Does isoxsuprine HCl facilitate the passage of the cervix in sheep?: a case series

N. TEKIN ONDER[a*], TAYGUN GÖKDEMIR[a], MUHAMMET CAN KILIÇ[a], OĞUZHAN ŞAHIN[a], M. BERK TOKER[B], SAVAŞ YILDIZ[a], YAVUZ ÖZTÜRKLER[a]

[a] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey
[b] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey

SUMMARY

The complexity of the sheep cervix limits non-surgical artificial insemination and embryo production technologies. For this reason, assisted reproduction techniques are generally performed with surgical methods in sheep. But it is said that surgical methods can hurt the health and welfare of animals and cause them to feel stressed in different ways. Because of these problems with surgical methods and some difficulties in the application phase, researchers are trying to come up with ways to help with reproduction that don't involve surgery. For the application of non-surgical assisted reproductive techniques in sheep, there is a need for successful relaxation of the cervix. Because of this, different tocolytic agents have been used before non-surgical methods of assisted reproduction. Isoxsuprine HCl is used to relax the uterus during procedures like simple dystocia, to prevent premature birth, embryotomies, and caesarean deliveries. It is also used to treat horse navicular disease and laminitis in modern veterinary medicine. Tocolysis usually sets in about 10-15 minutes after an intramuscular isoxsuprine HCl administration. The aim of the present study was to evaluate the effects of isoxsuprine HCl on cervical dilatation in ewes. In our study, it has been thought that isoxsuprine HCl, which is also a tocolytic agent, might be an alternative for non-surgical reproductive uses. The study was carried out on a total of 20 animals: Ten sheep were given cervical relaxation with isoxsuprine HCl and ten animals were given no tocolytic agent. The mean cervical transition time was 83.60±13.63 seconds in animals treated with isoxsuprine HCl and 168.22±20.83 seconds in the control group. A significant difference was found between the groups ($P<0.05$). The minimum transition time was found to be 19 seconds in the isoxsuprine HCl group and 30 seconds in the control group. Maximum transition times were found at 140 and 238 seconds, respectively. As a result, it was seen that isoxsuprine HCl can offer a good alternative in transcervical applications in sheep.

KEY WORDS

Ewe, Transcervical, Dilation.

INTRODUCTION

Artificial insemination and embryo transfer applications are applied surgically due to the complex structure and anatomical differences of the cervix uteri in sheep [1-4]. Alternative methods for these procedures are being developed in order to avoid complications such as adhesion and bleeding [5]. However, the applicability of transcervical applications in the luteal phase becomes difficult. The duration of the passage of the cervical rings and the experience of the operator are very important factors in the success of these procedures. It is necessary to avoid cervical bleeding that may occur due to force while passing the cervical rings. Because bleeding, adhesions, and infections that may occur for this reason may decrease the future fertility rate of that animal [6,7]. In order to perform assisted reproduction processes in small ruminants; prostaglandin E2 (PGE2), estradiol, and oxytocin applications have been carried out to obtain embryos from donor animals and transfer them to carrier animals by directly entering the cervix uteri [4,5,8,9].

Isoxsuprine HCl was first synthesized in 1956 by Moed and Van Dijk. Isoxsuprine HCl, which has been reported to have a spasmolytic effect 40 times more than papaverine, is used as a myometrial relaxant to prevent premature births in humans [8,10,11]. In cattle, Gregory and Rodrigues [12] applied isoxsuprine HCl 5 minutes before embryo transfer to relax the uterus. In ruminants, the recomended dose of 0.4-2 mg/kg is administered intramuscularly, and tocolytic effect begins within 10-15 minutes and uterine contractions triggered by -adrenergic receptors are suppressed [11,13].

According to our research, no study has been found using isoxsuprine HCl for the purpose of relieving and passing the cervix in sheep. In the present study, the passage time of the cervical canal was measured after isoxsuprine HCl application and without any application in the control group. The difference between the control group and the isoxsuprine HCl group was evaluated.

MATERIAL AND METHODS

The Scientific Ethical Committee (Kafkas University, Kars, Türkiye) has approved all issues concerning the experimental

Corresponding Author:
Nail Tekin Onder (nailtekinonder@gmail.com).

setups and evaluation techniques (2022-110). All Tuj breed ewes aged 2 to 5 years were kept in the same conditions at Kafkas University's Faculty of Veterinary Medicine in Kars, Turkey. Experiments were carried out during the non-breeding season, and ewes that had given birth at least once were used.
Before the presented study, at least 20 animals were treated, and the possibilities that could affect the cervical transit times were examined. The focus was on maximum efficiency during the presented study.

Experimental Design

This study was designed to evaluate the degree of relaxation of the sheep cervix after intra-muscular isoxsuprine HCl (Utelax® 50 mL, Sanovel, Turkey) injection. For this purpose, 10 animals were assigned to the isoxsuprine HCl (U) group, and 10 animals were assigned to the control group (C).

Sedation and Cervical Dilatation

The animal was restrained by a special restrainer for ewes. Then, 0.4 mg/kg of xylazine (Rompun® %2, Bayer, Turkey) was administered i.m. [14]. Considering the recommendation to use a double dose if anesthetic applications are made in the prospectus of the drug, 0.8 mg/kg of isoxsuprine HCl was administered by i.m. The operator waited for 10 minutes for the drug to take effect.

Preparation of Animal and Cervical Penetration

The perineal region of the animal was cleaned with povidone iodine. The tail was tied, and contamination was prevented. Lubricant was applied to the small speculum, the vulva was opened, and it was inserted into the vagina. The protocol used by Pereira et al. [9] was modified in the cervical penetration stage. The cervical entrance was grasped with forester ring forceps. The speculum was removed, and the cervix was pulled caudally. With the 1 mm diameter Bakes rosebud urethral sound dilator (Figure 1), the cervical rings were passed with gentle movements and gentle manipulations with the fingers through the vagina to the cervix. The stopwatch was started at the beginning of cervical passage and stopped when the cervix was passed (Figure 2). During the study, operations were carried out by a single operator.

Statistical Analysis

All data obtained from the study were analyzed using SPSS (20.0 for Windows; SPSS, Chicago, IL, USA). Data were represented as mean ± standard error (x±Sx). The Shapiro-Wilk test was used as a normality test. The statistical differences between the groups' means were determined by an Independent Sample T-Test. Differences with values of $P<0.05$ were considered statistically significant.

RESULTS

In the present study, the mean transit time of the cervix was found to be 83.60±13.63 seconds in the U group, and 168.22±20.83 in the control group (Table 1). The statistical analysis revealed a difference between the groups ($P<0.05$). In the trial study (before the presented study), we used Allis forceps, Pozzi forceps, and Forester ring forceps. It has been seen that Allis and Pozzi forceps cause a small amount of bleeding. There was no active bleeding when forester ring forceps were used, but redness was observed on the clipped tissue. In the trials, it was also clear that where the forester ring forceps are used to clip is important. This may make it harder for the operator to handle the situation or make the cervical transition process easier.

DISCUSSION

The complex structure of the cervix of sheep and the inability to control the cervical line with rectal manipulations limit

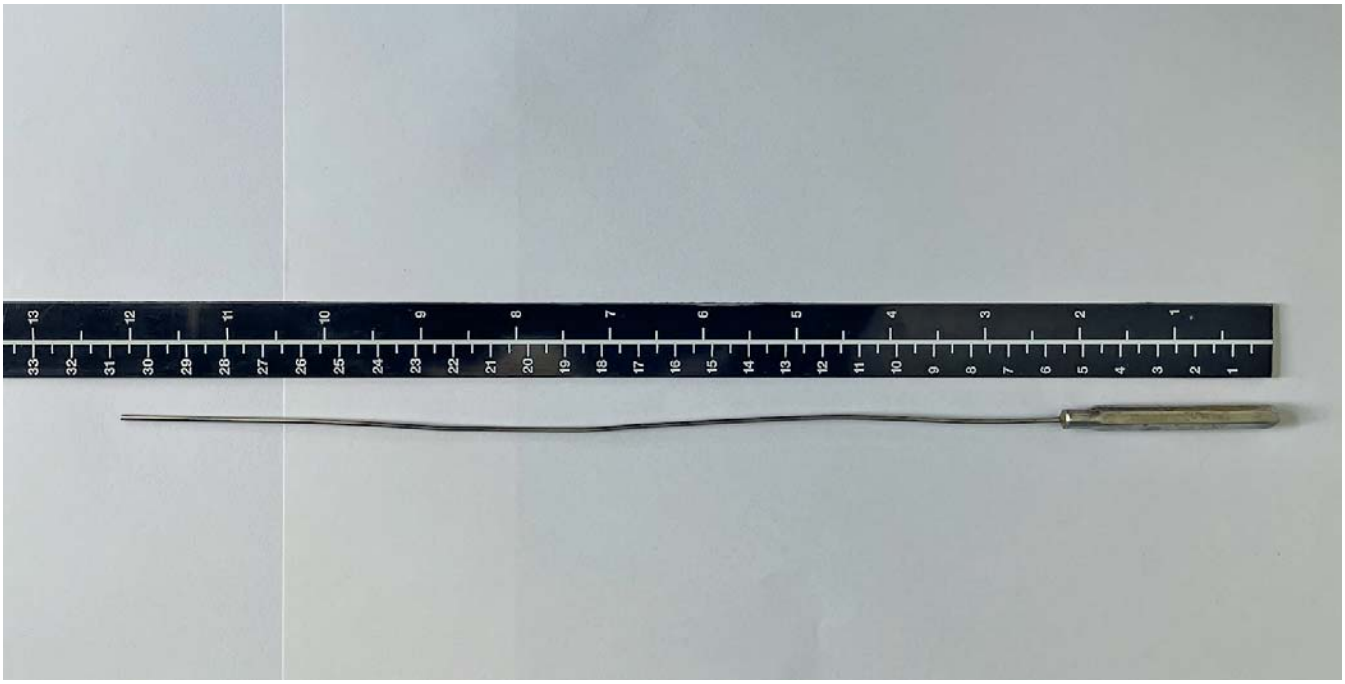

Figure 1 - 1 mm diameter bakes rosebud urethral sound dilator.

Figure 2 - Cervical passage of the cervix in the sheep.

Table 1 - Cervical transit time in seconds.

Groups	Transit Time (TT)	Minimum TT	Maximum TT
U	83.60±13.63[a]	19	140
C	168.22±20.83[b]	30	238

a and b: Values with different superscripts in the same column for each times are significantly different (P < 0.05).

the application of non-surgical assisted reproductive techniques, especially embryo production and transfer [5,15,16]. However, the use of different tocolitic agents that will facilitate the passage of the cervical line would increase the applicability of these techniques [17,18,19]. Furthermore, the use of these methods would have positive effects on animal welfare compared to surgical methods [5, 20].

In sheep, during labor, estrogen and oxytocin levels start to increase; as a result, cervical dilatation and an increase in uterine contractility occur [21]. Due to the cervical dilation effects of these hormones, they have been used in transcervical applications in sheep [4,7,22]. However, Stellflug et al. [23] reported that oxytocin applications before transcervical artificial insemination in sheep decreased the rate of pregnancy-specific protein B and lambing. According to King et al. [24], sheep that received laparoscopic and cervical insemination had a lower lambing rate than those that received oxytocin.

PGE2, which has an anti-inflammatory and relaxing effect on myometrial smooth muscles, is also used for cervical dilation in sheep [9,25]. It is also reported that PGE2 has positive effects on fertility [26]. On the other hand, it has been reported that isoxsuprine provides much better arterial and venous relaxation compared to PGE2 in horses [27,28]. In the studies of Gregory and Rodrigues (1986) [12] performed by applying isoxsuprine chloride before embryo transfer in cattle, it is seen that the pregnancy rate improved numerically, and the pregnancy rates in the control group and animals treated with isoxsuprine chloride were 39% and 48%, respectively.

Pozzi forceps and Allis forceps have been reported to be used in previous studies to hold and pull the cervix [5, 7, 9, 29]. In our study, in which we used forester ring forceps, it was observed that there was no active bleeding and a slight discoloration of the tissue. For this reason, the use of uterine forceps in transcervical applications seems to be more advantageous for holding and pulling the cervix.

According to the findings of our study, isoxsuprine HCl can be used successfully to provide cervical relaxation in sheep. In this context, we think that the effects of isoxsuprine HCl on pregnancy deserve to be examined in detail in the future.

ACKNOWLEDGEMENTS

None

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION SECTION

NTO designed and performed the experiment. NTO, YO, MBT wrote the manuscript. YO provided the isoxsuprine HCL for the experiment. TG, MCK, OS, SY provided assistance during the experiment. NTO and MBT performed the statistical analysis.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

References

1. Armstrong D.T., Evans G. (1983). Factors influencing success of embryo transfer in sheep and goats. Theriogenology. 19(1): 31-42.
2. Bruno-Galarraga M.M., Cueto M., Gibbons A.E., Pereyra-Bonnet F., Catalano R., Gonzalez-Bulnes A. (2014). Repeatability of superovulatory response to successive FSH treatments in Merino sheep. Small Rumin Res. 120(1): 84-89.
3. Santos J.D.R., Ungerfeld R., Balaro M.F.A., Souza-Fabjan J.M.G., Cosentino I.O., Brair V.L., Souza C.V., Pinto P.H.N., Bade A.L.C., Fonseca J.F., Brandão F.Z. (2020). Transcervical vs. laparotomy embryo collection in ewes: the effectiveness and welfare implications of each technique. Theriogenology. 153: 112-121.
4. Wulster-Radcliffe M.C., Costine B.A., Lewis G.S. (1999). Estradiol-17 β-oxytocin-induced cervical dilation in sheep: application to transcervical embryo transfer. J Anim Sci. 77(10): 2587-2593.
5. Fonseca J.F., Souza-Fabjan J.M.G., Oliveira M.E.F., Leite C.R., Nascimento-Penido P.M.P., Brandão F.Z., Lehloenya K.C. (2016). Nonsurgical embryo recovery and transfer in sheep and goats, Theriogenology. 86(1): 144-151.
6. Candappa I.B.R., Bartlewski P.M. (2014). Induction of cervical dilation for transcervical embryo transfer in ewes. Reprod Biol Endocrinol. 12(1): 1-9.
7. Fonseca J.F., Oliveira M.E.F., Brandão F.Z., Batista R.I., Garcia A.R., Bartlewski P.M., Souza-Fabjan J.M. (2019). Non-surgical embryo transfer in goats and sheep: the Brazilian experience. Reprod Fertil Dev. 31(1): 17-26.
8. Bhoi D.B. (2022) Tocolysis in farm animal reproduction. Livest Sci. 13, 41-47.
9. Pereira R.J.T.A., Sohnrey B., Holtz W. (1998). Nonsurgical embryo collection in goats treated with prostaglandin F2α and oxytocin. J Anim Sci. 76(2): 360-363.
10. Landesman R., Wilson K., Zlatnik F.J. (1966). The Myometrial relaxant properties of isoxsuprine and 2 methanesulfonamido derivatives. Obstet

Gynecol. 28(6): 775-783.
11. Menard L. (1984). Tocolytic drugs for use in veterinary obstetrics. Can Vet J, 25 (10): 389-393.
12. Gregory R.M., Rodrigues J.L. (1986). Pregnancy rates after embryo transfer using drugs that produce relaxation of the uterus. Theriogenology. 25(1): 157.
13. Vural H.A., Koenhemsi L., Gonul R., Yardibi H.D., Gulyasar T., Or E., Hosturk G.T., Barutcu B., Balci H. (2016). Effects of isoxsuprine hydrochloride on electrocardiographic and trace element status in sheeps. J Pak Med Assoc. 66(8): 938-942.
14. Kurtdede A., Börkü M.K., Gürkan M. (1988). Xylazine hydrochloride (rompun) ve ketamine hydrochloride (ketalar) uygulanan koyunlarda bazi klinik gözlemler. Lalahan Hay Araşt Enst Derg. 28(1): 42-50.
15. Kershaw C.M., Khalid M., McGowan M.R., Ingram K., Leethongdee S., Wax G., Scaramuzzi R.J. (2005). The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. Theriogenology. 64(5): 1225-1235.
16. Robinson J.J., McKelvey W.A.C., King M.E., Mitchell S.E., Mylne M.J.A., McEvoy T.G., Dingwall W.S., Williams L.M. (2011). Traversing the ovine cervix-a challenge for cryopreserved semen and creative science. Animal. 5(11): 1791-1804.
17. Prellwitz L., Zambrini F.N., Guimarães J.D., de Sousa M.A.P., Oliveira M.E.F., Garcia A.R., Esteves S.N., Bartlewski P.M. Souza-Fabjan J.M.G., Fonseca J.F. (2019). Comparison of the intravenous and intravaginal route of oxytocin administration for cervical dilation protocol and non−surgical embryo recovery in oestrous−induced Santa Inês ewes. Reprod Domest Anim. 54(9): 1230-1235.
18. Figueira L.M., Alves N.G., Batista R.I.T.P., Brair V.L., Lima R.R., Oliveira M.E.F., Fonseca J.F., Souza−Fabjan J.M.G. (2019). Pregnancy rate after fixed−time transfer of cryopreserved embryos collected by non−surgical route in Lacaune sheep. Reprod Domest Anim. 54(11): 1493-1496.
19. Leite C.R., Fonseca J.F., Fernandes D.A.M., Souza-Fabjan J.M.G., Ascoli F.O., Brandão F.Z. (2018). Cervical relaxation for non-surgical uterus access in Santa Inês ewes. Arq Bras Med Vet Zootec. 70: 1671-1679.
20. Morais M.C.C., Esteves L.V., Souza-Fabjan J.M., Oliveira M.E.F., Silva M.R., Brandão F.Z., Fonseca J.F. (2020). Factors affecting pregnancy rates for goat embryos recovered and transferred by transcervical route. Small Rumin Res. 192: 106215.
21. Leethongdee S. (2010). Development of trans-cervical artificial ınsemination ın sheep with special reference to anatomy of cervix. Suranaree J Sci Technol. 17(1): 57-69.
22. Figueira L.M., Alves N.G., Souza-Fabjan J.M.G., Oliveira M.E.F., Lima R.R., Souza G.N., Fonseca J.F. (2020). Preovulatory follicular dynamics, ovulatory response and embryo yield in Lacaune ewes subjected to synchronous estrus induction protocols and non-surgical embryo recovery. Theriogenology. 145: 238-246.
23. Stellflug J.N., Wulster-Radcliffe M.C., Hensley E.L., Cowardin E.A., Seals R.C., Lewis G.S. (2001). Oxytocin-induced cervical dilation and cervical manipulation in sheep: effects on laparoscopic artificial insemination. J Anim Sci. 79(3): 568-573.
24. King M.E., McKelvey W.A., Dingwall W.S., Matthews K.P., Gebbie F.E., Mylne M.J., Stewart E., Robinson J.J. (2004). Lamb in gratesand litter sizes following intrauterine or cervical insemination of frozen/thawed semen with or without oxytocin administration. Theriogenology. 62(7): 1236-1244.
25. Slater D.M., Astle S., Woodcock N., Chivers J.E., de Wit N.C., Thornton S., Newton, R. (2006). Anti-inflammatory and relaxatory effects of prostaglandin E2 in myometrial smoothmuscle. Mol Hum Reprod. 12(2): 89-97.
26. Niringiyumukiza J.D., Cai H., Xiang W. (2018). Prostaglandin E2 involvement in mammalian femalefertility: ovulation, fertilization, embryo developmentand early implantation. Reprod Biol Endocrinol. 16(1): 1-10.
27. Baxter G.M., Tackett R.L., Moore J.N. (1989). Reactivity of equine palmar digital arteries and veins to vasodilating agents. Vet Surg. 18(3): 221-226.
28. Erkert R.S., Macallister C.G. (2002). Isoxsuprine hydrochloride in the horse: a review. J Vet Pharmacol Ther. 25(2): 81-87.
29. Figueira L.M., Alves N.G., de Souza-Fabjan J.M.G., Batista R.I.T.P., da Cruz Morais M.C., de Lima R.R., Oliveira M.E.F., Fonseca J.F. (2020). Embryo yield and quality are associated with progestogen treatment during superovulation protocol in lactating Lacaune ewes. Theriogenology. 155, 132-138.