Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2014) 38: 418-424
© TÜBİTAK
doi:10.3906/vet-1302-35

Evaluation of ovsynch protocols for timed artificial insemination in water buffaloes in Bangladesh

Md. Nazmul HOQUE[1,*], Anup Kumar TALUKDER[2], Masuma AKTER[3], Mohammed SHAMSUDDIN[3]

[1]Department of Medicine, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

[2]Department of Gynecology, Obstetrics and Reproductive Health, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

[3]Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

Received: 16.02.2013 • **Accepted:** 03.03.2014 • **Published Online:** 17.06.2014 • **Printed:** 16.07.2014

Abstract: A total of 65 water buffaloes (groups A, B, and C) at ≥60 days postpartum with a body condition score (BCS) of ≥2.5 were selected to evaluate ovsynch protocols for timed artificial insemination (TAI). The group A buffaloes (n = 25) were treated with a simple ovsynch protocol (GnRH – Day 7 – PGF2α – Day 2 – GnRH –16 h – TAI). The group B buffaloes (n = 22) received PGF2α treatment 12 days before the initiation of simple ovsynch (PGF2α at Day –12 + simple ovsynch; modified ovsynch). The group C buffaloes (n = 18) were treated with a double ovsynch protocol (GnRH – Day 7 – PGF2α – Day 3 – GnRH – Day 7 – GnRH – Day 7 – PGF2α – 48 h – GnRH – 16 h – TAI). Milk P4 ELISA was used for tracking ovulation and conception rates. Ovulation rates were higher in buffaloes that received the double ovsynch treatment (group C; 83.3%) than those with simple ovsynch (group A; 72.0%; $P < 0.05$). The group C cows (44.4%) achieved a higher conception rate than the cows of groups A (28.0%) and B (36.4%) ($P < 0.05$) and multiparous buffaloes having BCS of ≥3.5 responded better to the ovsynch treatments than the primiparous ones ($P < 0.05$). The double ovsynch protocol increases both ovulation and conception rates in comparison to the simple and modified ovsynch protocols and is more effective in multiparous cows than in primiparous ones.

Key words: Ovsynch, timed artificial insemination, milk ELISA, water buffaloes

1. Introduction

Water buffaloes are one of the most important economic animals in many Asian countries, including Bangladesh, for milk and meat production. However, the buffalo farmers usually face challenges in detecting estrus as buffaloes have a tendency to show silent estrus. Furthermore, their delayed onset of puberty, seasonality, inability to show prominent estrus signs, and wide variation in duration of estrus hinder their better reproductive management and genetic improvement (1). The longer life span of buffaloes facilitates the assessment and improvement of their genetic and reproductive potential (2). Prolonged postpartum acyclicity and anestrus are also responsible for huge economic losses to buffalo breeders (3). The incidence of anestrus is higher (56.0%) in buffalo heifers than in cow heifers (36.0%) (4). To have one calf per year, the calving interval, lactation, and dry periods should be synchronous and pregnancy must be established within 90 days after calving (5). Therefore, there is a requirement for adoption of new technologies like ovsynch to overcome the issues. Ovsynch plays an important role in improving conception rates in farm animals. Ovsynch in buffaloes can result in a reliable and consistent synchronization of the stages of the estrous cycle and subsequently can increase the rates of ovulation and conception when combined with timed artificial insemination (TAI) (6). Artificial control of the estrous cycle can provide an efficient means to increase the reproductive capacity of buffalo by obviating the need for frequent visual inspections (7). Timed inseminations have the potential to improve genetic characteristics in buffaloes but widespread use of TAI in buffaloes is still limited due to a relatively poor expression of estrus behavior and lower conception rates (8). Thus, considering all of these factors, protocols such as ovsynch along with TAI have been developed (9). The ovsynch protocol is a sequence of GnRH and PGF2α treatments that can successfully synchronize ovulation in dairy cows and buffaloes and can also increase conception rates when combined with TAI

* Correspondence: nazmul.hoque90@gmail.com

(10). In buffaloes, 2 prominent protocols for ovsynch have been investigated during the last few years using GnRH + PGF2α after 7 days + GnRH after 36–48 h or progesterone-containing devices along with estradiol, pregnant mare's serum gonadotropin, and prostaglandins (11,12). The simple ovsynch protocol combines treatments of buffaloes with GnRH and PGF2α analogues (GnRH – Day 7 – PGF2α – Day 2 – GnRH – 16 h – TAI) (6). Moreover, ovsynch protocols have effectively been applied to cyclic and acyclic buffaloes (2,13) and primiparous and multiparous ones (14). In a previous study, Moreira et al. (15) reported that 2 treatments with PGF2α 14 days apart can increase the percentage of cows in early to mid-luteal phase and can also improve fertility in cycling cows if a simple ovsynch is initiated 12 days later (PGF2α at Day –12 + simple ovsynch; modified ovsynch). Ovulations due to the first GnRH injection of ovsynch treatment and fertility during the ovsynch treatments are dependent on the stages of the estrous cycle (16). The double ovsynch protocol is one of the well-established reproductive technologies in dairy cattle reproduction and it can also potentially improve fertility in water buffaloes if combined with TAI. The double ovsynch protocol includes 2 treatments with PGF2α 14 days apart followed by TAI at 64 h after the final PGF2α treatment. In a previous study, Souza et al. (17) reported higher rates of ovulation in cows with double ovsynch treatments (GnRH – PGF2α – GnRH – GnRH – PGF2α – GnRH – TAI). Standard ovsynch protocols like double ovsynch should be attempted to increase reproductive traits in anovular and subestrous buffaloes. However, the efficacy of different ovsynch protocols has not yet been studied in water buffaloes of Bangladesh. The study was, therefore, conducted to evaluate different ovsynch protocols in water buffaloes and their success on ovulation and conception rates under Bangladesh conditions.

2. Materials and methods

2.1. Buffalo cow selection and their management system

A total of 65 water buffaloes of indigenous type with a prolonged postpartum period (≥60 days) at random stages of their estrous cycle from 45 smallholder buffalo farms in the Mymensingh District of Bangladesh were included in this study. The buffaloes were divided into 3 groups: groups A (n = 25), B (n = 22), and C (n = 18). All buffaloes were reared under same climatic (subtropical) and management system since the farmers had low numbers (≤10) of buffaloes (6). The body condition scores (BCSs) of the buffaloes varied from 2.5 to 4.5 (on a 1.0–5.0 scale), but for our study we divided the cows into 2 groups in terms of BCS (BCS ≤ 3.0, n = 19; BCS ≥ 3.5, n = 46). The ages of the buffaloes ranged from 3 to 10 years and their parities ranged between 1 and 4.

2.2. Ovsynch treatment protocols and TAI

2.2.1. Simple ovsynch

The group A buffaloes (n = 25) were treated with intramuscular injection of a GnRH analogue (Fertazyl; Gonadorelin 500 µg, Intervet International BV, the Netherlands) at Day 0 followed by a PGF2α analogue (Gabrostim; Alfaprostol 8 mg, VETEM SpA, Italy) at Day 7, a second GnRH injection at Day 9, and TAI 16 h after the second GnRH injection using frozen semen from Mediterranean buffalo bulls (GnRH – Day 7 – PGF2α – Day 2 – GnRH – 16 h – TAI) (Figure 1) using a similar protocol to our previous study (6).

2.2.2. Modified ovsynch

The group B buffaloes (n = 22) received a luteolytic dose of PGF2α (Gabrostim; Alfaprostol 8 mg) 12 days before (Day –12) the initiation of simple ovsynch treatment (PGF2α at Day –12 + simple ovsynch) and TAI 16 h after the second GnRH injection (PGF2α – Day –12 – GnRH – Day 7 – PGF2α – Day 2 – GnRH – 16 h – TAI) (Figure 2) following a similar protocol reported earlier by Cordoba and Fricke (18).

2.2.3. Double ovsynch

The group C buffaloes (n = 18) were treated with a double ovsynch protocol in a manner similar to that established by Souza et al. (17). This group of buffaloes received treatment with intramuscular injection of a GnRH analogue (Fertazyl; Gonadorelin 500 µg) at Day 0 (treatment initiation day) followed by a PGF2α analogue (Gabrostim; Alfaprostol 8 mg) at Day 7, another GnRH injection 3 days after the PGF2α treatment, and finally another simple ovsynch treatment 7 day later along with TAI 16 h after the last GnRH injection (GnRH – Day 7 – PGF2α – Day 3 – GnRH – Day 7 – GnRH – Day 7 – PGF2α – 48 h – GnRH – 16 h – TAI) (Figure 3).

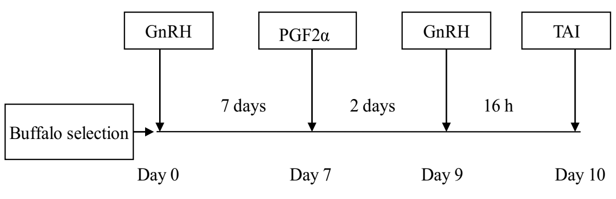

Figure 1. Ovsynch treatment of group A buffaloes (simple ovsynch).

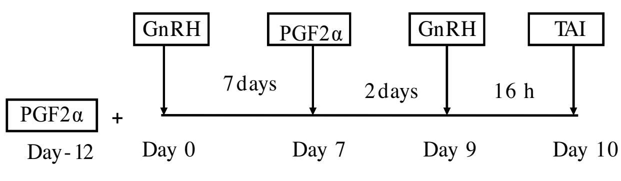

Figure 2. Ovsynch treatment of group B buffaloes (modified ovsynch).

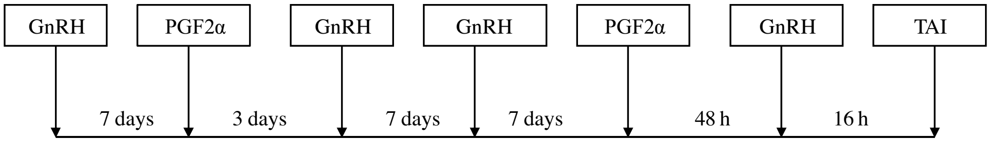

Figure 3. Ovsynch treatment of group C buffaloes (double ovsynch).

2.3. ELISA for milk progesterone

Postpartum luteal function in water buffaloes was monitored by determining progesterone concentration in milk with ELISA (19,20). A progesterone standard was prepared following the procedure established by Khan (19) at the Field Fertility Clinic Laboratory of Bangladesh Agricultural University.

2.4. Ovulation tracking and pregnancy diagnosis

Pregnancy diagnosis was made 22–24 days after TAI by measuring milk progesterone concentrations with ELISA. A progesterone concentration of ≥1.0 ng/mL of milk 22–24 days after TAI indicated pregnancy. Finally, real-time ultrasonography (Pharvision Micro V10, 3.5 MHz, Pie Medical, USA) was done at 40–45 days after breeding to confirm the pregnancy diagnosis (Figure 4).

2.5. Statistical analysis

Milk progesterone concentration along with intraassay and interassay coefficients of variation were determined by following procedures developed in other studies (6,19). The data were entered into Microsoft Excel 2003 and transferred into SPSS 11.5 for descriptive statistical analysis. A chi-square test was performed to see the effect of different ovsynch protocols on ovulation and conception rates. A paired t-test was also conducted to find out the effect of parity and BCS on ovulation and conception rates.

3. Results

Ovsynch treatments induced estrus signs of diverse intensity with bellowing, frequent urination, swelling of the vulva, and a pink-colored vestibule in all buffaloes of this study. The buffaloes had a variable degree of uterine tone and their cervices were open enough for easy passage of the AI gun during insemination. The proportions of cycling buffaloes exhibiting luteal functions were greater for cows receiving the double ovsynch treatment than for those in the simple and modified ovsynch groups.

In group A (simple ovsynch), 18 out of 25 treated buffaloes had a ≥1.0 ng/mL milk progesterone concentration (Table 1) at Days 10–12 after TAI and the ovulation rate was 72.0% (Table 2). A rise in progesterone concentration (≥1.0 ng/mL) at 10–24 days after AI indicated pregnancy in 7 cows (Table 1) and, therefore, the conception rate was 28.0% (7/25) in group A (Table 2). Accordingly, the ovulation rates were 77.3% (17/22) and 83.3% (15/18) in group B and C buffaloes, respectively (Table 2), and the conception rates were 36.4% (8/22) in

Table 1. Progesterone concentration (mean ± SD) at TAI and after TAI in buffalo cows of 3 ovsynch treatment groups.

Buffalo groups	Progesterone concentration (ng/mL) on:		
	Day 0	Days 10–12	Days 22–24
a. Buffaloes that did not respond to the ovsynch treatment.			
Group A (n = 7)	0.29 ± 0.16	0.78 ± 0.13	0.39 ± 0.11
Group B (n = 5)	0.23 ± 0.10	0.70 ± 0.07	0.65 ± 0.16
Group C (n = 3)	0.20 ± 0.10	0.77 ± 0.13	0.62 ± 0.16
b. Buffaloes that ovulated (responded to treatment) but did not get pregnant.			
Group A (n = 11)	0.44 ± 0.18	1.47 ± 0.30	0.72 ± 0.14
Group B (n = 9)	0.47 ± 0.25	1.29 ± 0.20	0.66 ± 0.20
Group C (n = 7)	0.53 ± 0.18	1.29 ± 0.20	0.70 ± 0.16
c. Buffaloes that ovulated and got pregnant.			
Group A (n = 7)	0.44 ± 0.19	1.79 ± 0.40	2.47 ± 0.30
Group B (n = 8)	0.64 ± 0.36	1.47 ± 0.33	2.14 ± 0.13
Group C (n = 8)	0.57 ± 0.24	1.54 ± 0.30	2.26 ± 0.19

Table 2. Ovulation and conception rate in group A, B, and C buffaloes after different ovsynch treatment protocols.

Treatment protocol	Total buffaloes	No. of buffaloes ovulated	Ovulation rate (%)	No. of buffaloes becoming pregnant	Conception rate (%)
Simple ovsynch	25	18	72.0[a]	7	28.0[a]
Modified ovsynch	22	17	77.3[ab]	8	36.4[ab]
Double ovsynch	18	15	83.3[b]	8	44.4[b]

[a,b]: Values within the same column differ statistically (P < 0.05).

group B buffaloes and 44.4% (8/18) in group C buffaloes (Table 2). Ovulation rates were higher in buffalo cows that received the double ovsynch treatment (group C; 83.3%) than those that received simple ovsynch (group A; 72.0%; P < 0.05). However, no marked differences were observed between the double and modified ovsynch groups (Group B, 77.3%; P < 0.05) (Table 2).

Importantly, the group C cows (44.4%) achieved a higher conception rate than the cows of group A (28.0%) and group B (36.4%) (P < 0.05) (Table 2). Real-time transrectal ultrasonography at Days 40–45 confirmed pregnancy in the buffaloes that showed indication of pregnancy at Days 10–12 and Days 22–24 according to the results of the milk ELISA test. Moreover, multiparous buffaloes showed better responses to all ovsynch treatments than the primiparous ones and this resulted in higher ovulation (86.5% vs. 47.6%; P < 0.05) and conception rates (40.7% vs. 21.4%; P < 0.05) (Table 3a). In all groups, the buffaloes with a good body condition score (BCS ≥ 3.5) responded better to the ovsynch treatments than the buffaloes with a poor body condition score (BCS ≤ 3.0) and had higher ovulation rates (88.1% vs. 49.2%; P < 0.05) and conception

Table 3. Response of buffaloes to different ovsynch protocols in terms of parity and BCS.

Treatment group	Criteria	No. of cows receiving treatment	No. of cows responding to treatment	Ovulation rate (%)	Conception rate (%)
a. Parity					
Group A (n = 25)	Primipara	7	3	42.8	14.3
	Multipara	18	15	83.3	33.3
Group B (n = 22)	Primipara	4	2	50.0	25.0
	Multipara	18	15	83.3	38.9
Group C (n = 18)	Primipara	4	2	50.0	25.0
	Multipara	14	13	93.0	50.0
Group A+B+C	Primipara	15	7	47.6[a]	21.4[a]
	Multipara	50	43	86.5[b]	40.7[b]
b. Body Condition Score (BCS)					
Group A (n = 25)	≤3.0	6	3	50.0	16.7
	≥3.5	19	15	79.0	31.6
Group B (n = 22)	≤3.0	8	3	37.5	12.5
	≥3.5	14	13	92.9	57.1
Group C (n = 18)	≤3.0	5	3	60.0	40.0
	≥3.5	13	12	92.3	46.1
Group A+B+C	≤3.0	19	9	49.2[a]	23.1[a]
	≥3.5	46	40	88.1[b]	44.9[b]

[a,b]: Values within the same column differ statistically (P < 0.05).

rates (44.9% vs. 23.1%; $P < 0.05$) (Table 3b). An ultrasonic image of a buffalo embryo at Day 40 of pregnancy is shown in Figure 4.

4. Discussion

Our investigations allowed us to declare that this is the first study done in water buffaloes where luteal activity was monitored after treatment with different ovsynch protocols to find out the most effective ones and also to determine the ovulation and conception rates after TAI. Estrus signs of varying intensity, indicating that the animals were cyclic but unable to show prominent estrus signs, were observed in all cows after different ovsynch treatments; thus, farmers could not detect their heat properly. In group A, 18 out of 25 buffaloes (72.0%) had ovulation from the dominant follicles of their ovaries. Treatments with GnRH at Day 0 and PGF2α at Day 7 induced ovulation of the dominant follicles and regression of the corpus luteum (CL), respectively, and, thereafter, the second administration of GnRH on Day 9 controlled the ovulation of the new dominant follicles. The surge of LH at a random stage of the estrous cycle was induced by the administration of GnRH, which subsequently caused luteinization of the predominant follicles (6). Researchers have found that ovsynch protocols can synchronize ovulation in 70.0% (21), 80.0% (6), and 90.0% (10) of buffalo cows. In 2 previous studies, Bridges et al. (22) and Baruselli et al. (23) reported that GnRH could induce ovulation in 60%–86.0% of treated cows and in 60.0% of postpartum buffaloes. A milk ELISA test done on Days 10–12 and Days 22–24 confirmed pregnancy in 7 out of 25 buffaloes (28.0%) of the simple ovsynch group and this conception rate (28.0%) is similar to the result of our previous study (6) with a similar protocol (30.0%). In another study, Paul and Prakash (10) reported 33.3% conception rates in Murrah buffaloes after TAI and 30.7% conception rates in buffaloes inseminated following spontaneous onset of estrus using a similar protocol. Neglia et al. (12) reported that buffaloes treated with different ovsynch treatment protocols could have 36%–57.0% conception rates.

Figure 4. Ultrasonic image of a buffalo embryo at Day 40 of pregnancy.

Luteal regression was induced 12 days before initiation of ovsynch treatment by the administration of a luteolytic dose of PGF2α in cows having a responsive CL. Hence, the proportion of cows responding to the ovsynch protocol was higher in group B than in group A of the present study, which was also reported by others (18). In group B, the ovulation rate was found to be 77.3% (17/22) after the modified ovsynch treatment, which is lower than the ovulation rate (90.5%) reported by Cordoba and Fricke (18) but higher than the ovulation rate (66.7%) observed by Souza et al. (17) using a similar protocol. In group B, the conception rate was 36.4% (8/22), which is higher than the result (30.7%) reported by Cordoba and Fricke (18) and lower than the result (41.7%) found by Souza et al. (17). Milk progesterone concentration did not differ significantly between the simple and modified ovsynch group buffaloes, but the proportion of cycling buffaloes exhibiting their luteal functions was greater for the cows that received the modified ovsynch treatment than for the buffaloes treated with the simple ovsynch protocol. This indicates that the administration of PGF2α 12 days before initiation of the simple ovsynch treatment shifted some buffaloes that would have been in the later stages of the estrous cycle into the early luteal phases of the estrous cycle.

In group C, ovulation occurred in 15 out of 18 buffaloes (83.3%) after treatment with the double ovsynch protocol, which is higher than the ovulation rate (78.1%) reported by Souza et al. (17). In this group, the conception rate was found to be 44.4% (8/18), which supports the results of Cunha et al. (24), who reported a 48.0% conception rate in dairy cows. In this study, the double ovsynch treatment increased pregnancies per timed insemination as compared to the simple and modified ovsynch protocols, which was also reported earlier in cows (49.7% vs. 41.7%) by Souza et al. (17). This indicates that the double ovsynch treatment more firmly synchronized the stages of the estrous cycles than the other ovsynch protocols. In an earlier study, Antal et al. (25) reported that conception could only be expected if the progesterone level at the time of insemination (Day 0) is low and gradually increased up to at least 24 days after AI. This phenomenon was true for the present study since the progesterone concentration of the pregnant cows was low (<1.0 ng/mL) at Day 0 (day of AI) and then gradually increased (≥1.0 ng/mL) until 10–24 days after breeding.

The buffalo cows with multiple parity showed better response to all the ovsynch protocols than the cows with single parity (primipara) and thus had higher ovulation and conception rates in this study. Ferreira et al. (26) reported that primiparous zebu cattle have lower conception rates than multiparous ones, which indicates that primiparous ones have some restrictions in ovsynch protocols with TAI.

In all groups, the buffalo cows with good body condition scores (BCS ≥ 3.5) responded better to the ovsynch treatments and achieved higher ovulation and conception rates. Conception rate in buffaloes receiving ovsynch protocols is influenced by BCS (a good efficiency of the ovsynch protocol is achieved when BCS is ≥3.5) and multiple parity (primiparous animals have lower conception rate than multiparous animals), as also reported in other studies (11,23,27). Derar et al. (28) found that 87.5% and 100.0% of buffalo heifers and buffalo cows, respectively, ovulated after the first GnRH injection of an ovsynch treatment. These results indicate that the success of different ovsynch protocols depends on the selection of buffaloes with good BCS and that parity also has a great influence on the improvement of the reproductive status of water buffaloes.

In conclusion, the results show that the double ovsynch protocol increases conception rates in comparison to the simple and modified ovsynch protocols and multiparous cows respond better than primiparous cows. However, further studies are needed to test the result of higher fertility with double ovsynch and to expound the physiological phenomena that enhance fertility with this protocol.

Acknowledgments
We sincerely acknowledge the United States Department of Agriculture (USDA) and the International Atomic Energy Agency (IAEA) for funding the project (grant number BG-ARS-121) entitled "Introduction of Herd Health Services for Sustainable Improvement of Dairy Production and Marketing through Farmers' Associations in Bangladesh" being implemented at the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh. The cooperation and the support of the buffalo farmers and attendants are also gratefully acknowledged.

References

1. Abdalla EB. Improving the reproductive performance of Egyptian buffalo cows by changing the management system. Anim Reprod Sci 2003; 75: 1–8.
2. Ali A, Fahmy S. Ovarian dynamics and milk progesterone concentrations in cycling and non-cycling buffalo-cows (Bubalus bubalis) during Ovsynch program. Theriogenology 2007; 68: 23–28.
3. El-Wishy AB. The postpartum buffalo: II. Acyclicity and anestrous. Anim Reprod Sci 2007; 97: 216–236.
4. Ullah N, Anwar M, Rizwan S, Murtaza S. Blood plasma progesterone concentrations in two different veins and comparison of progesterone concentrations and rectal palpation findings to determine ovarian cyclicity in the Nili-Ravi buffalo (Bubalus bubalis). Pak Vet J 2006; 26: 118–120.
5. McDougall S, Macmillan KL, Williamson NB. Factors associated with prolonged period of post partum anoestrus in pasture-fed dairy cattle. In: Proceedings of the XX World Buiatrics Congress; 1998. pp. 657–662.
6. Hoque MN, Talukder AK, Kamal MM, Jha AK, Bari FY, Shamsuddin M. Ovulation synchronization in water buffaloes guided by milk progesterone assay. J Embry Trans 2011; 26: 105–109.
7. Hussein MM, Amen K, Abdel-Moghney AF. Evaluation of Crestar® and modified Crestar programs for timed insemination in lactating Egyptian buffaloes (Bubalus bubalis) under intensive production system. In: 5th Scientific Conference; 2007. pp. 170–175.
8. Ohashi OM. Estrous detection in buffalo cow. Buffalo J 1994; 2: 61–64.
9. Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 1995; 44: 915–923.
10. Paul V, Prakash BS. Efficacy of Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes. Theriogenology 2005; 64: 1049–1060.
11. Berber RC de A, Madureira EH, Baruselli PS. Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (Bubalus bubalis). Theriogenology 2002; 57: 1421–1430.
12. Neglia G, Gasparrini B, Palo RD, Rosa CD, Zicarelli L, Campanile G. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean Buffalo cows. Theriogenology 2003; 60: 125–133.
13. De Rensis F, Ronci G, Guarneri P, Nguyen BX, Presicce GA, Huszenicza G, Scaramuzzi RJ. 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 2005; 63: 1824–1831.
14. Presicce GA, Senatore EM, Bella A, De Santis G, Barile VL, De Mauro GJ, Terzano GM, Steccob R, Parmeggiani A. Ovarian follicular dynamics and hormonal profiles in heifer and mixed-parity Mediterranean Italian buffaloes (Bubalus bubalis) following an estrus synchronization protocol. Theriogenology 2004; 61: 1343–1355.
15. Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 2001; 84: 1646–1659.

16. Cerri RLA, Rutigliano HM, Bruno RGS, Chebel RC, Santos JEP. Effect of artificial insemination (AI) protocol on fertilization and embryo quality in high-producing dairy cows. J Dairy Sci 2005; 88: 86 (abstract).

17. Souza AH, Ayres H, Ferreira RM, Wiltbank MC. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 2008; 70: 208–215.

18. Cordoba MC, Fricke PM. Evaluation of two hormonal protocols for synchronization of ovulation and timed artificial insemination in dairy cows managed in grazing-based dairies. J Dairy Sci 2001; 84: 2700–2708.

19. Khan AHMSI. Development of milk progesterone ELISA and its application at AI field services in cattle. PhD, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh, 2008.

20. Shamsuddin M, Bhuiyan MMU, Chanda PK, Alam MGS, Galloway D. Radioimmunoassay of milk progesterone as a tool for fertility control in smallholder dairy farms. Trop Anim Health Prod 2006; 38: 85–92.

21. Baruselli PS. Control of follicular development applied to reproduction biotechnologies in buffalo. In: Proceedings of the I Congresso Nazionale sull'Allevamento del Bufalo. Eboli, Italy; 2001. pp. 128–146.

22. Bridges A, Lake S, Lemenager R, Claeys M. Timed Artificial Insemination in Beef Cows: What are the Options? West Lafayette, IN, USA: Purdue University Cooperative Extension Service; 2012.

23. Baruselli PS, Carvalho NAT, Henriquez CEP, Amaral R, Nichi M, Reichert RH. Use of progesterone associated to "Ovsynch" protocol for timed artificial insemination in buffalo (Bubalus bubalis). In: Proceedings of the II Congresso Nazionale sull'Allevamento del Bufalo. Rome, Italy; 2003. pp. 265–268.

24. Cunha AP, Guenther JN, Maroney MJ, Giordano JO, Nascimento AB, Bas S, Ayres H, Wiltbank MC. Recent advances in ovulation synchronization and superovulation in dairy cattle. J Dairy Sci 2008; 91: 246.

25. Antal T, Faluhelyi S, Szabo I, Janaky T, Toth I, Faredine I, Laszlo F. Study of the relationship between milk progesterone profiles measured at the time of insemination and conception rate in buffalo. Acta Vet Hunga 1987; 35: 391–395.

26. Ferreira MBD, Lopes BC, Andrade IC, Conceicao VJ. Escore corporal e anestro pós-parto em primíparas zebu. Revista Brasileira Reproducao Animal 1997; 114–116 (in Portuguese).

27. Baruselli PS, Carvalho NAT, Reichert RH, Henriquez CEP, Nichi M. Pre-synchronization with GnRH 7 days before Ovsynch protocol for timed insemination in buffalo. In: Buffalo Symposium of Americas Proceedings; 2002. pp. 414–417.

28. Derar R, Hussein HA, Fahmy S, El-Sherry TM, Megahed G. The effect of parity on the efficacy of an ovulation synchronization (Ovsynch) protocol in buffalo (Bubalus bubalis). Anim Reprod 2012; 9: 52–60.