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**EFFECT OF ESTRADIOL AND DIFFERENT TYPE AND LEVELS OF OXYTOCIN
INJECTION ON DEPTH OF PENETRATION, CONCEPTION RATE AND LAMBING
RATE IN IRANIAN LORI-BAKHTIARI EWES**

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ABSTRACT

In this study three experiments were conducted on 84 Iranian fat tailed Lori-bakhtiari ewes (weighing 63 ± 1.8 kg) during anestrus season to determine: 1) effect of two type of OT injection (intravenous (i.v.) vs. intramuscularly (i.m.)) and three levels of OT (60, 80 and 100 IU) on depth of penetration; 2) effect of estradiol injection alone and estradiol plus OT together on depth of penetration and 3) effect of best level of OT from experiment one on cervical relaxation, conception rate and lambing rate. Cervix relaxation were examined before and 15 minutes after OT injection. Results showed the type of OT injection (i.v. or i.m.) had no effects on cervical

relaxation but levels of OT injection affected cervix dilation ($P < 0.05$), using of 80 and 100 IU OT induced cervix dilation compared to 60 IU OT. There were no differences between 80 and 100 IU OT. Estradiol injection alone did not affect cervix dilation ($P > 0.05$) but the effect of estradiol in combination with OT was significant and increased depth of penetration. Conception rate improved in ewes received OT compared to control ewes. In conclusion, high doses of OT (≥ 80 IU) could dilate the cervix and improve of pregnancy rate and lambing rate; and because there was no difference between types of injections we can use intramuscular injection of OT for cervix relaxation. Therefore using of OT because of its cheap price and results is a good way for simplify of transcervical artificial insemination and increase the pregnancy rate in ewes.

Keywords: Cervix, Estradiol, Ewes, Lambing rate, Oxytocin

INTRODUCTION

Artificial insemination is a good way for the use of superior rams in reproduction but the conception rates in ewes via cervical AI with frozen-thawed semen are poor [1]. Transcervical artificial insemination is a simpler technique for AI in sheep [2] but usually the anatomical structure of the ovine cervix prevents transcervical artificial insemination and embryo transfer which limited commercial use of these techniques in sheep [3].

In the cervix, there is a degree of natural relaxation during estrus that is associated with ovarian steroids and gonadotrophic hormones [4, 5] caused mainly by a rearrangement of cervical smooth muscle fiber and connective tissue. For successful transcervical artificial insemination the insemination pipette should be penetrated into cervix. Passage through the cervix

using modified inseminating pipettes has resulted in improvements in conception rate directly related to depth of penetration [6] but can cause significant trauma to the pelvic tissue of ewes where cervical dilation has not taken place [7].

There are three ways for reducing the physical effects of the ewe cervical rings: physically (e.g., attaching a hemostat to the external os and retracting the cervix to align the os and reduce obstructions to the uterus) [8], mechanically (e.g., by designing TC AI and ET equipment to passage the cervical canal), and chemically (e.g., dilating the cervix with exogenous PGE2 or oxytocin) [9-12]. Physical and mechanical methods maybe cause to trauma in cervix that could induce release of spermicidal or embryocidal compounds and therefore decreasing fertility of ewes [13]. On the other hand, if cervix

was dilated via estradiol and/or oxytocin injection, it is a good way for improving the nonsurgical artificial insemination and embryo transfer. Several studies have shown that in ewes at estrus, there is a pattern of natural cervical relaxation that enables greater penetration of the cervix with an insemination catheter [4, 5]. These findings suggest that the high concentrations of reproductive hormones during the periovulatory period, such as FSH and estradiol may mediate molecular and structural changes in the cervix in an oxytocin-mediated manner leading to enhanced relaxation. The aim of this study was to determine the effect of estradiol (E2) and/or different doses of oxytocin (OT) treatments on the cervical relaxation and conception rate of Iranian fat-tailed Lori-bakhtiari ewes during an estrous season.

MATERIALS AND METHODS

Experiments management

In experiment 1, 84 Iranian fat-tailed Lori-bakhtiari ewes with 63 ± 1.8 kg weight and 3-4 years old were randomly assigned into six groups that included two types of injection (intravenous (i.v.) vs. intramuscularly (i.m.)) and three levels of OT (60, 80 and 100 IU) in each type. They were kept indoors in groups of 8 to 10 ewes on straw bedding in open-sided pens. They were fed a commercial

concentrate diet with hay and water provided ad libitum. Cervical relaxation was examined before and 10 to 15 minutes after OT (Abureihan Company; 10 IU/ml, Iran) injection.

In the second experiment, ewes were received 100 µg i.v. estradiol (100 µg in 5 ml of 1:1 saline-ethanol, Abureihan Pharmacy; Vetaestrol, 10mg estradiol benzoate, Iran) and cervical relaxation was examined before and 10 to 15 minutes after injection then 12 h after estradiol injection, ewes were randomly allocated into six groups that included two types of injection (intravenous (i.v.) vs. intramuscularly (i.m.)) and three levels of OT (60, 80 and 100 IU) in each type and cervical relaxation was examined before and 10 to 15 minutes after OT injection.

In the third experiment, the estrous cycle of cycling ewes was synchronized using CIDR's (Easy-Breed™, CIDR®, New Zealand), inserted for 12 days, eCG (400 IU, Sanofi Animal Health, Libourne Cedex, France) was injected to ewes at the time of CIDR removal, then the ewes were equally assigned to two groups and received 0, 100 IU OT and transcervically inseminated 54 h after CIDR removal.

Measurement of the depth of cervical penetration

The device used to measure the depth of cervical penetration was a modified stainless steel cattle artificial insemination pipette[14]. The sheath was 42 cm long with a diameter of 3 mm while the inner plunger was 52 cm long with a diameter of 1.5 mm. Once inserted into the external opening of the cervix, the plunger was delicately pushed into the cervical canal and when resistance to its further passage was encountered the plunger was fixed to the sheath and the entire device removed. The depth of penetration was then measured to the nearest 0.1 mm on the plunger using a ruler.

Statistical analysis

The data were analyzed using the statistical software program SAS 9.1. GLM procedure was 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 and conception rate was analyzed via GENMOD procedure.

RESULTS AND DISCUSSION

Results showed the type of OT injection (i.v. or i.m.) had no effects on depth of cervical penetration and the proportion of ewes in which intrauterine penetration was achieved ($P > 0.05$). Depth of cervical penetration and the proportion of ewes in which intrauterine penetration was achieved affected by levels of OT injection ($P < 0.05$). The mean length

of the cervix in the ewe is about 4.5 cm [1, 15, 16] and in our experiments using of 80 and 100 IU OT increased the average depth of penetration of the cervix compared to 60 IU OT levels (4.50 vs. 1.03 cm, $P < 0.05$) in both type of injections, This would mean that in ewes with a cervix >3.4 cm in length the pipette would transverse the whole cervix to the uterus. The proportion of ewes with intrauterine penetration was consistently greater in 80 and 100 IU OT levels compared to 60 IU OT (90.47 vs. 16.66 %, $P < 0.05$). In agreement with our results Falchi et al. (2012) reported the average depth of penetration of the cervix was at least 50 to 60% of its length with injection of FSH, misoprostol and oxytocin even though oxytocin and PGE. Oxytocin (100 USP) given intravenously dilates the sheep cervix in 15 min [10, 13, 17] allowing intrauterine penetration through the cervix. In contrast with our result King et al (2004) reported that OT had no effect on cervix relaxation; OT 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 [18]. When oxytocin binds to its receptor, it activates a G protein that triggers a series of downstream pathways that leads to an increase in intracellular calcium (Ca^{2+}), caused by the opening of Ca^{2+} channels in the endoplasmic

reticulum[19]. The increase in intracellular Ca^{2+} is required to activate the enzyme phospholipase A2 which, when phosphorylated, mobilizes arachidonic acid (AA) from membrane phospholipids[20, 21]. Then AA is in its free form it acts as a substrate for cyclooxygenase-2 (COX-2) which catalyzes its conversion into prostaglandin H2. Prostaglandin H2 is the substrate for the action of specific prostaglandin synthases that convert prostaglandin H2 into a final prostaglandin (PG) product. In the cervix, we suggest that this is predominantly PGE2, acting through its receptors, which cause relaxation of cervical smooth muscle and the softening and dispersal of cervical extracellular matrix resulting in cervical dilation[22]. Stellflug et al. (2001) reported oxytocin induced cervical dilation and decreased the difficulty of passing a catheter through the cervix and into the uterus.

The high concentrations of reproductive hormones during the peri-ovulatory period, such as FSH and estradiol may mediate molecular and structural changes in the cervix in an oxytocin-mediated manner leading to enhanced relaxation[14]. High doses of E2 could induce estrus behavior, ovulation and increases the expression of OT receptors (OTR) in the lumen of the

cervix[23]. After estradiol and oxytocin treatment, the catheter could penetrate successfully through the cervical canal of ewe. The results of this study was similar to many of previous studies [9-12, 16] but in this experiment we used. We expected ewes treated with estradiol alone to show increased cervical relaxation and allow deeper cervical penetration or even intrauterine penetration but the depth of penetration was not affected by estradiol and in treated ewes with the combination of estradiol and OT depth of penetration was consistently greater compare to estradiol alone ($P < 0.05$). There was no difference between ewes treated with OT alone and the combination of estradiol and OT in depth of penetration ($P > 0.05$). In a general sense, our findings agree with those of Sayre and Lewis (1996) that reported the uterine responses were not altered in ewes with estrogen concentration increase, in contrast Wulster-Radcliffe et al. (1999) reported estradiol in combination with oxytocin induced cervical dilation. These differences may be explained mainly by individual differences in the technique of intracervical penetration that resulted in deeper average penetration of the cervix and possibly lower doses of OT used in this study compared to those.

The result of present study showed that using of OT could improve pregnancy rate. Cervix dilation and increase of sperm numbers in fertilization place could improve the chance of ovum/sperm contact and the lambing rate was improved. As results of present study showed this improvement can be associated with greater proportion of ewes with intrauterine penetration and deeper penetration which was achieved with OT injection. Eppleston, et al. (1994) reported increase in depth of penetration was associated with a 24% increase in fertility rate and in the context of sheep AI this is significant[24]. In contrast with our result King et al (2004) reported that 10 IU OT decreased the number of ewes lambing; this disagreement can be associated with lower OT dose which was used in King's study so that cervix could not completely dilate [18].

CONCLUSION

This study has shown in untreated ewes the pipette could only be passed on average 1.03 ± 0.05 cm into the cervical canal. With OT increased penetration of a further 3.47 cm was achieved. This would mean that in ewes with a cervix >3.4 cm in length the pipette would transverse the whole cervix to the uterus. High doses (≥ 80 IU) of OT promoted cervical relaxation, increased depth of penetration and the proportion of ewes

with intrauterine penetration resulted in higher lambing rate.

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REFERENCES

- [1] Salamon S, Maxwell WMC. Frozen storage of ram semen I. Processing, freezing, thawing and fertility after cervical insemination. *Anim Reprod Sci* 1995;37:185-249.
- [2] Wulster-Radcliffe MC, Lewis GS. 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* 2002;58:1361-71.
- [3] Wulster-Radcliffe MC, Wang S, Lewis GS. Transcervical artificial insemination in sheep: effects of a new transcervical artificial insemination instrument and traversing the cervix on pregnancy and lambing rates. *Theriogenology* 2004;62:990-1002.
- [4] Kershaw CM, Khalid M, McGowan MR, Ingram K, Leethongdee S, Wax G, et al. The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. *Theriogenology* 2005;64:1225-35.

- [5] Leethongdee S, Khalid M, Bhatti A, Ponglowhapan S, Kershaw CM, Scaramuzzi RJ. The effects of the prostaglandin E analogue Misoprostol and follicle-stimulating hormone on cervical penetrability in ewes during the peri-ovulatory period. *Theriogenology* 2007;67:767-77.
- [6] Salamon S, Maxwell WMC. Frozen storage of ram semen II. Causes of low fertility after cervical insemination and methods of improvement. *Anim Reprod Sci* 1995;38:1-36.
- [7] Campbell JW, Harvey TG, McDonald MF, Sparksman RI. Transcervical insemination in sheep: An anatomical and histological evaluation. *Theriogenology* 1996;45:1535-44.
- [8] Halbert GW, Dobson H, Walton JS, Buckrell BC. The structure of the cervical canal of the ewe. *Theriogenology* 1990;33:977-92.
- [9] Khalifa RM, Sayre BL, Lewis GS. Exogenous oxytocin dilates the cervix in ewes. *J Anim Sci* 1992;70:38-42.
- [10] Wulster-Radcliffe MC, Costine BA, Lewis GS. Estradiol-17 beta-oxytocin-induced cervical dilation in sheep: application to transcervical embryo transfer. *J Anim Sci* 1999;77:2587-93.
- [11] Sayre BL, Lewis GS. Cervical dilation with exogenous oxytocin does not affect sperm movement into the oviducts in ewes. *Theriogenology* 1996;45:1523-33.
- [12] Flohr SF, Wulster-Radcliffe MC, Lewis GS. Technical note: development of a transcervical oocyte recovery procedure for sheep. *J Anim Sci* 1999;77:2583-6.
- [13] Hawk HW. Sperm Survival and Transport in the Female Reproductive Tract. *J Dairy Sci* 1983;66:2645-60.
- [14] Falchi L, Taema M, La Clanche S, Scaramuzzi RJ. 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 peri-ovulatory period. *Theriogenology* 2012;78:376-84.
- [15] Evans G, Maxwell WMC. Salmon's artificial insemination of sheep and goats. Butterworths. 1987.
- [16] Stellflug JN, Wulster-Radcliffe MC, Hensley EL, Cowardin EA, Seals RC, Lewis GS. Oxytocin-induced cervical dilation and cervical manipulation in sheep: effects on laparoscopic artificial insemination. *J Anim Sci* 2001;79:568-73.
- [17] Naqvi SMK, Pandey GK, Gautam KK, Joshi A, Geethalakshmi V, Mittal JP. Evaluation of gross anatomical features of cervix of tropical sheep using cervical silicone moulds. *Anim Reprod Sci* 2005;85:337-44.

- [18] King ME, McKelvey WAC, Dingwall WS, Matthews KP, Gebbie FE, Mylne MJA, et al. Lambing rates and litter sizes following intrauterine or cervical insemination of frozen/thawed semen with or without oxytocin administration. *Theriogenology* 2004;62:1236-44.
- [19] Asselin E, Drolet P, Fortier MA. Cellular Mechanisms Involved during Oxytocin-Induced Prostaglandin F₂ α Production in Endometrial Epithelial Cells in Vitro: Role of Cyclooxygenase-2 1. *Endocrinology* 1997;138:4798-805.
- [20] Burns PD, Graf GA, Hayes SH, Silvia WJ. Effect of oxytocin on expression of cytosolic phospholipase A2 mRNA and protein in ovine endometrial tissue in vivo. *Domest Anim Endocrin* 2000;19:237-46.
- [21] Burns PD, Graf GA, Hayes SH, Silvia WJ. Cellular mechanisms by which oxytocin stimulates uterine PGF₂[α] synthesis in bovine endometrium: Roles of phospholipases C and A2. *Domest Anim Endocrin* 1997;14:181-91.
- [22] Kershaw CM, Scaramuzzi RJ, McGowan MR, Wheeler-Jones CP, Khalid M. The expression of prostaglandin endoperoxide synthase 2 messenger RNA and the proportion of smooth muscle and collagen in the sheep cervix during the estrous cycle. *Biol Reprod* 2007;76:124-9.
- [23] Ayad VJ, Leung ST, Parkinson TJ, Wathes DC. Coincident increases in oxytocin receptor expression and EMG responsiveness to oxytocin in the ovine cervix at oestrus. *Anim Reprod Sci* 2004;80:237-50.
- [24] Eppleston J, Salamon S, Moore NW, Evans G. The depth of cervical insemination and site of intrauterine insemination and their relationship to the fertility of frozen-thawed ram semen. *Anim Reprod Sci* 1994;36:211-25.

Table 1: Effect of different type and levels of OT injection on depth of penetration

Item	OT					
	iv			im		
	60	80	100	60	80	100
Cervical penetration (cm)						
Before oxytocin	0.78 ^a	0.55 ^a	0.89 ^a	0.96 ^a	0.98 ^a	0.67 ^a
After oxytocin	1.15 ^a	4.06 ^a	4.74 ^a	0.92 ^b	3.98 ^a	4.80 ^a
Total no. ewes	14	14	14	14	14	14
Uterine entered % (no)	14.2 (2) ^b	85.7(12) ^a	100(14) ^a	14.2 (2) ^b	85.7 (12) ^a	100 (14) ^a

Different letters indicate significant difference at P < 0.05

Table 2. Effect of estradiol (E) and estradiol in combination with different type and levels of OT injection (E+OT) on depth of penetration

Item	E	E+OT					
		iv			Im		
		60	80	100	60	80	100
Cervical penetration (cm)							
Before oxytocin	0.75	0.85 ^a	0.92 ^a	0.88 ^a	0.75 ^a	0.80 ^a	0.95 ^a
After oxytocin	0.82	1.88 ^b	4.67 ^a	4.84 ^a	1.05 ^b	4.51 ^a	4.75 ^a
Total no. ewes		14	14	14	14	14	14
Uterine entered % (no)		28.5 (4) ^b	100(14) ^a	100(14) ^a	14.2 (2) ^b	100 (14) ^a	100 (14) ^a

Different letters indicate significant difference at P < 0.05

Table 3. Effect of OT injection on depth of penetration, conception rate and lambing rate

Cervical penetration (cm)	Control	OT
Before oxytocin	0.85 ^a	0.92 ^a
After oxytocin	0.85 ^b	4.67 ^a
Total no. ewes	42	42
Uterine entered % (no)	16.66 (7) ^b	90.47(38) ^a
Conception rate % (no)	35.71 (15) ^b	50.0(21) ^a
Lambing rate % (no)	30.95 (13) ^b	42.8(18) ^a

Different letters indicate significant difference at P < 0.1