

Full Length Research Paper

Effect of estradiol and oxytocin on ovine cervical relaxation

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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

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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 entered/ 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 entered/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 anestrus 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 (6th 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 entries/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 transcervical 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.

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