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Improvement of bovine pregnancy rate through intravaginal biostimulation with penis like device

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Abstract

The aim of this study was to increase bovine pregnancy rate by intra-vaginal biostimulation penis like device (PLD). A total of 336 heifers/cows were AI, in which PLD was used in 193 animals. Animals were grouped as A (only AI) and B (PLD after AI). The overall pregnancy rate was 70.0%. Pregnancy rate in group B was significantly (P=0.004) higher than that of group A. Cows of >7.5 years old is showed lower pregnancy rate in group A, but higher in group B. The heifers were showed more responsive to PLD than others parity. The parity 5 or more in group B was responded to PLD and showed higher pregnancy rate than that of group A. It was found that age was significantly positive correlation parity whereas significantly negative correlation with breed. It is concluded that PLD will increase the pregnancy rate of cows through biostimulation on vagina.

Keywords: Biostimulation; Cows; Penis like device; Pregnancy rate

Introduction

The reproductive efficiency is the important characteristics for cattle farming. Subsequently, yearly calving is the key of reproductive efficiency. Recently, small and medium scale farmers are dependent on artificial insemination (AI) for their cattle breeding because of high cost involving in bull rearing. One of the major constrains of profitable dairying is low pregnancy rate in cows (Alam *et al.,* 1994; Shamsuddin *et al.,* 2001) which impair the reproductive efficiency of herds in Bangladesh (Paul *et al.,* 2011). The AI is one of the effective tools of assisted reproductive technologies. Economy of dairy farming largely depends on a good pregnancy rate after artificial insemination (AI) (Paul *et al.* 2011; Islam *et al.* 2021). In case of successful AI, the accurate heat detection by the farmer is mandatory, whereas bull detect the accurate heat in case of natural mating. For this, the conception rate in case of natural breeding is higher than that of AI. Although a series of factors are involved in natural mating to conception, there are three important factors that directly stimulate the process of breeding. These are bull's heat detection, intra-vaginal penile stimulation and cow's psychological satisfaction of copulation. The pulsatile hormonal secretion and activity, which is essential for ovulation to conception, markedly depends on those three factors. However, this characteristic activity is absent in case of AI that might is a cause of lower pregnancy rate in cows. Biostimulation is the term referred to the stimulatory effect of a male on estrus and ovulation through genital stimulation, olfactory pheromones, or other less well-defined external cues such as tactile, visual, olfactory and auditory (Chenoweth, 1983; Fiol *et al.* 2010). In bovine, biostimulation can be achieved by direct bull exposure (Rajput *et al.,* 2021) reduce the interval from calving to resumption of ovarian activity (Soto-Belloso *et al.,* 1997) hastened luteal function (Landaeta-Herna´ndez *et al.,* 2004; Fiol and Ungerfeld, 2016). Previous several studies (Randel *et al.,* 1975; Short *et al.,* 1979) were determined that pot AI clitorial massage had increased the conception rate of cows. To ameliorate this issue in case of AI, development of alternative means to produce intra-vaginal biostimulation like bull's penis is necessary that may eventually increase the bovine conception rate. Therefore, the aim of this study is to develop penis like device for intra-vaginal sensation during AI.

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Materials and methods

Study location

The research was conducted at Theriogenology and animal reproductive biotechnology laboratory, Department of Medicine, Surgery and Obstetrics, Faculty of Animal science and Veterinary Medicine, Patuakhali Science and Technology University, Barishal Campus.

Study area

The study areas were randomly selected in four different upazilas of Bangladesh. These were Sadar upazila of Barishal district, Keshobpur upazila of Jessore district, Bandor upazila of Narayangonj district and Mohadebpur upazila of Naogaon district of Bangladesh.

Preparation of penis like device (PLD)

The body frame of PLD was made stainless steel (SS) wire which was rapping by cotton and paper tape. The wire frame again fixed with SS pipe (length 3'') and handle (length 4''). Then, the wire frame was covered by synthetic latex, silicone and adhesive layer by layer to make it rubber like texture and softness. Finally, it was rapped with rolling of thin polythene (Figure 1). The total length of PLD is 18 inch. The diameter of body is 5'' at the base and gradually narrowing to 2'' at the tip.

Use of PLD

Before insert the device into the vagina, it was sterilized by 70% ethanol spray and lubricated by coconut oil. After insertion into vagina, it was pushed and pulled three to four times slowly for bio-stimulation. After using, the device was cleaned by fresh tube well water and sterilized again by 70% ethanol spray and was kept in the sac for future use.

Examination the effect of PLD after use

To examine the any reaction or inflammations of PLD, we inserted PLD randomly in ten (10) cows with estrous and ten (10) cows without in estrous. It was found that none of these cows showed any inflammations and discomfort during use it.

Animals

A total of 336 heifers/cows were AI, in which PLD was used in 193 animals. Age, body weight, breed, parity, reproductive health and previous calving history were recorded in a pre-prescribed format during AI. According to the age, animals were grouped into 1.5 to 3.5 years, 3.6 to 5.5 years, 5.6 to 7.5 years and 7.5 years old. Based on the number of calving or parity, animals were grouped into P0 (heifers), P1, P2, P3, P4 and ≥P5. The animals were divided on their body weight as 100 to 200 kg and 201 to 300 kg. On the basis of breed of animals, they were grouped into local (indigenous, humpless) and crossbred. According to the reproductive health status determined by observation and rectal palpation, animals were categorized as poor (regular estrus cycle and few amounts of clear mucosal secretion and narrow uterus), moderate (regular estrus cycle and adequate amount of clear mucosal secretion and narrow uterus) and good (regular estrus cycle and adequate amount of clear mucosal secretion and healthy uterus). On history of previous calving record, cows were grouped as normal and dystocia. The PLD was trialed by six professional AI technicians who were working in both government and private sector of the study areas. The animals were cared according to the departmental animal ethics guideline.

Experimental design

The PLD was inserted during AI in estrus cows/heifers for bio-stimulation. The cows/heifers were divided into two groups. The groups were as follows-

Group A: Cows/heifers were inseminated without intra-vaginal stimulation (Control).

Group B: Cows/heifers were inseminated with intra-vaginal stimulation with PLD.

Pregnancy diagnosis

The pregnancies of cows were diagnosed by observing not return to estrus rate at 20-22 days. The cows which were not observed estrus sign at 20-22 days were rechecked and confirmed by rectal palpation of reproductive organ at 60-90 days of post insemination.

Statistical analysis

The collected data was recorded and coded in Microsoft excel sheet. The rate was expressed as percentage (%). The logistic regression with odd ratio, chi-square test (χ^2) and Pearson correlations (2-tailed) coefficients were calculated by statistical program for social science (SPSS) software (SPSS® Version 22.0). The analysis of variance was done for test the significance. In case of all parameters, a statistical significance was considered at $P \leq 0.05$.

Results and discussion

The PLD was developed for post AI intra-vaginal bio-stimulation of estrous heifer/ cows. Any abnormalities or behavior

Table I. Comparison of pregnancy rate among group A and B

Group	Number of observation	Pregnancy rate (%)
A (Only AI)	143	89 (62.2)[a]
B (Use PLD after AI)	193	150 (77.7)[b]
Over all	336	239 (70.0)

Table II. Effect of different parameters on pregnancy rate

Parameters	Category	Total	No. of observation (N)		Pregnancy rate			
			Group A	Group B	Group A		Group B	
					N	%	N	%
Age (years)	1.5-3.5	109	50	59	33	66.0	46	78.0
	3.6-5.5	133	55	78	33	60.0	60	76.9
	5.6-7.5	68	22	46	14	63.6	35	76.1
	>7.5	26	16	10	9	56.3	9	90.0
Parity (number)	P0	25	14	11	9	64.3	11	100.0
	P1	70	34	36	22	64.7	26	72.2
	P2	88	39	49	23	59.0	42	85.7
	P3	52	19	33	10	52.6	25	75.8
	P4	36	11	25	8	72.7	18	72.0
	≥P5	65	26	39	17	13.7	28	71.8
Body weight (kg)	100-200	257	124	133	77	62.1	103	77.4
	201-300	79	19	60	12	63.2	47	78.3
Breed	Local	123	68	55	39	57.4	43	78.2
	Cross	213	75	138	50	66.7	107	77.5
Reproductive health	Poor	15	8	7	3	37.5	6	85.7
	Moderate	117	52	65	29	55.8	51	78.5
	Good	204	83	121	57	68.7	93	76.9

of cows after use of PLD were recorded. In this study, we could not found any abnormalities, abnormal behavior and complain from cattle owners. We also recorded the people perception regarding the use of this device. All of the people thought that it could useful and good technology for increasing cow's pregnancy. A total of 336 heifers/cows were AI, in which PLD was used in 188 animals. The overall pregnancy rate was 70.0%. The pregnancy rate of group A and B were 62.2 and 77.7%, respectively (Table I). The pregnancy rate of group B (with PLD) was significantly higher than that of group A. The overall pregnancy rate of our study is similar with the report (72.0%) of Howlader *et al.* (2019) and higher than that report (57.3%) of Paul *et al.* (2011) and Khan *et al.* (2015).

The pregnancy rate according to the different group of parameters is showed in table II and the logistic regression among the category of parameters has been shown in table III. According to age cows, the pregnancy rate of 1.5-3.5, 3.6-5.5, 5.6-7.5 and >7.5 years old cows in group A and B were 66.0 and 78.0, 60.0 and 76.9, 63.6 and 76.1, 56.3 and 90.0%, respectively. Our study has similarity with several studies. The pregnancy rate of group B was higher than that of group A in all age categories of cows. The cows of >7.5 years old is showed lower pregnancy rate in group A, but the rate is higher in group B. It may due to the old age of cows which is responded more in PLD bio-stimulation than control. Khatun *et al.* (2014) found a significantly decreased conception rate ($p<0.01$) was observed in age group more than 8 years than other groups. Howlader *et al.,* (2019) found that the cows aged <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%, 74.52% conception rate after first AI, respectively.

According to the parity of cows, the pregnancy rate of P0, P2, P3, P4 and ≥P5 in group A and B were 64.3 and 100, 64.7 and 72.2, 59.0 and 85.7, 52.6 and 75.8, 72.7 and 72.0, 13.7 and 71.8%, respectively. The heifers were more responsive to intra-vaginal bio-stimulation because of PLD may help them to get feelings of bull penis. The parity 5 or more in group B is responded to PLD and showed higher pregnancy rate than that of group A due to their old age. The pregnancy rate without bio-stimulation is showed higher than that of Paul *et al.* (2011) in Sirajgonj district, Al-amin *et al.* (2018) and Islam *et al.* (2021) demonstrated that the first service conception rate was studied from Heifer (Parity-0) to 9th parity. The PR/FAI of Parity-0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 were 29.3, 30.1, 28.1, 6.9, 27.4, 29.7, 29.4, 32.0, 50.0 and 20.0%, respectively.

According to the body weight of cows, the pregnancy rate of 100-200 and 201-300 kg body weight in group A and B were 62.1 and 77.4, 63.2 and 78.3%, respectively. The pregnancy rate within the groups had no differentness but differ between

Table III. Logistic regression of analysis

Variables	Category	Wald	P value	Odd ratio	95% CI for odd ratio	
					Lower Bound	Upper Bound
Group	A	8.502	.004	.468	.281	.780
	B	.	.	.	.	.
Age (years)	1.5-3.5	.278	.598	.749	.256	2.193
	3.6-5.5	.424	.515	.698	.237	2.059
	5.6-7.5	.039	.843	.896	.302	2.659
	>7.5	.	.	.	.	.
Parity (number)	P0	2.457	.117	2.597	.787	8.566
	P1	.301	.583	1.255	.557	2.828
	P2	1.006	.316	1.521	.670	3.453
	P3	.001	.975	.986	.403	2.412
	P4	.001	.971	.982	.356	2.705
	≥P5	.	.	.	.	.
Body weight (kg)	100-200	.030	.862	.945	.500	1.787
	201-300	.	.	.	.	.
Breed	Local	.575	.448	.805	.460	1.410
	Cross	.	.	.	.	.
Reproductive health	Poor	.637	.425	.620	.192	2.005
	Moderate	1.446	.229	.715	.414	1.235
	Good	.	.	.	.	.
Calving history	Normal	.100	.752	.669	.055	8.152
	Dystocia	.	.	.	.	.

Table IV. Correlations coefficient between different factors

Variables	Age	Parity	Body weight	Reproductive health	Breed	Calving history	Group
Age	1	.351**	-.035	-.074	-.216**	-.038	.004
Parity	.351**	1	.059	.189**	-.016	.004	.106
Body weight	-.035	.059	1	.043	.305**	.022	.208**
Reproductive Health	-.074	.189**	.043	1	.045	.017	.057
Breed	-.216**	-.016	.305**	.045	1	.006	.196**
Calving history	-.038	.004	.022	.017	.006	1	-.110*
Group	.004	.106	.208**	.057	.196**	-.110*	1

** Correlation is significant at the 0.01 level and *Correlation is significant at the 0.05 level

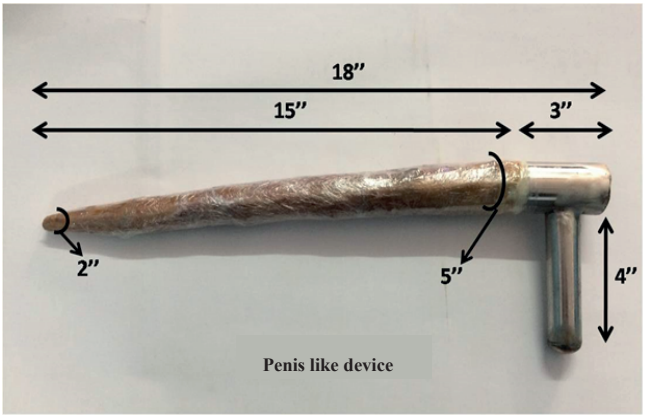

Fig. 1. Penis like device

the groups. Similarly, Paul *et al.* (2011) also was not found significant difference among the body weight, but Saacke *et al.* (1991) reported that the performance of heavier cows more than lighter counterparts. According to the breed of cows, the pregnancy rate of local and crossbred cows in group A and B were 57.4 and 78.2, 66.7 and 77.5%, respectively. The pregnancy rate was higher in crossbred cows than that of local breed within the group. Al-Amin *et al.* (2018) reported that the pregnancy rate was significantly (P<0.05) higher in local breed (62.0%) than in pure Friesian, Local x Friesian, Sahiwal x Friesian and Local x Jersey breed. Similarly, higher pregnancy rate in indigenous cows than in other groups was reported elsewhere (Japri *et al.,* 1997; Paul *et al.,* 2011). The higher pregnancy rate in local cows may be due to better adaptation to the local environment. In contrast, no difference was observed in pregnancy rate between local and crossbred cows in previous studies in Bangladesh (Shikder, 2011; Khatun *et al.,* 2014; Hossain *et al.,* 2015). According to the reproductive health, the pregnancy rate of poor, moderate and good in group A and B were 37.5 and 85.7, 55.8 and 78.5, 68.7 and 76.9%, respectively. The poor reproductive health cows were showed lower pregnancy rate in group A whereas relatively higher in group B. It is meant that the PLD increased the pregnancy rate of poor reproductive health cows after intra-vaginal bio-stimulation. According to the previous calving history, the pregnancy rate of normal and dystocia or abnormal in group A and B were 62.1 and 77.7, 66.7 and 0.0%, respectively.

In case of correlation coefficient between factors has shown in table IV. It was found that age was significantly positive correlation parity whereas significantly negative correlation with breed of animals. There was positive correlation between parity and reproductive health. The body weight of animals had significantly positive correlation with breed and

experimental group. The breed had significantly positive correlation coefficient with body weight and experimental group. The positive correlation signifies that the both variables move the same direction whereas negative is vice versa.

Stimulation pathways and mechanisms of response to male effect in cattle are not fully understood (Fiol and Ungerfeld, 2012). The detection and integration of olfactory signals in mammals occurs through the main olfactory system (MOS) and accessory olfactory system (AOS) pathways. Grus and Zhang (2008) stated the hypothesis that environmental cues are perceived by MOS, while specie-specific signals (e.g. pheromones) may be perceived by AOS. Thus, chemical signals associated to male effect may principally act through MOS and not AOS, and, as mentioned before, sensory signals other than olfaction appear to be involved in the response to males (Gelez and Fabre-Nys, 2006). Bull's sexual stimulation is a tactile effect which may relate with sensory nerve stimulation (neuro-endocrine). Physiological mechanisms involved in the response to biostimulation are not well understood, and most studies have been performed with puberty (Choudhary *et al.,* 2020) expression of oestrus (Roelofs *et al.,* 2008) postpartum cows (Landaeta-Herna´ndez *et al.,* 2004; Roelofs *et al.,* 2007; Tauck *et al.,* 2010). There is considerable evidence in a number of species of a stimulatory male effect on oestrus and ovulatory responses in females (Fraser, 1968). The genital tract like clitoris stimulation during AI favorably can influence pregnancy rates in cattle has been shown in several studies and, improved pregnancy rates by 6.3 to 7.5% in cows (Chenoweth, 1983; Ramiro *et al.,* 2020). Bull biostimulation hasten the reproductive efficacy (Choudhary *et al.,* 2020) and follicular development of anoestrus heifers (Fiol and Ungerfeld, 2016).

In conclusion, the intra-vaginal biostimulation with PLD significantly increase the pregnancy rate of cow's especially praiseworthy in aged cows. However, the hormonal assays of the experimental animals were not conducted in this study. Therefore, further study with hormonal assays is needed to determine the pathway of mechanism.

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Authorship contribution statement

AK Paul designed the experiment, supervised the study, analyzed the data and revised the final draft of manuscript. S Biswas and M Swarna were directly involved to do the experiment, collection of data and reviewed the literature and tabulated the data and written the draft of this manuscript.

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