JARB
Journal of Animal Reproduction and Biotechnology

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Original Article

Effects of number of frozen-thawed ram sperm and number of inseminations on fertility in synchronized ewes under field condition

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ABSTRACT The effects of the number of frozen-thawed ram sperm per single and double intra-cervical artificial insemination (AI) on fertility in ewes were studied. A total of 89 non-pregnant ewes were synchronized for oestrus with two doses of 100 μg $PGF_{2\alpha}$ (Cloprostenol) 9 days apart. The ewes were randomly assigned to one of four groups; D200 (n = 23; double AI with 200 × 10^6 sperm), S200 (n = 24; single AI with 200 × 10^6 sperm), D100 (n = 24; double AI with 100 × 10^6 sperm) and S100 (n = 18; single AI with 100 × 10^6 sperm). Ewes were inseminated within 12 to 18 h for single AI and, within 10 to 12 h and 16 to 18 h for double AI after the onset of oestrus. The onset of oestrus ranged from 28 to 76 h (54.33 ± 1.28 h). The high percentage (29.2%) of ewes showed oestrus between 51 to 60 h. The non-return rates were highest in group D200 (56.5%) and differed significantly ($p < 0.05$) from group S100 (11.1%). No ewes were pregnant in group S100, and the pregnancy rates among the remaining groups did not differ. The mean gestation period was 152.8 ± 0.5 days and no difference was observed among the groups. The lambing and multiple birth rates were 100% in group D200. The single and twin lambing was highest in group D100 (33.3%) and group D200 (83.3%), respectively. Only one triplet lambing and the highest lambing size (2.2 ± 0.2) was recorded in group D200. In conclusion, double AI with 200 × 10^6 sperm showed comparatively most practical for achieving high pregnancy rates and lambing performances in Bangladeshi ewes under field conditions.

Keywords: artificial insemination, frozen ram semen, sheep fertility, sperm number

INTRODUCTION

Sheep in Bangladesh are non-descript indigenous and small sized, called *Wera*. The breed probably originated from south-eastern sub-tropical humid region's (Turner, 1983). They are hair-wool type, greyish in appearance with black or white patches. The face, ear and feet are mostly light black. The mature body weight weighing 15-25 kg (Ewe) and 18-22 kg (Ram). They are capable of bi-annual lambing with litter size commonly 1-2, rarely 3

(Bhuiyan, 2006). Indiscriminate breeding, scarcity of superior rams and lack of scientific breeding program are the major constraints. Now a days, sheep rearing is gaining popularity in Bangladesh. Breeding programs for sheep is the major concern of farmers in Bangladesh which necessitates use of sustainable and locally appropriate reproductive techniques. Artificial insemination (AI) in conjugation with oestrus synchronization is extensively used in sheep industries in developed countries. It is one of the important assisted reproductive techniques (ARTs) to disseminate genetic traits of interest and increase progenies in a short time. Cryopreservation of semen allows more efficient use of superior rams (Menchaca and Rubianes, 2004; Tekin, 2006). Success of AI depends on the quality of semen (Tekin, 2006), oestrus synchronization, the number of sperm and timing of insemination (Hashem et al., 2015). To establish sheep AI program in Bangladesh, different types of extenders, both home-made (egg yolk tris-based) and commercial have been studied for ram semen preservation (Azizunnesa et al., 2014; Rekha et al., 2016a, 2016b). Studies were conducted with different analogues and doses of progesterone and prostaglandin to observe the oestrus response and pregnancy rates using chilled and frozen semen in indigenous ewes (Roy et al., 2014; Zohara et al., 2014; Rekha et al., 2016a, 2016b). However, semen freezing and AI in sheep in Bangladesh is limited to research stations. Acceptable results have been achieved using frozen-thawed semen abroad (Maxwell et al., 1995; Anel et al., 2003; Anel et al., 2006). Different doses of frozen sperm (50, 100, 200, 400, 500, and 800 $\times$ 10^6/mL) have been used in sheep AI (D'Alessandro et al., 2001; Paulenz et al., 2002; Nordstoga et al., 2009). AI in ewes is normally performed 12-24 h after the onset of oestrus for single insemination (Nordstoga et al., 2009), or 14-22 h after the onset of oestrus for double insemination (Paulenz et al., 2002). Achieving acceptable pregnancy rates is very important for the uptake of sheep AI services. To best of our knowledge, there are no reports on fertility in Bangladeshi ewes after using cryopreserved ram sperm under field condition in Bangladesh. Therefore, the objective of this study was to evaluate the effects of the number of cryopreserved ram sperm per single and double intra-cervical artificial insemination on fertility in Bangladeshi ewes.

MATERIALS AND METHODS

All procedures were approved by the Animal Experimental Ethics Committee, Department of Surgery and Obstetrics, Bangladesh Agricultural University (AEEC/DSO-BAU/02/2015) and were carried out from September 2016 to October 2017. The frozen ram semen was produced in the laboratory of DOS, BAU (N 24.73 latitude and E 90.44 longitude), and AI was performed at Trisal Upazilla (Sub-district), Mymensingh (N 24.34 latitude and E 90.23 longitude), Bangladesh. The area is elevated at 9 m above sea level and receives on average 174 mm of rainfall with the mean annual minimum and maximum temperatures ranges between 16.46 to 29.13°C, respectively.

Rams
The best ten selected indigenous rams ("Wera") based on semen quality evaluation were used as semen donors. The rams were aged 2 to 2.5 years, body weight was 18 to 24 kg, and scrotal circumference was 18 to 22 cm. The volume of semen was $\geq$ 0.5 mL, colour $\geq$ 3 (milky white), mass activity $\geq$ 4, motility $\geq$ 80%, viability $\geq$ 90%, concentration (10^6/mL) $\geq$ 2500, plasma membrane integrity (HOST) $\geq$ 85%, acrosome integrity $\geq$ 90%, and normal sperm $\geq$ 80%. The rams belonged to a project funded by Bangladesh Academy of Science and United States Department of Agriculture (BAS-USDA; LS-02) and reared at a research farm, DSO, BAU (Jha et al., 2018).

Chemicals
All chemicals used in this study were from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Semen collection and evaluation
Briefly, semen was collected using an artificial vagina (Jha et al., 2018). Soon after collection, the tube containing semen was immersed in a water bath at 35°C. Sperm motility (%; warming stage at 37°C) was estimated subjectively by placing a drop of semen 5 µL (100×) using a coverslip. Sperm concentration (10^6/mL) was calculated using a Neubauer counting chamber at a dilution of 1:200 (semen: buffered formal saline, 200×).

Semen dilution, freezing, and preservation
A home-made tris based extender (Tris 3.63 g, fructose 0.5 g, citric acid 1.99 g in 100 mL of deionized water) was

prepared and the stock was stored at 5°C. On the day of semen collection, the final extender was prepared by adding egg-yolk 20% v/v, penicillin G (sodium salt) 100,000 IU, dihydrostreptomycin sulfate 100 mg after which the extender was split into two fractions: fraction A (no glycerol) and fraction B (5% v/v glycerol).

Only ejaculates with volume $\geq$ 0.5 mL, sperm motility $\geq$ 80%, and sperm concentration $\geq$ 2500 × 10^6/mL were processed. Individual ram semen ejaculates were extended to two final concentrations of 400 and 800 × 10^6 spermatozoa/mL, in two fraction dilution step. Semen was diluted with fraction A at +35°C and cooling gradually (-0.25°C/min) from +35 to +5°C in 120 min. The samples were further diluted with fraction B at +5°C. The diluted semen was loaded into 0.25 mL French mini straws and were equilibrated at 5°C for 120 min. The straws were frozen in liquid nitrogen (LN2) vapor in a Styrofoam box using 3 step freezing technique developed in our laboratory (Jha et al., 2019). The straws were placed 9 cm above the surface of LN2 for 7.5 min (where the temperature decreased from +5°C to -80°C at 11.33°C/min), at 7 cm for 1.5 min (from -80°C to -120°C at 26.66°C/min), and at 5 cm for 1.5 min (from -120°C to -140°C at 13.33°C/min). Finally the straws were plunged into the LN2 (-196°C).

The semen straws were thawed in warm water at 37°C for 20 sec. Batches with sperm motility $\geq$ 50% were stored in Cryocan for AI purposes.

Ewes for AI and its management in the field

An interaction program in consultation with the District Livestock Services (DLS) was organized to acquaint the sheep farmers about the application and benefits of AI. Apparently mature, healthy and non-pregnant ewes (n = 89 out of 237) aged $\geq$ 16 to 30 month, body weight $\geq$ 15 to 18 kg, body condition score $\geq$ 3.5 to 4 and parity $\geq$ 1 to 3 were selected with the aid of ultrasound scanning (DRAMINSKI ANIMAL profi portable ultrasound scanner, Poland) using a 5.0 MHz trans-abdominal transducer. To prevent natural mating, the neighboring farmer's ewes were housed separately from the day of ultrasonography to non-return period. Feeding, grazing, watering, and housing remained as routinely done by the farmer.

Experimental design

The ewes (n = 89) were randomly allocated into 4 groups: D200 (n = 23; double AI with 200 × 10^6 sperm);

S200 (n = 24; single AI with 200 × 10^6 sperm); D100 (n = 24; double AI with 100 × 10^6 sperm); and S100 (n = 18; single AI with 100 × 10^6 sperm). The insemination was performed 12 to 18 h after the onset of oestrus for single AI, or 10 to 12 h and 16 to 18 h after the onset of oestrus for double AI.

Oestrous synchronization

Oestrous synchronization was done according to Zohara et al. (2014). All ewes were given two doses of 0.4 mL (100 μg) intramuscular (im) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (Cloprostenol, Ovuprost, Bayer New Zealand Ltd., Auckland, New Zealand) 9 days apart.

Artificial insemination

The ewes were positioned upright with their head down and hind quarter up. AI was performed using an eccentric inseminating pipette and insemination gun (Minitube, Slovakia) with the help of vaginal speculum and penlight. The semen was slowly deposited as deep as possible into the cervix. Insemination was performed by the same inseminator through the study.

Reproductive performance

The onset of oestrous was determined by monitoring every 6 h for 30 to 40 min from 12 to 96 h following the second $PGF_{2\alpha}$ treatment, with the help of a mature ram wearing an apron. Similarly, the non-return rates was determined by monitoring every 6 h for 30 to 40 min from 13 to 21 days after insemination, with the help of a mature ram wearing an apron (Olivera-Muzante et al., 2011). The non-returned ewes were checked for pregnancy by ultrasound 40 to 50 days after AI (Olivera-Muzante et al., 2011). The oestrus response (number of ewes showing oestrus/ number treated × 100), non-return rate (number of ewes not returning to oestrus/ number inseminated × 100), pregnancy rate (number of pregnant ewes/ number inseminated ×100), lambing rate (number of lambing ewes/ number of pregnant ewes × 100), multiple birth rates (number of ewes lambing twin or triplet/ total number of lambing ewes × 100), lambing size (number of total lambs/ number of lambing ewes × 100) and the female or male lamb rates (number of female or male lamb/ total number of lambs × 100) were recorded (Türk et al., 2008).

Data analysis

Excel (Microsoft Excel 2010) was used to record and calculate the frequency distribution of onset of oestrus, and SPSS (Version 20; IBM) was used for other data analysis. The gestation periods and lambing size were compared by one-way analysis of variance (ANOVA) followed by post hoc Tukey-HSD test. The other reproductive traits were compared by Chi-squared test. Data for gestation period and lambing size were presented as mean ± S.E.M. and a value $p < 0.05$ was considered significantly different.

RESULTS

Onset of oestrus and oestrus response rates

The frequency distribution of onset of oestrus in ewes following second $PGF_{2\alpha}$ treatment is shown in Fig. 1. The oestrus response rates was 100% (89/89) with the ewes coming into oestrus 28 to 76 h with a mean time of 54.33 ± 1.28 h after the second $PGF_{2\alpha}$ treatment. The highest percentages of ewes observed in oestrus between 51 to 60 h were 29.2% (26/89). Only 2.2% (2/89) of ewes showed onset of oestrus between 21 to 30 h.

Reproductive performances

1) Non-return rates, pregnancy rates, and gestation period
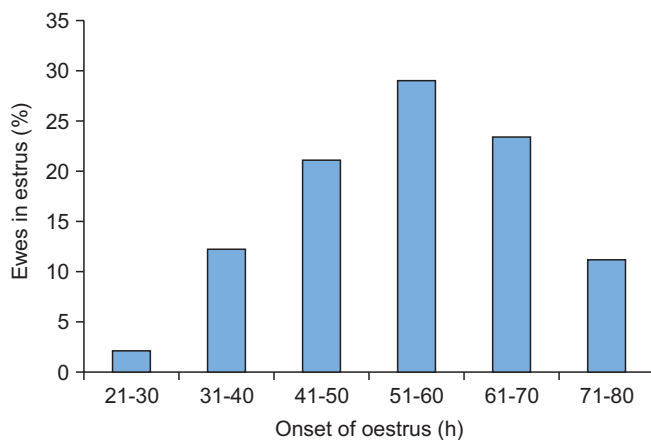Table 1 show the non-return and pregnancy rates. The

Fig. 1. Frequency distribution of onset of oestrus in ewes after second $PGF_{2\alpha}$ treatment. Selected non-pregnant ewes (n = 89) were treated with two doses of 0.4 mL (100 μg) intramuscular (im) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (Cloprostenol, Ovuprost) 9 days apart for oestrus synchronization. The ewes were monitored every 6 h for 30 to 40 min from 12 to 96 h following the second $PGF_{2\alpha}$ treatment, with the help of a mature ram wearing an apron.

non- return rate were higher in group D200 (56.5%) than group S100 (11.1%) ($p < 0.05$). None of the ewes were pregnant in group S100, and the pregnancy rates among the remaining groups varied insignificantly. The mean gestation period was 152.8 ± 0.5 days and no difference was observed among the groups.

2) Lambing performance
The lambing rates of each group were 100% (Table 1). The differences in multiple birth rates and the number of lambing among the groups were insignificant. The multiple birth rate was highest in group D200 (100%, 6/6) but differed insignificantly with group S200 (75%, 3/4) and group D100 (66.7%, 2/3). The single and twin lambing was highest in group D100 (33.3%, 1/3) and group D200 (83.3%, 5/6), respectively. The lambing size did not differ among the groups, however was highest in group D200 (2.2 ± 0.2) followed by group S200 (1.8 ± 0.3) and group D100 (1.7 ± 0.3). The male and female lambing rates differed insignificantly.

DISCUSSION

We evaluated the effects of the number of frozen-thawed ram sperm and the number of inseminations on fertility in synchronized ewes under field conditions in Bangladesh. We observed 100% estrous response and lambing rates. The overall reproductive performance was observed higher in the group inseminated twice with 200 × 10⁶ sperm; no ewes became pregnant in the group inseminated once with 100 × 10⁶ sperm.

The success of AI depends on the efficacy of the oestrus synchronization (Gungor et al., 2007; Hashem et al., 2015), quality of frozen semen (Tekin, 2006) and timing of AI (Hashemi et al., 2006). We used $PGF_{2\alpha}$ as an oestrus synchronizing agent which is believed to be a cost-effective luteolytic agent (Davis et al., 1980; Light et al., 1994).

Despite its wide variation in oestrus response (up to 100%) and onset of oestrus, (over a 4 day interval; Menchaca and Rubianes, 2004), the oestrus response rates in our study were in agreement with Öztürkler et al. (2003) and Zohara et al. (2014) who reported oestrous response 100% using Cloprostenol 75 μg and 100 μg/ ewe in Bangladeshi and Tushin ewes. In contrast, Trounson et al. (1976) reported oestrus response rates of 84% and 80% in ewes using 100 μg and 125 μg cloprostenol, respectively.

Table 1. Reproductive performances of ewes in different AI group

Reproductive parameters	Group			
	D200	S200	D100	S100
Number of ewes	23	24	24	18
Non-return rates (%)	56.5[a] (13/23)	41.7[a,b] (10/24)	25.0[a,b] (6/24)	11.1b (2/18)
Pregnancy rates (%)	26.1 (6/23)	16.7 (4/24)	12.5 (3/24)	0 (0/18)
Gestation period (days)				
Single (mean ± S.E.M.)	NA	154.0	152.0	NA
Twin (mean ± S.E.M.)	153.2 ± 0.4	152.0 ± 2.3	153.5 ± 0.5	NA
Triplet (mean ± S.E.M.)	151.0	NA	NA	NA
Lambing rates (%)	100 (6/6)	100 (4/4)	100 (3/3)	NA
Multiple birth rates (%)	100 (6/6)	75 (3/4)	66.7 (2/3)	NA
Number of lambs				
Single (%)	NA	25 (1/4)	33.3 (1/3)	NA
Twin (%)	83.3 (5/6)	75 (3/4)	66.7 (2/3)	NA
Triplet (%)	16.7 (1/6)	NA	NA	NA
Lambing size (mean ± S.E.M.)	2.2 ± 0.2	1.8 ± 0.3	1.7 ± 0.3	NA
Female lamb rates (%)	46.2 (6/13)	57.1 (4/7)	40.0 (2/5)	NA
Male lamb rates (%)	53.8 (7/13)	42.9 (3/7)	60.0 (3/5)	NA

Differences among values bearing different superscripts (a, b) are statistically significant ($p < 0.05$).

NA: Not applicable.

Selected non-pregnant ewes (n = 89) were randomly assigned to 4 groups: D200 (n = 23; double AI with 200×10^6 sperm), S200 (n = 24; single AI with 200×10^6 sperm), D100 (n = 24; double AI with 100×10^6 sperm) and S100 (n = 18; single AI with 100×10^6 sperm). The insemination was performed 12 to 18 h after the onset of oestrus for single AI, or 10 to 12 h and 16 to 18 h after the onset of oestrus for double AI. Ewes which did not return to oestrus after 13 to 21 days post insemination were submitted to ultrasonography between 40 to 50 days for confirmation of pregnancy. Reproductive parameters such as oestrus response (number of ewes showing oestrus/number of treated ewes × 100), non-return (number of ewes non-return to oestrus/number of inseminated ewes in each group × 100), pregnancy rates (number of pregnant ewes/number of inseminated ewes in each group × 100), lambing rates (number of lambing ewes/ number of pregnant ewes in each group × 100), multiple birth rates (number of ewes lambing twin or triplet/ total number of lambing ewes in each group × 100), lambing size (number of total lambs/ number of lambing ewes in each group × 100) and the female or male lamb rates (number of female or male lamb/ total number of lambs in each group × 100) were recorded. The reproductive traits were expressed as % except the gestation period and lambing size which were expressed as mean ± S.E.M.

*$p < 0.05$ was compared among the groups.

Hashem et al. (2015) and Menchaca et al. (2004) reported oestrus response rates 78.57% with 5 mg dinoprost tromethamine and 82.4 to 93.9% with 160 µg delprostenate in ewes. The mean time to onset of oestrus in our study was closer to Hashem et al. (2015), who reported 50.4 ± 7.3 h. The higher proportion of oestrus observed between 41 to 70 h was in agreement with Acritopoulou-Fourcroy et al. (1982), but differs with Menchaca et al. (2004) who reported between 25 to 48 h.

Variation in oestrus response is due to differences in season, the presence of ram in the herd (Moeini et al., 2009), body condition and management system (Yadi et al., 2011), nutritional condition and latitude (Martinez-T et al., 2011). The parity and breeds may also affect the oestrus response. A lower mean time of estrous than our result has been reported in the Corriedale breed both in nulliparous (39.6 ± 1.1 h) and multiparous (40.6 ± 0.5 h) ewes (Menchaca et al., 2004). The mean time to onset of oestrus could vary due to the follicular status of an individual ewe. A large healthy growing follicle at time of treatment will continue its development leading to oestrus and ovulation soon after $PGF_{2\alpha}$ administration. Similarly, while the largest follicle is in a regressing phase, a new follicle needs to emerge, and normally oestrus and ovulation will follow (Menchaca and Rubianes, 2004; Hashem et al., 2015).

The non-return rates depends upon the fertility of the ram. The non-return rates in our study ranged from 11.1 to 56.5%. The highest non-return rate of 56.5% in group D200 was comparable with that of Kerton et al. (1984) who reported 56%, and 31% after vaginal insemination. The variation in non-return rates could be due to the skill of the

technician, difference in flock conditions, and observation of the oestrus behavioural signs (Andersen Berg and Aamdal, 1991). Non-return rates is also associated with insemination being conducted too early; deleterious effects often occur both before and after maternal recognition of pregnancy (Olivera-Muzante et al., 2011). The rams used as donors in this study were selected based on semen evaluation (Jha et al., 2018), not on breeding value. However, it is ideal to select rams for AI based on breeding value after natural mating, which is an important index of fertility.

The highest pregnancy rates (26.1%) in this study was observed after double AI with 200×10^6 sperm. Rekha et al. (2016b) reported 20.8% pregnancy rate using frozen semen in Bangladesh ewes. However, the pregnancy rates in our study using double insemination with 100×10^6 sperm was comparable with Rekha et al. (2016b) who obtained 13.6%. This difference might be due to the higher number of sperm per insemination and double AI. The pregnancy rates may vary with the type of semen used, insemination technique and stage of oestrus. In sheep the AI dose (number of motile sperm/ insemination) has been reported to range from 50 to 200×10^6 and inseminated using single or double doses (6 to 8 h apart) between 12 to 24 h after the onset of oestrus (D'Alessandro et al., 2001; Nordstoga et al., 2009; Paulenz et al., 2009). The pregnancy rates was 85% after AI with fresh semen in natural oestrus (Meinecke and Meinecke-Tillmann, 1986), 88.4% with fresh semen inseminated into the cervix (Khalifa et al., 2008), 25 to 27.3% and 7.1 to 26.8% with chilled semen inseminated trans-cervical (Rekha et al., 2016a), 50.8% and 53.5% following vaginal and cervical insemination with chilled semen at 400×10^6 sperm in Sarda ewes (Branca et al., 1994), 60% and 64% following insemination with 100×10^6 sperm after vaginal and cervical insemination (Maxwell and Hewitt, 1986) and 59.3% with frozen semen inseminated trans-cervical (Wulster-Radcliffe and Lewis, 2002). The lambing rates in our study were 100%. In contrast, Tervit et al. (1984) reported lambing rates of about 69% and 64% following insemination with 225×10^6 sperm by cervical and vaginal insemination, respectively. The lambing rates and lambing size depends upon genetic potential, plane of nutritional, age, weight, and timing of AI (Downing and Scaramuzzi, 1991).

In conclusion, double AI with 200×10^6 sperm showed comparatively most practical for achieving high pregnancy rates and lambing performances in Bangladeshi ewes

under field conditions. This study provides some preliminary and important results that suggest room for further research with a greater number of ewes. We also suggest that such further research is also needed in appropriate knowledge transfer leading to highest technology adoption rates, including methods for in-depth training of sheep farmers and appropriate dissemination of this technology which will result in enhanced fertility.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

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REFERENCES

Acritopoulou-Fourcroy S, Pappas V, Peclaris G, Zervas N, Skoufis V. 1982. Synchronization of oestrus in ewes with Provera sponges/PMSG, prostaglandin F2α or the prostaglandin analogue, ICI 80996, and fertility following natural mating or artificial insemination. Reprod. Nutr. Dévelop. 22:345-354.

Andersen Berg K and Aamdal J. 1991. Artificial insemination with frozen semen in ewes at different times of the breeding season. Reprod. Domest. Anim. 26:27-30.

Anel L, Alvarez M, Martinez-Pastor F, Garcia-Macias V, Anel E, de Paz P. 2006. Improvement strategies in ovine artificial insemination. Reprod. Domest. Anim. 41 Suppl 2:30-42.

Anel L, de Paz P, Alvarez M, Chamorro CA, Boixo JC, Manso A, González M, Kaabi M, Anel E. 2003. Field and in vitro assay of three methods for freezing ram semen. Theriogenology 60:1293-1308.

Azizunnesa, Zohara BF, Bari F, Alam MGS. 2014. Effects of proportion of egg yolk and preservation time on chilled semen from indigenous rams. GSTF J. Vet. Sci. 1:18-26.

Bhuiyan AKFH. 2006. Livestock genetic resources in Bangladesh: preservation and management. International conference on livestock services, Chinese Academy of Agricultural Science (CAAS), Beijing, China, pp. 16-20.

Branca A, Cappai P, Dattene M, Gallus M, Ledda S, Loi P, Naitana S. 1994. A simplified method of artificial insemination for ewe lambs. In: Abstraacts of the XI Congresso Nazinale Societa Italiana Di Patologia e Di Allevamento Degli Ovini e Di Caprini. Perugia, Italy. pp. 317-318.

D'Alessandro AG, Martemucci AG, Colonna MA, Bellitti A. 2001. Post-thaw survival of ram spermatozoa and fertility after insemination as affected by prefreezing sperm concentration and extender composition. Theriogenology 55:1159-1170.

Davis A, Fleet I, Harrison F, Walker FM. 1980. Pulmonary metabolism of prostaglandin F2a in the conscious non-pregnant ewe and sow. J. Physiol. 301(Suppl), 86.

Downing JA and Scaramuzzi RJ. 1991. Nutrient effects on ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones in sheep. J. Reprod. Fertil. Suppl. 43:209-227.

Güngör Ö, Cenesiz M, Pancarci SM, Yıldız S, Kaya M, Kaçar C, Özyurtlu N, Gürbulak K. 2007. Effects of different intravaginal progesterone releasing devices on estrous synchronization and LH surge in fat-tailed ewes during non-breeding season. Med. Weter. 63:1316-1319.

Hashem NM, El-Zarkouny SZ, Taha TA, Abo-Elezz ZR. 2015. Oestrous response and characterization of the ovulatory wave following oestrous synchronization using PGF2α alone or combined with GnRH in ewes. Small Rumin. Res. 129:84-87.

Hashemi M, Safdarian M, Kafi M. 2006. Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. Small Rumin. Res. 65:279-283.

Jha PK, Alam M, Al-Mansur MA, Islam M, Bari F. 2018. Selection of breeding rams by evaluating semen quality. J. Appl. Anim. Sci. 11:9-20.

Jha PK, Alam MGS, Mansur AAL, Naher N, Islam T, Bhuiyan MU, Bari FY. 2019. Cryopreservation of Bangladeshi ram semen using different diluents and manual freezing techniques. Cryobiology 89:35-41.

Kerton D, McPhee S, Davis I, White M, Banfield J, Cahill L. 1984. A comparison of insemination techniques in Corriedale ewes. Proceedings of the Australian Society of Animal Production. 15, pp.701.

Khalifa TA, Lymberopoulos AG, El-Saidy BE. 2008. Testing usability of butylated hydroxytoluene in conservation of goat semen. Reprod. Domest. Anim. 43:525-530.

Light JE, Silvia WJ, Reid RC 2nd. 1994. Luteolytic effect of prostaglandin F2 alpha and two metabolites in ewes. J. Anim. Sci. 72:2718-2721.

Martinez-Tinajero JJ, Ruiz-Herluver I, Montanez-Valdez OD, Martinez-Priego G, Velasco-Zebadua ME, Izaguirre F. 2011. Reproductive performance in Dorper ewes synchronized at estrus during non breeding season in tropical conditions. J. Anim. Vet. Adv. 10:221-223.

Maxwell WM, Landers AJ, Evans G. 1995. Survival and fertility of ram spermatozoa frozen in pellets, straws and minitubes. Theriogenology 43:1201-1210.

Maxwell WMC and Hewitt LJ. 1986. A comparison of vaginal, cervical and intrauterine insemination of sheep. J. Agric. Sci. 106:191-193.

Meinecke B and Meinecke-Tillmann S. 1986. [Fertilization results in superovulated ewes and goats after transmural-intrauterine insemination controlled by laparoscopy]. Tierarztl. Prax. 14:35-41. German.

Menchaca A, Miller V, Gil J, Pinczak A, Laca M, Rubianes E. 2004. Prostaglandin F2alpha treatment associated with timed artificial insemination in ewes. Reprod. Domest. Anim. 39:352-355

Menchaca A and Rubianes E. 2004. New treatments associated with timed artificial insemination in small ruminants. Reprod. Fertil. Dev. 16:403-413.

Moeini MM, Alipour F, Moghadam A. 2009. The effect of hu-

man chorionic gonadotropin on the reproduction performance in Lory sheep synchronized with different doses of pregnant mare serum gonadotrophin outside the breeding season. Asian J. Anim. Vet. Adv. 4:9-15.

Nordstoga AB, Söderquist L, Ådnøy T, Paulenz H. 2009. Effect of different packages and freezing/thawing protocols on fertility of ram semen. Reprod. Domest. Anim. 44:527-531.

Olivera-Muzante J, Fierro S, López V, Gil J. 2011. Comparison of prostaglandin- and progesterone-based protocols for timed artificial insemination in sheep. Theriogenology 75:1232-1238.

Öztürkler Y, Çolak A, Baykal A, Guven B. 2003. Combined effect of a prostaglandin analogue and a progestagen treatment for 5 days on oestrus synchronisation in Tushin ewes. Indian Vet. J. 80:917-920.

Paulenz H, Adnøy T, Fossen OH, Söderquist L. 2009. Effect on field fertility of addition of gelatine, different dilution rates and storage times of cooled ram semen after vaginal insemination. Reprod. Domest. Anim. 45:706-710.

Paulenz H, Adnøy T, Fossen OH, Söderquist L, Berg KA. 2002. Effect of deposition site and sperm number on the fertility of sheep inseminated with liquid semen. Vet. Rec. 150:299-302.

Rekha A, Zohara BF, Bari F, Alam MGS. 2016a. Comparison of commercial Triladyl extender with a tris-fructose-egg-yolk extender on the quality of frozen semen and pregnancy rate after transcervical AI in Bangladeshi indigenous sheep (Ovis aries). Small Rumin. Res. 134:39-43.

Rekha A, Zohara BF, Bari FY, Alam MGS. 2016b. Comparisons of commercial Triladyl and locally manufactured extenders for the chilling of semen and their effects on pregnancy rates after transcervical AI in Bangladeshi Indigenous (Ovis aries) sheep. Anim. Reprod. 13:735-742.

Roy PK, Zohara BF, Nesa A, Paul AK, Bhuiyan MMU, Bari FY.

2014. Oestrus synchronization with Ovuprost® and Prostenol® in the indigenous ewes of Bangladesh. J. Emb. Trans. 29:149-155.

Tekin N, Uysal O, Akçay E, Yavaş İ. 2006. Effects of different taurine doses and freezing rate on freezing of row semen. Ank. Üniv. Vet. Fak. Derg. 53:179-184.

Tervit H, Goold P, James R. 1984. The insemination of sheep with fresh or frozen semen. In: Abstracts of the proceedings of the New Zealand Society of Animal Production. pp. 11-13.

Trounson AO, Willadsen SM, Moor RM. 1976. Effect of prostaglandin analogue Cloprostenol on oestrus, ovulation and embryonic viability in sheep. J. Agric. Sci. 86:609-611.

Türk G, Gür S, Sönmez M, Bozkurt T, Aksu EH, Aksoy H. 2008. Effect of exogenous GnRH at the time of artificial insemination on reproductive performance of Awassi ewes synchronized with progestagen-PMSG-PGF2alpha combination. Reprod. Domest. Anim. 43:308-313.

Turner HN. 1983. Origins of the CSIRO Booroola. Wool Technol. Sheep Breed. 31:10-13.

Wulster-Radcliffe MC and Lewis GS. 2002. Development of a new transcervical artificial insemination method for sheep: effects of a new transcervical artificial insemination catheter and traversing the cervix on semen quality and fertility. Theriogenology 58:1361-1371.

Yadi J, Moghaddam MF, Khalajzadeh S, Solati AA. 2011. Comparison of estrus synchronization by PGF2α, CIDR and sponge with PMSG in Kalkuhi ewes on early anestrous season. In: Abstracts of the International Conference on Asia Agriculture and Animal. Singapoore. pp. 61-65.

Zohara BF, Azizunnesa A, Faruk I, Alam MGS, Bari FY. 2014. Comparison of estrus synchronization by PGF2α and progestagen sponge with PMSG in indigenous ewes in Bangladesh. GSTF J. Vet. Sci. 1:27-37.