexual maturity (Jainudeen & Hafez, 2000; Mondal & Prakash, 2004), high percentage of animals with silent oestrus (Barile, 2005), the longer period between calving and the reduced ovarian activity during the hot months of the year (Singh *et al.*, 2000; De Rensis & Lopez-Gatius, 2007; Dimitrov *et al.*, 2009).

The different duration of oestrus (4 to 64 h) impedes the exact detection of ovulation and results in limited application of artificial insemination in buffaloes (Neglia *et al.*, 2003). That is why, a more efficient approach is oestrus induction and a fixed-timed artificial insemination (Presicce *et al.*, 2005). Such protocols allow the broader application of artificial insemination as a modern reproduction biotechnology (Baruselli *et al.*, 2007). Most protocols used for oestrus synchronization in buffaloes are adapted from similar studies performed in cattle (Pursley *et al.*, 1995).

The Ovsynch protocol for synchronization of the ovulation in buffaloes has been tested during different seasons (Berber *et al*., 2002; Baruselli *et al.,* 2003; Neglia *et al.,* 2003; De Rensis *et al*., 2005; Paul & Prakash, 2005; Carvalho *et al*., 2007; Warriach *et al.,* 2008). Neglia *et al*. (2003) observed a conception rate of 36% after using the Ovsynch programme. Paul & Prakash (2005) reported similar results – 33.3%. Warriach *et al*. (2008) established different conception rates in buffaloes, insemination after ovulation synchronization: 36.3% during the oestral and 30.4% during the anoestral season. With respect to season, Baruselli *et al*. (2003) demonstrated a considerable difference in conception rates: 48.8% and 6.9% during the oestral and anoestral seasons, respectively. Similarly, Carvalho *et al.* (2007) reported conception rates of 46.8% after using the Ovsynch protocol during the reproductive season. Unlike them, Berber *et al*. (2002) established pregnancy in 56.5% of treated animals.

To the best of our knowledge, there is no information about the application of oestrus synchronization protocols and scheduled insemination in Bulgarian Murrah buffaloes. The aim of our research was therefore to investigate the effect of oestrus induction and scheduled artificial insemination on conception rates in Bulgarian Murrah buffaloes in production conditions.

MATERIALS AND METHODS

The study was performed in 15 buffaloes from the Bulgarian Murrah breed, reared in a buffalo farm in central North Bulgaria (geographic coordinates 42.95 N, 25.13 I) in the beginning of the reproduction season: October and November 2009. The body condition score of animals was 3.5 (on a five-point scale, 1: emaciated; 5: fat) and their body weight – from 550 to 650 kg. The buffaloes were between 3 and 8 years of age, all of them lactating, fed and reared uniformly.

Ultrasonography was carried out with ultrasound Aloka SSD 500 Micrus (Aloka Co. Ltd, Tokyo, Japan), supplied with 5 MHz linear probe using a transrectal approach. The findings were documented by means of thermovideoprinter Mitsubishi P91 E (Tokyo, Japan).

The animals were divided into two groups depending on the ovarian follicle size: group I (n=8) with follicle size <10 mm and group II (n=7) with follicle size >10 mm.

The treatment for ovulation synchronization was performed as followed (Fig. 1): day 0 – intramuscular administration of 100 μg GnRH analogue (Gonadorelin, Gonavet Veyx®, Veyx-Pharma GmbH Soehreweg, Schwarzenborn, Germany), 7 days later – intramuscular administration of 500 μg $PgF_2\alpha$ analogue (Cloprostenol, PGF Veyx®

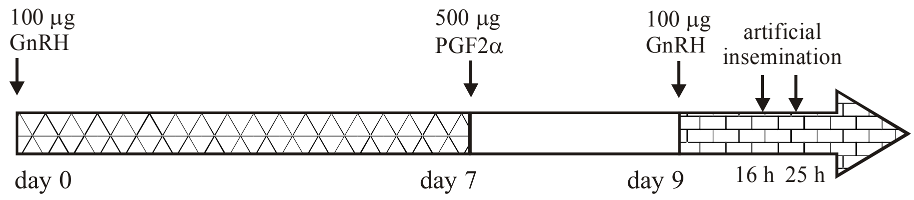

Fig. 1. Schematic representation of oestrus induction plan and timed artificial insemination in Bulgarian Murrah buffaloes.

Table 1. Results from oestrus induction and timed artificial insemination in Bulgarian Murrah buffaloes

Groups	Clinical signs of oestrus, % (n)			Conception rates, % (n)	Spontaneous oestrus after Ovsynch, % (n)
	Cervical passage	Uterine rigidity	Oestrus discharges		
Group I (n=8) follicle size < 10 mm	62.5 (5/8)	100 (8/8)	37.5 (3/8)	25.0 (2/8)	33.3 (2/6)
Group II (n=7) Follicle size >10 mm	85.7 (6/7)	100 (7/7)	71.4 (5/7)	57.1 (4/7)	66.7 (2/3)
Total (n=15) follicle size 6–11.5 mm	73.3 (11/15)	100 (15/15)	53.3 (8/15)	40.0 (6/15)	44.4 (4/9)

forte, Veyx-Pharma GmbH Soehreweg, Schwarzenborn, Germany). On the 9th day, a second injection of 100 µg GnRH analogue was done, followed by two artificial inseminations on hours 16 and 25 after the second GnRH infection. Insemination was carried out with plastic straws of frozen semen obtained from a certified buffalo bull.

The following clinical signs of oestrus were determined: uterine cervical penetration as per Stevenson *et al.* (1983), uterine muscle tone according to Loeffler *et al.* (1999). The reproduction traits conception rate and spontaneous oestrus manifestation in non-fertilized animals were also recorded.

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

The relative proportions of studied reproductive traits were determined. Data were statistically processed by StatSoft (Microsoft corp. Ink) software. Results were considered statistically significant at $P<0.05$.

RESULTS

The results from the study are presented in Table 1. By the day of artificial insemination, increased uterine tone was observed in 100% of animals from groups I and II.

The uterine cervix penetration was good in 73.3% (11/15) of all buffaloes, with significantly higher prevalence in group II (85.7%; 6/7) than in group I (62.5%; 5/8).

Oestral discharges from external genitalia were present in 53.3% (8/15) of animals included in the Ovsynch programme. The relative share of buffaloes exhibiting oestral discharge from group II was statistically significant ($p<0.05$) higher compared to group I – 71.4% (5/7) vs 37.5% (3/8), respectively.

The overall conception rate in both groups was 40% (6/15), tending to increase in group II – 57.1% (4/7) compared to 25% (2/8) in group I. Spontaneous oestrus within 40 days after the artificial insemination was observed in 44.4% (4/9) of non-fertilized buffaloes: 33.3% (2/6) in the first group and 66.7% (2/3) in the second group.

DISCUSSION

The presence of a follicle with a size >10 mm in ovaries at the time of inclusion of buffaloes in oestrus synchronization programmes is essential for obtaining good results (Presicce *et al*., 2005; De Rensis & Lopez-Gatius, 2007). This is due to the fact that small follicle size during the first GnRH treatment is related to a high probability of preliminary ovulation of the dominant follicle – between the $PgF_2\alpha$ analogue injection and the second GnRH administration (Baruselli *et al.,* 2003). A similar tendency was shown by Vasconcelos *et al.* (1999) in cows.

De Rensis *et al*. (2005) observed a relatively higher conception rate in animals with follicle size ≥10 mm on the first day of Ovsynch programme, compared to buffaloes with follicle size of <10 mm: 44% and 8%, respectively. A similar tendency was present in our study too. The better conception rate of 57.1% in group II compared to 25% in group I could be explained by the ovulation in more animals from group II after the second GnRH injection. In support of our hypothesis, Baruselli *et al.* (2003) reported ovulation in 60.6% of animals with average tertiary follicle size 9.5±1.7 mm and 39.4% in buffaloes with follicle size 6.7±2.4 mm. Therefore, we assume that ovarian follicle size could be used as a parameter for group formation before the first treatment.

In the view of some researchers, the uterine muscle tone determined by rectal palpation is a good criterion for conception in cattle after spontaneous oestrus (Bostedt *et al.,* 1976; Gordon, 1996). The presence of increased uterine tone in all animals suggested the presence of oestrogen-producing tertiary follicle in the ovaries of treated buffaloes. Berber *et al*. (2002) did not observe any relationship between this parameter and fixed-time insemination. Neglia *et al*. (2003) reported increased uterine tone in 88% of buffaloes included in a programme for ovulation synchronization and presence of tertiary follicle in one of ovaries of all animals.

In this study, vulvar discharge was observed in 53.3% (8/15) of all animals, whereas a good cervical penetration was registered in 73.3% (11/15). According to Loeffler *et al*. (1999), the presence of transparent discharge of uterine origin during insemination indicated increased fertility in cattle. Such secretion and oestral behaviour in buffaloes was observed in very rare occasions, both in spontaneous (Jainudeen & Hafez, 2000) and synchronized (Neglia *et al*., 2003) oestrus. In 31.5% of synchronized animals, Berber *et al*. (2002) established vulvar discharge and therefore considered this sign to be unreliable for detection of oestrus and ovulation. According to our results, oestral discharges were found out in more buffaloes with follicle size >10 mm and considering the conception rates in this group, we assume that this parameter is a trait of complete ovulation as confirmed by the higher relative share of animals which exhibited clinical signs of spontaneous oestrus after the synchronization.

The data for cervical penetration in artificial insemination indicated that this parameter was not important with regard to conception, similarly to what was reported by Stevenson *et al*. (1983).

The total conception rate of both groups – 40% (6/15), is similar to those reported by Neglia *et al*. (2003), Paul & Prakash (2005) and Warriach *et al.* (2008), but differed from data of Berber *et al*. (2002), Baruselli *et al*. (2003) and

Carvalho *et al.* (2007). This could be explained by the fact that the beginning of our studies matched the onset of the physiological reproductive season in buffaloes, starting with decrease of daylight hours. In support of the opinion of De Rensis & Lopez-Gatius (2007) about the relationship between follicle size at the time of the first treatment and the synchronization of ovulation by the end of the Ovsynch protocol, our study showed a tendency towards increased relative share of fertilized animals in group II (57.1% or 4/7) as compared to group I (25% or 2/8).

In conclusion, follicle size could be used as a criterion in selecting candidates for oestrus and ovulation synchronization in Bulgarian Murrah buffaloes. Oestrus-related discharges were observed in considerably higher number of animals with follicle size > 10 mm. This oestrus synchronization and fixed-time insemination protocols could be successfully used in Bulgarian Murrah buffaloes.

REFERENCES

Barile, V. L., 2005. Improving reproductive efficiency in female buffaloes. *Livestock Production Science*, **92,** 183–194.

Baruselli, P. S., E. H. Madureira, V. H. Barnabe, R. C. Barnabe & R. C. A. Berber, 2003. Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (*Bubalus bubalis*). *Brazilian Journal of Veterinary Research and Animal Science*, **40**, 431–442.

Baruselli, P. S., N. A. T. Carvalho, L. U. Gimenes & G. A. Crepaldi, 2007. Fixed-time artificial insemination in buffalo. *Italian Journal of Animal Science*, **6** (Suppl. 2), 107–118.

Berber, R. C. A., E. H. Madureira & P. S. Baruselli, 2002. Comparison of two ovsynch protocols (GnRH vs LH) for fixed timed insemination in buffalo (*Bubalus bubalis*). *Theriogenology*, **57**, 1421–1430.

Bostedt, H., H. Bonengel, D. Gunzler, H. Reissenger, F. Hofer & K. Brunold, 1976. Ovarian changes and measurement of electrical resistance of vaginal secretions in the cow during estrous. *Tierärztliche Umschau*, **31**, 211–215.

Carvalho, N. A. T., M. Nichi, C. E. P. Henriquez, C. A. Oliveira & P. S. Baruselli, 2007. Use of human chorionic gonadotropin (hCG) for fixed-time artificial insemination in buffalo (*Bubalus bubalis*). *Animal Reproduction*, **4**, 98–102.

De Rensis, F. & F. Lopez-Gatius, 2007. Protocols for synchronizing estrus and ovulation in buffalo (*Bubalus bubalis*): A review. *Theriogenology*, **67**, 209–216.

De Rensis, F., G. Ronci, P. Guarneri, B. X. Nguyen, G.A. Presicce, G. Huszenicza & R. J. Scaramuzzi, 2005. Conception rate after fixed time insemination following ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (*Bubalus bubalis*). *Theriogenology*, **63**, 1824–1831.

Dimitrov, M., Y. Ilieva, Ts. Peeva, A. Atanassov, P. Georgiev, S. Yotov & A. Antonov, 2009. Some reproductive characteristics in buffalo cows of the Bulgarian Murrah breed. *Bulg. J. Vet. Med.*, **12**, Suppl. 1, 141–145.

Gordon, I., 1996. Controlled Reproduction in Cattle and Buffalo. CABI Publishing, Wallingford UK

Jainudeen, M. R. & E. S. Hafez, 2000. Cattle and buffalo. In: *Reproduction in Farm Animals,* 7th edn, ed. B. Hafez & E. S. E. Hafez, Lippincott Willams & Wilkins, Baltimore, pp. 167–170.

Loeffler, S. H., M. J. Vries, Y. H. Schukken, A. C. Zeeuw, A. A. Dijkhuizen, F. M. Graaf & A. Brand, 1999. Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Theriogenology*, **51**, 1267–1284.

Mondal, M. & B. S. Prakash, 2004. Effects of long-term growth hormone-releasing factor administration on plasma growth hormone, luteinizing hormone and progesterone profiles in growing female buffaloes (*Bubalus bubalis*). *Reproduction in Domestic Animals*, **39**, 333–339.

Neglia, G., B. Gasparrini, R. Di Palo, C. De Rosa, L. Zicarelli & G. Campanile, 2003. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean buffalo cows. *Theriogenology*, **60**, 125–133.

Paul, V. & B. S. Prakash, 2005. Efficacy of the Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes (*Bubalus bubalis*). *Theriogenology*, **64**, 1049–1060.

Presicce, G. A., E. M. Senatore& G. De Santis, A. Bella, 2005. Follicle turnover and pregnancy rates following oestrus synchronization protocols in Mediterranean italian buffaloes (*Bubalus bubalis*). *Reproduction in Domestic Animals*, **40**, 443–447.

Pursley, J. R., M. O. Mee & M. C. Wiltbank, 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology*, **44**, 915–923.

Singh, J., A. S. Nanda & G. P. Adams, 2000. The reproductive pattern and efficiency of female buffaloes. *Animal Reproduction Science*, **60–61**, 593–604.

Stevenson, J. S., M. K. Schmidt & E. P. Call, 1983. Estrous intensity and conception rates in Holsteins. *Journal of Dairy Science*, **66**, 275–280.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley & M. C. Wiltbank, 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrus cycle in lactating dairy cows. *Theriogenology,* **52**, 1067–1078.

Warriach, H. M., A. A. Channa & N. Ahmad, 2008. Effect of oestrus synchronization methods on oestrus behaviour, timing of ovulation and pregnancy rate during the breeding and low breeding seasons in Nili-Ravi buffaloes. *Animal Reproduction Science*, **107**, 62–67.

Paper received 29.06.2010; accepted for publication 08.04.2011

Correspondence:

Dr. Anatoli Atanasov
Department of Obstetrics, Reproduction and Reproductive Disorders,
Faculty of Veterinary Medicine,
6000 Stara Zagora, Bulgaria
e-mail: a_stefanov@mail.bg