African Journal of Biotechnology Vol. 11(11), pp. 2803-2806, 7 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3154
ISSN 1684–5315 © 2012 Academic Journals

*Full Length Research Paper*

# Effect of estradiol and oxytocin on ovine cervical relaxation

**Reza Masoudi[1]*, Hamid Kohram[2], Ahmad Zare Shahne[2] and Seyed Danial Moein Ale Davoud[1]**

[1]Young researchers club, Rudehen branch, Islamic Azad University, Rudehen, Iran.
[2]Department of Animal Sciences, Faculty College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Accepted 16 December, 2011

**The aim of this study was to examine the effect of estradiol (E2) and oxytocin (OT) treatments on the cervix dilation of three breeds of Iranian ewes. Cervix dilation was measured by penetration of scaled bovine catheter into the cervix of the ewes. In experiment 1, 60 Zell ewes were equally assigned to three groups in the breeding season. At first, the ewes received 100 µg intravenous (i.v) E2. 12h later, the ewes received 150, 100, and 50 IU OT. In experiment 2, 27 Zandi ewes were equally assigned to three groups in late breeding season and after jugular E2 injection, received 100, 80, and 60 IU OT. In Experiment three, 15 Lori-bakhtiari ewes were equally assigned to three groups during anestrous season and received 100, 80, and 60 IU OT after jugular E2 reception. Cervix dilation were examined before and 10 to 15 min after E2 and OT injection. This study indicates that just E2 had no effect on cervix dilation but injection of E2 and more than 80 IU OT can completely dilate the cervix of Iranian ewes ($P < 0.05$). Reproductive seasons (anestrous, late breeding season and breeding season) have no significant effect on induced cervix dilation by E2 and OT. In conclusion, this is good procedure for improve cervix relaxation and perform transcervical artificial insemination and transcervical embryo transfer in ewes.**

**Key words:** Ewes, Estradiol, Oxytocin, Cervical dilation.

## INTRODUCTION

Artificial insemination (AI) is a good way for the use of superior rams in reproduction but the conception rates in sheep following cervical AI with frozen-thawed semen are poor (Salamon and Maxwell, 1995). Nowadays, laparoscopy is a commercial procedure for intrauterine artificial insemination in ewes, but this technique has not been successful in sheep industry because it is costly, time consuming and require technical proficiency (Evans and Maxwell, 1987). The most effective procedure for embryo recovery is laparotomy but surgical embryos and ovum collections have some problems like laparoscopy. Laparotomy often causes the formation of post-operative adhesions in the uterus, oviducts and ovaries, thus inducing a reduction in embryo recovery rate after repeated surgery (Torres and Sevellec, 1987). Transcervical artificial insemination is the simpler technique for AI in ewes (Wulster-Radcliffe and Lewis, 2002), but usually the anatomical structure of the ovine cervix prevents transcervical artificial insemination and embryo transfer which limited commercial use of these techniques in ewes. The ovine cervix is long and fibrous tubular that composed of connective tissue with an inner epithelial and outer serosal layers. The lumen is very convoluted and forms four to seven cervical rings that act as a physical barrier to external contaminants (Fukui and Roberts, 1978). Its means that the length of ewes are 6.5 to 6.7 cm and these dimensions were influenced by breeds, parity, age and physiological state (More 1984, Halbert et al., 1990a).

We want to dilate ovine cervix by using estradiol (E2) and oxytocin (OT). It is a good way for improving the nonsurgical artificial insemination and embryo transfer if the ovine cervix was dilated. In this study, we use E2 and

*Corresponding author. E-mail: Rezamasoudi@ut.ac.ir.

**Table 1.** The depth of penetration into the cervix of Zell ewes.

| Cervical penetration (cm) | E2 (100 µg) | OT | | | ± SEM | Mean |
|---|---|---|---|---|---|---|
| | | 50 | 100 | 150 | | |
| Before estradiol | 0.68 | | | | ± 0.09 | 0.68 |
| After estradiol | 0.78 | | | | ± 0.13 | 0.78 |
| Before oxytocin | | 0.88[a] | 0.75[a] | 0.85[a] | ± 0.11 | 0.81 |
| After oxytocin | | 1.88[b] | 4.67[a] | 4.84[a] | ± 0.33 | 3.79 |
| Uterine entried/ no. of ewe (%) | | 5/20 (25) | 15/50 (75) | 16/20 (80) | | |

[a, b] Values with different superscripts in the same row differ ($P < 0.05$).

**Table 2.** The depth of penetration into the cervix of Zandi ewes.

| Cervical penetration (cm) | E2 (100 µg) | OT | | | ± SEM | Mean |
|---|---|---|---|---|---|---|
| | | 60 | 80 | 100 | | |
| Before estradiol | 0.75 | | | | ± 0.14 | 0.75 |
| After estradiol | 0.92 | | | | ± 0.19 | 0.92 |
| Before oxytocin | | 1.08[a] | 0.95[a] | 0.89[a] | ± 0.17 | 0.97 |
| After oxytocin | | 2.03[b] | 4.77[a] | 5.04[a] | ± 0.41 | 3.84 |
| Uterine entried/no. of ewe (%) | | 3/9 (33.3) | 7/9 (77.77) | 8/9 (88.88) | | |

[a, b]Values with different superscripts in the same row differ ($P < 0.05$).

different doses of exogenous OT to examine these effects on cervix relaxation of Iranian ewes.

## MATERIALS AND METHODS

### Animals' management

Three breeds of Iranian ewes are used in this study. First group was 60 Zell ewes with the average of 3.5 years old and 43 kg weight at the breeding season. Second group include 27 Zandi ewes with the average of 3.5 years old and 50 kg weight at the late breeding season and in the third group we examined the cervix dilation of 15 Lori-bakhtiari ewes with the average of three years old and 65 kg weight during anestrous season. The ewes received CIDR (EAZI-Breed™, CIDR®, New Zealand) for 12 days before all experiments. Seven days after CIDR removal, the injection of OT was started.

In the first group, Zell ewes (n = 60) were assigned equally to three groups. At first ($6^{th}$ night after CIDR removal at 22 o'clock), they received jugular E2 (100 µg in 5 ml of 1:1 saline-ethanole) (Abureihan Pharmacy; Vetaestrole, estradiol benzoate) and 12 h later, 150, 100 and 50 IU OT (Abureihan Pharmacy; 10 UPS units/ml, Iran) intravenously respectively. In second group, 27 Zandi ewes were assigned equally to three groups and received 100, 80 and 60 IU of OT. And the third group, 15 Lori-bakhtiari ewes were assigned equally to three groups and received the treatments same as Zandi groups.

### Cervix relaxation measurement

Cervix relaxation in all ewes were measured before and 10 to 15 min after E2 and OT injection by a scaled bovine catheter with 40 cm length and 4 mm in diameter. The difference in penetration before and after OT injection was considered as cervix penetration.

### Statistical analysis

The SAS (9.1) GLM procedure were used to determine the effect of OT on cervix dilation. When F-tests were significant, the DUNCAN option in GLM was used to separate means.

## RESULTS AND DISCUSSION

The results are show in Table 1. Exogenous E2 and OT (upper doses than 80 IU) induced cervix relaxation in all ewes. E2 could not dilate the cervixes alone ($P > 0.05$) but the effect of E2 and OT combination on cervical relaxation were significant ($P < 0.05$) and higher doses had better effects on cervix relaxation.

Table 1 indicates that E2 treatment had no effect on cervical dilation but injection of 100 and 150 IU OT, 12h after E2, could induce cervical relaxation in Zell ewes. Table 2 exhibits that just E2 injection had no effect on cervical dilation but injection of 80 and 100 IU OT, 12 h after E2 injection caused cervical relaxation in Zandi ewes. Table 3 shows that E2 injection had no effect on cervical dilation but injection of 80 and 100 IU OT, 12 h after E2, could induce cervical relaxation in Lori-bakhtiari ewes.

This penetration is described as the amount of entry of catheter to the second and third rings of the cervix (Naqvi et al., 2005). According to morphological studies, internal cervical rings are the main barriers to AI catheter penetration (Kershaw et al., 2005). The second and third rings are commonly not in alignment with the first ring but the AI pipette rarely penetrates more than 1 cm into the cervical

**Table 3.** The depth of penetration into the cervix of Lori-bakhtiari ewes.

| Cervical penetration (cm) | E2 (100 µg) | OT | | | ± SEM | Mean |
|---|---|---|---|---|---|---|
| | | 60 | 80 | 100 | | |
| Before estradiol | 0.81 | | | | ± 0.04 | 0.81 |
| After estradiol | 1.0 | | | | ± 0.09 | 1.0 |
| Before oxytocin | | 0.98[a] | 0.85[a] | 1.02[a] | ± 0.17 | 0.95 |
| After oxytocin | | 2.18[b] | 5.00[a] | 4.95[a] | ± 0.48 | 4.04 |
| Uterine entried/no. of ewe (%) | | 2/5 (40) | 5/5 (100) | 5/5 (100) | | |

[a, b]Values with different superscripts in the same row differ ($P < 0.05$).

canal (Halbert et al., 1990b, Kershaw et al., 2005). The main barrier for insemination catheter penetration is the second and/or third rings of the cervical canal. High doses of E2 could induce estrus behavior, ovulation and increases the expression of OT receptors (OTR) in the lumen of the cervix (Ayad et al., 2004). After estradiol and oxytocin treatment, the catheter was able to penetrate successfully through the cervical canal into the ovine uterus. The results of the present study are similar to previous studies (Khalifa et al., 1992, Sayre and Lewis, 1996; Flohr et al., 1999; Wulster-Radcliffe et al., 1999; Stellflug et al., 2001), but in this experiment we used lower doses of OT. Deepest time for cervical penetration was 9.54 ± 4.32 min which is similar to previous studies on the cervix which dilated adequately within 10 min (Khalifa et al., 1992, Sayre and Lewis, 1996).

OT increased COX-2 mRNA expression in cervical canal that causes to prostaglandin E2 synthesis in cervix during the estrus phase (Kershaw-Young et al., 2009; Kershaw-Young et al., 2010). Treatment of ewes with more than 80 IU OT, seven days after estrus, resulted to complete cervix relaxation in Iranian ewes. The least dose for complete dilation of the cervix is 50 UPS units of OT that induce uterine contraction with 60% uterine entry rate and they declare that 200 USP units of OT had 100% uterine entry rate with cervix dilation (Sayre and Lewis, 1996). According to studies, the uterine response to the OT is highest when estrogen concentrations had been increased (McCracken et al., 1984). However, Sayre and Lewis (1995) reported that the uterine responses were not altered in ewes with estrogen concentration increased or E2:P4 ratio. In King et al. (2004) research, OT had no effect on cervix relaxation. OT dose which was used in King's study was 10 IU so that cervix was not able to dilate because of the use of the low dose (King et al., 2004). In this study E2 and high doses (≥ 80 IU) of OT could dilate the cervix of three breeds of ewes in breeding season, transition period and anestrous season. According to the results of this study, we could use E2 and OT for transcrvical artificial insemination and transcervical embryo recovery in Iranian ewes. E2 and OT are cheap and the using of them could simplify and improve intrauterine artificial insemination in ewe than laparoscopy but it is important that these effects on conception rate were determined.

## Conclusion

In conclusion, intravenous injection of E2 and OT is an effective procedure for cervix relaxation. After cervical softening, the problem of cervical rings were solved and via cervical canal we could perform artificial insemination and embryo collection in ewes. Therefore, using of OT is a good way for simplify of transcervical artificial insemination and embryo transfer.

## REFERENCES


Ayad VJ, Leung ST, Parkinson TJ, Wathes DC (2004). Coincident increases in oxytocin receptor expression and EMG responsiveness to oxytocin in the ovine cervix at oestrus. Anim. Reprod. Sci. 80: 237-250.

Evans G, Maxwell WMC (1987). Salmon`s artificial insemination of sheep and goats. Butterworths.

Flohr SF, Wulster-Radcliffe MC, Lewis GS (1999). Technical note: development of a transcervical oocyte recovery procedure for sheep. J. Anim. Sci. 77: 2583-2586.

Fukui Y, Roberts EM (1978). Further studies on non-surgical intrauterine technique for artificial insemination in the ewe. Theriogenology, 10: 381-393.

Halbert GW, Dobson H, Walton JS, Buckrell BC (1990a). The structure of the cervical canal of the ewe. Theriogenology, 33: 977-992.

Halbert GW, Dobson H, Walton JS, Buckrell BC (1990b). A technique for transcervical intrauterine insemination of ewes. Theriogenology, 33: 993-1010.

Kershaw-Young CM, Khalid M, McGowan MR, Pitsillides AA, Scaramuzzi RJ (2009). The mRNA expression of prostaglandin E receptors EP2 and EP4 and the changes in glycosaminoglycans in the sheep cervix during the estrous cycle. Theriogenology, 72: 251-261.

Kershaw-Young CM, Scaramuzzi RJ, McGowan MR, Pitsillides AA, Wheeler-Jones CP, Khalid M (2010). The effect of estradiol on COX-2, EP2, and EP4 mRNA expression and the extracellular matrix in the cervix of the hypogonadotrophic, ovariectomized ewe. Theriogenology, 73: 620-628.

Kershaw CM, Khalid M, McGowan MR, Ingram K, Leethongdee S, Wax G, Scaramuzzi RJ (2005). The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. Theriogenology, 64: 1225-1235.

Khalifa RM, Sayre BL, Lewis GS (1992). Exogenous oxytocin dilates the cervix in ewes. J. Anim. Sci. 70: 38-42.

King ME, McKelvey WAC, Dingwall WS, Matthews KP, Gebbie FE, Mylne MJA, Stewart E, Robinson JJ (2004). Lambing rates and litter sizes following intrauterine or cervical insemination of frozen/thawed

semen with or without oxytocin administration. Theriogenology, 62: 1236-1244.
McCracken JA, Schramm W, Okulicz WC (1984). Hormone receptor control of pulsatile secretion of PGF2[alpha] from the ovine uterus during luteolysis and its abrogation in early pregnancy. Anim. Reprod. Sci. 7: 31-55.
More J (1984). Anatomy and histology of the cervix uteri of the ewe: new insights. Acta Anat. (Basel), 120: 156-159.
Naqvi SMK, Pandey GK, Gautam KK, Joshi A, Geethalakshmi V, Mittal JP (2005). Evaluation of gross anatomical features of cervix of tropical sheep using cervical silicone moulds. Anim. Reprod. Sci. 85: 337-344.
Salamon S, Maxwell WMC (1995). Frozen storage of ram semen I. Processing, freezing, thawing and fertility after cervical insemination. Anim. Reprod. Sci. 37: 185-249.
Sayre BL, Lewis GS (1996). Cervical dilation with exogenous oxytocin does not affect sperm movement into the oviducts in ewes. Theriogenology, 45:1523-1533.
Stellflug JN, Wulster-Radcliffe MC, Hensley EL, Cowardin EA, Seals RC, Lewis GS (2001). Oxytocin-induced cervical dilation and cervical manipulation in sheep: effects on laparoscopic artificial insemination. J. Anim. Sci. 79: 568-573.
Torres A, Sevellec C (1987). Repeated superovulation and surgical recovery of embryos in ewe. Reprod Nutr Dev. 24: 859-863.
Wulster-Radcliffe MC, Costine BA, Lewis GS (1999). Estradiol-17 beta-oxytocin-induced cervical dilation in sheep: application to transcervical embryo transfer. J. Anim. Sci. 77: 2587-2593.
Wulster-Radcliffe MC, 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.