Asian Journal of Biology

2(1): 1-8, 2017; Article no.AJOB.31950

Synchronization of Estrus Using Ovsynch Protocol and Fixed Timed Artificial Insemination (FTAI) in Indigenous Dairy Buffaloes: An Effective Buffalo Breeding Program in Bangladesh

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SKR and MRG designed the study. Author MRG wrote the protocol and author SKR carried out the experiments. Author MRG performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2017/31950
Editor(s):
(1) David E. Martin, Founder and Director, Evolv BioVet, Inc, Chapel Hill, North Carolina, USA.
(2) Tulay Askin Celik, Department of Biology, University of Adnan Menderes, Turkey.
Reviewers:
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(2) O. Wai Sum, The University of Hong Kong, Hong Kong.
(3) Duygu Kaşıkcı, Suleyman Demirel University, Isparta, Turkey.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/18317

Original Research Article

Received 31st January 2017
Accepted 20th March 2017
Published 24th March 2017

ABSTRACT

Background: Estrus synchronization may be a tool to overcome silent estrus and breeding seasonality, the major factors limit the reproductive efficiency of indigenous buffalo (*Bubalus bubalis*) in Bangladesh. The present study was carried out to evaluate estrus responses and conception rate in response to the Fixed Time Artificial Insemination (FTAI) of indigenous buffaloes (n=97) with Ovsynch protocol.

Materials and Methods: Buffaloes (morning and evening groups) were synchronized in three different seasons of Bangladesh. Estrus was synchronized according to Ovsynch protocol (a sequence of GnRH - $PGF2\alpha$ - GnRH treatments). Each animal received a single artificial

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insemination with frozen semen from Mediterranean buffalo bulls at 18 to 20 hours (fixed timed AI) following second GnRH injection. Diagnosis of conception was performed by real-time transrectal ultrasonography using an ultrasound scanner on Day 40 post-AI.

Results: Ovsynch treatment induced the estrus signs in all buffaloes. The conception rate was 56.70% (55/97) and pregnancy rate was 31.96% (31/97) in indigenous river type buffaloes of Bangladesh. Though the difference of conception and pregnancy rate was insignificant ($p>0.05$) among seasons, the conception rate was varied from 44% to 64.87% and the pregnancy rate varied from 28% to 35.14% in different seasons of Bangladesh. The conception rate was also varied depending on the time of administration of hormones as well as the time of insemination. The conception rate was the highest (64.87%) in late autumn (Hemonto) season when the temperature was low (17-22°C) and the higher in morning group (61.54%).

Conclusion: The Ovsynch protocol effectively synchronized estrus in indigenous river type buffaloes with a reasonable conception rates after FTAI. Estrus synchronization and FTAI could be applied for effective breeding program of buffalo in Bangladesh, though further studies are needed to increase the conception rate.

Keywords: Buffalo; conception; estrus synchronization; FTAI; ovsynch protocol.

1. INTRODUCTION

Buffalo has a significant role in the agricultural economy of developing countries [1]. Buffaloes play a prominent role in rural livestock production in Asia and Bangladesh in particular [2], though reproductive performance of indigenous buffaloes is not convincing in Bangladesh. Silent estrus and seasonality of breeding are two important factors responsible for poor reproductive efficiency in buffaloes [3,4], especially during hot summer months [5]. Several programs have been practiced nowadays to improve the reproductive performances of animals. Estrus synchronization protocols dramatically improve reproductive success by offering the possibility of planning the application of assisted reproductive technologies and allowing producers to breed more cows in less time by reducing the length of breeding and calving seasons [6]. It is also practiced in cyclic animals to bring a large group of animals into heat at a prearranged time for efficiency in artificial insemination (AI) and embryo transfer practice [7]. Successful synchronization protocol requires a clear knowledge of ovarian secretory functions in relation to control of follicle development, luteal phase of the cycle and ovulation [8]. A novel synchronization protocol named Ovsynch was developed [9] in cows, which requires a three injection schedule (GnRH-PGF2α-GnRH) for synchronization of ovulation. The technique was successfully carried out in cycling Indian buffaloes [10] for synchronization of ovulation and fixed timed AI.

The Ovsynch protocol has successfully synchronized ovulation in lactating dairy cows, resulting in fertility to timed AI (TAI) that was similar to that of cows inseminated after detection of estrus and became popular for oestrus synchronization in cattle with an acceptable fertility over the last decade [11]. Numerous variations of the protocol have also been tested and developed to meet demands of different physiological situations [12,13]. The GnRH injection, at any phase of estrous cycle, results in release of high amount of LH that ovulates an existing dominant follicle [14] or causes lutenization of non viable follicles, and starts emergence of a new follicular wave 2 or 3 days later [15]. With the ovulation or lutenization of the dominant follicles, P4 levels will remain high; therefore PGF2α is given on day 7 to induce luteolysis and promote the ovulation of the follicle of the new wave of follicular growth. The second GnRH injection is recommended after 48 h of PGF2α injection for better synchronization of ovulation and to allow the fixed timed AI [16]. The use of protocol does not require the identification of estrus, allow for the increase use of AI at fixed time and are more efficient in cattle and buffalo for increasing re productivity and also used in breeding farms for genetic improvement of animals.

As silent estrus and seasonal breeding are the major limiting factors and no report of estrus synchronization in buffalo reproduction (at Farm level with large population) yet in Bangladesh, establishment of estrus synchronization associated to FTAI (the major development of reproductive biotechnologies) with an acceptable fertility could be of significant relevance in indigenous buffalo reproduction in Bangladesh. Therefore, the objective of the present study was to determine the efficacy of the Ovsynch protocol for synchronization of estrus and to determine conception and pregnancy rates to FTAI of buffalo reproduction in Bangladesh.

2. MATERIALS AND METHODS

2.1 Buffalo Selection and Management

The study was carried out on 97 indigenous river type buffaloes of Lal Teer Livestock Research and Breeding Farm (the only private buffalo farm in Bangladesh), "Lal Teer Animal Breeding House", situated at Tangail (a district in the central region of Bangladesh, lies between 24° 01′ and 24° 47′ north latitudes and between 89° 44′ and 90° 18′ east longitudes; https://en.wikipedia.org/wiki/Tangail_District), Bangladesh, selected randomly irrespective of milk yield and body weight. The age of the buffaloes ranged from 4 to 8 years, their parity ranged between 2 and 5 and body condition score was from 2.5 to 4.0 (1.0 to 5.0 scale) according to Anitha et al. [17]. The buffalo cows had no history of peri-parturient disorders and calved normally during their previous parturitions. The buffaloes were fed on rice straw, cut-and-carry grasses and milling by-products as concentrate (crushed rice and/or sometimes mustard oil cake) with a mineral mixture, and salt. Fresh tap water was available *ad libitum*. All the selected buffaloes were dewormed by using Triclabendazole 900 mg and levamisole 600 mg/tablet (Tablet Renadex®, Animal Health Division, Renata Limited, Dhaka, Bangladesh) one month before beginning of the experiment. The buffaloes used were free from any apparent anatomical, physiological or reproductive disorders. However, all buffaloes were vaccinated against four major diseases namely Anthrax, Foot and mouth disease (FMD), Black quarter (BQ) and Haemorrhagic septicemia (HS). The buffaloes were kept under loose housing conditions in clean, hygienic paddocks with brick flooring, asbestos roofing, and sufficient space for the free movement of the animals.

2.2 Ovsynch Treatment and Fixed Timed AI

Estrus was synchronized in three different seasons of Bangladesh; namely in Spring (mid February to mid April; 20-32°C), Rainy/ Monsoon (mid June to mid August; 24-30°C) and Late Autumn/ Hemonto (mid October to mid December; 17-22°C). Estrus was synchronized according to Ovsynch protocol by the administration of a GnRH analogue on Day 0 (Gonadorelin 500 µg, Ovurelin®, BOMAC, New Zealand), followed by PGF2α treatment (Cloprostenol 500 µg, Ovuprost®, BOMAC, New Zealand) on Day 7 and a second GnRH treatment (Gonadorelin 250 µg) at 48 hour after PGF2α treatment (on Day 9) (Fig. 1). On Day 7, buffaloes were divided into two groups (morning group, 7.00 AM, n=52 and evening group, 7.00 PM, n=45). All treatments were given as intramuscular injections. The frozen semen was imported from Italy (COFA, Agricultural Cooperative Society, Orezola, Tidolo sigh of Cremona, Italy). Post-thaw sperm motility was 50-60%. Fixed timed AI was done without considering the onset of prominent estrous signs as there is no relationship between estrus stage and conception rate in the PGF2a treatment [18]. Each buffalo received a single artificial insemination with frozen semen (AI straw gun) from Mediterranean buffalo bulls at 18 to 20 hours (fixed timed AI) following second GnRH injection.

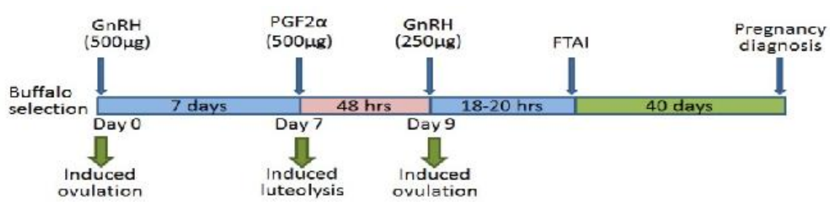

Fig 1. The Ovsynch treatment protocol for estrous synchronization of indigenous dairy buffaloes

The Ovsynch treatment protocol includes the administration of a GnRH analogue on Day 0 (Gonadorelin 500 µg, Ovurelin®, BOMAC, New Zealand), followed by PGF2α treatment (Cloprostenol 500 µg, Ovuprost®, BOMAC, New Zealand) on Day 7 and a second GnRH treatment (Gonadorelin 250 µg) at 48 hour after PGF2α treatment (on Day 9). FTAI was performed at 18 to 20 hours following second GnRH injection and conception was diagnosed by the application of real-time transrectal ultrasonography using an ultrasound scanner (YSVET0206 Veterinary Ultrasound Machine, Guangzhou Yueshen Import & Export Co. Ltd., China) on Day 40 post-insemination

2.3 Conception and Pregnancy Diagnosis

Conception was diagnosed by the application of real-time transrectal ultrasonography using an ultrasound scanner (YSVET0206 Veterinary Ultrasound Machine, Guangzhou Yueshen Import & Export Co. Ltd., China) on Day 40 (Day 40 is the 40th day after FTAI) post-insemination. Buffaloes were considered pregnant only those were not become in estrus after AI and finally calved the newborn with the normal range of gestation.

2.4 Statistical analysis

The conception and pregnancy rates obtained in different experimental groups were compared and analyzed using Chi square test [19] with the help of SPSS statistics version 20.

3. RESULTS

Ovsynch treatment induced estrus signs 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, swollen vulva and clear mucus hanging from the vulva. Bellowing and frequent urination were observed in all buffaloes. No buffaloes were examined for standing estrus by buffalo bull.

The conception rate was 56.70% (55/97) in indigenous river type buffaloes that was confirmed by transrectal ultrasonography on Day 40 post-AI. Ultrasonic image of a buffalo embryo on Day 40 of pregnancy is depicted in Fig. 2. The conception rate was varied from 44% to 64.87% in different seasons of Bangladesh (Table 1), though the difference among seasons was insignificant (p>0.05). The conception rate was the highest in late autumn (Hemonto) season when the temperature was low (17-22°C) and the time of breeding season of buffalo. The conception rate was also varied depending on the time of administration of hormones as well as the time of insemination (Table 2). The conception rate was the higher (61.54%) in morning group than evening group (51.11%). The overall pregnancy rate was 31.96%, though the rate varied from 28% to 35.14% in different seasons of experimental breeding (Table 1) in this study.

4. DISCUSSION

To the best of our knowledge this is the first study conducted on estrus synchronization in indigenous river type water buffaloes (at Farm level with large population) using Ovsynch protocol in Bangladesh. This study was designed to evaluate the efficacy of the Ovsynch protocol for estrus (ovulation) synchronization and conception rate to FTAI in indigenous river type water buffaloes.

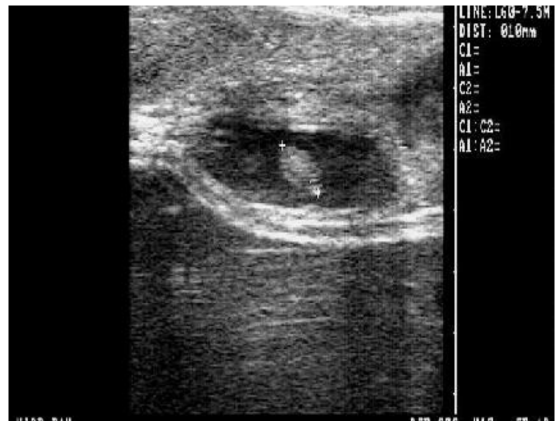

Fig 2. Ultrasonic image of an indigenous buffalo embryo on Day 40 post-AI

The reproductive function of buffaloes is a major factor determining the economic significance of this animal species [20]. Incidence of anestrus is higher (56.0%) in buffalo heifers than cow heifers (36.0%) [21]. Reproductive efficiency of buffaloes is hampered by inherent prolonged inter-calving interval [22]. In this study, ovsynch treatment induced estrus signs in all buffaloes. This indicated that animals were cyclic but estrus was not detected due to their nature of showing silent estrus behaviour. Silent estrus is a common problem in buffaloes even under good management and non-stressful periods of the year [23]. The silent estrus results in difficulties to determine the exact time for AI. That is why, in buffaloes, oestrous induction and a fixed-timed AI is regarded as more efficient approach. In this experiment, using FTAI with Ovsynch protocol, conception rate was 56.70%. In the study of Berber et al. [24] in cross-bred (Murrah x Mediterranean buffaloes) and Bartolomeu et al. [25] in Brazilian buffaloes, conception rates of 55.6–64.2% were reported after TAI, which are similar to present investigation. Reasons for conception rate difference in response to the same treatment in different studies may be attributed to differences in breed and condition of environment. In that regard, substantial variations in conception rates have also been reported in cattle treated with this protocol. In beef cows, conception rates exceeding 60% were obtained by Roy and Twargiramungu [26],

Table 1. Conception and pregnancy rate after Ovsynch treatment and FTAI in indigenous buffaloes in different seasons of Bangladesh

Season	Conception rate (%)	Pregnancy rate (%)
Spring (mid February to mid April; 20-32°C) (n=25)	44.00[a]	28.00[a]
Rainy/ Monsoon (mid June to mid August; 24-30°C) (n=35)	57.14[a]	31.43[a]
Late Autumn/ Hemonto (mid October to mid December; 17-22°C) (n=37)	64.87[a]	35.14[a]
Overall	56.70	31.96

a superscript in the same column shows no significant difference (p>0.05)

whereas in dairy cows, conception rates ranged from 40 to 55% [9,27]. This first study using Ovsynch protocol in Bangladesh reported the conception rate that agrees the reports of previous studies in different countries demonstrate that the use of Ovsynch protocol for FTAI is feasible in buffalo reproduction in Bangladesh.

Table 2. Conception rate in respect of the time of administration of hormones and insemination in indigenous buffaloes

Time of ovulation induction treatment and insemination	Conception rate (%)
Morning group (n=52)	61.54[a]
Evening group (n=45)	51.11[a]

a superscript in the same column shows no significant difference (p>0.05)

Breeding season influenced the conception rate (CR). In present study, the highest conception rate was in late autumn (Hemonto) season (64.87%) and the lowest rate was in spring season (44%) of Bangladesh. The differences of CR among different seasons were insignificant (p>0.05). Baruselli et al. [28] and Warriach et al. [29] reported that reproductive performance of buffaloes during the breeding season (autumn and winter) presented higher CR than reproductive performance of buffaloes during the off breeding season. Serena [30] found higher conception (pregnancy) rate during autumn compared to transitional period (January to March/spring) when the author performed a study on efficiency of FTAI in Mediterranean Italian buffalo after Ovsynch synchronization in two seasons. Mohan et al. [31] also observed the effect of temperature on conception rate and the authors found that the conception rate was 26% in summer season whereas, conception rate was 40% in winter season for buffaloes treated with Heatsynch protocol. Good conception rate in late autumn/ Hemonto season may be due to buffaloes were in breeding season and the temperature was lower compared to other seasons. In contrast, high temperatures of spring may be the reason for the lower conception rate obtained in our study. Reduced sexual activity in the buffalo has been associated to bio meteorological factors such as day length, ambient temperature, relative humidity and rainfall [32]. The heat stress is associated with increased uterine temperature and reduced blood supply to the uterus in cows. These changes inhibit embryonic development and increase embryonic loss [33].

Considering the time of administration of hormones as well as the time of insemination, 61.54% buffaloes were conceived in morning group, whereas 51.11% were conceived in evening group. Though the difference was not significant, it is important to increase success rate of Ovsynch protocol and in the point of view of economy of breeding farm. Srivastava and Sahni [34] reported that buffaloes showed more oestrous activity in the morning (06:00–07:30 hour) than in the afternoon (14:00– 15:30 hour) or during the night (22:00–23:30 hour). However, the lower temperature and humidity at morning than that of evening during ovulation induction treatment and insemination might be a favorable condition for obtaining higher conception or pregnancy rate in morning group than that of evening counterpart.

The reproductive seasonality of the dairy buffalo is the physiological factor that exerts the greatest impact on the economic activity. The overall pregnancy rate was 31.96% but it varied in different seasons of breeding (from 28.00% in spring to 35.14% in late autumn/ Hemonto season). Paul and Prakash [10] conducted Ovsynch protocol for synchronization of ovulation in Indian Murrah buffaloes and the pregnancy rates was 33.3% for TAI and was 30.7% for buffaloes inseminated following spontaneous estrus. Frares et al. [35] observed the pregnancy rate was 37% of the Brazilian buffaloes submitted to Heatsynch protocol for synchronization of estrus and induction of ovulation followed by FTAI. The achievement of present study was similar to some extent in success of buffalo reproduction in earlier studies. The reason for difference in pregnancy rates among studies may be due to differences in breed of buffaloes, ovulation induction protocols and agro-climatic conditions of study areas in different studies. The 31.96% pregnancy rate resulted in the concentration of parturitions and subsequently in improved milk production in the period when milk availability was beneficial for the small local producers of Bangladesh. However, effective measures should be taken to improve embryo survival and ultimately for establishment of a high rate of pregnancy to make breeding programs successful.

5. CONCLUSION

In conclusion, the Ovsynch protocol effectively synchronized estrus in indigenous river type buffaloes with a reasonable conception rates after FTAI. Estrus synchronization and fixed timed insemination could be applied in buffaloes for overcoming the estrus detection and breeding seasonality problems in Bangladesh, though more studies are needed to increase the conception rate. However, season and the time of day should be considered during administration of hormones for Ovsynch protocols and insemination. An improved post-conception management needs to practice to achieve the higher pregnancy rate in indigenous buffalo. Therefore, this study clearly indicates the opportunity for practical application of the Ovsynch protocol for estrus synchronization and FTAI for successful breeding program in indigenous river type buffaloes in Bangladesh. It will be important to further elucidate the factors that can contribute to increase pregnancy in dairy buffaloes so that strategies can be developed to optimize fertility to synchronization and AI during periods of reduced reproductive activity in Bangladesh.

ACKNOWLEDGEMENT

Authors would like to thank the farm (Lal Teer Livestock Research and Breeding Farm, Mymensingh, Bangladesh) owner as he allowed us to perform the study in his farm and for providing financial help to buy the semen, hormone and drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:http://dx.doi.org/10.1590/S1516-89132013000400007

Peer-review history:
The peer review history for this paper can be accessed here:
http://prh.sdiarticle3.com/review-history/18317