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Research Article

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Effect of timing of synchronization and follicular status on the pregnancy rate of Bangladeshi Water buffaloes

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Abstract

The purposes of this study were to determine the effects of follicular status (number and size) at beginning of estrus induction, the timing of estrus synchronization and insemination (morning vs. evening), season (decreasing day length vs. increasing day length) in pregnancy rate of synchronized Bangladeshi water buffaloes. A total of 79 lactating indigenous water buffalo cows were used and synchronized with modified Ovsynch protocol (GnRH+$PGF_{2\alpha}$+GnRH+GNRH) for estrus detection. Buffaloes were divided into two groups as Group-1 (n=54) and Group-2 (n=25) according to day length. Each group was subdivided as AM and PM according to the time of administration of hormones for synchronization protocols. AI was performed using frozen-thawed semen. Pregnancy was confirmed after watching viable embryos at day 40 days post-AI by trans-rectal ultrasonography. The overall pregnancy rate was 56.25% in this study. A higher pregnancy rate (77.7%) was observed in buffaloes of Group-1 where synchronization was conducted in the morning in September (during decreasing day length). Follicular size at the time of AI significantly ($P < 0.05$) affected the pregnancy rate. The findings of the study suggest that follicular size, time of AI and season should be considered during estrus induction for the improvement in the pregnancy rate of water buffaloes when inseminated with frozen-thawed semen.

Keywords: Buffaloes, Follicular size, Synchronization, Artificial Insemination, Pregnancy rate

INTRODUCTION

Buffalo is known to be a seasonally polyestrous animal. The duration of the estrous cycle in buffalo ranges from 17 to 26 days with a mean of around 21 days (Jainudeen and Hafez, 1993). Artificial insemination (AI) in buffaloes has limited use worldwide due to the difficulties in estrus detection and in finding an adequate moment for this procedure due to covert estrus. Herd management and estrus detection are important issues in AI programs for buffalo (Singh *et al.*, 2000). These limitations are intensified during the hot season when fertility decreases abruptly. The breeding frequency in buffaloes is highest during the winter, decreased in autumn and spring, and is lowest in the summer due to increasing daylight and heat (Shah, 1988). Adverse environmental conditions, nutrition and irregularities in the secretion of ovarian steroid hormones (Nanda *et al.*, 2003) affect buffalo's reproduction. The period of postpartum anestrous is usually longer in buffalo than in cattle un-

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der comparative management conditions (Jainudeen and Hafez, 1993). These considerations indicate a need for estrus synchronization using fixed-timed AI for the implementation of breeding programs in buffalo (Ali and Fahmy, 2007) and it has been reported as an efficient approach for estrus synchronization in buffaloes elsewhere (Presicce *et al.*, 2005). Estrus synchronization allows the wider application of AI as modern reproduction biotechnology in buffaloes (Baruselli *et al.*, 2003).

There is limited study on estrus induction and fixed-time AI in buffaloes of Bangladesh. Previously, Hoque (2009) and Saha (2012) had conducted studies on the application of estrus synchronization protocols using Ovsynch (GnRH+PGF2α+GnRH) and TAI in Bangladeshi river type buffaloes. In this experiment, an extra GnRH injection (3rd dose of GnRH) was performed at the time of AI in buffaloes synchronized using Ovsynch protocol. This study was conducted to observe the effects of timing of induction of estrus and AI and follicular status (number and size) at the inception of estrous induction and pregnancy.

MATERIALS AND METHODS

The study was conducted at a breeding farm (Lalteer Livestock Ltd.), Bhuapur, Tangail from September 2012 to April 2013.

Animal selection and their management

A total of 107 buffaloes were examined during the study period. Of 107 buffaloes a total of 79 buffaloes were selected after monitoring of ovarian follicles with transrectal ultrasonography. Buffaloes containing follicle size ¡4mm were discarded from this study. All buffaloes were reared under a semi-intensive system. The buffaloes were between 3.5 and 7 years of age. The animals were fed with paddy straw, concentrate mixture, cut-and-carry grass and milling by-product according to their body weight and milk production. Vitamin premixes (Powder Renavet-DB®, Renata Animal Health, Mirpur, Dhaka, Bangladesh) were also supplied to the buffaloes with concentrate. All the selected buffaloes were dewormed by using Nitroxyline (Bolus Nitronex®, Renata Animal Health, Mirpur, Dhaka, Bangladesh) 7 days before the beginning of the experiment.

Estrus induction and AI

Buffaloes were divided into two groups as Group-1 (n=54) and Group-2 (n=25) according to day length when the protocol was conducted. IN Group-1, buffaloes were induced from September to October (decreasing day length) and induction was performed in buffaloes of Group-2 from February to March (increasing day length). For estrus induction in buffaloes of both groups, 500 µg Gonadorelin (5ml Ovurelin®, Bomac Laboratories Ltd., New Zealand) was administered at the intramuscular route (i/m) followed by i/m administration of 500 µg Cloprostenol (2ml Ovuprost®, Bomac Laboratories Ltd., New Zealand) at 7 days interval. Forty-eight hours after Prostaglandin F2α analogue administration, 150 µg of GnRH analogue was also administered at the i/m route. At 18-20 hours after the second gonadorelin injection, AI in buffaloes was carried out by a trained AI technician using frozen-thawed semen and 3rd dose of gonadorelin injection was given in a small amount. The frozen semen was imported from Italy (Italian Buffalo Breeding Agency, Italy). Buffaloes in both groups were divided into two subgroups namely AM and PM groups according to the timing of day for estrus induction and insemination. In buffaloes of AM sub-group, the protocol was conducted at 7 .00 am, and injection was given at 5.00 pm in buffaloes of the PM sub-group.

Transrectal-Ultrasonography

B-mode digital Ultrasound system (Vet Eickemeyer Magic 5000) with transrectal probe 7.5 MHz, (Probe type: C20615S), was used transrectally to monitor the ovaries and pregnancy. The number and Diameter of follicles were measured by ultrasonography during 1st injection of GnRH and the time of AI. before insemination. Pregnancy diagnosis was performed 40 days after AI and data were recorded. Confirmed pregnant buffaloes were recorded after watching viable embryos visible on the viewing screen of the ultrasound machine.

Statistical analysis

A Chi-square test was used to analyze the pregnancy rate. An unpaired T-test was used to observe the overall difference in pregnancy rates in the two groups. All statistical analysis was performed using the Statistical Package for Social Science, SPSS (17.0) system for windows and $P < 0.05$ was considered as a significant value.

RESULTS

The effect of some factors such as time of synchronization protocol, seasonal effect (decreasing day length or increasing day length) and time of AI (morning or evening) in Bangladeshi water buffaloes inseminated with frozen-thawed semen was studied and overall pregnancy rates obtained in response to these factors are given in Table 1 and Figure 1. The overall pregnancy rate was 56.25% in this study. The pregnancy rate was significantly ($P < 0.05$) higher when TAI was performed during September-October (72.5%). When compared, the overall pregnancy rate was higher (62.5%) in buffaloes who received induction treatment and insemination in the morning than that of their evening counterpart (48.5%). The pregnancy rate was (77.7%) in Group-1 and (46.7%) in Group-2 when the modified Ovsynch protocol started in the morning and values were significantly ($P < 0.05$) higher in comparison to that of the evening sub-groups.

Table 1: Effects of day length and time of AI and synchronization protocol on the pregnancy rate in water buffaloes

Factor	**Pregnancy rate (%)**
AI at decreasing day length (September-October)	72.5*
AI at increasing day length (February-March)	40.0
At the morning	62.5*
At the evening	48.5

* Significant ($P < 0.05$)

Table 2: Number (Mean ± SD) of follicles (FL) and corpus luteum (CL) at the time of selection in pregnant and non-pregnant buffaloes of two groups

Groups	**Buffaloes**	**Left ovary**		**Right ovary**	
		FL	**CL**	**FL**	**CL**
Group-1	Pregnant	3.82 ± 0.22	1.00 ± 0.17	3.95 ± 0.24	1.33 ± 0.16
	Non pregnant	3.27 ± 0.35	1.00 ± 0.20	3.33 ± 0.39	1.00 ± 0.16
Group-2	Pregnant	2.90 ± 2.18	0.00	2.10 ± 0.57	0.00
	Non pregnant	2.27 ± 1.39	0.00	2.07 ± 1.22	0.00

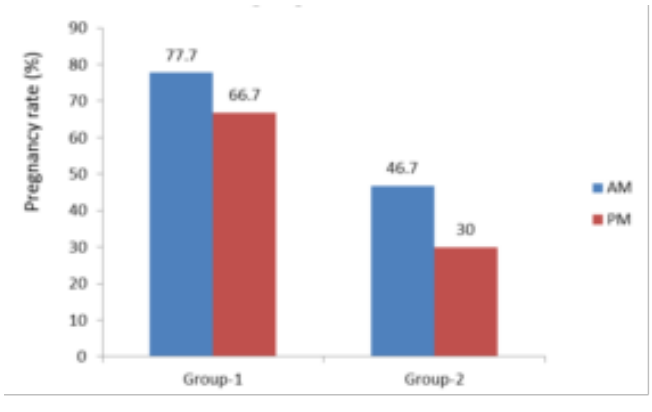

Figure 1: Pregnancy rate obtained in buffaloes of AM and PM subgroups within the group.

We considered and monitored ovarian follicular status (number and size), uterine status, number of follicles (FL) and corpus luteum (CL) with trans-rectal ultrasonography during the first administration of GnRH and results are presented in Table 2. During AI and we did not find any significant ($P > 0.05$) difference as regards follicles and corpus luteum numbers in pregnant and non-pregnant buffaloes of both groups (Table 2).

Table 3: Follicular size (Mean ± SD) at the starting of Protocol (day 0) and at the time of AI (day 10) in the buffaloes

Buffaloes	**Follicular size (mm)**	
	At the starting of protocol (day 0)	**At the time of AI (day 10)**
Pregnant	4.75	8.5*
Non pregnant	5.43	6.4

* Significant ($P < 0.05$)

Table 3 represents values of the follicular size observed at different times during synchronization protocol in pregnant and non-pregnant buffaloes. The result showed no significant ($P > 0.05$) effect of follicular size and number at the starting of protocol on the pregnancy rate.

But follicular size present at the time of AI varied significantly ($P < 0.05$) between pregnant and non-pregnant buffaloes.

DISCUSSION

Covert estrus is a prevalent condition in buffaloes (Abdallah, 2003) and estrus detection is one of the key limits for satisfactory reproductive performance. Cyclic buffaloes are not identified by farmers even under appropriate management and during non-stressful times of the year, due to their habit of expressing silent heat. Therefore, in the current investigation, the modified Ovsynch protocol was adopted in indigenous water buffaloes.

The current study clearly stated that when estrus was triggered during decreasing day duration using a combination of GnRH analogue and PGF2 7 days apart, followed by GnRH analogue administration and AI utilizing frozen-thawed semen, 72.25% of buffaloes became pregnant. When compared to the results of other studies, which ranged from 27.2 to 42.4 percent (Baruselli *et al.*, 1997; Irikura *et al.*, 2003; Neglia *et al.*, 2003; Paul and Prakash, 2005), the conception rate observed in this study is regarded high.

The obtained pregnancy rate of this study matches that of Berber *et al.* (2002), who found a 56.5% of pregnancy rate in buffaloes. At the time of AI, we employed a third GnRH injection. The first GnRH injection can successfully synchronize a new follicular wave 1-3 days after therapy (Neglia *et al.*, 2003; Ali and Fahmy, 2007), and this wave leads to the establishment of a new dominant follicle, which could be due to the low P4 concentrations. Sub-luteal circulating P4 levels have been linked to a higher frequency of LH pulses and a longer dominant follicle growth phase (Bridge and Fortune, 2003). By lysis of both the cyclic CL and the CL resulting from ovulation of the dominant follicle, the subsequent injection of PGF2 enhances the percentage of synchronized animals (Pursley *et al.*, 1995).

It has been proposed that high P4 levels at the time of PGF2 administration may be a crucial factor in improving insemination conception rates (De Rensis *et al.*, 2005). The second dosage of GnRH is injected to ovulate the preovulatory follicle at a particular moment to improve ovulation synchrony (Wiltbank, 1998). On day 9 of the treatment, the second GnRH injection generates an induced LH surge, which leads to ovulation of the dominant follicle and the creation of a new CL (Senger, 2003). The third dose of GnRH may boost LH surge, ovulation, and fertilization in a synergistic manner. Differences in pregnancy rates between studies could be due to differences in buffalo breeds, follicular number and size, estrus induction protocols, and agro-climatic conditions of study areas in different studies, emphasizing the importance of selecting an appropriate estrus induction protocol.

Buffaloes in Group 1 were treated for estrus induction in September-October, while buffaloes in Group 2 were treated for estrus induction in February-March, and the results showed a significant difference in pregnancy rates between the two groups (72.5% and 40.0%). Using the Ovsynch procedure during the breeding season in buffaloes, Carvalho *et al.* (2007) reported a conception rate of 46.8%. When compared, Warriach *et al.* (2008) found no change in buffalo conception rates depending on the season of estrus induction. In contrast, Baruselli *et al.* (2003) found a significant difference in buffalo conception rates between estrus induction seasons. The variation in conception rates between studies based on the season of estrus induction could be attributed to the existence of a stressful environment with high temperatures and humidity. When comparing the time of day of administration of hormones and AI, we observed higher pregnancy rates in buffaloes who received hormones in the morning. The lower temperature and humidity might be a favorable condition for obtaining a higher pregnancy rate in AM group than that of the PM counterpart as described by Mollah (2013).

In the present study, B-mode modern ultrasonography was used for the determination of follicular status and pregnancy of buffaloes. Applications of ultrasound in research on buffalo reproduction have been documented in Bangladesh (Hoque, 2009). Results confirmed a significant ($p < 0.05$) impact of follicular size present at the beginning of the protocol. The time to ovulation after GnRH injection relies upon the diameter of the largest follicle at the time of injection, which is a figuring out thing for a successful synchronization of ovulation and high conception rates (Hussein *et al.*, 2002; De Rensis *et al.*, 2005). Moreover, the stage of follicular development (growing or regressing) is known to affect its response to GnRH treatment (Dharani *et al.*, 2010).

CONCLUSIONS

The findings of the study reveal that synchronization protocol can be successfully used for selective breeding programs of Bangladeshi Water buffaloes. The time of day should be considered during the administra-

tion of hormones for synchronization protocols. Moreover, this study suggests that a good knowledge about follicular wave dynamics studied with transrectal ultrasonography could facilitate in designing the proper synchronization protocol for the enhancement of reproductive efficiency in river buffaloes.

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