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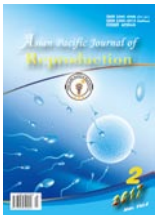

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Cervical dilation and improvement of reproductive performance in fat-tailed ewes via cervical dilator treatments

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ABSTRACT

Objective: To determine the effect of different cervical dilators on cervical dilation and reproductive performance of fat-tailed ewes. **Methods:** In experiment 1 140 ewes were divided into seven groups with seven different treatments as following: 10 mL normal saline (control group), 100 IU oxytocin (OT group), 100 μg estradiol and 100 IU oxytocin (E_2+OT group), 5 mL relaxin (R group), 2 mL sensiblex (SEN group), 200 μg misoprostol (MIS group) or 200 μg dinoprostone (DIN group). In experiment 2, artificial insemination was applied for evaluation of reproductive performance in experimental groups. **Results:** In experiment 1, the highest cervical dilation was observed in OT (90%) and E_2+OT (100%) groups (P<0.05), while no significant differences was found among DIN, MIS, SEN and R groups (80%, 75%, 70% and 65%, respectively). In addition, the lowest cervical dilation was observed in control group. Experiment 2 found no significant differences among control, OT and E_2+OT groups. The highest pregnancy rate, parturition rate and lambing rate were observed in OT groups (60%, 60% and 70%, respectively) and E_2+OT groups (65%, 60% and 70%, respectively) compared to SEN, R, MIS and DIN groups (P<0.05). **Conclusions:** Oxytocin treatment alone or with estradiol could be used as a suitable dilator for improving reproductive efficiency during artificial insemination in fat-tailed ewes.

1. Introduction

Artificial insemination (AI) in sheep is a valuable tool for amplification of good sire in commercial stock. Nowadays, AI uses fresh and frozen semen but with almost no satisfactory results. This phenomenon is mainly related to the winding shape of ewes' cervix and lack of efficient methods[1]. Although laparoscopy and laparotomy have been used as efficient methods for artificial, they have some limitations including high cost, time consuming and special equipment[2,3]. Therefore, changes in the physiological characteristics of cervix seem to be an efficient method to improve the reaching of sperm to fertilization site.

Cervix in ewes is a long fibrosis tubular structure containing around 5 rings[4]. According to the histological studies, the interior rings are the most important obstacle against insemination pipette[5]. To overcome this problem, two strategies have been recorded. The first one is a physico-mechanichal method[6] that is based on physical stress to the cervix or transfer of sperm by a flexible or semi-flexible pipette[6-10] that may injure the cervix and produce

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some anti-sperm secretions leading to lower sperm viability, and consequently lower conception rate[11,12]. In recent years, hormonal strategy has been proposed to improve the efficacy of AI. Oxytocin and prostaglandin E (PGE) are effective hormones on cervix dilation[8,13,14]. Oxytocin and E_2 have no effects on luteal phase and have minimum injury when pipette is crossing the cervix during AI[8]. Oxytocin administration dilates the cervix and thus facilitates the passage of the pipette through the cervix and allow semen deposition intra uterus[8].

In our records, no attempt has been made to evaluate the efficacy on AI of the systematic comparison of oxytocin, estradiol and oxytocin, relaxin, sensiblex, misoprostol and dinoprostone. Furthermore, we applied AI technique for the first time to assess the effects of these treatments on the reproductive performances. Therefore, this study was conducted to determine the effect of different cervical dilators on cervical dilation and reproductive performance of fat-tailed ewes.

2. Materials and methods

2.1. Experiments design

One hundred forty Iranian fat-tailed Zandi ewes with (55±5) kg weight and 3 to 4-year-old were used to determine the effects of different cervical dilators on cervical dilation and reproductive performance.

2.2. Experiment Ⅰ

All ewes received controlled internal drug release (CIDR) (Easy-Breed™, CIDR®, New Zealand) for 12 d. After CIDR removed about 2 d, they were divided into 7 equal groups (20 ewes per group). Each group received one of the following treatments: 1) 10 mL saline (control group). 2) 100 IU oxytocin (Abureihan Pharmacy; 10 IU/mL oxytocin, Iran) (OT group). 3) 100 μg estradiol (Abureihan pharmacy, 2 mg/mL estradiol benzoate, Iran) and 12 h later 100 IU oxytocin (E_2+OT group). 4) 5 mL relaxin (Sina Pharmacy; 1 g/10 mL Methocarbamol, Iran) (R group). 5) 2 mL sensiblex (Veyx Pharma-GmbH; 40 mg/mL solution Denaverine hydrochloride) (SEN group). 6) 200 μg misoprostol (PGE_1 analogue, Abureihan Pharmacy, Iran) (MIS group). 7) 200 μg dinoprostone (PGE_2 analogue, Abureihan Pharmacy, Dinoproston, PGE_2 analogue, Iran) (DIN group). The method, time and dosages of hormonal applications were used according the instruction of company and their effective time of each hormone, specifically (Table 1).

2.3. Experiment Ⅱ

To evaluate the reproductive performance, ewes were intramuscularly received 500 IU eCG (Sanofi Animal Health, Libourne Cedex, France) at the time of CIDR removal and divided into 7 equal groups for AI according to Table 1. All ewes were inseminated 54 h after CIDR removal.

Table 1
Experimental procedure to determine the cervical dilators effects on cervical dilation and reproduction performance.

Groups	Treatments	Dosage	Cervicaldilation measurement	AI
1	Saline	10 mL	20 min after injection	54 h after CIDR removal
2	Oxytocin	100 IU	20 min after injection	20 min after injection
3	Estradiol/ Oxytocin	100 μL/ 100 IU	20 min after OT injection	20 min after OT injection
4	Relaxin	5 mL	40 min after injection	40 min after injection
5	Sensiblex	2 mL	40 min after injection	40 min after injection
6	Misoprostol	200 μg	5 h after pessary insertion	5 h after pessary insertion
7	Dinoprostone	200 μg	5 h after pessary insertion	5 h after pessary insertion

2.4. Cervix relaxation measurement

Cervical dilation was measured by a scaled AI pipette (40 cm length and 4 mm diameter). The difference in penetration before and after injections and pessary insertion in each group was considered as cervical penetration.

2.5. Sperm collection and dilution

Semen was collected using artificial vagina from six mature Zandi rams. To eliminate individual differences, the semen was pooled and then diluted 1:1 (v:v) with skim milk and finally loaded in straws (0.25 mL). Semen samples with at least 60% progressive motility was selected for insemination.

2.6. AI and pregnancy diagnosis

AI was transcervically accomplished at the time of cervical dilation, 54 h after CIDR removal. For control group, the routine method was applied for AI. Pregnancy diagnosis was performed by an ultrasound unit (falco 100, premedical) equipped with a 3.5 MHz sectorial transducer probe in day 50 post insemination.

2.7. Statistical analysis

The SAS (9.1) GLM procedure were used to determine the effect of treatments on cervix dilation. When *F*-tests were significant, the DUNCAN option in GLM was used to separate means and reproductive parameters were analyzed via GENMOD procedure.

3. Results

3.1. Experiment 1: Cervical dilation

Table 2 shows the results of cervical dilation in ewes treated with various hormonal groups. Cervical dilation has been improved in OT, E_2+OT, DIN, MIS, SEN and R groups.

The highest rate of cervical dilation were observed in OT (90%)

Table 2
Effects of cervical dilator treatments on cervical dilation, and different cervical dilators on pregnancy, pariturition, lambing and twinning rate of Iranian fat tailed Zandi ewes.

Treatments	Control	OT	E_2+OT	R	SEN	MIS	DIN
Penetration (cm)[#]	0.59	3.90[*]	4.10[*]	3.11[*]	3.28[*]	3.32[*]	3.56[*]
Open Cervixes (*n*)[#]	0/20	18/20	20/20	13/20	14/20	15/20	16/20
Open Cervixes (%)[#]	0	90	100	65	70	75	80
Pregnancy rate(%)	(10/20)50	(12/20)60	(13/20)65	(2/20)10[*]	(2/20)10[*]	(5/20)25[*]	(6/20)30[*]
Parturition rate (%)	(9/20)45	(12/20)60	(12/20)60	(1/20)5[*]	(2/20)10[*]	(4/20)20[*]	(4/20)20[*]
Lambing rate (%)	(10/20)50	(14/20)70	(14/20)70	(1/20)5[*]	(2/20)10[*]	(4/20)20[*]	(4/20)20[*]
Twinning rate (%)	(1/9)11.11	(2/12)16.66	(2/12)16.66	(0/1)0	(0/2)0	(0/4)0	(0/4)0

The number in parentheses indicates the number of ewes/total in each group. [*]P<0.05 compared with control group. R: Relaximol, SEN: Sensiblex, MIS: Misoprostol, DIN: Dinoproston. [#]SEM=0.19.

and E_2+OT (100%) groups (P<0.05). In pairwise comparison on OT and E_2+OT groups, no significant difference was observed between them. Furthermore, no significant differences were observed among DIN, MIS, SEN and R groups (80%, 75%, 70% and 65%, respectively), and their rate of cervical dilation were lower than OT and E_2+OT groups'. Saline had no effect on cervical dilation.

3.2. Experiment 2: Reproduction performance

Table 2 shows the percentage of reproductive performance traits of ewes treated with different cervical dilators. OT and E_2+OT treatments have improved pregnancy rate (60% and 65%, respectively) compared to R (10%), SEN (10%), MIS (25%) and DIN (30%) groups (P<0.05). Pregnancy rate was not significantly higher in OT and E_2+OT groups compared to control group. Moreover, there were no significant differences between OT group and E_2+OT group. The pregnancy rate of R, SEN, MIS and DIN groups was lower than control group (P<0.05). Group OT and E_2+OT (60%) have similar parturition rate, which was the highest one in comparison to other groups' (R: 5%, SEN: 10%, MIS: 20% and DIN: 20%). However, parturition rates of R, SEN, MIS, and DIN groups were lower than control group (45%).

OT or E_2+OT treatment led to significant improvement in lambing rate, which was 70% in comparison to groups R (5%), SEN (10%), MIS (20%), and DIN (20%) (P<0.05). However, there were no significant differences among OT, E_2+OT and control groups in lambing rate. Lambing rate in R, SEN, MIS, and DIN groups was significantly lower than control groups (P<0.05).

4. Discussion

The deeper intra cervix insemination in ewes allows more spermatozoa to reach fertilization site and subsequently increase the fertilization rate, and ultimately improve pregnancy rates[15,16]. However, unique structure of ewes' cervix acts as a barrier against AI gun and embryo recovery catheter which causes drooping of fertility rates following AI and embryo transfer[8]. Hormonal treatment to change the physiological characteristics of cervix seems to be an efficient method for increment of sperm chance for fertilization. Misoprostol pessary which used 48 h after sponge removal led to cervix opening in 100% of ewes at the time of AI[17]. In this study, using misoprostol and dinoprostone could make cervical dilation and penetration of insemination gun into uterus of 75% and 80% ewes. However, the lambing rate in these groups was lower compared to OT, ET+OT and control group.

Furthermore, relaxin treatment did not have beneficial effects on reproductive parameters. Similarly, Akinbami *et al.*[18] observed that oocyte recovery rate and pregnancy rate of ewes with relaxin treatment was depressed, mainly because relaxin causes uterus and cervix to unclench too much that hampers sperm oocyte interaction. Hence, low fertilization rate might explain the lower lambing rate of R (5%) and SEN (10%) in our study.

Among various cervical dilators, OT and E_2+OT had the highest significant rate of pregnancy, parturition rate and lambing rate compared to other groups. This answer may be explained by effects of oxytocin that initiates collagenolytic enzyme process resulting in cervical dilation[19]. Previous studies showed that E_2+OT or only OT[13,20–22] caused cervical dilation in ewes. It has been proven that 400 IU OT led to dilation of cervix, but the injection of this amount of hormone right before the insemination may have some problems. In this study, 100 IU OT increased dilation of the cervix successfully, which reduced the cost of cervical dilation treatment. It has been previously proven that E_2+OT have no negative effects on luteal activity. Meanwhile, their application reduces the cervix damage during insemination and transcervical embryo transfer, and increase the functional speed during embryo recovery[8,23]. Estradiol enhances the effect of OT through increasing OT receptors and improving cell responsiveness to OT. Estradiol up-regulates cyclooxygenase mRNA and EP4 expression in cervix, and may cause cervical dilation by either increasing the production of PGE_2 or activating its receptors. After E_2 injection for 24-28 h, it has been shown that the amount of cyclooxygenase gene expression increased[24]. Moreover, oxytocin increases uterine constrictions with no deleterious effects on sperm transfer into the oviduct. The minimum dosage needed for dilating the cervix is 50 IU[12]. Also, PGE_2 remodels the extracellular matrix of the cervix which ultimately lead to the process of cervical dilation[25,26]. PGE_2 exerts its action mainly through the expansion of smooth muscle and increasing the production of glycosaminogly can by activating EP4 and EP2 receptors in the cervix. Hyaluronan and water molecules accumulation between collagen fibrils could lead to dispersion of collagen fibrils, which reduces the cervix resistance[27]. On the other hand, low molecular weight of hyaluronan affects vascularization, increases leukocytes penetration and stimulates cervix biochemical changes[28]. As leukocytes accumulate in the cervix, the neutrophils contain excessive amount of collagenase

and elastase, which are important for collagen fibrils dispersion and cervical dilation.

Furthermore, OT could increase sperm transfer through the female reproductive tract[29] and consequently more spermatozoa arrive to fertilization site. Moreover, OT injection during estrus elevate ovulation rate[30] so that the rate of sperm/oocyte fertilization increases.

From the results of our study, it could be concluded that E_2+OT causes cervical dilation in Zandi ewes, which provides possibility of non-surgical intrauterine AI through cervix in ewes. Besides, the results of fertility traits showed that E_2+OT treatment improves pregnancy, parturition and lambing rates of ewes.

Conflict of interest statement

We declare that we have no conflict of interest.

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