

*Full Length Research Paper*

## Effects of exogenous oxytocin on cervical penetration of Iranian ewes

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The objective of this study was to determine the effect of exogenous oxytocin (OT) treatments on the cervix dilation of three breeds of Iranian ewes. Cervix dilation was measured by transcervical passage of scaled bovine catheter into the uterus of the ewes. Fifty four healthy Zandi ewes were equally assigned to six groups in the late breeding season. Thirty healthy Lori-bakhtiari breed ewes were equally assigned to six groups during anestrus. The ewes received 100, 80 and 60 IU of intravenous (i.v) and intramuscular (i.m) OT. In the breeding season, 120 healthy Zell breed ewes were equally assigned to six groups and received 150, 100 and 50 IU of i.v and i.m OT. Cervix dilation was examined before OT injection 10 to 15 min following treatment. This study indicates that injection of more than 80 IU OT can completely dilate the cervix of Iranian ewes ( $P < 0.05$ ) and the reproductive seasons (anestrus, late breeding season and breeding season) and the type of injection (i.v or i.m) has no significant effect on induced cervix penetration by OT. Therefore, this dose is the applicable dose for the cervix dilation and transcervical artificial insemination and embryo recovery in Iranian ewes.

**Key words:** Iranian ewes, oxytocin, cervical dilation, transcervical artificial insemination, transcervical embryo transfer.

### INTRODUCTION

The importance of artificial insemination (AI) is the use of superior rams for reproduction. However, the conception rates in sheep following cervical AI with frozen-thawed semen are weak (Salamon and Maxwell, 1995). Therefore, AI fertility and lambing rate should be improved with frozen-thawed semen in sheep. Laparoscopy artificial insemination is the commercial procedure that is used for intrauterine 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 simpler technique for

ewes AI and ET are transcervical artificial insemination and embryo transfer (Wulster-Radcliffe et al., 1999; Wulster-Radcliffe and Lewis, 2002) but the anatomical structure of the cervix in ewes usually prevents intrauterine artificial insemination and transcervical embryo transfer which limit commercial use of these techniques in ewes (Wulster-Radcliffe et al., 1999). Usually, ewes have a long and fibrous tubular cervix that is composed of connective tissue with inner epithelial and outer serosal layers (More, 1984). The lumen is very convoluted and forms 4 to 7 cervical rings that act as a physical barrier to external materials (Fukui and Roberts, 1978). The female sheep have a long cervix with the mean length of 6.5 to 6.7 cm that is influenced by breeds, parity, age and physiological state (More, 1984; Halbert et al., 1990a).

Lastly, the most effective procedure for embryo

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**Abbreviations:** OT, Oxytocin treatments.

**Table 1.** The depth of penetration into the cervix of oxytocin treated Zandi ewes seven days after CIDR treatment at the late breeding season.

Cervical penetration (cm)	Oxytocin (IU)						± SEM	Mean
	i.v			i.m				
	60	80	100	60	80	100		
Before oxytocin	0.8 <sup>a</sup>	0.5 <sup>a</sup>	0.9 <sup>a</sup>	0.6 <sup>a</sup>	0.8 <sup>a</sup>	0.7 <sup>a</sup>	± 0.05	0.71
After oxytocin	2.85 <sup>ab</sup>	4.26 <sup>a</sup>	4.81 <sup>a</sup>	0.62 <sup>b</sup>	3.95 <sup>a</sup>	4.55 <sup>a</sup>	± 0.53	3.5
Uterine entered/no. of ewe (%)	5/9 (55.55)	7/9 (77.77)	8/9 (88.88)	1/9 (11.11)	7/9 (77.77)	7/9 (77.77)		

<sup>a, b</sup> Values with different superscripts in the same row differ ( $P < 0.05$ ).

recovery was laparotomy but surgical embryo and ovum collection have some problems like laparoscopy (Cognie et al., 1999). Laparotomy often cause 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). Some researchers invented a transcervical pipette for transcervical artificial insemination and embryo transfer but conception rate and embryo transfer rate via those pipette were poor (Halbert et al., 1990b; Wulster-Radcliffe and Lewis, 2002; Wulster-Radcliffe et al., 2004). Many studies had shown that OT (a peptide hormone) could increase cervix dilation (Khalifa et al., 1992; Sayre and Lewis, 1996; Flohr et al., 1999). Now, if we can increase the cervix penetration of ewes by oxytocin (OT), it is a good way for nonsurgical artificial insemination and embryo transfer.

In this study, we used 3 breeds of Iranian ewes (Zandi, Lori-bakhtiari and Zell) in different seasons of reproduction (breeding, transition and anestrus) to examine the effect of different doses of exogenous OT (i.v/i.m) on cervix penetration of Iranian ewes in Iran.

## MATERIALS AND METHODS

In this study, we use three breeds of Iranian ewes. We examined the cervix penetration of 54 Zandi ewes  $3.5 \pm 0.5$  years old and  $50 \pm 2.5$  kg in weight at the late breeding season, 30 Lori-bakhtiari ewes  $3 \pm 0.5$  years old and  $65 \pm 2.5$  kg in weight at the anestrus season and 120 Zell ewes  $3.5 \pm 0.5$  years old and  $42 \pm 1.5$  kg in weight at the breeding season. All the ewes received 0.33 g of progesterone (Easy-Breed™, CIDR®, New Zealand) for 12 days before all experiments. Seven days after CIDR removal, the injection of OT was started because we wanted to show the effect of just OT (without estradiol effect on OT receptors increasing at estrous time) on cervix penetration. The study was performed in Tehran with moderate temperature between 20 and 32°C on the experiment period.

The Zandi ( $n = 54$ ), Lori-bakhtiari ( $n = 30$ ) and Zell ( $n = 120$ ) ewes were assigned equally to six groups of treatment ( $n = 9$ ,  $n = 5$  and  $n = 20$  each group, respectively). Three of these groups in Zandi and Lori-bakhtiari breeds received 60, 80 and 100 IU of OT (Abureihan Pharmacy; 10 UPS units/ml, Iran) intravenously and the other three groups received the same doses intramuscularly. In Zell breed, the ewes received 50, 100 and 150 IU of OT.

## Assessment of cervix penetration

For measurement of cervix penetration, the ewes were kept in a sheep box and cervix penetration in all ewes was measured before and 10 to 15 min after OT injection by a scaled bovine artificial insemination catheter with 40 cm length and 4 mm diameter. The vagina was opened via an ovine speculum and the pipette was penetrated to cervix. Before OT injection, the pipette penetration to cervix was low but after OT injection, the penetration was increased. The penetration (cm) difference between 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

Table 1 presents the results of the effect of exogenous OT on cervix penetration of Zandi ewes at the late breeding season. In this breed and season, OT could dilate the cervix of Zandi ewes and the doses of 80 and 100 IU were useful for cervix dilation and the difference between these doses was not significant. There was no difference between i.v and i.m injection unless in 60 IU OT where i.v injection had higher percentage of cervix penetration. 60 IU of i.m OT was not effective for cervical penetration.

Table 2 presents the results of the effect of exogenous OT on cervix penetration of Lori-bakhtiari ewes at the anestrus season. In this breed and season, like the Zandi breed, OT could dilate the cervix of Lori-bakhtiari ewes and the doses of 80 and 100 IU were useful for cervix dilation and the difference between these doses were not significant and there was no difference between i.v and i.m injection. 60 IU of OT was not effective for cervix penetration.

Table 3 shows the results of the effect of exogenous OT on cervix penetration of Zell ewes in breeding season. In this experiment, doses of 100 and 150 IU dilated the cervix of ewes and the difference between

**Table 2.** The depth of penetration into the cervix of oxytocin treated Lori-bakhtiari ewes seven days after CIDR treatment at the anestrous season.

Cervical penetration (cm)	Oxytocin (IU)						± SEM	Mean
	i.v			i.m				
	60	80	100	60	80	100		
Before oxytocin	0.6 <sup>a</sup>	0.5 <sup>a</sup>	0.9 <sup>a</sup>	1 <sup>a</sup>	0.7 <sup>a</sup>	0.7 <sup>a</sup>	± 0.04	0.73
After oxytocin	1.15 <sup>b</sup>	4.66 <sup>a</sup>	5.51 <sup>a</sup>	1.02 <sup>b</sup>	4.95 <sup>a</sup>	5.26 <sup>a</sup>	± 0.71	3.75
Uterine entered/no. of ewe (%)	1/5 (20)	4/5 (80)	5/5 (100)	0/5 (0)	4/5 (80)	5/5 (100)		

<sup>a, b</sup> Values with different superscripts in the same row differ (P<0.05).

**Table 3.** The depth of penetration into the cervix of oxytocin treated Zell ewes seven days after CIDR treatment in breeding season.

Cervical penetration (cm)	Oxytocin IU						± SEM	Mean
	i.v			i.m				
	50	100	150	50	100	150		
Before oxytocin	0.5 <sup>a</sup>	0.7 <sup>a</sup>	0.9 <sup>a</sup>	0.8 <sup>a</sup>	0.7 <sup>a</sup>	0.5 <sup>a</sup>	± 0.04	0.6
After oxytocin	1.3 <sup>b</sup>	4.44 <sup>a</sup>	4.51 <sup>a</sup>	1.00 <sup>b</sup>	4.85 <sup>a</sup>	4.47 <sup>a</sup>	± 0.29	3.42
Uterine entered/no. of ewe (%)	2/20 (10)	15/20 (75)	15/20 (75)	0/20 (0)	16/20 (80)	18/20 (90)		

<sup>a, b</sup> Values with different superscripts in the same row differ (P<0.05).

these doses was not significant and there was no difference between i.v and i.m injection. 50 IU of OT could not dilate the cervixes of Zell ewes in breeding season.

## DISCUSSION

In this study, using OT caused the increase of penetration of scaled bovine artificial insemination pipette to the cervix of Iranian 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). Morphological studies of the cervix showed that internal cervical rings are the main barriers to AI catheter (Kershaw et al., 2005). The second and third rings are commonly out of alignment with the first ring then they are in the misdirection from the central lumen and the AI pipette is rarely penetrated more than 1 cm into the cervix (Halbert et al., 1990b; Kershaw et al., 2005). High doses of E2 could induce estrus behavior, ovulation and increase the expression of OT receptors (OTR) in the lumen of the cervix (Ayad et al., 2004). After OT treatment, the catheter could penetrate successfully through the cervical canal and into the uterus of ewes but this response was dose dependent and lower dose than 80 IU could not dilate the cervix successfully. The results of this study is similar to that of 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 applied lower doses of OT. The deepness of the catheter penetration decreased in with time. Time to deepest cervical penetration was  $9.54 \pm 4.32$  min which is same with previous studies where the cervix dilated adequately within 10 min (Khalifa et al., 1992; Sayre and Lewis, 1996). Cervical manipulation and use of inflexible transcervical pipette could damage the cervix and release spermicidal or embryocidal compounds and therefore decrease fertility (Hawk, 1983) but use of OT has no any harmful effect on cervix, sperm and oocyte (Sayre and Lewis, 1996, 1997; Stellflug et al., 2001).

OT via increase of the cyclo-oxygenase-2 mRNA expression induces prostaglandin E2 production from cervix during the estrus phase (Shemesh et al., 1997). Prostaglandin E2 could increase cervical penetration by acting on EP2 and EP4 receptors in cervical canal (Myne et al., 1992; Kershaw-Young et al., 2009). Then, OT induced cervical penetration is by acting on prostaglandin E2. Treatment of ewes with 80 or 100 IU of OT could induce cervical penetration. Sayre and Lewis (1996) showed that the least dose for the complete dilation of the cervix is 50 IU of OT that induce uterine contraction with 60% uterine entry rate and they showed that 200 USP units of OT had 100% uterine entry rate with cervix dilation. Our study in anestrous season showed that 100 IU OT could dilate cervical canal too. In King et al. (2004) research, OT had no effect or decreased the number of ewes lambing. Oxytocin dose which was used in King's study was 10 IU so that cervix could not dilate because of

the use of the low dose (King et al., 2004). In this study, high doses ( $\geq 80$  IU) of OT could dilate the cervix of every three breeds of ewes in every three seasons of reproduction. With regard to these results, we could use this hormone for transcervical artificial insemination and embryo recovery in ewes. OT is a cheap hormone and its use could simplify and improve intrauterine artificial insemination in ewe than laparoscopy but the pregnancy examination is necessary to determine its effects on conception rate.

## Conclusion

In conclusion, intravenous and intramuscular injection of OT is effective for cervical penetration of three breeds of Iranian ewes in different reproductive seasons. After cervical dilation, the problem of cervical rings was solved and we could perform artificial insemination and embryo collection in ewes via cervix canal. Therefore, using OT is a good way to simplify artificial insemination and embryo transfer via cervical canal.

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