Veterinary Integrative Sciences 2022; 20(2): 309-316. DOI; 10.12982/VIS.2022.024

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Factors affecting the pregnancy rate of Bangladeshi ewes following Laparoscopic Artificial Insemination (LAP-AI)

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Abstract

The study was conducted to determine the effects of different factors on increased pregnancy rate of Bangladeshi ewes through LAP-AI. A total of 24 indigenous ewes and 4 rams were selected. Frozen semen with a concentration of 100×106 spermatozoa/ 0.25 ml straws were produced. The selected ewes were synchronized using two doses of PGF2α @ 175µg per ewe at 9 days intervals intramuscularly. Estrus was confirmed by the vasectomized ram. The LAP-AI was performed at two different times from the onset of estrus, two angles of placement on the laparoscopic cradle, and two different puncture sites. Pregnancy was confirmed by ultrasonography on days 45-50 following insemination. The pregnancy rate of ewes 2.5-4 years of age and 16-20 kg bodyweight was higher (75% vs. 41.67%) than ewes 1-2 years and 9-13 kg. However, this was not significant ($p>0.05$). The pregnancy rate of ewes having BCS >3 was significantly higher ($p<0.05$) (78.57% vs. 30%) than BCS 2-2.5. The 20-24 hours insemination time from the onset of estrus yielded a significantly higher ($p<0.05$) (75% vs. 25%) pregnancy rate than 12-16 hours. The pregnancy rate was higher (66.67% vs. 50%) at 45° than 30°angle of placement of ewes on the laparoscopic cradle. However, there was no significant difference ($p>0.05$). The puncture point, 4-5 cm away from the udder resulted from a significantly higher pregnancy rate ($p<0.05$) (83.33% vs. 33.33%) than 7-10 cm. The present study indicates that the pregnancy rates following LAP-AI using frozen semen could be increased if the significant factors are considered.

Keywords: Artificial Insemination, Factors, Frozen semen, Laparoscopic, Pregnancy rate, Sheep

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Funding; The authors want to acknowledge the University Grant Commission (UGC), Bangladesh for funding the research project and also the National Science and Technology (NST), Bangladesh for providing the fellowship.

Article history; *received manuscript: 12 December 2021,*
revised manuscript: 18 January 2022,
accepted manuscript: 24 February 2022,
published online:25 February 2022
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Artificial insemination is one of the most significant breeding tools accepted globally for the genetic improvement of animal species through the male line. Now it is possible to obtain a higher pregnancy rate following AI, even with poor quality frozen semen through the development of semen cryopreservation and estrus synchronization. However, ewes bred with frozen semen typically have a lower pregnancy rate, 23-48% following TC-AI (Maxwell and Hewitt, 1986; Kumar and Naqvi, 2014) than LAP-AI, 50-80% (Maxwell and Hewitt, 1986; Haresign 1990; Bari and Haresign, 1998) due to the convoluted and tortuous nature of the cervix and the presence of 4–7 cervical rings (Kershaw *et al*., 2005; Kaabi *et al*., 2006) which prevents deep AI. In LAP-AI semen is deposited directly into the lumen of the uterine horns bypassing the cervix of the ewe (Sathe, 2018). It has a distinct advantage over TC-AI because sperm cells can be deposited fairly close to the site of fertilization. The success of LAP-AI depends on some fundamental factors (Anel *et al*., 2005; Duran 2018; Alejandro, 2019). The pregnancy rate with chilled semen was high (55-64%) following TCAI (Azizunnesa, 2016). However, the pregnancy rate with frozen semen was low (25% to 37%) both at the research and field level (Jha *et al*., 2019). The present study was designed to increase the pregnancy rate using frozen semen following LAP-AI by managing the potential factors which may affect the pregnancy rates in Bangladeshi ewes.

Most of the Bangladeshi sheep are indigenous, with few crossbreds, having short estrus length and are capable of bi-annual lambing and multiple births (Bhuiyan, 2006). Indigenous sheep are inferior to produce hides and wool. Their average body weight 15 to 25 kg and are adapted to hot humid climate (Mukherjee, 2000). The ewe reach puberty around the age of 5-12 months (Mukherjee, 2000). Their body is grey, with black or white patches, and the face, ear, and feet are mostly light black. Under traditional feeding systems, the sheep are raised on harvested or fallow lands, roads, and canal sides and also graze on aquatic weeds.

The effects of frozen-thawed sperm dose and diluent type on pregnancy rate of indigenous ewes were established by Azizunnesa (2016) and Jha *et al.* (2020). It is urgent to determine the influence of other factors before implementing the technique in the field. In Bangladesh, the moving of farming systems from individual to commercial style will require the LAP-AI application in the field in the near future. The present study was undertaken to determine the age, body weight, and BCS of ewe, insemination time, angle of placement of ewe on the laparoscopic cradle, and puncture site on increased pregnancy rate through LAP-AI.

MATERIALS AND METHODS

Study area

The study was performed at Sheep Research Farm and Reproduction Laboratory, under the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, from January 2020 to April 2021. All experimental procedures were performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee (AWEEC) [Date of permission: 26/12/2019, License no: AWEEC/BAU/2019(54)].

Selection and grouping of the animal

Four Bangladeshi indigenous rams and twenty-four ewes were selected randomly and bought from the local market. The ewes were divided in to two groups, A and B (Figure 1). Each group contained 12 animals. The age, bodyweight and parity of the ewes of group A were 1-2 years, 9-13 kg and 1st -3rd parity and 2.5-4 years, 16-20 kg and 4th- 6th parity, respectively for group B. The BCS of ewes was measured following the method of Russel et al. (1969), which was varied from 2- >3 on a scale of 1-5 at 0.5 intervals. Group A and B were again sub-divided into four groups A1, A2, and B1, B2 respectively (Figure 1)

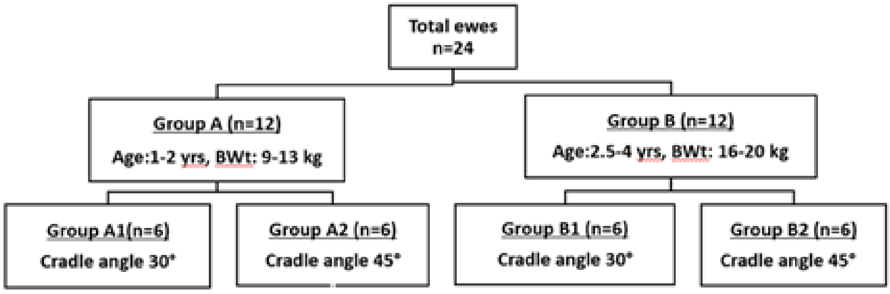

Figure 1 Experimental design of LAP-AI for pregnancy rate determination in ewes; 24 Bangladeshi ewes were selected and divided into groups, A and B. The age and bodyweight of the ewes of group A were 1-2 years and 9-13 kg, and 2.5-4 years and 16-20 kg respectively for group B. Group A and B were again sub-divided into four groups A1, A2, and B1, B2 respectively. The ewes were restrained in a laparoscopic cradle in dorsal recumbence with the rear legs lifted and fortified to an angle of 30° for groups A1 and B1 and 45º for groups A2 and B2 for performing LAP-AI.

Synchronization and detection of estrus in ewes

The ewes were synchronized for estrus using Ovuprost™ (Cloprostenol sterile injection, Bayer Newzealand Ltd) @ 175µg (0.8 ml) per ewe two doses at nine days interval following deep intramuscular injection. Estrus was detected by using vasectomized rams and observing ewes' genitalia by the trained personnel.

Laparoscopic artificial insemination

The synchronized ewes underwent LAP-AI with frozen semen containing 100×10^6 sperm/ml in 0.25 ml semen straw. Before LAP-AI, the frozen semen straws were produced by three-step freezing technique (Jha *et al*., 2019) and semen had at least 50% of sperm motility stored in liquid N2 can at -196°C. The frozen semen straws were thawed at 37°C for 15-30 seconds, and LAP-AI was performed at 12-16 hours and 20-24 hours from the onset of estrus in ewes.

The synchronized ewes were kept fasted and restricted access to water for 12 hours before AI. The ewes were pre-medicated with Acepromazine maleate @ 0.25mg/kg body weight IM and sedated with Xylazine @ 0.22mg/kg body weight IV after 15 minutes of premedication. The lower abdominal region of the ewes was clipped, shaved, and disinfected with povidone-iodine just in front of the mammary gland. The ewes were restrained in a laparoscopic cradle in dorsal recumbence with the rear legs lifted and fortified to an angle of 30° for groups A1 and B1 and 45º for groups A2 and B2 (Figure 1). Two punctures were made with 7mm and 5mm trocar cannula on either side of the midline either 4-5 cm (Jha *et al*., 2020) or 7-10 cm (El-Badry *et al*., 2014) anterior to the mammary gland of the ewe. The trocar was withdrawn, and the abdomen was inflated with CO_2. The laparoscopic tube was inserted into the left-hand cannula for viewing the genitalia. After viewing the bifurcation of the uterine horn, the inseminating pipette was inserted through the right-hand cannula, and each uterine horn was intra-luminally inseminated. All animals were kept under observation and good food was provided two times per day after AI. Broad-spectrum antibiotic, antihistaminic and anti-inflammatory drugs were injected intramuscularly to the inseminated ewes daily for 5 days.

Pregnancy diagnosis

All inseminated ewes were monitored for non-return to estrus with the help of vasectomized ram on 17-21 days following insemination. The inseminated ewes were subjected to ultrasonography for pregnancy confirmation, using trans-abdominal ultrasonic transducer 5.0 MHz, on 45-50 days following insemination by the vet.

Pregnancy rate (%) = Number of ewes pregnant ×100/Number of ewes inseminated

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) 20.0. Significant ($p<0.05$) differences of pregnancy rates between groups of animals were calculated by logistic regression model.

RESULTS

The effects of different factors on pregnancy rate through LAP-AI are placed in Table 1. Ewes were divided into two groups according to their age, body weight and parity vis. 1-2 years of age, 9-13 kg body weight and 1^{st}– 3^{rd} parity versus 2.5-4 years, 16-20 kg and 4^{th}-6^{th} parity respectively. Pregnancy rates for both groups were 41.67% and 75% respectively. The pregnancy rate was higher (75% vs. 41.67%) for the group of ewes of 2.5-4 years of age, 16-20 kg body weight and 4^{th}-6^{th} parity. There was no significant difference ($p>0.05$) of age and body weight of ewes on pregnancy rate. There were two groups of ewes according to BCS ie. BCS 2-2.5 and BCS >3. Pregnancy rates for both groups were 30% and 78.57%, respectively. The pregnancy rate following LAP-AI was significantly higher ($p<0.05$) (78.57%) of ewes having BCS>3 compared to BCS 2-2.5. Eight ewes were inseminated at 12-16 hours and sixteen were inseminated 20-24 hours from the onset of estrus. The pregnancy rates were 25% and 75%, respectively following LAP-AI. The pregnancy rate of ewes was significantly higher ($p<0.05$) (75%) when insemination was performed at 20-24 hours from the onset of estrus. The pregnancy rates were 50% and 66.67% following 30° and 45° angle of placement. Although, the pregnancy rate was higher (66.67% vs. 50%) at 45° angle of placement, there was no significant difference ($p>0.05$) between the two groups. The pregnancy rates of ewes for the sites of trocar puncture, 4-5 cm and 7-10 cm away from the udder were 83.33% and 33.33% respectively. The pregnancy rate was significantly higher ($p<0.05$) (83.33%) when puncturing 4-5 cm than 7-10 cm away from the udder.

Table 1 Effect of different factors on pregnancy rate following LAP-AI in indigenous ewes

Factors	Groups	No. of ewes inseminated	Pregnancy rate	Odds ratio	95% confidence intervals	Chi square p- value
Age	1-2 yrs	12	41.67%	Reference	-	0.098
	2.5-4 yrs	12	75%	4.2	0.738-23.90	
Body weight	9-13 kg	12	41.67%	Reference	-	0.098
	16-20 kg	12	75%	4.2	0.738-23.90	
BCS	2-2.5	10	30%	Reference	-	0.017
	>3	14	78.57%	8.556	1.332-54.949	
Insemination time	12-16 hr	8	25%	Reference	-	0.019
	20-24 hr	16	75%	9	1.268-63.89	
Angle of placement	30°	12	50%	Reference	-	0.408
	45°	12	66.67%	2	0.384-10.409	
Puncture site	4-5 cm	12	83.33%	Reference	-	0.013
	7-10 cm	12	33.33%	0.1	0.014-0.693	

DISCUSSION

It has been considered that the pregnancy rate of ewes after LAP-AI is affected by many factors (Anel *et al*., 2005; Duran 2018; Alejandro, 2019). Among them, ewe age, body weight, BCS, AI timing, angle of placement of ewe on the laparoscopic cradle and site of puncture were investigated concerning pregnancy rate after laparoscopic AI using frozen-thawed semen. The pregnancy rate of ewes 2.5-4 years of age and 16-20 kg bodyweight was higher (75% vs. 41.67%) than ewes 1-2 years and 9-13 kg. The pregnancy rate of ewes having BCS >3 was significantly higher ($p<0.05$) (78.57% vs. 30%) than BCS 2-2.5. The 20-24 hours insemination time from the onset of estrus yielded a significantly higher ($p<0.05$) (75% vs. 25%) pregnancy rate than 12-16 hours. The pregnancy rate was higher (66.67% vs. 50%) at 45° than 30°angle of placement of ewes on the laparoscopic cradle. The puncture point, 4-5 cm away from the udder resulted from a significantly higher pregnancy rate ($p<0.05$) (83.33% vs. 33.33%) than 7-10 cm.

In this study, the higher pregnancy rate (75%) was found for the group of ewes 2.5-4 years of age and 16-20 kg body weight. This is according to the other published works. The pregnancy rate ($p<0.05$) in aged merino ewes was higher than ewe hoggets following LAP-AI in Argentine Patagonia (Moses, 1997). A higher pregnancy rate of 69.9% was found in nulliparous Suffolk ewes less than 3 years old in Japan (Fukui *et al*., 2010). In this study, a higher pregnancy rate was found in ewes having higher body weight. This is similar to Fukui *et al*. (2010) said that, compared to lighter ewes, ewes with higher body weight have higher pregnancy rate due to higher ovulation rate.

A high body condition score has been associated with an increase in ovulation rate (Gonzalez *et al*., 1997). In this study, the pregnancy rate was significantly higher ($p<0.05$) in ewes group having BCS >3 because ewes having BCS >3, show better reproductive performance, normal estrus cycle with better fertility and heavier lamb born while estrous cycle is shorter and more irregular in ewes having BCS 2-2.5 (Aliyari *et al*., 2012). Higher BCS ensures cholesterol availability to maintain ovarian follicle function and favors earlier resumption of ovarian activity (Oliveira Filho *et al*., 2010) and consequently enhances pregnancy rate. This study is similar to Fukui *et al*. (2010) stated that ewes with a BCS >3 are expected to have a higher pregnancy rate through LAP-AI.

For successful LAP-AI, the knowledge of the duration of estrus and the ovulation time is required to know (Gourley and Reise, 1990). Ovulation in sheep normally occurs 24 hours after the onset of estrus. Once ovulation is completed, the ova remain highly fertile for 12-24 hours. On the other hand, frozen-thawed semen survives in the ewe tract maintaining its capacity to fertilize ova for approximately 18-35 hours. So, the time of insemination is more important for frozen-thawed semen than fresh semen due to capacity for survival (Robinson *et al*., 1989) and the optimum time for LAP-AI of ewes is close to the time of ovulation when using frozen-thawed semen (Haresign, 1990). However, it is concluded that the most preferable time of insemination is at the end of estrus in ewes through laparoscopic AI (Gourley and Reise, 1990; Haresign, 1990) because when the ovulation is performed at an early age before ovulation it may increase embryo mortality (Maxwell *et al*., 1984). In this study, a significantly higher ($p<0.05$) pregnancy rate (75%) was found in ewes which were inseminated 20-24 hours from the onset of estrus. The study report is similar to Naher *et al*. (2017) and Azizunnesa (2016), who reported a pregnancy rate of 75%-77.3% following LAP-AI, inseminated 20-24 hours from the onset of estrus.

The angle of placement of ewe on the laparoscopic cradle is an important factor that correlates with the ease of performance of insemination through the intrauterine route. The pregnancy rate was higher (66.7% vs. 50%) in ewes which were placed at 45° angle compared to 30° in this study because the reduction in the size of rumen and bladder due to off feed and drink place less pressure on the diaphragm when the animal is placed at 45° angle (Gourley and Reise, 1990). The present study result is within the range of (Jha *et al*., 2020) who reported a pregnancy rate 63.2-72% using 100×10^6 semen by placing animals at a 45° angle due to better visualization of reproductive organs and ease performance of AI.

In this study, the sites of trocar puncture were 4-5 cm and 7-10 cm away from the udder during performing LAP-AI. The pregnancy rate was significantly higher (83.33%) in ewes which were punctured 4-5 cm away from the udder, facilitating the clear visualization of the uterine horn near the puncture site. This result is similar to Naher *et al*. (2017) and Jha *et al*. (2020). This result is not matched with Dovenski *et al*. (2012) and El-Badry *et al*. (2014), found higher pregnancy rate when puncturing ewes 7-10 cm away from the udder. Due to the smaller reproductive organs of smaller size Bangladeshi ewes punctures had been made at a short distance ie. 4-5 cm to visualize the uterus.

CONCLUSION

In the present study, the pregnancy rate following LAP-AI was excellent (83.33%). The most important factors for increasing the pregnancy rate following LAP-AI were the site of puncture, BCS of ewe, and time of insemination. If we could implement Laparoscopic AI in the field by considering the significant factors it will definitely increase pregnancy rate in ewes. This will ensure the acceptability of AI by the sheep farmers due to the increased production of quality sheep within a shorter period of time.

When preparing ewe lots for LAP-AI, we recommend that ewes having BCS less than 3 should not be used and insemination time should be strictly maintained when using frozen semen to get a higher pregnancy rate.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

AUTHOR CONTRIBUTIONS

Farida Yeasmin Bari- Conceptualization and design of study
Suravi Akter- Investigation and manuscript preparation
Amit Saha- Data analysis
Md. Golam Shahi Alam- Interpretation of the study
Mohammad Asaduzzaman- Manuscript revision, editing and finalization.

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How to cite this article;

Suravi Akter, Mohammad Asaduzzaman, Amit Saha, Md. Golam Shahi Alam, Farida Yeasmin Bari. Factors affecting the pregnancy rate of Bangladeshi ewes following Laparoscopic Artificial Insemination (LAP-AI). Veterinary Integrative Sciences. 2022; 20(2): 309- 316.