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Estrus synchronization of native buffaloes using PGF2α and GnRH based protocols in Bangladesh

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Abstract

The inherent low reproductive efficiency of buffalo results in lower productivity. In buffalo overt signs of estrus are not as pronounced as in cattle, which hinders the reproductive efficiency of this species. The purpose of this study was to evaluate the effectiveness of GnRH and PGF2α-based estrus synchronization procedures in native buffaloes. About 16 indigenous buffaloes were equally grouped into two and treated with GnRH and PGF2α. In the GnRH-based treatment group of eight (8) buffaloes, the 1st 5ml GnRH injection was administrated initially (Day 15). Nine (09) days after the administration of the 1st GnRH injection, 5ml PGF2α was injected (Day 6) which was followed by the 2nd 5ml GnRH injection two (02) days after PGF2α administration (Day 4). In the case of PGF2α treated group, an initial injection of 5ml PGF2α was administrated to initiate the synchronization process (Day 15) which was followed by the 2nd injection of 5ml of PGF2α 12 days after the administration of 1st PGF2α injection (Day 4). In the GnRH-based treatment group, all buffalo cows (n=8) were inseminated artificially 16 hours after the last GnRH administration. In the PGF2α treated group, buffaloes (n=8) were inseminated artificially 90 hours after the last PGF2 α administration. Visual monitoring of estrus signs and rectal examination during AI were used to determine the estrus response rate. All buffaloes were examined rectally for confirmation of pregnancy at 60 days post-insemination. Non-significant differences ($p>0.05$) were found between GnRH and PGF2α treated group in terms of responses of estrus synchronization (75% vs. 62.5%) and conception rate (37.5% vs. 25%) respectively. In conclusion, both the GnRH and PGF2α based protocols could be used to synchronize the estrus of indigenous buffaloes in Bangladesh.

Key words: Buffalo, overt signs of estrus, estrus synchronization, artificial insemination.

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Introduction

Seasonality nature in breeding, late maturity, trivial estrus expression, lengthy inter-calving intervals and impaired ovarian activity during non-breeding season reduce the reproductive efficiency of female

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buffalo which is responsible for lower productivity in this species (Madan *et al.*, 1996; Singh *et al.*, 2000). Additionally, the success rate of artificial insemination (AI) in water buffalo is low because of poor detection of estrus, variable estrus duration (4-64 hours), and difficulty in determining the ovulation time (Barkawi *et al.*, 1993; Ohashi, 1994; Baruselli *et al.*, 2001). The overt signs of the estrus in buffaloes are not as pronounced as in cattle, which hinders the reproductive efficiency of this species (Arthur *et al.*, 1996). High reproductive efficiency is important for achieving the maximum economic benefits which could significantly enhance the standard of living of small holder in Bangladesh (Alam and Ghosh, 1991).

Estrus synchronization is an important tool to control breeding of animals for silent/not pronounced estrus and anestrus. Estrous cycle synchronization and artificial insemination AI can be applied to improve fertility of livestock. Poor expression of estrus signs and anestrus postpartum period during the non-breeding season which increase days open and obstruct yearly calving can be addressed with Estrus synchronization combined with artificial insemination (Roy and Prakash, 2009; Baruselli *et al.*, 2013; Kumar *et al.*, 2012). In the first month of the breeding season, when estrus synchronization is utilized, 50-75 % of pregnancies can be achieved without detecting detection. (Baruselli *et al.*, 2013; Crudeli and de La Sota, 2011). It has been shown to use in both natural and manipulative breeding for example super ovulation and embryo transfer technique (Bari *et al.*, 1999).

These approaches of the estrus synchronization have become more frequently used in buffalo due to its success in cattle. (Colazo and Mapletoft, 2014; Bridges and Lake, 2011). Estrus synchronization technique has been used in buffaloes for many years, yet there are still considerable limitations. Because of few numbers of buffalo per farmer, low awareness on the part of farmers and, poor success of estrus synchronization regimens particularly during non-breeding season, this bio-technology has not been adopted in developing countries. Despite having 2-3 follicular wave in each estrous cycle of buffalo, follicles recruited in each follicular wave is fewer than that is in cattle which compromise the success of synchronization techniques in buffalo (Gimenes *et al.*, 2009; Baruselli *et al.*, 2013 and Campanile *et al.*, 2010).

In the past decades, several protocols for synchronizing estrus and ovulation have been invented. GnRH and PGF2α based protocols are used successfully in several species for estrus synchronization. The GnRH and PGF2α based strategies have been demonstrated to be effective in synchronizing estrus in cattle and buffaloes. (Lamb *et al.*, 2001, 2004; Odde 1990 and Amaya *et al.*, 2007). However, little is known about the efficacy of these protocols in indigenous buffaloes of Bangladesh.

Here estrus synchronization procedure was performed using GnRH and PGF2α based protocols to compare the efficacy of these two protocols in indigenous buffaloes in the Jamalpur region of Bangladesh.

Materials and Methods

Animals

The experiment was conducted on 16 cyclic female native buffaloes at the Jamalpur district. The body condition score of the buffaloes ranged from 2.5 to 3.0 where 1.0 is for very Lean and 5.0 is for very fatty. The estimated age and live weight of the buffaloes were 4.5 years and 200 kg, respectively. Average number of calving of the buffaloes ranged from 2 to 3. The health status of the experimental animals was clinically checked. Any sort of morphometric/developmental defect or genetic abnormality or any unusual perparturient events were absent that could influence the synchronization response.

Drugs and biologics

The drugs used in this study were synthetic PGF2α, Ovuprost (a PGF2α analogue, each ml contains 250 μg Cloprostenol as sodium, Bayer New Zealand Limited) and synthetic GnRH, Ovurelin (a GnRH analogue, each ml contains 100 μg Gonadorelin as acetate, Bayer New Zealand Limited).

Estrus synchronization

Before beginning the synchronization techniques, the buffalo cows underwent a thorough examination by trans-rectal palpation to check their reproductive health. Throughout the experimental period, estrus synchronization was initiated at the same time of day and was done using two distinct procedures. The buffalo cows were divided into two groups of eight (8) animals randomly.

GnRH based protocol

In GnRH based treatment group of eight (8) buffaloes to synchronize the ovulation the 1st 5ml GnRH injection was administrated initially (Day 15). Nine (09) days after the administration of 1st GnRH injection, 5ml PGF2α was injected for the regression of resulting Corpus luteum (Day 6) which was followed by 2nd 5ml GnRH injection two (02) days after PGF2α administration to improve ovulation synchrony (Day 4)

PGF2α based protocol

In the 2nd group of eight (08) buffaloes, PGF2α was employed solely to synchronize estrus. In this procedure to lyse the present CL an initial injection of 5ml PGF2α was administrated to initiate the synchronization process (Day 15) which was followed by 2nd injection of 5ml of PGF2α 12 days after the administration of 1st PGF2α injection (Day 4)

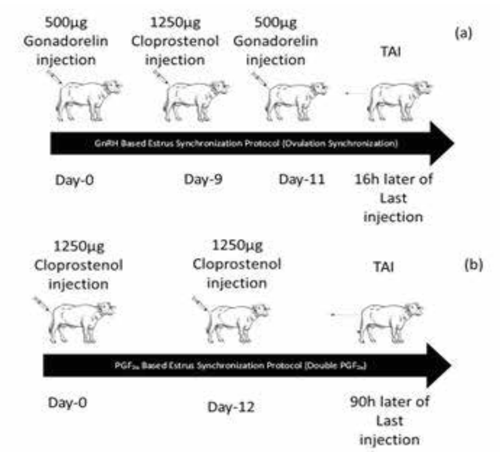

Fig. 1. Protocols of estrus synchronization; (a) Estrus synchronization using GnRH based protocol. 1st 5ml GnRH injection was administrated initially (Day 15). Nine (09) days after the administration of 1st GnRH injection, 5ml PGF2α was injected (Day 6) which was followed by 2nd 5ml GnRH injection two (02) days after PGF2α administration (Day 4) and finally AI was performed hours later of last injection (b) Estrus synchronization by double PGF2α based protocol. To lyse the present CL an initial injection of 5ml PGF2α was administrated to initiate the synchronization process (Day 15) which was followed by 2nd injection of 5ml of PGF2α 12 days after the administration of 1st PGF2α injection (Day 4) and AI was performed 90 hours later of last injection.

Route of injection administration
All the injections were administrated intramuscularly in the anterior half of the neck and the injection sites were thoroughly cleaned by alcohol solution before administration.

Artificial insemination
In the GnRH based protocol group, all 8 animals were artificially inseminated 16 hours after the last GnRH administration and in case of double PGF2α based protocol group, all the animals were inseminated artificially 90hrs after the last PGF2α administration. Frozen semen straws of buffalo bulls were purchased from the Department of Livestock Services (DLS) and used for AI. After microscopically inspecting the semen quality all the inseminations were performed by an experienced AI worker.

All the treated animals were observed closely for estrus signs such as reddish and swollen vulvar lips, mucus discharge from vaginal wall, moist-congested vulvar mucus membrane, frequent urination, feed intake and cervix opened. Transrectal palpation was done to check the pregnancy at 60 days after insemination

Statistical analysis
Data were analyzed by independent t- test using SPSS version 25.0. Differences at $p < 0.05$ were considered statistically significant

Results

Estrus Responses
Considering the estrus signs observed during AI, estrus rates in terms of estrus synchronization were recorded 75% (6 out of 8 animals) and 62.5% (5 out of 8 animals) in GnRH and PGF2α based protocols, respectively (Figure 2). However, the results revealed no significant difference between the two protocols ($p>0.05$).

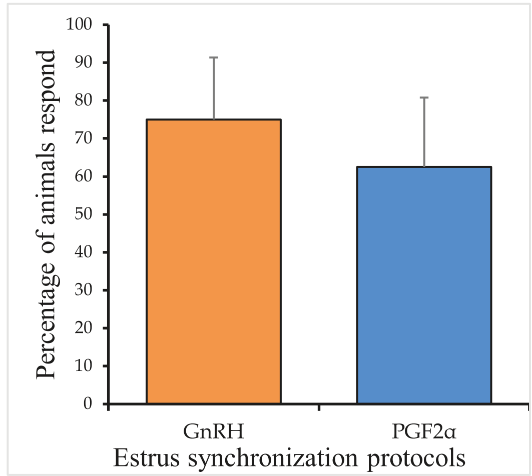

Figure 2. Responses of estrus synchronization in buffaloes

Conception rates of buffaloes synchronized with GnRH and PGF2α based protocols
The present study showed that conception rates in buffaloes were 37.5% and 25% in GnRH and PGF2α based protocol, respectively (Figure 3). However, the results revealed no significant difference between the two protocols ($p>0.05$).

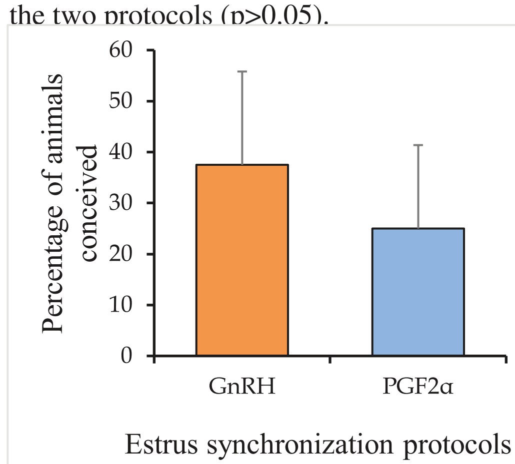

Figure 3. Conception rate of estrus synchronized buffaloes

Discussion

In the present study, PGF2α and GnRH-based protocols induced estrus signs in 62.5% and 75% of buffaloes, respectively. The presence and status (size and dominance) of the dominant follicle (DF) and corpus luteum (CL) during PGF2 injection determine the success rate of PGF2 α treatment to bring the animal to estrus. PGF2α would be effective in most buffaloes with a CL and DF of less than 1.0 cm while buffaloes with a DF and CL greater than 1.0 cm do not react well (Brito *et al.,* 2002). None of the 12 postpartum acyclic buffaloes treated with PGF2α responded to the treatment, suggesting that the results of estrus synchronization using PGF2α in pre-pubertal buffalo heifers and postpartum anestrus buffaloes may not be ideal (Honparkhe *et al.,* 2008) possibly due to the absence of active CL on which PGF2α would have to be effective. In addition, numerous factors control the effects of PGF2α, such as the day after parturition (Usmani, 2001), nutritional state (Hussein and Abdel-Raheem, 2013), and others. An experiment by Singh and Madan (2000) showed that to induce estrus in buffaloes, the nutritional state and present follicular and luteal activity seem to be crucial. It seems that a significant factor influencing success with PGF2α treatment is the season. When buffalo heifers received a single PGF2α injection during the low breeding season, their estrus rates were 60%, but during the breeding season, they were 86.6% (Chohan *et al.,* 1993). The PGF2 α-based protocol used in this study for estrus synchronization produced results that were similar to previous findings of Jindal *et al.* (1988), Siregar *et al.* (2015), El-Belely *et al.* (1995) and Hassan *et al.* (2016). Jindal *et al.* (1988) did an experiment where they synchronized the estrus in lactating buffaloes and buffalo heifers by giving PGF2α intramuscularly at the dose rate of 25 mg (5ml) per animal following two different schedules and found 90% of the buffalo cows and 100% of the buffalo-heifers came in estrus following a single injection of PGF2α given after palpation of a mature CL whereas, this response was only 80% and 67.67% in randomly cycling lactation buffaloes and buffalo heifers, respectively, which were given double injections of PGF2 α at an interval of 11 days. El-Belely *et al.* (1995) observed the responses of estrus after two PGF2α treatments. The impact of a double PGF2α injection was assessed in 48 suboestrous buffalo cows which had palpable CL. Within 5 days following the second PGF2α injection, 37 (77%) of the 48 cows under study displayed oestrus. Hassan *et al.* (2016) showed that after receiving two doses of PGF2α spaced 11 days apart, a 70% response rate was observed in Egyptian buffalo heifers.

In GnRH-based protocol, a new wave of follicular growth is triggered when a dominant follicle ovulates, which is facilitated by the administration of the first GnRH injection. When first GnRH is administered, the largest follicle may regress or continue to grow and eventually ovulate (Day and Geary, 2005). More accurate regulation of ovarian follicular growth and estrus is achieved by administering PGF2α seven days later while a second injection of GnRH two days later ensures ovulation. The existence of a DF at the time of the first GnRH injection is necessary for the protocol to be successful (De Rensis and Lopez-Gatius, 2007). Additionally, the DF stage affects ovulation as well. The growth and regression phases of the first wave DF in buffaloes are considered as Day 6 and Day 10 of the estrus cycle, respectively and during these phases, 75.0 and 16.67% of buffaloes ovulate in response to GnRH (Dharani *et al.,* 2010). The size of the DF is likely to affect the largest follicle's ovulation during the 1st GnRH administration. (De Rensis *et al.,* 2005). During the first GnRH injection, 100% of adult buffaloes and 87.5% of heifers ovulated when the largest follicle measured more than 8 mm (Derar *et al.,* 2012). The results of estrus synchronization in GnRH-based protocol in this study were comparable to other findings of Nazmul *et al.* (2014), and Rathore *et al.* (2017). Nazmul et al. (2014) found a 72% ovulation rate with GnRH-based protocol in water buffaloes in Bangladesh. Rathore *et al.* (2017) carried out a study to evaluate the effectiveness of synchronization protocols based on gonadotropic hormone (GnRH) and found an 82% estrus response rate in terms of estrus synchronization. The present study's results were in line with all of the previously mentioned literature's findings. In this study, it was observed that both GnRH and PGF2α based protocols are usable in buffalo to induce estrus.

Kharche and Srivastava (2001) demonstrated that in buffalo estrus and conception rates obtained after PGF2α treatment are low compared to cattle. The average conception rate is 50-60% in natural service and half of the value (25-30%) is found in artificially inseminated buffaloes (jainudeen, 1986) whereas the value is almost double in cattle. The most likely causes of these variations are overt signs of estrus that make it difficult to detect estrus in these animals and the poor body condition that frequently affects follicular events in postpartum buffalo cows. The season at which the prostaglandins are administered was found to be a vital factor along with days after parturition and feeding management that control the effects of prostaglandins (Usmani, 2001; Hussein and Abdel, 2013). In the low breeding season when buffalo heifers were administered a single PGF2α injection, these showed estrus rates of 60% and poor conception rates (22.8 - 25.6%) (Chohan *et al.,* 1993) in contrast in the breeding season estrus rates of 86.6% and conception rates ranging from 47.8 to 53% had been observed (Purohit *et al.,* 2019). In the current experiment conception rates in buffaloes were found 37.5% (3 out of 8 animals) and 25% (2 out of 8 animals) in GnRH and PGF2α based protocols, respectively. These results were consistent with some previous reports. Hassan et al. (2016) found a 35% conception rate in Egyptian buffalo heifers treated with PGF2α double injection protocols and timed artificial insemination which was consistent with the present study. There were some studies in which the conception rates were higher than the present study. The Jaffrabadi buffaloes treated with two injections of prostaglandins in 11 days followed by artificial insemination were shown to achieve a 49% conception rate (Ahlawat *et al.,* 2015).

With results of estrus rates ranging from 41.6 to 91.9% and conception rates from 11.11 to 68.8%, the GnRH-based protocols have been applied to buffaloes in several different countries (Purohit *et al.,* 2019). Age and season of treatment have some effect on buffaloes treated with GnRH-based protocols. Buffalo heifers treated with GnRH-based protocols to synchronize estrus resulted in decreased conception rates (18.8-40%) while in adult buffaloes the conception rate was comparatively better (Presicce *et al.,* 2005; Irikura *et al*., 2003). It has been demonstrated that there is a positive correlation between breeding season and the effectiveness of GnRH-based protocols considering estrus and conception rates (Estrus rates 87.5% versus 36.3% and conception rates 40% versus 11.1%) (Jabeen *et al.,* 2013; Warriach *et al.,* 2008 and Baruselli *et al.,* 2002)

Conclusions

In conclusion, both GnRH and PGF2 α-based protocols successfully synchronized estrus in domestic water buffalo despite low conception rates after artificial insemination. Estrus synchronization along with timing AI could be used to overcome the problem of estrus detection and the seasonal breeding nature of buffaloes in Bangladesh, but further research is needed to increase conception rates. However, to get optimum results before administering hormones in both protocols and AI, feeding regimen, season, and time of day must be considered. To gain higher pregnancy rates in domestic buffaloes, post-conception management needs to be improved. Hence, this experiment neatly demonstrates the potential of the practical application of GnRH and PGF2α-based estrus synchronization protocols and artificial insemination for the successful breeding of domestic buffaloes in Bangladesh.

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