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Research Article

Enhancement of the Pregnancy Rate of Buffalo Cows Through Intra-Vaginal Bio-Stimulation with Penis-Like Device in the Coastal Area of Bangladesh

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Abstract | The study aimed to enhance the rate of pregnancy of water buffalo by intra-vaginal bio-stimulation after AI. The study was conducted from August 2022 to July 2023 in the selected coastal area of Bangladesh. Sums of 75 adult female buffaloes were selected. Age, breed, parity, and others were recorded during selection. The animals were grouped into three experimental groups (group A: natural service, group B: conduct AI only, group C: using penis-like device-PLD following AI) and each group consisted of 25 animals. It was found that the average rate of pregnancy was 46.6%. The rate pregnancy in group A (64.0%) was higher than that of other groups. However, among the AI, the rate of pregnancy in group C (44.0%) was comparatively higher than that of group B (32%). This study also found that the pregnancy rate had a significant (P<0.05) result on parity, reproductive health, and previous records of calving difficulties. It could be concluded that the use of a penis-like device helps to raise the rate of pregnancy of cows subsequent to AI. However, the PLD must be modified to make the device more convenient and useful in buffalo cows. In further study, it is suggested to modify the device with the clitoral massage.

Keywords | Buffalo, Bio-stimulation, Penis-like device, Pregnancy rate, Coastal, Risk factors

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INTRODUCTION

Livestock is an indispensable sector of the economy of Bangladesh that plays a vital role in sustaining rural economic growth. Approximately 1.90% of gross domestic product (GDP) is derived from livestock industry (BBS, 2022). Among the various livestock species, buffalo is one of the most important and is widely distributed throughout the country (Rahman *et al*., 2018). The whole buffalo inhabitants in Bangladesh are 1.471 million, which

are mostly reared in household continuation farming as well as bathan (free range rearing of animals at fallow land and river-basin area) in the coastal region (DLS, 2022). Buffalo's populaces in Bangladesh are typically the native type that covers swamp and riverine types which are distributed all over the country. Nevertheless, about 40% of the entire buffalo are in the coastal area, Jamuna-Brahmaputra and Meghna-Ganga floodplains measured to be the buffalo prone (Faruque *et al*., 1990). Coastal buffaloes are salinity tolerant and capable of roaming in saline water. While buffaloes exhibit relative resistance to infectious diseases, they are prone to various reproductive disorders. These include delayed onset of puberty, suboptimal estrus expression, longer postpartum ovarian quiescence, and notably lowered pregnancy rates, especially under artificial breeding conditions (Gordon, 1996).

Issues related to artificial breeding conditions in buffaloes are multifaceted. Challenges arise in ensuring optimal reproductive outcomes due to factors such as inadequate estrus expression, suboptimal synchronization protocols, and difficulties in achieving successful conception. Additionally, the handling and management of semen, as well as the intricacies of the artificial insemination process, present further hurdles (Balhara *et al*., 2022; Kumar *et al*., 2023). These challenges collectively contribute to reduced pregnancy rates and hinder the efficiency of artificial breeding programs in buffaloes. To overcome these issues, require a complete understand of buffalo reproductive physiology and the development of tailored management strategies to optimize breeding success under artificial conditions. In Bangladesh, anestrus and heat detection in buffalo cows are the major problems of buffalo reproduction. Factors such as delayed puberty, seasonal breeding, long calving intervals, and inadequate estrus detection hampered the reproductive efficiency of female buffaloes. However, higher fertility could be achieved through better feeding and management practices (Qureshi *et al*., 2007). Both natural mating and artificial insemination system are prevalent in farming practices in Bangladesh, although artificial insemination adoption remains limited. This is largely due to challenges such as seasonal breeders, poor estrus sign, lower pregnancy rate, and variability of estrus length in buffaloes. Moreover, buffalo also tend to show heat signs primarily at night, which poses difficulties for farmers in observation. Additionally, environmental factors, such as the tropical climate, influence estrus behavior, with high ambient temperatures reducing sexual activity during the day (Jainudeen and Hafez, 1977) and shortening the estrus period (Gill *et al*., 1973) with the occurrence of silent estrus commonly observed during the summer. This can lead to a higher incidence of silent estrus, particularly during the hot summer season. These adverse effects of heat stress make estrus detection much more difficult in buffalo, thereby leading to poor reproductive performance and impeding economic growth. Despite its effectiveness in breed up gradation, AI remains unpopular in buffaloes due to their less estrus behavior compared to cows, as well as the i.e., short-day breeding tendencies. Furthermore, the scarcity of buffalo bulls, compounded by the prevalent practice of free-range island rearing, further limits the widespread of adoption of AI in buffalo breeding programs. During the estrus cycle of buffalo cows, the farmers are looking for bull buffalos instead of AI for breeding purposes. This preference is partly due to the interval of 30 hours between standing estrus and ovulation in buffaloes, a critical factor for successful of artificial insemination (Warriach and Ahmad, 2008). In field conditions, the am-pm rule of insemination, originally developed for cattle (Trimberger, 1948), which is greatly followed in buffaloes too. Therefore, the buffaloes are to be bred 12 hours after the detection of estrus. However, the onset of estrus signs as an alternative of the onset of standing estrus has been incorrectly considered as the rule for buffaloes. This incorrect time of breeding is credible for poor fertility and it is justified by the reality that there is a period of 8 to 10 hours between the beginning of estrus signs and the commencement of standing estrus. This indicates buffaloes should be inseminated 12 hours after the detection of standing sign of estrus (detection by bulls/teasers bull) or 18 to 24 hours after the start of estrus signs.

In natural breeding, the pregnancy rate is higher than artificial insemination. It happens because the bull can recognize the suitable time of estrus of buffalo cows, the volume of the semen, the concentration of semen as well as the bio-stimulation of the penis during mating (Biswas *et al*., 2022). There is considerable evidence in numerous species of a stimulatory male factor on estrus and ovulatory reactions in female animals. This male factor of stimulation during mating has been termed bio-stimulation (Fraser, 1968). Bio-stimulation may accelerate the commencement of puberty in females, influence cyclicity to recommence in females undergoing seasonal or lactational anestrus and vary times related with estrus and ovulation. Additionally, in certain species like cats and rabbits, the act of penile intromission provides vital genital stimulation necessary for ovulation (Clemens and Christensen, 1975). The genital tract such clitoris stimulation at artificial insemination may influence the rate of pregnancy in cattle as explored in several studies and increase pregnancy rates by 6.3 to 7.5% in cows (Chenoweth, 1983; Ramiro *et al*., 2020). Previously, it is used a penis-like device (PLD) after artificial insemination for intra-vaginal sensation and got a higher pregnancy rate in cows (Biswas *et al*., 2022). However, the use of a PLD during artificial insemination may act as bio-stimulation and increase the pregnancy rate of buffalo cows in the coastal area of Bangladesh. In this study, we had

used the penis-like device for intra-vaginal bio-stimulation immediately following artificial insemination in buffaloes. As far as our knowledge extends, there has been no prior investigation into the application of intra-vaginal bio-stimulation with artificial insemination in buffaloes. Therefore, aim of this study to enhance the pregnancy rate of water buffaloes through intra-vaginal bio-stimulation following artificial insemination.

MATERIALS AND METHODS

This study was carried out at the Theriogenology and Reproductive Biotechnology Laboratory under the Department of Medicine, Surgery and Obstetrics, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Bangladesh.

Anthelmentics, vitamins and minerals

(LT vet® 2g bolus) as anthelmintic medicine, (Vita AD_3E® syrup 100 ml bottle and Rena Sel-E® syrup 100 ml bottle) as vitamin minerals were purchased from Acme Animal Health Limited, Bangladesh and Reneta Animal Health Limited, Bangladesh. The LT vet® bolus contained triclabendazole 900 mg and levamisole 600 mg per 2000 mg bolus and it was administered @ one bolus per 70-80 kg body weight of animals. The Vita AD_3E® syrup contained Vitamin A @ 10000000 IU, Vitamin D3 @ 2000000 IU, and Vitamin E @ 2000 mg per ml, and it was administered @ 10 ml orally per buffalo cow for 30 days. The Rena Sel-E® syrup contained Vitamin E 80mg, Selenium 0.6mg per ml and it was used @ 10 ml orally per buffalo cow for 30 days.

Penis like device (PLD)

The intra-vaginal bio-stimulation was performed with a penis-like device (PLD) (Figure 1), which was prepared according to the description by Biswas *et al.* (2022).

Figure 1: Penis like device (PLD).

Study area and period

The study was conducted in different coastal regions of Bangladesh which were Bhola Sadar Upazila in Bhola district (Latitude: 22° 41' 25.08"N, Longitude: 90° 39' 9.00" E) and Bauphal Upazila in Patuakhali district (Latitude: 22° 25' 45.12" N, Longitude: 90° 30' 50.04" E). The laboratory work was conducted in the Theriogenology and Animal Reproductive Biotechnology Laboratory, Department of Medicine, Surgery and Obstetrics, Patuakhali Science and Technology University, Bangladesh for the period of July 2022 to June 2023.

Selection and management of buffalo heifers/cows

A total of 180 buffalo heifers/cows were selected simple random sample method from the study area for the experiment based on the research communication, health status, calving interval, farmer's accountability etc. Different types of inspections were performed to select buffalo heifers/cows. Finally, a total of 75 animals were selected and experiments were done on animals in the study area. The body condition of all animals was suitable to conceive without observing any abnormalities of estrus signs and mucosal secretion. During the selection of animals, AI Technician (AIT), breed, parity, age, and body condition score (BCS) were recorded in a pre-tested data recording format. The reproductive health of animals was confirmed by per rectal palpation of the uterus and ovary. After that, anthelmintics, vitamins, and mineral syrup were supplied to the farmer for the selected buffalo cows/heifers. The anthelmintic, LT Vet® was advised to administer @ one bolus per 75 kg body weight of animals. It was suggested to inform us when the buffalo heifer/cows come to estrus. Then after observing the sign of estrus, experimental procedures were performed. Most of the buffaloes were reared in the bathan system. Farmers were brining their animals for grazing from early morning up to afternoon and then return home. Farmers were supplied few amount of mixed concentrate (rice polis, wheat bran, broken rice, and oil cake) per animal. It was also advised to feed their buffalo heifer/cows a sufficient amount of concentrate and green grass for maintaining good reproductive health.

Grouping of animals

The grouping of animals was done according to the category of the different parameters. The age was determined as previously described by Banerjee (2010) grouped 2.5 to 3.5, 3.6 to 4.5, 4.6 to 5.5, 5.6 to 6.5 and ≥ 6.6 years old. In category of breed, buffaloes were divided into Local (Non-descriptive indigenous/ deshi breed), crossbred and Murrah. According to the parity, it was categorized as Parity 0 (not yet calved), Parity 1, 2, 3 and ≥ 4. According to the BCS, they were classified as BCS 2 to 2.5, 3 to 3.5 and 4. The reproductive health was determined as previously described by Biswas *et al.* (2022). The reproductive health status determined by observation and rectal palpation, animals were categorized as good, moderate and poor.

Artificial insemination technician (AIT)

Hasan Ali, Khalilur Rahman and Habibullah were appointed as AI technicians in the study area. Hasan Ali and Khalilur Rahman worked in the Bhola district and Habibullah was in the Patuakhali district. According to the AI technician, the animals were divided into three groups AIT-1, AIT-2 and AIT-3. These technicians were trained up from government and non-government institution as well as conducting artificial insemination in both cows and buffalo for long time at that study areas.

Preparation of penis-like device (PLD)

The penis-like device was prepared as described in a report by Biswas *et al.* (2022) is showed in Figure 1.

Experimental design

The study was designed as five experimental groups as follows:

Group A (25): It was a control group in which buffalo cows/heifers were bred naturally as usual procedure without applying intra-vaginal bio-stimulation.

Group B (25): In this group, buffalo cows/heifers were inseminated by AI technicians after observing heat signs without applying intra-vaginal bio-stimulation.

Group C (25): In this group, AI was conducted after observing estrus signs and intra-vaginal bio-stimulation was applied through PLD following artificial insemination to enhance the ovulation and increase sperm swimming.

Estrus detection and breeding

The estrus of buffalo cows was detected by observing signs of estrus such as hanging of vaginal mucus and standing to be mounted. It was also noticed, excessive bellowing, vulvar swelling, restlessness and temporary teat engorgement. Sometimes rectal palpation was performed and coiled and tonus uterus was indicated as estrus signs in case of silent heat. Congested vulva and clear mucus streaming were also considered as estrus signs. In this study, natural service and AI were conducted.

Bio-stimulation through PLD

The intra-vaginal bio-stimulation was given by PLD (Figure 1). It was used according to a previous study by Biswas *et al.* (2022). In brief, firstly it was taken out carefully from the sac and sterilized with 70% ethanol spray. Before inserting into the vagina it was lubricated by coconut oil. The PLD device was carefully inserted into the vagina. Additional care was taken to prevent any type of discomfort during insertion. After insertion into the vagina, it was pressed and pulled three to four times smoothly for bio-stimulation. The device was removed carefully. After use, the device was cleaned with clean water and sanitized with 70% ethanol spray, and kept in the sac for future use.

Pregnancy diagnosis

The pregnancy of animals was recorded by non-return to estrus sign and ultrasonography at 28-30 days. The confirmatory diagnosis was done by rectal palpation of the reproductive organ between 60-90 days of post-service.

Statistical analysis

The collected data was recorded and coded in an Excel sheet. The rate was expressed as a percentage (%). The analysis of variance was calculated by SPSS statistical Software (version 20.0). Analysis of Variance (ANOVA test) was considered significant at a level of $P < 0.01$ and $P < 0.05$. The data were decoded, entered, and sorted accordingly using MS Excel. The data were then transferred to the SPSS software for descriptive analysis. Initially, the data were sorted and cross-checked for duplication and/or missing values. The missing values for each variable were excluded from the analysis (Anon, 1996).

$$\text{Pregnancy rate (\%)} = \frac{\text{Total no. of buffalo heifer/cows pregnant}}{\text{Total no. of buffalo heifer/cows were breed}} \times 100$$

RESULTS AND DISCUSSION

Intra-vaginal bio-stimulation effect in buffalo cows

The rates of pregnancy in groups A, B and C were 64.0, 32.0 and 44.0%, respectively which is showed in Figure 2. It was found that the rate of pregnancy in group A (52.0 %) was higher than to other groups. The use of PLD (group C) simultaneously after AI was found to have a higher pregnancy rate (44.0%) of buffalo heifers/cows than that of only AI (group B). In this study, we found that the overall rate of pregnancy was 46.4%, which is similar to Riaz *et al.* (2018). The overall rate of pregnancy in the study was higher than reported by Yousuf *et al.* (2015) and Hoque (2014) whose were found 41.3%, and 28.0%, respectively. In this study, we used a PLD device that gives bio-stimulation and hastens the ovulation process, The buffalo cows were approachable to intra-vaginal bio-stimulation due to PLD may possibly help them to get feelings of bulls penis therefore it was found a relatively higher pregnancy rate. The intra-vaginal bio-stimulation helps to get feelings of the bull penis, triggering ovulation and stimulate cyclic activity. Consequently, this enhances the pregnancy rate of buffalo heifers/cows (Biswas *et al.*, 2022). In group C, the buffalo heifers/cows were inseminated after observing the sign of estrus and immediately after AI, an intra-vaginal bio-stimulation was given with PLD. The genital tract such as clitorial stimulus at AI favorably may influence pregnancy rates in cattle which is shown in several studies and improved pregnancy rates by 6.3 to 7.5% in cows (Chenoweth, 1983; Ramiro *et al.* 2020). Group B showed a lower pregnancy rate (32.3%) than the other group. Group A showed a higher pregnancy rate (64.0%)

than the other group. In Group A, the buffalo heifers/cows were serviced naturally after observing the sign of estrus and no intravaginal bio-stimulation was applied. Biswas *et al.* (2022) reported that the PLD stimulation increases the rate of pregnancy in cows during bio-stimulation to the vagina. Bull penis's stimulation hastens the reproductive effectiveness according to Choudhary *et al.* (2020) and follicular development of anoestrus heifers (Fiol and Ungerfeld, 2016). The PLD was recognized for post-AI intra-vaginal bio-stimulation of estrus cows. No abnormalities or behavior of heifers/cows after applying of PLD were recorded. In this study, they could not find any abnormalities, abnormal behavior, or complaints from buffalo owners. It is noted that the people's perceptions concerning the use of this device. Farmers were believed that it might be a useful tools and good technology for increasing the bubaline pregnancy rate. Biswas *et al.* (2022) studied on cows and determined overall pregnancy rate was 70.0% also showed that the pregnancy rate (77.7%) of group B (with PLD) was significantly higher than the pregnancy rate (62.2%) of group A. Therefore, we also found a higher pregnancy rate in buffalo after using the PLD device following AI.

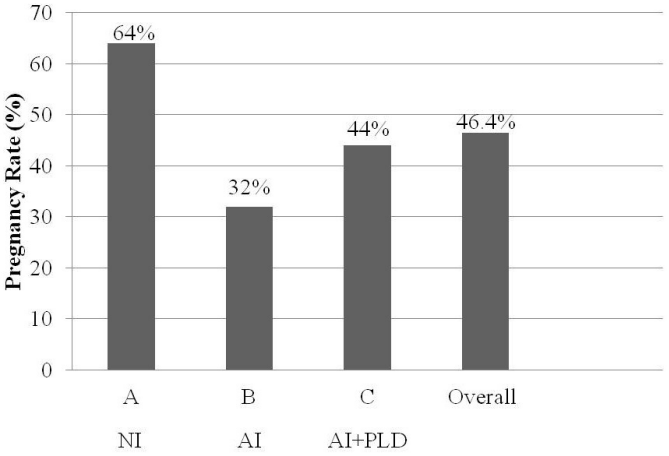

Figure 2: Pregnancy rate in different interventions.

Analysis of risk factors associated with a pregnancy rate

The pregnancy rate of different risk factors is showed in Table 1.

Table 1: Factors affecting the pregnancy rate.

Factors	Category	Total	Number	Pregnancy rate (%)	P value	Group wise pregnancy rate (%)		
						A	B	C
Breed	Indigenous	56	29	51.79*	0.680	13 (23.2)	7 (12.5)	9(16.07)
	Cross	12	4	33.33		3 (25.0)	1(8.34)	0(0.0)
	Murrah	7	2	28.58		0 (0.0)	0(0.0)	2(28.5)
Age (years)	2.5 to 3.5	15	5	33.34	0.083	2 (13.3)	2(13.3)	1(6.6)
	3.6 to 4.5	17	8	47.05		4(23.5)	2(11.7)	2(11.7)
	4.6 to 5.5	21	13	61.90*		5(23.8)	4(19.0)	4(19.0)
	5.6 to 6.5	10	3	30.00		2(20.0)	0(0.0)	1(10)
	> 6.5	12	6	50.00		3(25.0)	0(0.0)	3(25.0)
Parity (number)	P0	14	4	28.57	0.025	2(14.2)	2(14.28)	0(0.0)
	P1	17	7	41.17		3(17.6)	2(11.7)	2(11.7)
	P2	22	15	68.18*		6(27.2)	4(18.1)	5(22.7)
	P3	15	5	33.33		3(20.0)	0(0.0)	2(13.33)
	≥P4	7	4	57.14		2(28.5)	0(0.0)	2(28.5)
Body condition score (BCS)	2 to 2.5	35	15	42.85	0.741	6(17.1)	4(11.4)	5(14.2)
	3 to 3.5	21	10	47.61		5(23.8)	1(4.7)	4(19.0)
	≥4	19	10	52.63		5(26.3)	3(15.7)	2(10.5)
Reproductive health (RH)	Good	36	16	44.45	0.042	9(25.0)	3(8.3)	4(11.1)
	Moderate	24	19	79.16*		7(29.1)	5(20.8)	7(29.1)
	Poor	15	0	0.00		0(0.0)	0(0.0)	0(0.0)
Calving difficulties (CD)	Yes	11	0	0.00	0.000	0(0.0)	0(0.0)	0(0.0)
	No	64	35	54.68		16(25)	8(12.5)	11(17.1)
AI technician (AIT)	AIT 1	28	14	50.00	0.330	6(21.4)	4(14.2)	4(14.2)
	AIT 2	27	13	48.14		6(22.2)	3(11.1)	4(14.8)
	AIT 3	20	8	40.00		4(20)	1(5.0)	3(15.0)

Breed

The significantly ($p<0.05$) highest pregnancy rate is shown in the indigenous breed (51.79%), among cross breed (33.33%) and Murrah breed (28.58%) is shown in Table 1. It is assumed that indigenous buffalo showed a higher pregnancy rate due to better adjustment to the environment. Our observation aligns with those of Karim *et al.* (2013) who reported a higher pregnancy rate (43.02%) in indigenous buffalo. The crossbred buffalo exhibited significantly lower pregnancy rates compared to indigenous and Murrah buffalo. Similarly, in the case of cows, a higher pregnancy rate was found in indigenous cows (Paul *et al.*, 2011). In contrast, no difference was observed in pregnancy rate between local and cross-bred buffalo cows in previous studies in Bangladesh (Shikder, 2011; Khatun *et al.*, 2014; Hossain *et al.*, 2015).

Age

The significantly ($p<0.05$) highest pregnancy rate was found at the age of 4.6 to 5.5 years old (61.90%) is shown in Table 1. At the same time, we also found that subsequently declining the pregnancy rate at the age from 5.6 to 6.5, and gain increase at more than 6.5 years which are supported by Hamid *et al.* (2016) who observed that the pregnancy rates at 3.6-4.5, 4.5-5.6, 7-8, and 9 years old were 47.9, 67.8, 55.6, and 33.3%, respectively. Therefore, these findings also agreed with Singh *et al.* (2015). Khatun *et al.* (2014) found a decreased conception rate which was in age groups more than 8 years. Howlader *et al.* (2019) found that the cows aged in < 2.5, 2.5 to 3.5, 3.6 to 4.5, 4.6 to 6, > 6 years found 50, 71.93, 78.06, 85.49 and 74.52% conception rate after first AI, respectively.

Parity

In this study, the pregnancy rate was found in buffaloes with second parity (63.64%) compared to buffaloes with other parities is shown in Table 1. Our observations are consistent with those reported by Chebel *et al.* (2004), Grimard *et al.* (2006) and Tebug *et al.* (2011). Additionally, Bhagat and Gokhale (1999) noted a gradual increased pregnancy rate increased gradually from the 1st to the 4th parity, followed by a decreased in the subsequent parities. Spalding *et al.* (1975) reported a significantly increased pregnancy rate was found in both parity 2 ($P \leq 0.02$, 95% CI 1.51, 58.3) and Parity 3 ($P \leq 0.02$, 95% CI 1.5, 124.6) in this investigation and pregnancy rate was 73-75% in case of natural breeding.

Body condition score (BCS)

In this study, it was found that there is no significant ($p>0.05$) difference among the BCS. Buffaloes with BCS 3 to 4 had more likelihood of getting pregnant than others is shown in Table 1. It was partially agreed with (Kumar *et al.*, 1997; Shamsuddin *et al.*, 2013).

Reproductive health (RH)

The buffaloes with moderate RH was shown significantly ($p<0.05$) higher pregnancy rate (79.16%) than that of others. It was also found that buffalo with poor RH was not pregnant (0.0%) which support the observation of Sachan *et al.* (2015) and Biswas *et al.* (2022). Biswas *et al.* (2022) determined that the pregnancy rates of poor, moderate, and good in groups A and B were 37.5 and 85.7, 55.8 and 78.5, 68.7 and 76.9%, respectively, which agreed with this study. The poor reproductive health cows showed a lower pregnancy rate in group A whereas relatively higher in group B. It is observed that the stimulation with PLD increased the pregnancy rate of moderate reproductive health heifers/cows after intra-vaginal bio-stimulation. Mufti *et al.* (2010) also found that reproductive disorders in the remaining 15% of the heifer/cows were an important cause of reduced pregnancy rates which was partially supported by this study.

Calving difficulties

The significantly ($p<0.001$) highest pregnancy rate (54.68%) was found in the case of the absence of calving difficulties (CD). Calving difficulties affect the pregnancy rate. Our observation aligns with those Biswas *et al.* (2022) who reported that calving difficulties negatively impact reproductive efficacy and pregnancy rates in bovine. The correlation between CD and pregnancy rate was found highly significant ($p \leq 0.001$).

AI technician (AIT)

There is no significant variation of pregnancy rate is found in the factors of AIT. The experience of AIT 1, AIT 2, and AIT-3 were 5, 3 and 2.5 years, respectively. The pregnancy rate achieved by AI technician-1, who inseminated th buffalo heifers/cows, was higher compared to that of AI technician-2 and AI technician-3 attributed to differences in their experience. The observation is consistent with findings reported by Biswas *et al.* (2022) and Paul *et al.* (2011) regarding cow, where they found a higher pregnancy rate in cases handled by AIT -1, whose experience surpassed that of others.

Limitations and further study

The animals of the study were not in same management system. It was in field condition. The device was not adopted with the thermoregulation system. Notably, hormonal assay was not conducted in this study. Further study with hormonal assays is needed to determine the pathway of mechanism as well as modified the device with thermoregulation system.

CONCLUSIONS

It is concluded that the use of penis-like devices after

artificial insemination increases the pregnancy rate of buffalo heifers/cows. It is also found that parity, reproductive health and calving difficulties are considered as the risk factors of buffalo reproduction in the coastal area.

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NOVELTY STATEMENT

The authors have developed a penis-like device for intra-vaginal bio-stimulation in buffalo to provide the natural feelings of coitus to increase pregnancy rate subsequent artificial insemination. It is the first report in buffalo.

AUTHOR'S CONTRIBUTION

Ashit Kumar Paul and Md. Fakruzzaman designed the experiment, supervised the study, analyzed the data and revised the final draft as well as submit the manuscript. Anup Sarker directly involved for conducting the experiment, collection of data, reviewing the literature and preparing the draft manuscript. Dibyendu Biswas and MAM Yahia Khandoker supervised and tabulated the data. Gautam Kumar Deb, SM Jahangir Hossain and Md. Ashadul Alam collected the fund and supervised the whole project.

Conflict of interest

The authors have declared no conflict of interest.

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