Ovulation Synchronization in Water Buffaloes Guided by Milk Progesterone ELISA

Md. Nazmul Hoque[1], Anup Kumar Talukder[1,2,*], Md. Mostofa Kamal[1,3], Ajit Kumar Jha[1], Farida Yeasmin Bari[1] and Mohammed Shamsuddin[1]

[1]*Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh*

[2]*Department of Medicine and Surgery, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Barisal 8210, Bangladesh*

[3]*Department of Livestock Services, Government of the People's Republic of Bangladesh, Dhaka 1215, Bangladesh*

ABSTRACT

Ovulation synchronization (ovsynch) has proved to increase the number of insemination in cattle by overcoming the problems of heat detection. The aim of this study was to do ovsynch in water buffaloes where heat detection is a major reproductive problem and to determine the conception rates after timed artificial insemination (TAI). Twenty cyclic buffaloes at ≥60 days postpartum were selected by examining 24 unobserved estrus buffaloes based on milk progesterone assay (progesterone concentration ≥1.0 ng/ml) from the Mymensingh district of Bangladesh. Ovsynch treatment regimen was started irrespective of the stage of estrous cycle. Gonadorelin (500 μg) was injected intramuscularly at Day 0 followed by Alfaprostol (8 mg) at Day 7. A second injection of Gonadorelin was given at Day 9 and TAI was done with frozen semen from Mediterranean buffalo bulls at 16~20 hours of the second Gonadorelin injection. Milk progesterone ELISA at Day 10~12 post AI confirmed ovulation in 16 out of 20 (80%) buffaloes (progesterone concentration ≥1.0 ng/ml). High progesterone concentration (≥1.0 ng/ml) at Day 10~12 and Day 22~24 of AI showed pregnancy in six out of 20 (30%) buffaloes. Pregnancy was further confirmed by ultrasonography at Day 40 in these six buffaloes. In conclusion, ovsynch followed by TAI could be applied in cyclic buffaloes for overcoming the estrus detection problems; however, more studies are needed to increase the conception rate.

(Key words : water buffaloes, ovulation synchronization, milk progesterone assay)

INTRODUCTION

Water buffaloes are very important for local milk production in Bangladesh. Estrus behavior in buffalo is hardly clear and the estrus signs, even when showed, are not reliable and thus ovulation can not be predicted causing major economic loss to buffalo breeders (Singh *et al.*, 2000; El-Wishy, 2007). It has been reported that GnRH administration in buffaloes induces ovulation in 60~86% of the treated animals (Aboul-Ela *et al.*, 1985). However, the presence of dominant follicles at the time of treatment is a pre-requisite for a successful induction of ovulation (Baruselli, 2001; De Rensis *et al.*, 2005). To promote the use of AI in buffaloes, different hormonal treatments are scheduled to obtain estrus synchronization and, in particular LH peak and ovulation synchronization, to perform timed AI (Barile, 2005).

The milk progesterone concentration is a good marker to determine the functional status of corpus luteum (CL) and the ovarian activity in ruminant farm animals (Ucar *et al.*, 2004). The failure of buffaloes to show overt estrus signs, together with the wide variation in duration of estrus is a major constraint to the proper adoption of AI for genetic improvement in buffaloes (Baruselli *et al.*, 2003). This requires an ovsynch that results in reliable and consistent synchronization of the stage of the estrous cycle and ovulation associated with a relatively high pregnancy rate when combined with timed AI in buffaloes. Therefore, the present study was conducted to do ovulation synchronization in buffalo cows to overcome the problems

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* Correspondence : E-mail : anupbau@gmail.com

in estrus detection and to determine the conception rates after timed artificial insemination in buffaloes assisted with milk progesterone ELISA.

MATERIALS AND METHODS

1. Animal Selection and Management

Twenty-four buffaloes of indigenous river type at ≥60 days postpartum with history of anoestrus were selected randomly for the experiment from 24 farm families belong to the Mymensingh district of Bangladesh. The body condition score of the buffalo cows varied from 2.5 to 4.0 (1.0 to 5.0 scale). The age of the buffaloes ranged from 4 to 10 years and their parity ranged between 2 and 5. The buffalo cows had no history of peri-parturient disorders and calved normally during their previous parturitions. The buffaloes were milked two times daily with their calves used for stimulating milk letdown. Calves survived on residual milk after the hand milking. Control weaning was not practiced. The buffaloes were fed on rice straw, cut-and-carry grasses and milling by-products as concentrate (crashed rice and/or sometimes mustard oil cake) with limited grazing on roadside and community land. Only at lactation period, 0.5 to 1.5 kg concentrate (rice or wheat bran and mustard oil cake) per day with few vitamin-mineral supplements (Vitamin A, D, E and Ca, Mg and P premix) was supplied to each buffalo.

2. Milk Progesterone ELISA

Postpartum ovulation and luteal function in buffaloes were studied by determining progesterone profiles in milk. Milk progesterone concentration of ≥1.0 ng/ml milk was referred as a luteal stage (Shamsuddin *et al.*, 2006) and <1.0 ng/ml was considered as non-luteal stage (Ali and Fahmy, 2007). Two milk samples were collected at 10 days apart before starting ovsynch treatment protocol to monitor ovarian cyclicity. Milk samples were collected in a 20 ml screw-capped plastic tube containing Bronopol® preservative (8 mg tablet /40 ml of milk, 2-bromo-2-nitro-1, 3-propanediol, D&F Control Systems, Inc. Dublin, California) from the teats of individual buffalo at their morning milking (between 9.0 am to 11.0 am). Whole milk samples were stored at 4℃ for maximum of two days and were defatted by centrifugation at 150 g for 30 minutes at 4℃. The fat-free milk samples were preserved at −80℃ until analysed by ELISA.

3. Ovsynch treatment and timed AI

Among 24 unobserved estrus buffaloes, 20 cyclic buffaloes were subjected for ovsynch protocol by the administration of a GnRH analogue at Day 0 (Gonadorelin 500 μg, Fertagyl®, Intervet International BV), followed by $PGF_{2\alpha}$ treatment (Alfaprostol 8 mg, Gabrostim®, VETEM SpA., Porto Empedocle-Italy) on Day 7 and a second GnRH treatment at 48 hour after PGF2α (Day 9) (Fig. 1). All treatments were given as intramuscular injections in the shoulder region. However, animals subjected to receive ovsynch treatment regimen regardless of stages of their estrous cycle. Ovsynch treated buffaloes were inseminated irrespective of the onset of prominent estrus signs. Each animal received a single artificial insemination with frozen semen from Mediterranean buffalo bulls at 16 to 20 hours (timed AI) following second GnRH injection. From all ovsynch treated buffaloes another three milk samples were collected at Day 0 (day of AI), Day 10~12, and Day 22~24 of post breeding and milk progesterone ELISA was done accordingly to detect ovulation and pregnancy by determining

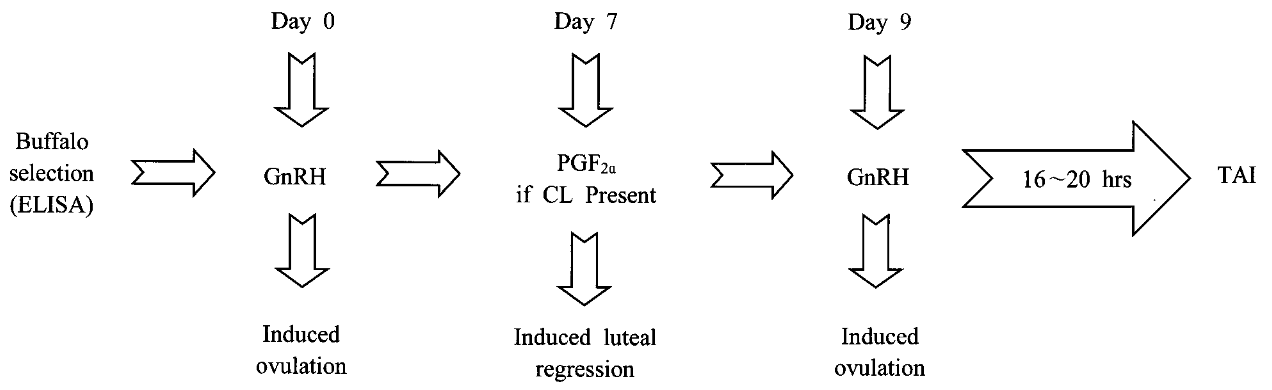

Fig. 1. The ovsynch treatment protocol for cyclic buffalo cows.

the rise of progesterone concentrations in milk.

4. PreGnancy Diagnosis

Early non-pregnancy at Day 22~24 was made by measuring progesterone concentrations with milk ELISA (Shamsuddin *et al.*, 2006). Finally pregnancy diagnosis was confirmed by the application of real-time transrectal ultrasonography using an ultrasound scanner (PharVision MicroV$_{10}$, Classic Medical Supply, Inc., USA) at Day 40 post-insemination.

5. Statistical Analysis

The data on milk progesterone ELISA were entered into Microsoft Excel pre-customized data application software to calculate the progesterone concentration for each sample and intra-assay co-efficient of variation. The inter-assay co-efficient of variation was calculated by the formula:

$$\frac{\text{Standard deviation of multiple estimates of a sample}}{\text{Mean of the multiple estimates}} \times 100$$

RESULTS

Two milk progesterone assays 10 days apart of 24 unobserved estrus buffaloes at ≥60 days postpartum confirmed true anoestrus in only four buffaloes (17%) whereas all the remaining 20 (83%) were sub-fertile that means animals were cyclic but farmers could not detect estrus (Fig. 2).

Ovsynch treatment induced estrus signs of varied intensity in all buffaloes. All buffaloes had variable degree of uterine tone on rectal palpation during AI. Cervix was open enough for easy passages of AI gun in all buffaloes. All the animals had pink vestibule and swollen vulva. Bellowing and frequent urination were observed in all buffaloes. No buffaloes were examined for standing estrus by buffalo bull.

High progesterone level (≥1.0 ng/ml) indicated ovulation in 16 of 20 (80%) buffaloes on Day 10~12 after insemination (Table 1(a), Table 1(b)). High progesterone level (≥1.0 ng/ml) on Day 10~12 and Day 22~24 after insemination indicated pregnancy in six of 20 (20%) buffaloes (Table 1(a)). Transrectal ultrasonography at Day 40 confirmed pregnancy in these six buffaloes that showed indication of pregnancy by milk progesterone assay. Ultrasonic image of a buffalo embryo at Day 40 of pregnancy is depicted in Fig. 3. Low progesterone concentrations (<1.0 ng/ml) in four out of 20 buffalo cows (20%) on Day 10~12 and Day 22~24 indicated non responsiveness to ovsynch treatment (Table 1(c)). These results point out that the synchronized ovulation was 80% (16/20) and conception rate was 30% (6/20) in cyclic buffaloes.

DISCUSSION

In Bangladesh, perhaps this is the first ever study in water buffaloes in which luteal activity was monitored after treating with GnRH-PGF$_{2\alpha}$-GnRH for ovulation synchronization, commonly known as 'ovsynch'. In this study, milk progesterone assays of 24 buffaloes at ≥60 day's postpartum revealed 4 (17%) buffaloes as acyclic and 20 (83%) buffaloes as cyclic. The farmers were not able to detect them in estrus. Incidence of anoestrus is higher (56.0%) in buffalo heifers than cow

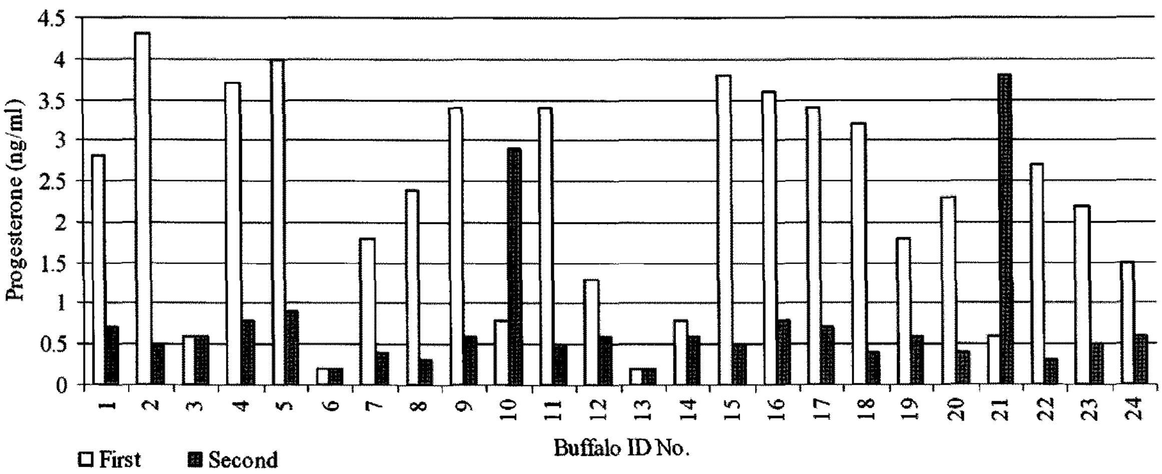

Fig. 2. Milk progesterone concentration in 24 buffaloes that had ≥60 days postpartum (two milk samples at 10 days interval).

Table 1. Progesterone profile in buffaloes following ovsynch treatment and timed AI

Buffalo ID No.	Progesterone concentration (ng/ml) on		
	Day 0 (Day of AI)	Day 10~12	Day 22~24
(a) Buffaloes that ovulated (responded to treatment) and get pregnant			
4	0.5	1.1	1.5
9	0.3	2.4	2.8
15	0.7	2.8	3.2
17	0.6	2.2	1.4
22	0.8	2.5	1.8
24	0.4	1.6	1.8
(b) Buffaloes that ovulated (responded to treatment) but not pregnant			
1	0.2	1.6	0.7
2	0.4	1.8	0.6
5	0.2	1.5	0.7
7	0.5	2.1	0.8
10	0.2	2.3	0.7
11	0.4	1.5	0.6
12	0.1	1.7	0.3
19	0.4	1.6	0.3
20	0.1	2.4	0.8
21	0.3	2.4	0.6
(c) Buffaloes did not respond to ovsynch treatment			
8	0.1	0.4	0.6
16	0.4	0.9	0.5
18	0.4	0.7	0.8
23	0.3	0.5	0.4

heifers (36.0%) (Ullah *et al.*, 2006). Reproductive efficiency of buffaloes is hampered by inherent prolonged inter-calving interval (Singh *et al.*, 2000). In this study, ovsynch treatment induced estrus symptoms of variable intensity in all buffaloes. This indicates that animals were cyclic but farmers could not detect estrus due to their nature of showing silent heat. Silent estrus is a common problem in buffaloes even under good management and non-stressful periods of the year (Abdalla, 2003). The silent estrus results in difficulties to determine the exact time for AI.

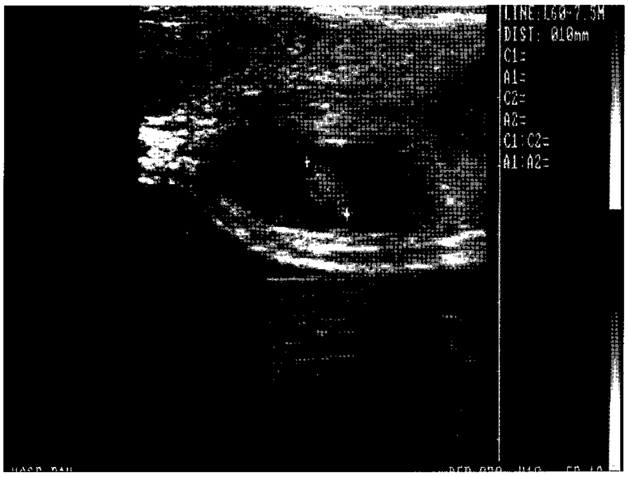

Fig. 3. Ultrasonic image of a buffalo embryo at Day 40 post AI.

In the current study, 16 out of 20 (80%) buffaloes showed ovulation from the dominant follicles of their ovaries. Ovulation of the dominant follicles were induced by the administration of GnRH at Day 0 and regression of CL was induced by the administration of $PGF_{2\alpha}$ at Day 7 and thereafter, the control of ovulation of the new dominant follicles were induced by a second time administration of GnRH at Day 9. The administration of GnRH leads to an LH surge during any stage of the estrous cycle, which promotes ovulation of dominant follicles or induces luteinization and/or atresia of pre-dominant follicles (Thatcher *et al.*, 1993). In buffalo, the ovsynch protocol has observed to synchronize ovulation in 70% (Baruselli, 2001) and 90% (Paul and Prakash, 2005) of animals with conception rates during breeding season averaging between 33% (Paul and Prakash, 2005) and 60% (Baruselli, 2001), respectively. The ovulation in response to GnRH treatment in postpartum buffaloes was 60% (Baruselli *et al.*, 2003).

In this experiment, using TAI with ovsynch protocol, 6 buffaloes out of 20 cyclic buffaloes were confirmed as pregnant giving a conception rate of 30%. Paul and Prakash (2005) found conception rates of 33.3% for TAI and 30.7% for buffaloes inseminated following spontaneous estrus using GnRH-$PGF_{2\alpha}$-GnRH protocol. In postpartum lactating dairy cows, Momcilovic *et al.* (1998) reported conception rates of 33% using TAI with the ovsynch protocol. The conception rates in buffaloes subjected to AI utilizing the ovsynch regimen ranged between 36 and 57% (Neglia *et al.*, 2003). However, in ovsynch programme first service conception rate did not differ with ovulation intervals (Wittke *et al.*, 2003). A conception can be expected only if the progesterone level by the time of insemination is low (Antal *et al.*, 1987). This was true in the present study as the progesterone concentration of the pregnant cows was at a low level at the day of AI (Day 0) and then increased to a high level at Day 10~24. These findings suggest that a quantitative milk progesterone assay could be used in ovsynch protocol in estimation of early pregnancy in buffaloes as also reported earlier by Ucar *et al.* (2004).

In conclusions, ovulation synchronization and timed insemination could be applied in cyclic buffaloes for overcoming the estrus detection problems; however, more studies are needed to increase the conception rate.

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