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Management of pregnancy failure in cows with vaginal stimulation and hormone therapy

Biswas S, Swarna M, Biswas D and Paul AK*
Department of Medicine, Surgery and Obstetrics, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Babugonj Campus, Barishal-8210, Bangladesh

Abstract

The focus of this study is to improve the pregnancy rate of cattle using different interventions with the analysis of associated risk factors. A total of 180 cows with pregnancy failure after one insemination were selected, which showed normal oestrous behaviour and vaginal mucus discharge. The animals were divided into six groups: A [control: with only Artificial Insemination (AI)]; B [penis-like device (PLD) used after AI]; C [intramuscular injection (im) of 2 ml prostaglandin-$F_{2\alpha}$ at day 7 of the oestrous cycle and AI at observed oestrus], D [AI at natural oestrus followed by 2.5 ml GnRH im, repeated 4-6h after AI], group E [As group C and PLD used after AI], group F [as D and PLD used after AI]. The overall pregnancy was 55.6%. Group F had the highest pregnancy rate (70.0%). Higher pregnancy rates were in older animals of more than 4.6 years of age with parity of more than two. A significant correlation was found between age and parity; age and Artificial Insemination Technician (AIT); Body Condition Score (BCS) and breed; Reproductive Health (RH) and AIT. It is suggested that the use of hormonal treatments (with or without PLD) improved the pregnancy rate in cattle. (*Bangl. vet*. 2022. Vol. 39, No. 1 - 2, 16 – 25)

Introduction

In Bangladesh, great efforts have been made to improve the performance of local breeds through AI programmes with semen of exotic dairy breeds (Paul *et al.,* 2011). The major restriction of profitable dairy farming is the low pregnancy rate (Shamsuddin *et al*., 2001, Paul *et al.,* 2011). The success of the AI programme depends on regular evaluation of the reproductive performance of cows. Pregnancy failure can be due to abnormal uterine environment, infection, anovulation, genetic predisposition, incorrect timing of insemination, poor quality of semen, faulty AI technique, embryonic death, heat stress or metabolic disease (Noakes *et al.,* 2001). GnRH can improve the pregnancy rate in primiparous cows (Kaim *et al.,* 2003) as it generates follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the follicular phase. GnRH and $PGF_2\alpha$ treatment in cows with pregnancy failure might improve pregnancy rate (Paul *et al.,* 2015). Vaginal stimulation occurs during natural

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*Corresponding author:- E-mail: akpaul2008@gmail.com; ashitpaul@pstu.ac.bd
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matting (Rajput *et al*., 2021), and bull exposure is reducing the interval from calving to onset of ovarian activity with the improvement of luteal function (Landaeta-Herna´ndez *et al.,* 2004; Fiol and Ungerfeld, 2016). At AI, there is less stimulation. The present study was designed to develop an alternative means to produce vaginal stimulation like natural service for improving the pregnancy rate in cows, and to evaluate the pregnancy rate of heifers/cows after AI with or without vaginal stimulation and hormone therapy.

Materials and Methods

Study area

The study was conducted in three districts, Mohadebpur Upazila (sub-district) in Naogaon district (Latitude: 24°55′16.72″N, Longitude: 88°44′24.34″E,), Babugonj Upazila in Barishal district (Latitude: 22°46′19.43″N, Longitude: 90°19′55.42″E) and Nazirpur Upazila in Pirojpur district (Latitude: 22°47′26.67″N, Longitude: 89°56′48.45″E).

Preparation of penis-like device (PLD) and use

The body frame of PLD was made of stainless steel (SS) wire, wrapped with cotton and paper tape. The frame was fixed to SS pipe (Length 7.62 cm) and handle (Length 10.16 cm) and covered by layers of synthetic latex, silicone, and adhesive to produce a rubber-like texture. Finally, it was wrapped with thin polythene. The total length of PLD was 18 inches, the diameter of the body was 5 inches at the base, tapering to 2 inches at the tip (Biswas *et al.,* 2022).

Before inserting the device into the vagina, it was sterilized with 70% ethanol spray and lubricated by coconut oil. After insertion into the vagina, it was pushed and pulled three to four times slowly for stimulation.

Animals and management

A total of 180 local (non-descriptive indigenous/desi) and crossbred (Local × Sahiwal & Local × Holstein-Friesian) cows and heifers were used. Heifers/cows were selected with a history of pregnancy failure once after AI, and showing normal oestrous signs and vaginal mucus discharge. Data were gathered from the selected animals about Artificial Insemination Technician (AIT), breed, age, parity, and Body Condition Score (BCS) using pre-tested questionnaires. The reproductive health (RH) of animals was checked by manual palpation of the genital organs per rectum and grouped as described by Biswas *et al.* (2022). Briefly, the RH status was grouped as (a) Good: Regular oestrous cycle with clear mucous discharge (b) Moderate: Regular oestrous cycle with cloudy mucous discharge and (c) Poor: Regular oestrous cycle and scanty mucous secretion. Anthelmintics, vitamins, and mineral supplementation were given. Most farmers reared animals in a semi-intensive system and allowed their animals grazing from early morning to noon and fed 4 - 5 kg green grasses mixed with 2-3 kg straw daily as an evening meal. Some animals were supplied with 150 gm of mixed concentrate (rice polish, wheat bran, broken rice, and oil cake) (Paul *et al.,* 2011).

Grouping of animals

Frozen bull semen from Advanced Chemical Industries (ACI), Bangladesh Rural Advancement Committee (BRAC) and Department of Livestock (DLS) were used.

Animals were grouped into six groups, each of 30 animals as follows-

Group A: control group in which **c**ows/heifers were inseminated with no treatment.

Group B: cows/heifers were inseminated with vaginal stimulation with PLD.

Group C: AI was not done at oestrus. The animals were rechecked 10 days after oestrus to confirm the presence of corpus luteum (CL) in the ovary per rectum. If CL was present two ml prostaglandin analogue (Ovuprost®) were injected im and AI was done at the next oestrus.

Group D: Heifer/cows were inseminated after observing oestrus and injected im 2.5 ml GnRH (Ovurelin®) immediately after AI followed by second AI 4-6 hrs later.

Group E: Animals were examined 10 days after oestrus to confirm the presence of CL in the ovary per rectum. Two ml prostaglandin analogue (Ovuprost®) were injected im. AI was done at the next oestrus. Vaginal stimulation was done with a PLD.

Group F: The heifer/cows were inseminated at oestrus and 2.5 ml GnRH (Ovurelin®) injected im immediately after AI and a second insemination was done within 4-6 hrs of first AI. Vaginal stimulation was done with a PLD.

Oestrus detection

The oestrus was detected by observing signs (drooling of mucus and standing to be mounted) 10 - 20 minutes twice daily by the farmers and rectal palpation of the uterus by the graduate research assistant.

Pregnancy diagnosis

Pregnancy diagnosis was done by rectal palpation 60 - 90 days after insemination.

Statistical analysis

The data were recorded and coded in an Excel sheet. The pregnancy rates are expressed as a percentage (%). The analysis of variance was done using SPSS statistical Software (version 20.0). Differences were considered significant at a level of $P<0.01$ and $P<0.05$. The data were decoded, entered, and sorted using MS Excel, and transferred to the analytical software SPSS for descriptive analysis. Descriptive analysis expressed as multiple logistic regression was done to measure the strength of association between breed of, age, parity, and body weight of cows or heifers, breed of bull, season of insemination and AI technician (Anon, 1996). The outcome variable was the pregnancy rate.

$$\text{Pregnancy rate} = \frac{\text{Total no. of heifer/cows pregnant}}{\text{Total no. of heifer/cows receiving AI}} \times 100$$

Results and Discussion

Effects of procedures on pregnancy rate

The pregnancy rate of groups A, B, C, D, E, and F were 33.3, 50.0, 60.0, 53.3, 70.0, 66.7, and 55.6%, respectively (Fig. 1). Group E animals had highest pregnancy rate. Okon *et al*. (2015) stated that prostaglandins are involved in LH release, ovulation, and luteolysis. Due to their luteolytic action, $PGF_2\alpha$ can be useful for oestrus synchronization. Group F showed a higher pregnancy rate (66.7%) than other groups except for group E. These results are consistent with Biswas *et al.* (2022), who reported that vaginal stimulation and GnRH increased the ovulation rate. Group C showed a higher pregnancy rate (60.0%) than other groups except for E and F. Group D showed a higher pregnancy rate (53.3%) than the other groups except for E, F, and C. Roth *et al.* (2021) suggested that GnRH shortly after onset of oestrus can stimulate normal LH surge and ovulation, permit normal fertilization, and improve the pregnancy rate. Group B showed a higher pregnancy rate than the other groups except for E, F, C, and D. No abnormalities or behaviour of heifers/cows after the use of PLD were recorded. Biswas *et al.* (2022) worked on 336 heifers/cows with AI, in which PLD was used in 188 animals. The overall pregnancy rate was 70.0%. The pregnancy rate (77.7%) of group B (with PLD) was significantly higher than the pregnancy rate (62.2%) of group A, which agrees with Biswas *et al.* (2022). The pregnancy rate of group C was 60.0%. Amjad *et al.* (2006) reported some improvement of pregnancy rate (66.6%) after using only $PGF_2\alpha$, but reduced pregnancy rate was recorded after using $PGF_2\alpha$ with PLD. Kiam *et al.* (2003) observed that injection of GnRH analogues at the onset of oestrus slightly increased the pregnancy rates from 41.3 to 55.5% across seasons when only GnRH was used, and the pregnancy rate declined after using GnRH with PLD in group D (53.3%).

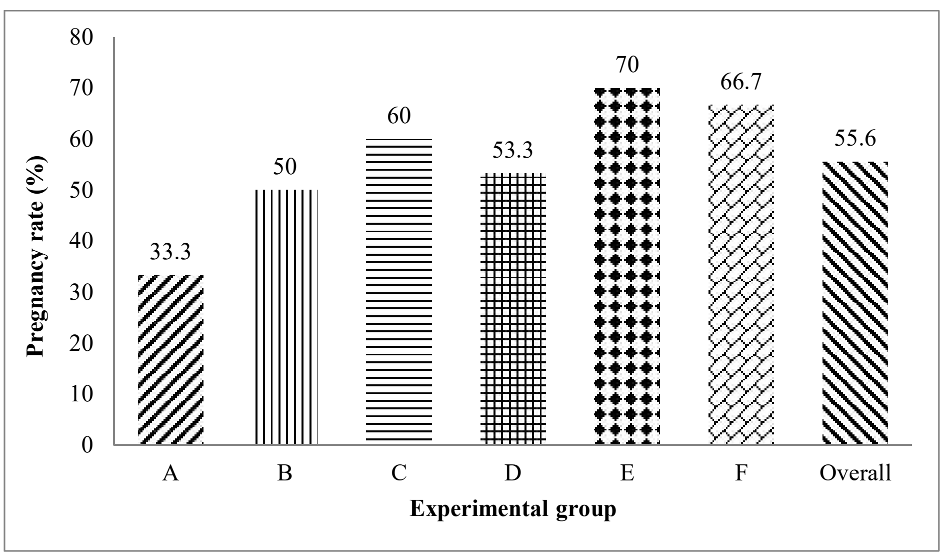

Fig. 1: Pregnancy rate in different interventions

Risk factors and pregnancy rate

The pregnancy rate associated with different risk factors is shown in Table 2. Analysis of risk factors is shown in Table 3. Correlation-coefficients between different factors is shown in Table 3.

Age

The pregnancy rate for heifer/cows aged 1.5 to 2.5, 2.6 to 3.5, 3.6 to 4.5, 4.6 to 5.5, and 5.6 years or more were 30.4, 30.6, 55.3, 77.5, and 73.5% respectively (Table 1). The correlation between age and pregnancy rate was highly significant (P≤ 0.001). In this study, the multiple logistic regressions showed no significant correlation with age (Table 2). The age between 4.5 to 5.5 years old heifers/cows is showed more chances (5.3 times) of becoming pregnant than that other age groups. Among all animals, age was highly positively correlated with parity (.0861**) and the performance of AIT (.212**) was negatively correlated with the breed (-.174*) due to the advancement of age of animals (Table 3). These findings were a little higher and lower than that of the findings reported by Paul *et al.* (2011). The highest pregnancy rate was found at the age of 4.6 to 5.5 years old. Khan *et al.* (2015) observed that the pregnancy rate at 2.5-3, 3.5-5, 6, 7-8, and 9 years old were 57.9, 77.8, 65.6, 46.7, and 31.3%, respectively. Therefore, these findings agreed with Khan *et al*. (2015).

Breeds

The pregnancy rate of local breed and crossbred heifer/cows were 60.8 and 53.5%, respectively, but there was no significant (P>0.05) difference between them (Table 1). The crossbred cattle might feel stress in the tropical environment. The association between the breed of heifer/cows and pregnancy rate was not significant (P>0.05). The breed was negatively correlated with age (-0.174*), and parity (-.0175*), respectively and positively correlated with BCS (0.209**). Hence breed affects the pregnancy rate. Khan *et al.* (2015) observed the pregnancy rate of local, Friesian, and Sahiwal were 63.8, 57.1 and 52.6%, respectively. In the case of local breed and cross-bred animals, the present results agree with Hossain *et al.* (2016).

Parity

The pregnancy rate of heifer/cows with parity 0,1,2,3, and 4 or more were 37.1, 41.5, 65.0, 63.9, and 75.0, respectively (Table 1). The association was highly significant (P≤0.001). Multiple logistic regression showed no significant difference between parities 0, 3, and 4, but significant difference (P≤0.05) between parities 1, and 2. Parity was positively correlated with age (.861**) and negatively correlated with breed (-.175*). The highest pregnancy rate was in parity 4 and above (75.0%). The pregnancy rate was 37.1% in parity 0 and increased to parity 4 or above (Jiménez *et al*., 2011). The low pregnancy rate in parity 1 might be due to nutritional stress (Paul *et al*., 2015).

Table 1: Pregnancy rate in heifers and cows given different treatments, and age, breed, parity, body condition score, reproductive health, and AI technician

Parameters	Category	Total	Number	Pregnancy rate (%)	P value	Pregnancy rate of experimental group n (%)					
						A	B	C	D	E	F
Age (years)	1.5 to 2.5	23	07	30.4	0.000	0 (0.0)	1 (4.3)	2 (8.7)	2 (8.7)	1 (4.3)	1 (4.3)
	2.6 to 3.5	36	11	30.6		1 (2.8)	1 (2.8)	1 (2.8)	2 (5.6)	3 (8.3)	3 (8.3)
	3.6 to 4.5	47	26	55.3		1 (2.1)	3 (6.4)	6 (12.8)	3 (6.4)	8 (17.0)	5 (10.6)
	4.6 to 5.5	40	31	77.5		2 (5.0)	5 (12.5)	6 (15.0)	7 (17.5)	8 (20.0)	3 (7.5)
	≥5.6	34	25	73.5		2 (5.9)	1 (2.9)	4 (11.8)	8 (23.5)	6 (17.6)	4 (11.8)
Breed	Local	51	31	60.8	0.378	4 (7.8)	4 (7.8)	4 (7.8)	4 (7.8)	7 (13.7)	8 (15.7)
	Cross	129	69	53.5		6 (4.7)	11 (8.5)	14 (10.9)	12 (9.3)	14 (10.9)	12 (9.3)
Parity (number)	P0	35	13	37.1	0.000	1 (2.9)	2 (5.7)	1 (2.9)	4 (11.4)	1 (2.9)	4 (11.4)
	P1	41	17	41.5		0 (0.0)	1 (2.4)	5 (12.2)	2 (4.9)	4 (9.8)	6 (14.7)
	P2	40	26	65.0		0 (0.0)	2 (5.0)	6 (15.0)	4 (10.0)	7 (17.5)	7 (17.5)
	P3	36	23	63.9		1 (2.8)	4 (11.1)	5 (13.9)	3 (8.3)	4 (11.1)	6 (16.7)
	≥P4	28	21	75.0		2 (7.1)	5 (17.9)	5 (17.9)	7 (25.0)	1 (3.57)	1 (3.57)
Body Condition Score (BCS)	2.5 to 3.0	80	46	57.5	0.933	5 (6.2)	8 (10.0)	6 (7.5)	10 12.5)	8 (10.0)	9 (11.3)
	3.5	31	15	48.3		2 (6.5)	1 (3.2)	2 (6.5)	2 (6.5)	5 (16.1)	3 (9.7)
	≥4	69	39	56.5		3 (4.3)	5 (7.2)	9 (13.0)	4 (5.8)	8 (11.6)	10 (14.5)
Reproductive Health (RH)	Good	78	37	47.4	0.114	3 (3.8)	4(5.1)	7 (8.9)	6 (7.7)	7 (8.9)	10 (12.8)
	Moderate	88	55	62.5		5 (5.68)	9 (10.2)	11 (12.5)	9 (10.2)	13 (14.8)	8 (9.1)
	Poor	14	8	57.1		0 (0.0)	2 (14.3)	1 (7.1)	2(14.3)	1(7.1)	2 (14.3)
AI Technician (AIT)	AIT 1	60	34	56.7	0.855	2 (3.33)	5(8.33)	7 (11.7)	6(10.0)	8(13.3)	6 (10.0)
	AIT 2	60	31	51.7		3 (5.0)	4(6.7)	6 (10.0)	5(8.3)	7(11.7)	6 (10.0)
	AIT 3	60	35	58.3		5 (8.3)	4 (6.7)	5 (8.3)	7 (11.7)	6 (10.0)	8 (13.3)

Table 2: Analysis of risk factors

Variables	Category	Wald	Sig.	Odd ratio	95.0% C.I. for odd ratio	
					Lower	Upper
Age (years)	1.5 to 2.5	6.775	0.148	1		
	2.6 to 3.5	0.021	0.885	1.099	0.308	3.923
	3.6 to 4.5	2.485	0.115	5.391	0.664	43.798
	4.6 to 5.5	0.010	0.919	1.078	0.251	4.630
	≥5.6	2.021	0.155	2.365	0.722	7.744
Breed	Breed	0.019	0.891	0.947	0.436	2.060
Parity (number)	Parity0	14.024	0.007	1		
	Parity 1	7.785	0.005	0.177	0.053	0.598
	Parity2	9.186	0.002	0.170	0.054	0.535
	Parity3	1.743	0.187	0.468	0.151	1.445
	Parity4	0.854	0.355	0.582	0.184	1.835
BCS	2.5 to 3.0	0.077	0.962	1		
	3.5	0.043	0.837	1.086	0.497	2.374
	≥4	0.005	0.944	0.964	0.350	2.655
RH	Good	1.288	0.525	1		
	Moderate	0.535	0.465	0.612	0.164	2.284
	poor	0.019	0.890	0.912	0.249	3.347
AIT	AIT 1	0.052	0.974	1		
	AIT 2	0.010	0.922	1.043	0.450	2.418
	AIT 3	0.052	0.820	1.105	0.468	2.611
Study group	Group A	10.552	0.061	1		
	Group B	7.200	0.007	0.202	0.063	0.650
	Group C	3.736	0.053	0.319	0.100	1.016
	Group D	0.570	0.450	0.644	0.205	2.019
	Group E	1.584	0.208	0.477	0.150	1.511
	Group F	0.045	0.832	0.882	0.276	2.813
	Constant	5.332	0.021	8.279		

Body condition score (BCS)

The pregnancy rate of animals with body condition scores (BCS) 2.5 to 3.0, 3.5 and 4 or more were 57.5, 48.3, and 56.5% respectively (Table 1). The association was not significant ($P<0.05$). Multiple logistic regression showed no significant effect of BCS. BCS was positively correlated with the breed (0.209^{**}) and negatively correlated with reproductive health (-0.197^{**}). The highest pregnancy rate in cattle with BCS 2.5 to 3.0

(57.5%) partially agrees with Nazhat *et al.* (2021) who observed that heifer/cows with poor body condition scores and loss of weight have lower pregnancy rate. Wathes *et al.* (2007) reported that heifer/cows with a BCS ≥3.0 took 3 weeks longer to conceive than cows with a BCS of 2.75 to 3.5, in agreement with this study.

Table 3: Correlation coefficients between different factors

	Age	Breed	Parity	BCS	RH	AIT
Age	1	-0.174*	0.861**	0.087	0.104	0.212**
Breed	-0.174*	1	-0.175*	0.209**	-0.062	-0.015
Parity	0.861**	-0.175*	1	0.058	0.096	0.025
BCS	0.087	0.209**	0.058	1	-0.197**	-0.010
RH	0.104	-0.062	0.096	-0.197**	1	0.208**
AIT	0.212**	-0.015	0.025	-0.010	0.208**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Reproductive health (RH)

The pregnancy rate of cattle with good, moderate, and poor RH were 47.4, 62.5, and 57.1, respectively, (Table 1). The association was not significant. Multiple logistic regression showed no significant correlation between reproductive health and body condition score (BCS). Reproductive health was correlated with AIT (.208**) and negatively correlated with BCS (-.197**) (Table 3). PLD increased the pregnancy rate of poor reproductive health heifers/cows. Mufti *et al.* (2010) found that reproductive disorders in heifer/cows were an important cause of reduced pregnancy rates, which was partially supported by this study.

AI technician

The pregnancy rates of animals with the performance of AI technicians 1, 2, and 3 were 56.7, 51.3, and 58.3%, respectively (Table 1). Experience of AI technicians can affect the success of AI. AIT 1, 2, and 3 had 9, 12, and 14 years of experience, respectively. The pregnancy rate was higher for the more experienced AI technician but the difference was not significant. The AIT was highly correlated with age (.212**) and RH (.208**) (Table 3). This study agreed with the report of Hassan *et al.* (2003); Shamsuddin *et al.* (2001); Hossain *et al.* (2016); Paul *et al.* (2011).

In conclusion**,** the penis-like device increased the pregnancy rate of heifers/cows. A farm with a good management system needs to be implemented for more accuracy of the study. Therefore, further study with hormonal assays is needed to determine the pathway of the mechanism of pregnancy with PLD stimulation.

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Conflict of interest

The authors have declared no conflict of interest.

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