DOI: http://dx.doi.org/10.22034/ari.2016.107489

Archives of Razi Institute, Vol. 72, No. 1 (2017) 33-41

Original Article

Effects of estradiol and oxytocin injection on the efficiency of artificial insemination in Iranian *Zel* ewes during the breeding season

Seifi-Jamadi [1], A., Masoudi [1], R., Hosseinzadeh Aski [4], A.R., Kohram [*1,3], H., Sharafi [4], M., Moein Aledavood [2], S.D., Sadeghpanah [5], A.

1. Department of Animal Sciences, College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran
2. Department of Animal Sciences, Faculty of Agriculture, Islamic Azad University, Ghaemshahr Branch, Ghaemshahr, Iran
3. Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran
4. Department of Poultry Sciences, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
5. Animal Sciences Research Institute, Agricultural Research, Education and Extension Organization, Tehran, Iran

Received 12 April 2016; accepted 03 May 2016
Corresponding Author: hamid_kohram@yahoo.com

ABSTRACT

In sheep industry, pregnancy rate after artificial insemination (AI) declines due to the complex anatomy of the cervix in ewes, such that it might prevent effective intrauterine insemination. At estrus, cervical relaxation occurs to some degree in ewes, which is regulated by the changes in the levels of reproductive hormones. This study aimed to evaluate the effects of estradiol and intravenous (IV) or intramuscular (IM) oxytocin injection at different doses on the cervical opening and pregnancy rate of Iranian Zel ewes during the breeding season. For this purpose, three experiments were conducted on 120 ewes (3-4 years old, weighing 47±2.5 kg). In the first experiment, ewes were equally assigned to two groups to receive estradiol (100-200 µg). After 12 h, each group was equally divided into six subgroups (n=20) and received 50, 100 and 150 IU oxytocin via IV and IM injection. Cervical opening was measured before and 15 min and 12 h after estradiol injection and 20 min after oxytocin administration. In the second experiment, we only assessed the effect of oxytocin administration on cervical opening similar to the first experiment. In the third experiment, controlled internal drug release (CIDR) was used in all the ewes for 12 days to induce estrus synchronization. Afterwards, the ewes received 550 IU intrauterine equine chorionic gonadotropin at the time of CIDR removal. Before AI, ewes were equally categorized into three groups (n=40); the first group was considered as control, and the other two groups received 100 IU oxytocin via IM or IV injection. At 54 h after CIDR removal, all ewes were inseminated transcervically using diluted fresh semen. Pregnancy was detected via ultrasound 50 days after insemination, and lambing and twinning rates were measured after parturition. Results of the first and second experiment indicated that estradiol injection had no effect on cervical opening ($P>0.05$), while the administration of 100 or 150 IU oxytocin (IV or IM) could dilate the cervix with or without estradiol ($P<0.05$). Furthermore, administration of 100 IU oxytocin (IV or IM) in the third experiment improved pregnancy and lambing rates compared to the control group ($P<0.05$); however, it had no effect on the twinning rate of the ewes ($P>0.05$). According to the results, IV or IM injection of oxytocin could improve the pregnancy rate in Iranian Zel ewes through the dilation of cervical canal. Therefore, it is suggested that this method be applied to enhance the pregnancy rate of ewes during the breeding season.

Keywords: Cervical dilation, Estradiol, Oxytocin, Pregnancy rate, Artificial insemination

Les effets de l'estradiol et de l'injection d'ocytocine sur l'efficacité de l'insémination artificielle chez les brebis Zel iraniennes pendant la saison reproductrice

Résumé: Dans l'industrie ovine, l'insémination artificielle (IA) engendre une diminution du taux de grossesse en raison de l'anatomie complexe du col de l'utérus chez la brebis. Ce facteur peut ainsi fortement entraver l'insémination intra-utérine. Durant le cycle œstral des brebis, la relaxation du col de l'utérus survient à différents niveaux et est principalement régie par les changements du taux d'hormones reproductrices. Cette étude avait pour objectif d'évaluer les effets de l'estradiol et de l'injection intraveineuse (IV) ou intramusculaire (IM) d'ocytocine (à différentes doses), sur l'ouverture du col et le taux de grossesse chez les brebis Zel iraniennes lors de la saison de reproduction. A cet effet, 3 expériences ont été menées sur 120 brebis âgées de 3 à 4 ans et pesant 47 ± 2,5 kg. Dans un premier temps, les brebis ont été reparties équitablement entre deux groupes avant de recevoir chacune 100 à 200 µg d'estradiol. Après 12 heures, chaque groupe a été divisé en 3 groupes de 20 brebis afin d'évaluer l'effet de l'injection IV ou IM d'ocytocine à 3 doses différentes (50, 100 et 150 IU). Le niveau d'ouverture du col de l'utérus a été mesuré avant l'injection ainsi que 15 mn et 12 h après l'injection d'ocytocine. Dans la deuxième expérience, l'effet d'une administration exclusive d'ocytocine sur l'ouverture du col de l'utérus a été évalué. Enfin, pour la troisième expérience, uneméthode de libération contrôlée de médicament (controlled internal drug release ou CIDR) a été utilisée sur 12 jours afin de synchroniser le cycle œstral. Ensuite, les brebis ont reçu 550 IU de gonadotrophine chorionique équine en injection intra-utérine durant la suppression du CIDR. Avant l'IA, les brebis ont été réparties équitablement entre 3 groupes (n=40), un groupe témoin et deux groupes d'essai recevant 100 IU d'ocytocine par injection IM ou IV. Les brebis ont reçu une insémination transcervicale avec une dilution de sperme frais 54 h après la suppression du CIDR. Afin de détecter les grossesses, des ultrasons ont été utilisés 50 jours après insémination les taux d'agnelage et de de naissance gémellaire ont été mesurés après parturition. Les résultats des deux premières expériences montrent que l'injection estradiol n'a pas d'effet sur l'ouverture du col de l'utérus (P>0,05), alors que l'administration de 100 et 150 IU d'ocytocine peut induire la dilatation du col avec ou sans estradiol (P>0,05). De plus, l'administration de 100 IU d'ocytocine (IV ou IM) lors de la troisième expérience, augmente les taux de grossesse et d'agnelage comparativement au groupe témoin (P>0,05). Cependant le taux de naissance gémellaire restait inchangé chez les brebis traitées (P>0,05). En résumé, nos résultats indiquent qu'une injection IV ou IM d'ocytocine peut améliorer le taux de naissance des brebis Zel iraniennes en provoquant la dilatation du canal cervical. L'application de cette méthode est donc recommandée pour augmenter le taux de grossesse des brebis lors de la saison de reproduction.

Mots clés: Dilation cervicale, Estradiol, Ocytocine, Taux de grossesse, insémination artificielle

INTRODUCTION

In transcervical artificial insemination programs for sheep, ewes are often inseminated with fresh semen, which results in the pregnancy rate of 40-60% (Anel et al., 2005). However, use of fresh semen in artificial insemination (AI) programs is associated with certain limitations, such as lack of rams with special traits and the collection, preservation, transportation, and insemination of fresh semen. AI with frozen semen is effective in maximizing the use of proved rams and controlling infections in herds. Nevertheless, current findings are indicative of the low pregnancy rate in transcervical insemination with frozen semen. Low rate of pregnancy arises from the inability of the sperm to pass through the cervix of ewes due to their complex cervix (Lightfoot and Salamon, 1970). On the other hand, lack of efficient approaches for AI in sheep restricts the use of frozen semen for the insemination of ewes. Scientific reports have proposed various methods to increase the pregnancy rate following AI with frozen semen; one of these methods is laparoscopic insemination. Currently, laparoscopic insemination and embryo transfer are associated with a high pregnancy rate in domestic animal herds. However, these methods are costly and time-consuming, requiring adequately trained personnel and use of sedative drugs. Laparotomy is another approach in this regard, which is

commonly used for embryo collection and is associated with certain complexities, such as the high risk of the adhesion of uterus, ovaries and oviduct (Torres and Sevellec, 1987). Sheep cervix is anatomically composed of cellular and extracellular segments. The cellular part includes the epithelia, smooth muscles, and fibroblast cells. The extracellular segment is composed of collagen fibers and elastin, which are consolidated with a matrix of proteoglycans, glycosaminoglycans and water (Cabrol et al., 1987). Moreover, the cervix canal in sheep mainly contains 4.9±0.1 rings (Halbert et al., 1990). According to histological studies, the internal cervical ring is the most important barrier against the entry of the AI pipette (Kershaw-Young et al., 2009), whereas the second or third ring are not positioned in a straight line toward the cervix, which is the main cause of the inability of insemination instruments to penetrate into the cervix and uterus. Cervix is located at a distance of 3.1±0.9 mm from the eccentric ring (Halbert et al., 1990). This property is associated with the reduced rate of pregnancy in transvaginal AI with frozen semen. On the other hand, deep discharge of semen in the cervix has been shown to increase the possibility of pregnancy in ewes (Eppleston et al., 1994; Anel et al., 2006). Therefore, to achieve a successful pregnancy in ewes, the insemination pipette should pass through the cervical barrier without causing injuries. To date, three main approaches have been introduced to facilitate the entry of insemination pipettes into the cervix, the first and second of which are physical and mechanical, respectively (Halbert et al., 1990; Buckrell et al., 1994; Wulster-Radcliffe and Lewis, 2002). In the mechanical method, the insemination pipette is specially designed to cross the cervix (Halbert et al., 1990; Wulster-Radcliffe and Lewis, 2002); however, according to the findings of Wulster-Radcliffe et al. (2004), use of this pipettes has no positive effects on the rates of pregnancy and lambing (Wulster-Radcliffe et al., 2004). Some AI pipettes and guns have been designed for easy passage into the cervical canal and uterus; however, the connection between the AI pipette and cervical rings may cause cervical injuries, stimulating the release of certain components that are lethal for the sperm and embryo, which ultimately leads to poor fertility (Hawk, 1983; Sayre and Lewis, 1997). In this context, the third approach involves the use of chemical elements, such as hormones (e.g., estradiol, prostaglandins and oxytocin), for cervical opening (Mylne et al., 1992). Although prostaglandin E is able to dilate the cervix, it is not considered a cost-effective approach (Mylne et al., 1992). On the other hand, since estradiol and oxytocin may not affect luteal activity, use of these hormones may reduce the risk of cervical scarring in transcervical insemination and embryo transfer. Furthermore, intravenous injection of oxytocin has been reported to be effective in the cervical opening of ewes, facilitating AI, and embryo collection, while it could improve the reproduction efficiency of sheep as well (Wulster-Radcliffe et al., 2004). Zel ewes are an Iranian breed of small sheep with an expensive meat in Iran. This breed is native to the regions near the Caspian Sea, and increasing the efficiency of their reproduction is considered greatly economical. This study aimed to evaluate the effects of estradiol and oxytocin administration on the reproductive performance of Zel ewes during the breeding season.

MATERIALS AND METHODS

Experimental design. In this study, ewes aged 3-4 years old with mean weight of 47±2.5 kg were involved in three experiments. The first experiment was carried out in two sections, each of which was performed at a different time. In the first section, ewes (n=120) were equally divided into two groups and received estradiol (100-200 µg) (Aburaihan Pharmaceutical Company; 2 mg/ml estradiol benzoate, Iran). At 12 h after estradiol administration, ewes were equally assigned to three subgroups (n=20) and received 50, 100 and 150 IU oxytocin via intravenous (IV) injection into the jugular vein (Aburaihan Pharmaceutical Company; 10 IU/ml oxytocin, Iran). After two weeks, the second section of the experiment was conducted similar to the first section using a different administration mode for

oxytocin. In this section, oxytocin was administered at three doses (50, 100 and 150 IU) via intramuscular (IM) injection into the hip muscle (Table 1). The second experiment was performed four weeks after the first experiment. In this stage, ewes were equally divided into six groups (n=20). First three groups received 50, 100 and 150 IU oxytocin via IV injection and the other three groups received 50, 100 and 150 IU oxytocin via IM injection (Table 2). After two weeks, the third experiment was carried out in order to induce estrus synchronization. For this purpose, controlled internal drug release (CIDR) was used for 12 days, and all the ewes (n=120) received 550 IU equine chorionic gonadotropin (Sanofi Animal Health, Libourne Cedex, France) immediately after CIDR removal. Afterward, ewes were equally assigned to three groups (n=40), and group one was administered with saline as control. The other two groups were administered with 100 IU oxytocin via IM (group two) and IV injection (group three). At 54 h after CIDR removal, transcervical insemination was performed on all the ewes using fresh semen (Figure 1).

Measurement of cervical dilation. In the first experiment, cervical dilation was measured using a sheep speculum and scaled AI pipette (to penetrate into the cervix) at four intervals: before administration, 15 min and 12 h after estradiol injection (before oxytocin injection), and 20 min after oxytocin administration. In the second experiment, we investigated the effect of oxytocin injection on cervical opening before and 20 min after oxytocin administration. Depth of penetration was measured using an insemination pipette before and 10-15 min after hormone injection. If the pipette penetrated into the cervix, cervical opening was confirmed (i.e., opened cervix). Cervixes with a little penetration or without penetration was considered

Table 1. Hormone therapy in first experiment

Experiment 1												
Section	Section 1						Section 2					
Estradiol	100 mg (n=120)						200 mg (n=120)					
Injection mode	Intramuscular (IM)			Intravenous (IV)			IM			IV		
Oxytocin (IU)	50	100	150	50	100	150	50	100	150	50	100	150
Cervical dilation measurement	Before injections 15 min after estradiol injection 12 h after estradiol injection 20 min after oxytocin injection											

Table 2. Hormone therapy in second experiment

Experiment two												
Injection mode	IM			IV			IM			IV		
Oxytocin (IU)	50	100	150	50	100	150	50	100	150	50	100	150
Cervical dilation measurement	Before injections 15 min after estradiol injection 12 h after estradiol injection 20 min after oxytocin injection											

Table 3. Effect of estradiol administration (100-200 µg) on cervical dilation (Mean±SE)

	E2	
Item	100 µl	200 µl
Before injection (cm)	0.37±0.19	0.59±0.26
15 min after injection (cm)	0.68±0.27	0.80±0.29
12 h after injection (cm)	0.67±0.12	0.78±0.18

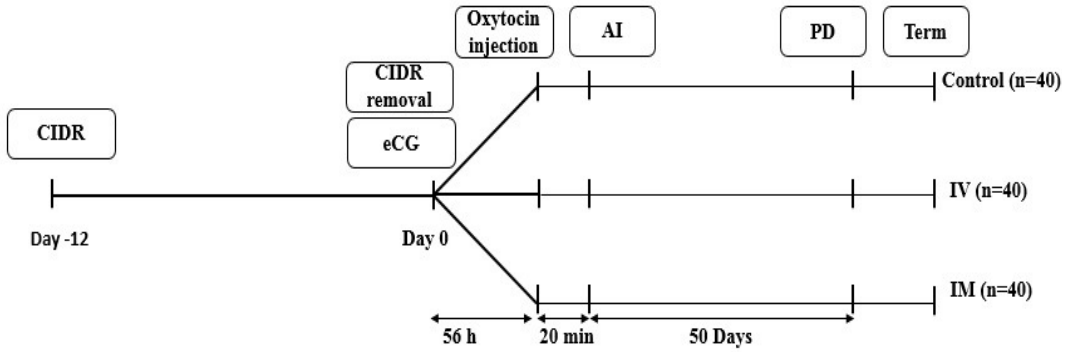

Figure 1. Schematic view of the third experiment
(**AI:** artificial insemination; **CIDR:** controlled internal drug release; **eCG:** equine chorionic gonadotropin; **IV:** intravenous; **IM:** intramuscular; **PD:** pregnancy diagnosis)

Table 4. Mean of pipette penetration 24 h after estradiol injection and 20 min after oxytocin injection in experiment 1

	E2													
E2 dose	100 mg							200 mg						
Injection type	IM			IV			M±SEM	IM			IV			M±SEM
OT doses (IU)	50	100	150	50	100	150		50	100	150	50	100	150	
Before OT (cm)	0.75	0.65^{d}	0.74^{d}	0.57	0.72^{d}	0.60^{d}	0.67±0.12	0.55	0.85^{d}	0.75^{d}	0.68	1.00^{d}	0.86^{d}	0.78±0.18
After OT (cm)	0.73^{b}	4.85^{ac}	4.92^{ac}	1.08^{b}	4.52^{ac}	3.47^{ac}	3.47±0.33	0.68^{b}	4.86^{ac}	4.96^{ac}	1.36^{b}	5.01^{ac}	4.78^{ac}	3.61±0.33
Opened cervix (%)	(3/20) 15%	(17/20) 85%	(17/20) 85%	(5/20) 25%	(16/20) 80%	(15/20) 75%		(3/20) 15%	(16/20) 80%	(17/20) 85%	(6/20) 30%	(18/20) 90%	(16/20) 80%	

^{a,b}: significant difference in rows (P<0.05); ^{c,d}: significant difference in columns (P<0.05)

Table 5. Effect of oxytocin administration on cervical opening in second experiment

Item	IM			IV			M±SEM
OT doses (IU)	50	100	150	50	100	150	
Before OT (cm)	0.62	0.72^{d}	0.45^{d}	0.55	0.45^{d}	0.77^{d}	0.59±0.21
After OT (cm)	0.71^{b}	4.88^{ac}	5.08^{ac}	0.63^{b}	4.44^{ac}	4.46^{ac}	3.37±0.29
Opened cervix (%)	(0/20), 0	(16/20), 80	(18/20), 90	(2/20), 10	(15/20), 75	(15/20), 75	

Numbers in parentheses indicate the number of ewes/total in each group;
^{a,b}: significant difference in rows (P<0.05);
^{c,d}: significant difference in columns (P<0.05)

Table 6. Effect of oxytocin administration on reproductive parameters in third experiment

	Control	100 (IV)	100 (IM)
Pregnancy rate (%)	(16/40), 40^{b}	(24/40), 60^{a}	(28/40), 70^{a}
Lambing rate (%)	(18/40), 45^{b}	(24/40), 60^{a}	(30/40), 75^{a}
Twinning rate (%)	(2/40), 5	(0/40), 0	(2/40), 5

Numbers in parentheses indicate the number of ewes/total in each group; ^{a,b}: significant difference in rows (P<0.05)

closed. Correspondingly, after the injections, the ewes were categorized into two groups of opened or closed cervix.

Semen collection and dilution. In this study, semen was collected from Zel rams using an artificial vagina (IMV, France). Collected semen was diluted in 1:1 (v:v) with skimmed milk and loaded into 0.25 ml straws. For primary evaluations, total sperm motility was measured using a light microscope, and samples with progressive motility of more than 60% were selected for insemination.

Artificial insemination and pregnancy diagnosis. In the control group, AI was performed routinely. As for the other two groups, the ewes were inseminated transcervically 20 min after oxytocin injection. Pregnancy was diagnosed 50 days after insemination via ultrasound using a device equipped with a 3.5 MHz sector probe (Pie Medical, Falco 100). Moreover, rates of lambing and twinning were calculated after the parturition of the ewes.

Statistical analysis. Data analysis was performed in SAS software version 9.1. In addition, data of cervical dilation were analyzed using the GLM procedure, and reproductive data set (percentage) was examined through the GENMOD procedure.

RESULTS

Cervical opening and pipette penetration. According to the results of this study, estradiol had no effect on the cervical opening of ewes at 15 min and 12 h after administration (Table 3), while the injection of 100 and 150 IU oxytocin at 12 h after estradiol administration led to cervical opening ($P>0.05$) (Table 4). In the groups receiving 100 and 150 IU oxytocin, complete cervical opening was observed 20 min after the injection, and the route of injection (IV or IM) had no significant effect in this regard ($P>0.05$) (Table 6).

Pregnancy and lambing rates. Results of the third experiment in this study indicated that rates of pregnancy and lambing significantly improved in the groups treated with 100 IU oxytocin compared to the control group ($P<0.05$), while oxytocin administration had no significant effect on the twinning rate of the ewes (Table 5).

DISCUSSION

Some chemicals and hormones are used to facilitate the passage of AI pipette and embryo transfer catheters through the cervix of sheep breeds. Moreover, it has been proposed that use of OVUGEN vaginal suppositories (containing the follicle-stimulating hormone) at 54 and 60 h after progesterone withdrawal could facilitate the AI pipette passage in ewes (Leethongdee et al., 2007). On the other hand, prostaglandin E (Mylne et al., 1992) and misoprostol suppositories (prostaglandin E analogue) have been shown to be able to dilate the cervix in ewes at 54 h after progesterone withdrawal, thereby facilitating the transvaginal penetration of pipettes or catheters. It is noteworthy that these drugs are often administered at 48 h after progesterone withdrawal (Akinbami et al., 1990; Leethongdee et al., 2007). Effects of estradiol on cervical opening are mediated through the regulation of COX-2 and EP4 mRNA expression in the cervix. Estradiol upregulates prostaglandin E receptors. At 20-48 h after estradiol administration, expression of cyclooxygenase increases (Shemesh et al., 1997). Additionally, use of prostaglandin E seems to open the cervix through the remodeling of the cervical extracellular matrix (Stys et al., 1981; Ledger et al., 1983). Effects of prostaglandin E on EP2 and EP4 receptors induce smooth muscle relaxation and biosynthesis of glycosaminoglycans. Hyaluronan is known as the major glycosaminoglycan in the cervix (Kershaw-Young et al., 2009). Accumulation of hyaluronan and water molecules in collagen fibers leads to the disruption of collagen fibers and reduction of cervical strength (El Maradny et al., 1997). However, low-molecular-weight hyaluronan is considered as an influential factor in vascularization, increased leukocyte infiltration, and stimulation of biochemical changes in the cervix (Perry et al., 2010). Interleukin 8 (IL-8) plays a key role in the entry of neutrophils into tissues. Neutrophils contain large

portions of collagenase and elastase, which are the essential enzymes involved in cervical opening. In the current study, treatment of ewes with human IL-8 at estrus resulted in cervical opening and increased the penetration of the transcervical pipette into the cervix (Croy et al., 1999). Progesterone inhibits the expression of IL-8 genes in the cervix; as such, overexpression of IL-8 at estrus is associated with high estradiol and low progesterone levels (Mitchell et al., 2002). In the present study, results of the first and second experiment indicated that estradiol had no significant effect on cervical opening. According to the findings of the current research, it seems that during the breeding season, estradiol cannot moderate the mechanisms of cervical opening, while in non-breeding seasons, estradiol could be effectively administered to open the cervix in sheep; further investigation is required in this regard. This finding is inconsistent with the results obtained by Wulster-Radcliffe et al. (2004), who claimed that estradiol injection could dilate the cervix in ewes, facilitating intrauterine deposition of embryos in an embryo transfer program. In the study by Kumaresan et al. (2013), it was proposed that injection of estradiol along with cloprostenol resulted in complete cervical dilation in cows. On the other hand, findings of another study in this regard indicated that estradiol administration had no significant effect on cervical opening (Masoudi et al., 2012a). Differences in the aforementioned findings regarding the effect of estradiol on cervical dilation could be attributed to the breeding season, injected doses, and combination of estradiol with other hormones. Low-molecular-weight hyaluronan is a glycoprotein that increases with heat. This glycoprotein could improve pipette penetration by 3.4 mm, which is excellent for breeds with a small cervix (Perry et al., 2010). Oxytocin is a hormone that initiates a series of collagenolytic process, which ultimately lead to cervical opening. According to the results of the current study, oxytocin injection (IV or IM) could dilate the cervix and facilitate semen deposition in the posterior portion of the uterus, thereby increasing the rate of pregnancy and decreasing the costs of AI through saving time and resources. Our findings were consistent with the results of previous studies in this regard, which demonstrated that administration of estradiol with oxytocin (Flohr et al., 1999; Stellflug et al., 2001) or oxytocin alone could be effective in cervical dilation (Sayre and Lewis, 1997; Viudes-de-Castro et al., 2009; Masoudi et al., 2012b).

Results of the present study indicated that cervical opening could be occured following the administration of oxytocin at low doses with or without estradiol. In previous studies, oxytocin was injected intravenously, while in the current research, we also used IM injection for this purpose since IM administration of hormones is easier than IV injection. However, our findings were indicative of no significant difference between IV and IM oxytocin administration in terms of cervical opening in ewes during the breeding season. Therefore, it seems that in large-scale AI, IM injection of oxytocin is more simple, rapid, and economic than IV administration. According to the literature, oxytocin has no negative effects on sperm transfer in the reproductive tract and sperm-oocyte fusion in mammals. Minimal dose of oxytocin required for cervical opening has been determined at 50 IU (Sayre and Lewis, 1997). Moreover, it has been proposed that estradiol could stimulate the expression of oxytocin receptors. Results of the first and second experiment in the present study showed that IV or IM administration of 100 IU oxytocin leads to cervical opening. On the other hand, in the third experiment, AI was carried out following oxytocin injection, and the pregnancy rate in the two groups receiving 100 IU oxytocin (IV and IM) was observed to be higher compared to the control group. According to previous studies in this regard, oxytocin injection leads to cervical opening and deeper sperm infusion in the cervix or uterus, which enhances the rate of pregnancy in ewes (Eppleston et al., 1994; Anel et al., 2006). On the other hand, oxytocin administration could improve sperm transfer from the cervix (Ayad et al., 2004), thereby increasing the sperm count in the fertilization site and possibility of sperm-

oocyte fusion (Stellflug et al., 2001; Wulster-Radcliffe and Lewis, 2002).

According to the results of this study, administration of 100 IU oxytocin led to cervical dilation in Iranian Zel ewes. Moreover, no significant difference was observed between the IM and IV administration of oxytocin in this respect. Therefore, as a cervical dilator, oxytocin injection could be applied in order to improve the rates of pregnancy and lambing in sheep husbandry.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Akinbami, M.A., Meredith, S., Warren, J.E., Jr., Anthony, R.V., Day, B.N., 1990. Cervical dilation, conception rate, and concentrations of progesterone and estradiol-17B in postpartum ewes treated with porcine relaxin. Theriogenology 34, 927-940.

Anel, L., Alvarez, M., Martinez-Pastor, F., Garcia-Macias, V., Anel, E., de Paz, P., 2006. Improvement strategies in ovine artificial insemination. Reprod Domest Anim 41 Suppl 2, 30-42.

Anel, L., Kaabi, M., Abroug, B., Alvarez, M., Anel, E., Boixo, J.C., *et al.*, 2005. Factors influencing the success of vaginal and laparoscopic artificial insemination in churra ewes: a field assay. Theriogenology 63, 1235-1247.

Ayad, V.J., Leung, S.T., Parkinson, T.J., Wathes, D.C., 2004. Coincident increases in oxytocin receptor expression and EMG responsiveness to oxytocin in the ovine cervix at oestrus. Anim Reprod Sci 80, 237-250.

Buckrell, B.C., Buschbeck, C., Gartley, C.J., Kroetsch, T., McCutcheon, W., Martin, J., *et al.*, 1994. Further development of a transcervical technique for artificial insemination in sheep using previously frozen semen. Theriogenology 42, 601-611.

Cabrol, D., Dallot, E., Cedard, L., Sureau, C., 1987. Pregnancy-related changes in the distribution of glycosaminoglycans in the cervix and corpus of the human uterus. European Journal of Obstetrics and Gynecology and Reproductive Biology 20, 289-295.

Croy, B.A., Prudencio, J., Minhas, K., Ashkar, A.A., Galligan, C., Foster, R.A., *et al.*, 1999. A preliminary study on the usefulness of huil-8 in cervical relaxation of the ewe for artificial insemination and for embryo transfer. Theriogenology 52, 271-287.

El Maradny, E., Kanayama, N., Kobayashi, H., Hossain, B., Khatun, S., Liping, S., *et al.*, 1997. The role of hyaluronic acid as a mediator and regulator of cervical ripening. Human Reproduction 12, 1080-1088.

Eppleston, J., Salamon, S., Moore, N.W., Evans, G., 1994. The depth of cervical insemination and site of intrauterine insemination and their relationship to the fertility of frozen-thawed ram semen. Animal Reproduction Science 36, 211-225.

Flohr, S.F., Wulster-Radcliffe, M.C., Lewis, G.S., 1999. Technical note: development of a transcervical oocyte recovery procedure for sheep. J Anim Sci 77, 2583-2586.

Halbert, G.W., Dobson, H., Walton, J.S., Buckrell, B.C., 1990. A technique for transcervical intrauterine insemination of ewes. Theriogenology 33, 993-1010.

Hawk, H.W., 1983. Sperm Survival and Transport in the Female Reproductive Tract. Journal of Dairy Science 66, 2645-2660.

Kershaw-Young, C.M., Khalid, M., McGowan, M.R., Pitsillides, A.A., Scaramuzzi, R.J., 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.

Kumaresan, A., Chand, S., Suresh, S., Mohanty, T.K., Prasad, S., Layek, S.S., *et al.*, 2013. Effect of estradiol and cloprostenol combination therapy on expulsion of mummified fetus and subsequent fertility in four crossbred cows. Vet Res Forum 4, 85-89.

Ledger, W.L., Ellwood, D.A., Taylor, M.J., 1983. Cervical softening in late pregnant sheep by infusion of prostaglandin E-2 into a cervical artery. J Reprod Fertil 69, 511-515.

Leethongdee, S., Khalid, M., Bhatti, A., Ponglowhapan, S., Kershaw, C.M., Scaramuzzi, R.J., 2007. The effects of the prostaglandin E analogue Misoprostol and follicle-stimulating hormone on cervical penetrability in ewes during the peri-ovulatory period. Theriogenology 67, 767-777.

Lightfoot, R.J., Salamon, S., 1970. Fertility of ram spermatozoa frozen by the pellet method. I. Transport and viability of spermatozoa within the genital tract of the ewe. J Reprod Fertil 22, 385-398.

Masoudi, R., Kohram, H., Shahne, A.Z., Davoud, S.D.M.A., 2012a. Effect of estradiol and oxytocin on ovine cervical relaxation. African J Biotechnol 11, 2803-2806

Masoudi, R., Kohram, H., Shahne, A.Z., Davoud, S.D.M.A., Sharif, A.A., 2012b. Effects of exogenous oxytocin on cervical penetration of Iranian ewes. African J Biotechnol 11, 2398-2401.

Mitchell, S.E., Robinson, J.J., King, M.E., McKelvey, W.A., Williams, L.M., 2002. Interleukin 8 in the cervix of non-pregnant ewes. Reproduction 124, 409-416.

Mylne, M.J., McKelvey, W.A., Fernie, K., Matthews, K., 1992. Use of a transcervical technique for embryo recovery in sheep. Vet Rec 130, 450-451.

Perry, K., Haresign, W., Wathes, D.C., Khalid, M., 2010. Intracervical application of hyaluronan improves cervical relaxation in the ewe. Theriogenology 74, 1685-1690.

Sayre, B.L., Lewis, G.S., 1997. Fertility and ovum fertilization rate after laparoscopic or transcervical intrauterine artificial insemination of oxytocin-treated ewes. Theriogenology 48, 267-275.

Shemesh, M., Dombrovski, L., Gurevich, M., Shore, L.S., Fuchs, A.-R., Fields, M.J., 1997. Regulation of bovine cervical secretion of prostaglandins and synthesis of cyclooxygenase by oxytocin. Reproduction, Fertility and Development 9, 525-530.

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, 568-573.

Stys, S.J., Dresser, B.L., Otte, T.E., Clark, K.E., 1981. Effect of prostaglandin on cervical compliance in pregnant ewes. American Journal of Obstetrics & Gynecology 140, 415-419.

Torres, S., Sevellec, C., 1987. Repeated superovulation and surgical recovery of embryos in the ewe. Reprod. Nutr. Dévelop. 27, 859-863.

Viudes-de-Castro, M.P., Salvador, I., Marco-Jimenez, F., Gomez, E.A., Silvestre, M.A., 2009. Effect of oxytocin treatment on artificial insemination with frozen-thawed semen in Murciano-Granadina goats. Reprod Domest Anim 44, 576-579.

Wulster-Radcliffe, M.C., Lewis, G.S., 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.

Wulster-Radcliffe, M.C., Wang, S., Lewis, G.S., 2004. Transcervical artificial insemination in sheep: effects of a new transcervical artificial insemination instrument and traversing the cervix on pregnancy and lambing rates. Theriogenology 62, 990-1002.