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

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Summary


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 nondprimiparous 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 E) 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 follows (Fig. 1): day 0 – intramuscular administration of 100 µg GnRH analogue (Gonadorelin, Gonavet Veyx®, Veyx-Pharma GmbH Soehreweg, Schwarzendorn, Germany), 7 days later – intramuscular administration of 500 µg PgF2α analogue (Cloprostenol, PGF Veyx®)
forte, Veyxd-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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Clinical signs of oestrus, % (n)</th>
<th>Conception rates, % (n)</th>
<th>Spontaneous oestrus after Ovsynch, % (n)</th>
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<tbody>
<tr>
<td></td>
<td>Cervical passage</td>
<td>Uterine rigidity</td>
<td>Oestrus discharges</td>
</tr>
<tr>
<td>Group I (n=8) follicle size &lt; 10 mm</td>
<td>62.5 (5/8)</td>
<td>100 (8/8)</td>
<td>37.5 (3/8)</td>
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<tr>
<td>Group II (n=7) follicle size &gt;10 mm</td>
<td>85.7 (6/7)</td>
<td>100 (7/7)</td>
<td>71.4 (5/7)</td>
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<tr>
<td>Total (n=15) follicle size 6–11.5 mm</td>
<td>73.3 (11/15)</td>
<td>100 (15/15)</td>
<td>53.3 (8/15)</td>
</tr>
</tbody>
</table>

**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 PgF₂α 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.

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Paper received 29.06.2010; accepted for publication 08.04.2011

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