IJAS Iranian Journal of Applied Animal Science

Determinants of Trans-Cervical Artificial Insemination Success in Synchronized Ewes Using Frozen Semen

Research Article

N. Naher[1], S. Arif[1], M. Hossain[1], N. Sultana Juyena[1] and F. Yeasmin Bari[1*]

[1] Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Received on: 15 Nov 2022
Revised on: 13 Jan 2023
Accepted on: 25 Feb 2023
Online Published on: Sep 2023

*Correspondence E-mail: faridabari06@gmail.com

Online version is available on: www.ijas.ir

ABSTRACT

The study was undertaken to observe the effects of cervical penetration depth, estrus signs intensity and vaginal electrical resistance on conception rate in ewes following trans-cervical artificial insemination with frozen semen. Twenty-four ewes were synchronized using 100 µg Prostaglandin $F_2\alpha$ intramuscularly, twice at 9 days interval. Estrus behavior of ewes with teaser ram was observed to determine the estrus onset and intensity. Vaginal electrical resistance was recorded immediately before insemination using electrical heat detector. A 200 µg tablet, Misoprostol was administered intra-vaginally in 10 ewes 11 hours before insemination, and cervical penetration depth was measured by centimeter graduated steel rod. Conception rate was confirmed by ultrasonography at 40-42 days of insemination. The cervical penetration depth was significantly increased ($P<0.05$) in Misoprostol-treated ewes compared to non-treated ones (3.54±0.10 *vs.* 0.47±0.04 cm, respectively). Similarly, conception rate was also higher following Misoprostol treatment (60.0 *vs.* 28.6%, respectively) but no significant difference. Significantly higher ($P<0.05$) conception rate was found in ewes with high estrous signs intensity compared to medium (70.0 *vs.* 22.2%) and low intensity (70.0 *vs.* 20.0%), respectively. Conception rate was tended to be higher (58.3 *vs.* 37.5%) in low vaginal electrical resistance group (230-280 Ω) compared with the high group (281-330 Ω). Higher conception rate in Misoprostol-treated ewes indicates that the grater cervical penetrability during semen deposition ensures the success of TCAI with frozen semen. Estrus signs intensity and low vaginal electrical resistance could be used as a valuable indicator to select ewes for successful trans-cervical artificial insemination (TCAI) through obtaining higher conception rate.

KEY WORDS cervical penetration depth, conception rate, estrus signs intensity, TCAI, VER.

INTRODUCTION

The development of livestock sector has been a significant priority in Bangladesh for last two decades. Sheep are one of the important livestock species of Bangladesh, especially in the char-island areas where people suffer from extreme poverty. Sheep rearing facilitates poor farmers to meet up their protein requirement in diet and helps in earning extra capital by selling them in the local market and employment facilities. However, the genetic potential and productivity of our indigenous sheep are declining due to lack of proper breeding systems (Islam *et al.* 2021). Moreover, there is also scarcity of high quality breeding ram. Hence, Artificial Insemination (AI) with frozen semen can serve as a fundamental tool for the advancement of sheep farming industry in these low income areas through genetic improvement of indigenous sheep. Fukui *et al.* (2010) have attempted to increase conception rate in ewes using frozen semen by laparoscopic intrauterine insemination (LAPAI) method. But, high cost, requirement of highly qualified staff, and

disturbing nature of insemination technique limit the use of LAPAI in local sheep farms (Aisen *et al*. 2000). Trans-cervical artificial insemination (TCAI) with frozen semen could be the most convenient technique to improve the conception rate in indigenous ewes. Unfortunately, this latter technique (TCAI) is not still accepted by the sheep farmers due to low conception rate obtained by researcher. Indeed, Mansur *et al.* (2018) obtained 25.0-26.7% conception rate in indigenous ewes with TCAI using frozen-thawed semen and claimed that this technique is influenced by many factors. The achievement of higher pregnancy rate of AI depends on the quality of cryopreserved semen, proper estrus detection, and mastership the insemination technique itself. In fact, performing TCAI seems to be difficult because of convoluted cervical anatomy in most ewes that limits transcervical passage of inseminating pipette (Leethongdee *et al*. 2010). Kumar and Naqvi (2014) reported that the depth of insemination is positively correlated with pregnancy and lambing rate. Several attempts were taken to induce cervical softening or dilation with hormonal treatment *viz.* oxytocin (Khalifa *et al*. 1992), prostaglandin (Leethongdee *et al.* 2007; Bartlewski and Candappa, 2015), oestradiol (Owiny *et al*. 1992) corazol (Gunduz *et al*. 2010) for softening or dilating the ovine cervix and thus increasing the insemination depth. Among them, a synthetic analogue of prostaglandin E1, Misoprostol is commonly used to induce cervical softening (Li *et al.* 2005). Leethongdee *et al.* (2007) obtained 5-6 cm cervical penetration depth using 1 mg Misoprostol as a cervical relaxant for Welsh Mountain sheep. Although oxytocin could increase the cervical penetration rate in ewes, it is possible that fertility following oxytocin treatment and TCAI may be adversely affected (Sayre and Lewis, 1997). Moreover, poor estrus detection rate in ewes also results in low pregnancy rate following TCAI. The intensity of estrus signs following estrus synchronization facilitate accurate estrus detection and select ewes to perform AI to optimize the conception rate (Kozdrowski *et al.* 2006; Garcia *et al.* 2011). Furthermore, it has been demonstrated that the lowest impedance of the vaginal mucous membrane in ewes occurs just before estrus due to high level of estrogen and remains low for 24 to 48 hours (Masia *et al.* 2007). Thus, values of Vaginal Electrical Resistance (VER) during estrus assist in proper timing of AI to optimize the conception rate in ewes following estrus induction (Theodosiadou and Tsiligianni, 2015). A preliminary study (Naher *et al*. 2016) was conducted in Bangladesh Agricultural University to observe the pregnancy rate in ewes with frozen semen using different AI technique. The result of that pilot study triggers us to find out the key factors compromising the conception in our local ewes following trans-cervical artificial insemination with frozen semen. However, the study of Naher *et al.* (2016), include a few number of ewes and did not consider the breeding season, management of ewes or ewe related parameter i.e. previous parity, body condition score (BCS), body weight. Hence, the present study was carried out following the methods used in the preliminary trial of Naher *et al.* (2016) to observe the consistency of that finding considering ewe related parameters, semi-intensive management, breeding season and intensive monitoring of experimental ewes. Therefore, the main objectives of this study were to determine the effects of cervical penetration depth, estrus signs intensity and VER on conception rate in synchronized ewes following TCAI technique with frozen-thawed semen.

MATERIALS AND METHODS

Ethical approval

The research was designed following the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The study design on ewes was approved by the Animal Experimental Ethics Committee (AEEC), Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. The experiment was conducted at the Sheep Research Farm of Bangladesh Agricultural University, Mymensingh during the period of October 2016-January, 2017 (breeding season for local sheep). Sheep were reared under semi-intensive system where they were allowed to graze on pasture land around 8 hours daily and fed with 200 gm. of concentrates mixture (wheat bran, crushed maize, soya bean meal, dry fish mineral, DCP powder and table salt) per animal per day. Regular deworming at 3 months intervals with broad spectrum anthelmintic and vaccination (PPR vaccine) were performed to the selected ewes.

Ewe selection

Non-pregnant multiparous (ewes between 1st and 2nd parity) indigenous ewes (n=24) were selected from the sheep farm for the study. Our non-descript indigenous sheep called Wera are small sized hair-wool type, greyish in appearance with black or white patches. The face, ear and feet are mostly light black (Jha *et al*. 2020). The age, body weight, BCS and parity of selected ewes were between 612.8 ± 12.6 months, 19.20 ± 0.35 kg, and 2.92 ± 0.35, respectively.

Estrus synchronization and detection

Estrus was synchronized by intra-muscular injection of 100 µg Prostaglandin $F_2\alpha$ (Ovuprost, Bayer, New Zealand) (0.4 mL) per ewe, twice at 9 days apart. Behavioral estrus signs of ewes were detected by the use of teaser ram (teasers are prepared through vasectomy of normal breeding rams). Two teasers were introduced into the ewe flock following one day of second injection for a total period of 48 hours

and estrus ewes were identified observing the teasing activity of teasers. Visual observation was made at 2 hours interval daily from 8.00 a.m. to 5.00 p.m., spending 30 minutes for each inspection to identify estrus ewes.

Observing estrus intensity

The intensity of estrus was determined and graded according to Ortman (2000). Presence of following parameters after introduction of teaser rams in ewe flock were considered for determining estrus intensity: 1) the ewe-ram seeking activity, 2) active immobilization followed by mating, 3) ewe's tail fanning and 4) movement of ewes' head towards male. Estrus intensity was graded as low (when at least parameter no. 2 was present), medium (when at least two parameters were observed and definitely one was no. 2) and high (when all 4 parameters were present).

Cervical ripening treatment and measuring cervical penetration depth

Among 24 selected ewes, 10 were treated with two tablets of Misoprostol (Cytomis, 200 µg tablet; Incepta Pharmaceuticals Ltd. Bangladesh) intra-vaginally 10-11 hours before AI as a means of cervical softening and relaxation treatment, as described by Ustuner *et al.* (2018). A centimeter (cm) graded steel rod of 25 cm length, 0.25 cm diameter having round end (modifying the technique of Leethongdee *et al.* (2007), who used AI gun plunger for measuring penetration depth) was introduced slowly into the cervix for measuring penetration depth immediately before AI in term of easiness of AI gun passes.

Recording VER

Electrical Resistance of vaginal mucosa in ewes were measured before TCAI with the aid of electronic heat detector (DRAMINSKI, Owocowa 17, Poland) following the technique of Theodosiadou *et al.* (2014).

Post-thaw semen evaluation

Artificial insemination was done using frozen ram semen produced in the reproduction laboratory of Surgery and Obstetrics department, BAU. Post-thaw semen quality was assessed by motility percentage, plasma membrane integrity and viability of the sperm as described by Rekha *et al.* (2016). The motility percentage was determined by eye-estimation of the proportion of spermatozoa moving progressively straight forward at higher magnification (400X) of Phase-contrast microscope. Plasma membrane integrity of the sperm was assessed by using hypo-osmotic swelling test (HOST+ve) whereas the viability of sperm was determined using eosin-nigrosin stain. The mean values (±SE) of post-thaw motility, functional integrity and viability were 51.0 ± 4.2, 58.4 ± 6.2 and 63.8 ± 4.9%, respectively.

TCAI in ewes

TCAI was performed within 14- 22 hours of onset of estrus with frozen semen. Afterwards positioning the ewes in dorso-head down position with hind quarter up, a sterile vaginal speculum was inserted into vagina. The cervical opening was visualized using pen-torch light. A commercially available insemination pipette having an eccentrically tip (Minitube, Germany) loaded with 0.25 mL thawed semen straw was introduced into the cervix and gently advanced forward through the cervical rings. Semen was then expelled from the pipette as deep as possible within the cervix.

Pregnancy diagnosis

Pregnancy was confirmed by ultrasonography using a digital trans-abdominal ultrasonic transducer 5.0 MHz (Model Magic 5000, Art NO. 303700, Germany) within 40-42 days of TCAI.

Conception rate (%)= number of ewes conceived × 100 / number of ewes inseminated

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SPSS 20.0 (SPSS, 2011). Chi-square test was performed to compare the conception rate. The difference between the groups was considered significant, when P value was less than 0.05 ($P<0.05$).

RESULTS AND DISCUSSION

The depth of cervical penetration (before and after ripening treatment with Misoprostol) and its effect on conception rate are presented in Table 1. Cervical penetration depths were 0.34 ± 0.09 and 0.36 ± 0.08 cm in Misoprostol treated and non-treated groups immediately before estrus synchronization; whereas, the depths were 3.54 ± 0.10 and 0.47 ± 0.04 cm immediately before AI, respectively. Cervical penetration depth was significantly higher ($P<0.05$) in treatment compared to non-treatment group immediately before AI. The depth was also significantly increased ($P<0.05$) after treatment than before (3.54±0.10 *vs.* 0.34±0.09 cm, respectively). The conception rate tended to be higher (60.0 *vs.* 28.6%) in treatment compared to non-treatment group but there was no significant difference ($P>0.05$). Twenty four ewes are allotted into 3 group *viz.* high, medium and low estrus sings intensity following the published report of Ortman (2000). The conception rates were 70.0, 22.2 and 20.0% in synchronized ewes with high, medium and low estrus intensity, respectively (Table 2).

Table 1 Depth of cervical penetration and conception rate

Ewe group	Cervical penetration depth immediately before estrus synchronization (cm) (Mean±SE)	Cervical penetration depth immediately before AI (cm) (Mean±SE)	Conception rate (%)
Misoprostol treated (n=10)	0.34±0.09[b]	3.54±0.10[a]	60.0 (6/10)
Non-treated (n=14)	0.36±0.08[b]	0.47±0.04[b]	28.6 (4/14)

AI: artificial insemination.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 2 Effects of estrus intensity on conception rate

Estrus intensity	No. of ewes inseminated	No. of ewes conceived	Conception rate (%)
High	10	7	70.0[a]
Medium	9	2	22.2[b]
Low	5	1	20.0[b]

The means within the same rows with at least one common letter, do not have significant difference (P>0.05).

Significantly higher (P<0.05) conception rate was obtained in those ewes showing high estrus intensity than in those with medium and low intensity of estrus.

On the basis of VER value (Ω) before AI, the twenty-four ewes were allocated into 3 group *viz.* 230-280 Ω, 281-330 Ω and 331-380 Ω. Effects of VER on conception rate of ewes are presented in Table 3. The conception rates were higher (50.0 *vs.* 37.5%, respectively) in VER group 230-280 Ω compared with VER group 281-330 Ω, but the difference was not significant. None of the four ewes in high VER group (331-380 Ω) conceive after AI.

A successful TCAI technique greatly depends on the ability of AI gun to pass deeply into the cervix as it can facilitate intrauterine semen deposition (Donovan *et. al.* 2004). Salamon and Maxwell (1995) reported a positive relationship between the site of semen deposition in the genital tract of ewes and fertility rates achieved. Buckrell *et al.* (1994) reported that there was significant linear increase in fertility as the depth of insemination increased (6.6-12.2% per cm past the cervical os). The findings from the present study revealed that the depth of cervical penetration increased significantly following misoprostol (PGE1 analogue) treatment in estrus induced ewes. Significantly higher (P<0.05) cervical penetration was observed in misoprostol treated ewes compared to non-treated ewes. Furthermore, post-treatment cervical penetration depth was significantly higher (P<0.05) from pre-treatment cervical penetration within the treatment group (misoprostopl treated ewes). Consequently, increased cervical penetration depth (3.54±0.10 cm) in misoprostopl treated ewes allowed the deposition of semen deeper in the cervix, which resulted in higher conception rate (60.0%). This finding is consistent with the result of Rashidi and Cedden (2013) who found 68.2% conception rate when cervical penetration depth was increased following Misoprostol administration. As PGE_1 analogue, Misoprostol causes dilatation of cervical canal and then semen can be deposited beyond 3.5 cm, which might result in higher pregnancy rate (Leethongdee *et al.* 2010). Bartlewski and Canadappa (2015) also stated that the 12 to 24 hour pretreatment with Prostaglandin E_2 (Cervidili) can improve cervical penetration depth as a result the total time required for TCAI significantly reduced. Conversely, Falchi *et al.* (2012) did not find any significant effect on the depth of cervical penetration following intracervical administration of oxytocin and / or misoprostol and the differences in the results may occur due to difference in sheep breed and estrus induction method. Although the exact mechanism by which Misoprostol relaxes the cervix of ewes is unknown, Leethongdee *et al.* (2010) proposed that the dilatation of cervical canal dilation occurs during estrus through the activation of prostaglandin synthesis via an increase in cyclooxygenase-2 (COX-2) in the fibroblast and smooth muscle cells following intra-cervical application of Misoprostol.

In this study, a major effect of estrus signs intensity was observed on conception rate. Conception rate was significantly higher (P<0.05) in the ewes with high intensity of estrus expression compared to medium and low intensity (70.0 *vs.* 22.2% and 70.0 *vs.* 20.0%, respectively). This result is in agreement with the study of Ferraz *et al.* (2017) who obtained higher conception rate in high estrus intensity. High estrogen level during estrus decreases uterine pH which causes reduced sperm metabolism and motility. As a result, sperm viability, motility and the speed of sperm transportation in the female genital tract is increased until ovulation (Ferraz *et al.* 2017). This may be the cause of higher conception rate in ewes exhibiting high estrus intensity.

Table 3 Effects of vaginal electrical resistance (VER) on conception rate

Vaginal electrical resistance (VER) (Ω)	No. of ewes inseminated	No. of ewes conceived	Conception rate (%)	Level of significance
230-280	12	7	58.3	
281-330	8	3	37.5	NS
331-380	4	0	0	

NS: non significant.

On the other hand, According to Perry *et al.* (2005), the higher conception rate of females expressing high estrus signs intensity might have occurred due to higher ovulation rate and better synchronization of ovulation compared to females with low estrus signs intensity.

For obtaining higher conception rate, accurate estrus detection is mandatory (Yamauchi *et al.* 2009). Visual observation following introduction of "teaser" rams remains the only available method for estrus detection in ewes. Monitoring changes in vaginal resistance could be a reliable alternative to visual observation of estrus in ewes. It is well known that vaginal resistance changes throughout the estrous cycle and its lowest values occur around estrus due to increased levels of NaCl (sodium chloride) in the vaginal mucosa as an effect of high estrogen level (Fehring, 1997). Therefore, the lowest VER value might be reliable tool for estrus detection (Theodosiadou *et al.* 2014). With respect to these considerations, one of the aims of the present study was to know the effects of VER on pregnancy rate. We obtained that the percentage of conception was higher (58.3%) in lower VER (vaginal electrical resistance) group (230-280 Ω) compared to higher one. This observation confirms the finding of Theodosiadou *et al.* (2014) and Naher *et al.* (2016) who obtained higher conception rate in ewes inseminated with low electrical impedance of cervical mucus.

CONCLUSION

Depth of cervical penetration during TCAI with frozen semen had a significant impact on the conception rate in ewes. To upsurge conception rate, high estrus intensity and low VER values during estrus could be used as effective tools for selecting indigenous ewes to be inseminated. Therefore, cervical penetration depth, intensity of induced estrus signs and values of vaginal electrical resistance could be considered as key determinants for TCAI success in ewes.

ACKNOWLEDGEMENT

The authors are grateful to Bangladesh Academy of Sciences- The United States Department of Agriculture (BAS-USDA; LS-02) for financial support as well as Dr. Pankaj Kumar Jha for laboratory support.

REFERENCES

Aisen E.G., Alvarez H.L., Venutrino A. and Garde J.J. (2000). Effect of trehalose and EDTA on cryoprotective action of ram semen diluents. *Theriogenology*. **53(5),** 1053-1061.

Bartlewski P.M. and Candappa I.B.R. (2015). Assessing the usefulness of prostaglandin E2 (Cervidil) for transcervical artificial insemination in ewes. *Theriogenology*. **84,** 1594-1602.

Buckrell B.C., Buschbeck C., Gartley C.J., Kroetsch T., McCutcheon W., Martin J., Penner W.K. and Walton J.S. (1994). Further development of a Trans-cervical technique for artificial insemination in sheep using previously frozen semen. *Theriogenology*. **42,** 601-611.

Donovan A., Hanrahan J.P., Kummen E., Duffy P. and Boland M.P. (2004). Fertility in the ewe following cervical insemination with fresh or frozen–thawed semen at a natural or synchronised oestrus. *Anim. Reprod. Sci*. **84(3),** 359-368.

Falchi L., Taema M., La Clanche S. and Scaramuzzi R.J. (2012). The pattern of cervical penetration and the effect of topical treatment with prostaglandin and/or FSH and oxytocin on the depth of cervical penetration in the ewe during the periovulatory period. T*heriogenology*. **78,** 376-384.

Fehring R.J. (1997). A comparison of the ovulation method with the CUE ovulation predictor in determining fertile period. *J. Am. Assoc. Nurs. Pract.* **8,** 461-466.

Ferraz P.A., Loiola M.V., Rodrigues A.S., Lima M.C., Bittencourt T.C. and Ribeiro Filho A.D. (2017). The effect of the intensity of estrus expression on the follicular diameter and fertility of Nellore cows managed under a FTAI program. *Ciên. Anim. Bras*. **18,** 1-9.

Fukui F., Kohno H., Okabe K., Katsuki S., Yoshizawa M., Togari T. and Watanabe H. (2010). Factors affecting the fertility of ewes after intrauterine insemination with frozen-thawed semen during non-breeding season. *J. Reprod. Dev*. **56(4),** 460-466.

Garcia E., Hultgren J., Fällman P., Geust J., Algers B., Stilwell G., Gunnarsson S. and Rodriguez-Martinez H. (2011). Oestrous intensity is positively associated with reproductive outcome in high-producing dairy cows. *Livest. Sci*. **139(3),** 191-195.

Gunduz M., Turna O., Cirit U., Ucmak M., Tek C., Sabuncu A. and Bacinoğlu S. (2010). Lambing rates and litter size following carazolol administration prior to insemination in Kivircik ewes. *Anim. Reprod. Sci*. **118(1),** 32-36.

Islam S.S., Hasan M.S., Ghosh N., Islam M.S. and Islam M.M. (2021). Prospects and Problems of Indigenous Sheep Production in South Western Coastal Regions of Bangladesh. *J. Agric. Sci. Sri Lanka*. **16(1),** 54-66.

Jha P.K., Alam M.G.S., Mansur M.A.A., Talukder M.R.I., Naher N., Rahman A.K.M.A., Hall D.C. and Bari F.Y. (2020). Eff-

ects of number of frozen-thawed ram sperm and number of inseminations on fertility in synchronized ewes under field. *J. Anim. Reprod. Biotechnol*. **35(2),**190-197.

Khalifa R., Sayre B. and Lewis G. (1992). Exogenous oxytocin dilates the cervix in ewes. *J. Anim. Sci.* **70,** 38-42.

Kozdrowski R., Twardoń J., Dejneka G.J. and Dzięcioł M. (2006). Influence of oestrus intensity and level of physical constitution on results of artificial insemination in cattle. *MedWet*. **62(9),** 1038-1040.

Kumar D. and Naqvi S.M.K. (2014). Effect of time and depth of insemination on fertility of Bharat Merino sheep inseminated trans-cervical with frozen-thawed semen. *Anim. Reprod. Sci.* **56(3),** 801-806.

Leethongdee S., Kershaw-Young C.M., Scaramuzzi R.J. and Khalid M. (2010). Intra-cervical application of Misoprostol at estrus alters the content of cervical hyaluronan and the mRNA ex pression of follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR) and cyclooxygenase-2 in the ewe. *Theriogenology*. **73(9),** 1257-1266.

Leethongdee S., Khalid M. and Bhatti A. (2007). The effects of prostaglandin analogue misoprostol and follicle-stimulating hormone on cervical penetration in ewes during the peri-ovulatory period. *Theriogenology*. **67,** 767-777.

Li Y.T., Kuo T.C., Kuan L.C. and Chu Y.C. (2005). Cervical softening with vaginal Misoprostol before intrauterine device insertion. *Int. J. Gynaecol. Obstet.* **83,** 67-68.

Mansur M.A.A., Alam M.G.S., Jha P.K., Rimon M.A., Naher N. and Bari F.Y. (2018). Intra-cervical artificial insemination conception rate using frozen semen at field level in indigenous sheep of Bangladesh. *Asian J. Med. Biol. Res*. **4(1),** 55-62.

Masia F., Mayorga I., Mara L., Stelletta C., Chessa F., Casu S. and Dattena M. (2007). Monitoring oestrus in synchronized sarda ewes by vaginal mucus impedance assay: A preliminary study. Pp. 202-212 in Preoc. 23th Sci. Meet. European Embryo Trans. Assoc. Alghero, Italy.

Naher N., Juyena N.S., Jha P.K., Talukder M.R.I., Alam M.G.S. and Bari FY. (2016). Factors influencing the pregnancy rate in indigenous ewes following artificial insemination (AI) using frozen semen. *Bangladesh Vet*. **33(2),** 33-38.

Ortman R. (2000). Monitoring of estrus cycle of ewes by ram-seeking behavior. *Small Rumin. Res*. **37,** 73-84.

Owiny J., Fitzpatrick R. and Spiller D. (1992). Changes in the extensibility of the ovine cervix uteri following infusion of estradiol-17β at term. *Small Rumin. Res.* **7,** 75-83.

Perry G.A., Smith M.F., Lucy M.C., Green J.A., Parks T.E., Macneil M.D., Andrew J.R. and Thomas W.G. (2005). Relationship between follicle size at insemination and pregnancy success. *Natl. Acad. Sci. USA*. **14,** 5268-5273.

Rashidi M. and Cedden F. (2013). Trans-cervical artificial insemination in ewes during out of breeding season. *Maced. J. Anim. Sci*. **3(2),** 143-146.

Rekha A., Zahora B.F., Bari F.Y. and Alam M.G.S. (2016). 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.

Salamon S. and Maxwell W.M.C. (1995). Frozen storage of ram semen and processing, freezing, thawing and fertility after cervical insemination. *Anim. Reprod. Sci.* **37,** 185-249.

Sayre B. and Lewis G. (1997). Fertility and ovum fertilization rate after laparoscopic or transcervical intrauterine artificial insemination of oxytocin-treated ewes. *Theriogenology*. **48,** 267-275.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc., USA.

Theodosiadou E. and Tsiligianni T. (2015). Determination of the proper time for mating after oestrous synchronisation during anoestrous or oestrous by measuring electrical resistance of cervical mucus in ewes. *Vet. Med.* **60(2),** 87-93.

Theodosiadou E., Amiridis G.S. and Tsiligianni T.H. (2014). Relationship between electrical resistance of cervical mucus and ovarian steroid concentration at the time of artificial insemination in ewes. *Reprod. Biol*. **14,** 234-237.

Ustuner H., Ustuner B., Toker M.B., Alcay S., Demir K., Sagirkaya H. and Nur Z. (2018). Administration time of misoprostol affects fertility rate in artificially inseminated Kivircik ewes with frozen-thawed ram semen. *Anim. Reprod*. **15(2),** 156-160.

Yamauchi S., Nakamura S., Yoshimoto T., Nakada T., Ashizawa K. and Tatemoto H. (2009). Prediction of estrous cycle and optimal insemination time by monitoring vaginal electrical resistance (VER) in order to improve the reproductive efficiency of the Okinawan native Agu pig. *Anim. Reprod. Sci.* **113,** 311-316.