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Mechanical and Pharmacologic Applications of Artificial Insemination in Ewes

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1. Introduction

Artificial insemination (AI) is the keys to the efficient transfer of genetical knowledge from rams to ewes and to ensure proper productive traits of theirs. The efficient insemination of high-quality semen is very important in an artificial insemination of ewe. Artificial insemination is also part of the biotechnological studies. The insemination process itself may, in fact, cause of poor fertility or reduce of frozen semen quality.

Insemination process is regulated by a complex interplay of inseminator, semen, and genital tract structure of ewe. Although there is general agreement that cervix is of critical importance in the initiation and improvement of semen in mammals during insemination, the role of cervical canal in the regulation of frozen sperm transport in the ewes is stil problematic.

Because of the size and shape of the external os and the tortuous structure of the cervical canal, intrauterine insemination of ewes generally carries out with frozen sperm. Transcervical AI is applied using specially designed inseminating equipment and manipulation of the cervix using forceps. The number of reports in which transcervical deposition of semen has been achieved is relatively low and there are concerns about the potential trauma involved. The role of cervix in the transport of frozen sperm in the ewe is not entirely clear. Determination of the causes for the generally low fertility obtained following cervical deposition of frozen semen is still an important subject for AI procedure for achieving acceptable pregnancy rates in ewes.

A previous work observed that oxytocin treatment induced cervical dilation and decreased the difficulty of passing a catheter through the cervix and into the uterus. It decreases in fertility have been associated with cervical manipulation.

For a long time, the standard procedure with fresh semen when inseminating ewes has been to deposit the semen in the external os of the cervical canal. However, recently several groups have reported differences in pregnancy rates when ewes were bred with artificial insemination supported by air pump, oxytocin, transcervical, etc.

There are many methods of artificial insemination of sheep. Specialization is concentrating or limiting one's focus to part of the whole methods of artificial insemination. Studies are guiding to the acquisition of new practice knowledge and skills. Artificial insemination is a powerful tool that provides common genetic information and deep insight into the insemination process that is at the heart of every embryo formation.

The earliest idea of artificial insemination definitely was a transfer of sperm from male to female. That is, based on the sperm form of fresh or chilled. In time, researchers observed that the fertility changes that produced physiologic reactions in ewes also are formed changes in the computable traits of sperm. Among these traits are the volume of a sperm, the number of sperm at a moment of fertilization, the physiological changes of a sperm in the female genitalia, and the transfer way of a sperm to female. Each of these traits can generate the basis for an effective action of an artificial insemination (Khalifa et al., 1992; Sayre and Lewis, 1996; Wulster-Radcliffe and Lewis, 2002; Candappa et al., 2009).

Donovan et al. (2004) reported higher pregnancy rates using fresh compared to frozen-thawed semen but found no differences in pregnancy rate following natural or synchronized estrus. The reason for the variation in fertility among ewe breeds following cervical AI with frozen-thawed semen may be due to differences in sperm transport through the cervix and uterus or due to early embryo mortality (Fair et al., 2005).

The place of deposition of frozen-thawed semen has a key effect on fertilization rate. In consequence, considerably advanced fertility is usually achieved with laparoscopic AI via frozen-thawed ram semen after transcervical or cervical insemination (Fair et al., 2005).

There are two obvious methods for struggle with the physical characteristics of the ovine cervix: set straight the servix and increase the diameter of the cervical lumen; or redesign TCAI (Trans-Cervical Artificial Insemination) equipment, or modify of embryo transfer catheter to invent the tight, convolute configuration of the cervix. Methods for straightening (such as, attaching a hemostat to the external cervical os and retracting the cervix) and dilating the cervix (chemically with PGE2 or oxytocin, etc., or mechanically) are effective (Khalifa et al., 1992; Sayre and Lewis, 1996; Wulster-Radcliffe et al., 1999; Wulster-Radcliffe and Lewis, 2002; Candappa et al., 2009; Gunduz et al., 2010).

Thus it is tried to give information about the last development of artificial insemination in the ewes.

2. Cervix antomy and function

The ewe cervix is a long and fibrous tubular organ. It contains connective tissue with an outer serosal layer and inner luminal epithelium. Because the lumen is the presence of 4-7 cervical rings that caudal opening of its provide a physical barrier to external contaminants and convolute and tortuous structure catheter entrance is somewhat more difficult than in the cow.

These cervical rings constitute the greatest obstacle against TCA. The first, second and third ring in lumens does not take place in same line, ensuing in the inseminating pipette being misdirected apart from the central lumen. In addition, the first ring is the most difficult will be further than 1 cm, can practically insemination pipette. Cervical canal length ranges between 2.5 and 10.5 cm according to breed, age, parity and physiological state. These major changes in length of the channel affect the success of in TCAI. The mean number of cervical rings is approximately 5 with a range of two-seven rings per cervix. (Halbert et al., 1990; Campbell et al., 1996; Wulster-Radcliffe and Lewis, 2002; Kaabi et al., 2006).

The external cervical os between ewes has in different location in the vagina. The cervical os in sheep makes to protrude into the vagina and in some animals completely obscure

Five types of external os were identified in the vagina (Halbert et al., 1990). (Fig. 1):

1. The Duckbill: two opposing folds of cervical tissue protruding into the vagina with a central horizontal slit like external os.
2. The Slit: no protrusions into the anterior vagina with a slit like opening at the os of the cervix giving entry to the cervical canal.

3. The Rose: a cluster of cervical folds protruding into the anterior vagina obscuring the external os.
4. The Papilla: a papilla protruding into the anterior vagina with the external os at its apex
5. The Flap: one-fold of cervical tissue protruding into the anterior vagina and completely or partially overlaying the external os creating the appearance of a flap.

Kershaw et al. (2005) found that the spreading of external os types differed with age. In particular, the rose type os is more common in adult ewes than ewe lambs and the reverse is true for the papilla type os.

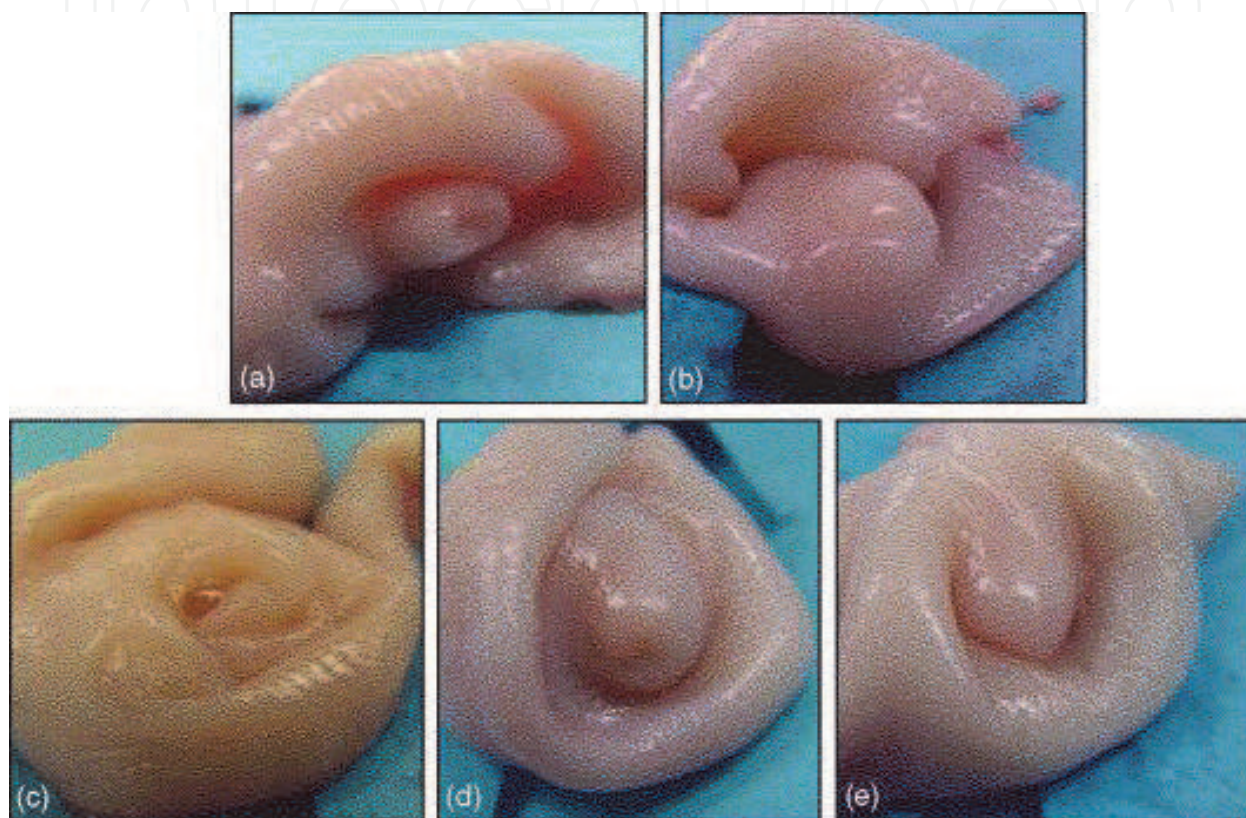


Fig. 1. The classification of the appearance of the external os of the ewe (a) duckbill, (b) slit, (c) rose, (d) papilla, and (e) flap. from Kershaw et al., 2005 .

Cervical penetration measuring is evaluated the penetration of the insemination pipette as shallow, middle and deep without being informed about the group to which the inseminated ewe belonged (shallow: <10mm; mid: 10–20mm; or deep: >20mm, using the colored tip of the plastic sheath as reference (Gunduz et al., 2010).

3. Transcervical AI method

There generally are 3 AI techniques 1) vaginal insemination, 2) the laparoscopic intrauterine insemination 3) cervical insemination that have been used in the sheep industry and newly developed fourth technique, trans-cervical artificial insemination (Leethongdee, 2010).

Transcervical AI (TCAI) is seemed as a potential alternative to laparoscopic AI. The basis of this technique, a AI catheter is passed through cervix for sperm to leave the uterus. This technique is used in other animals, but they are not used due to the difference of the sheep cervix. There is as a degree of natural cervical relaxation in oestrus and trans-cervical

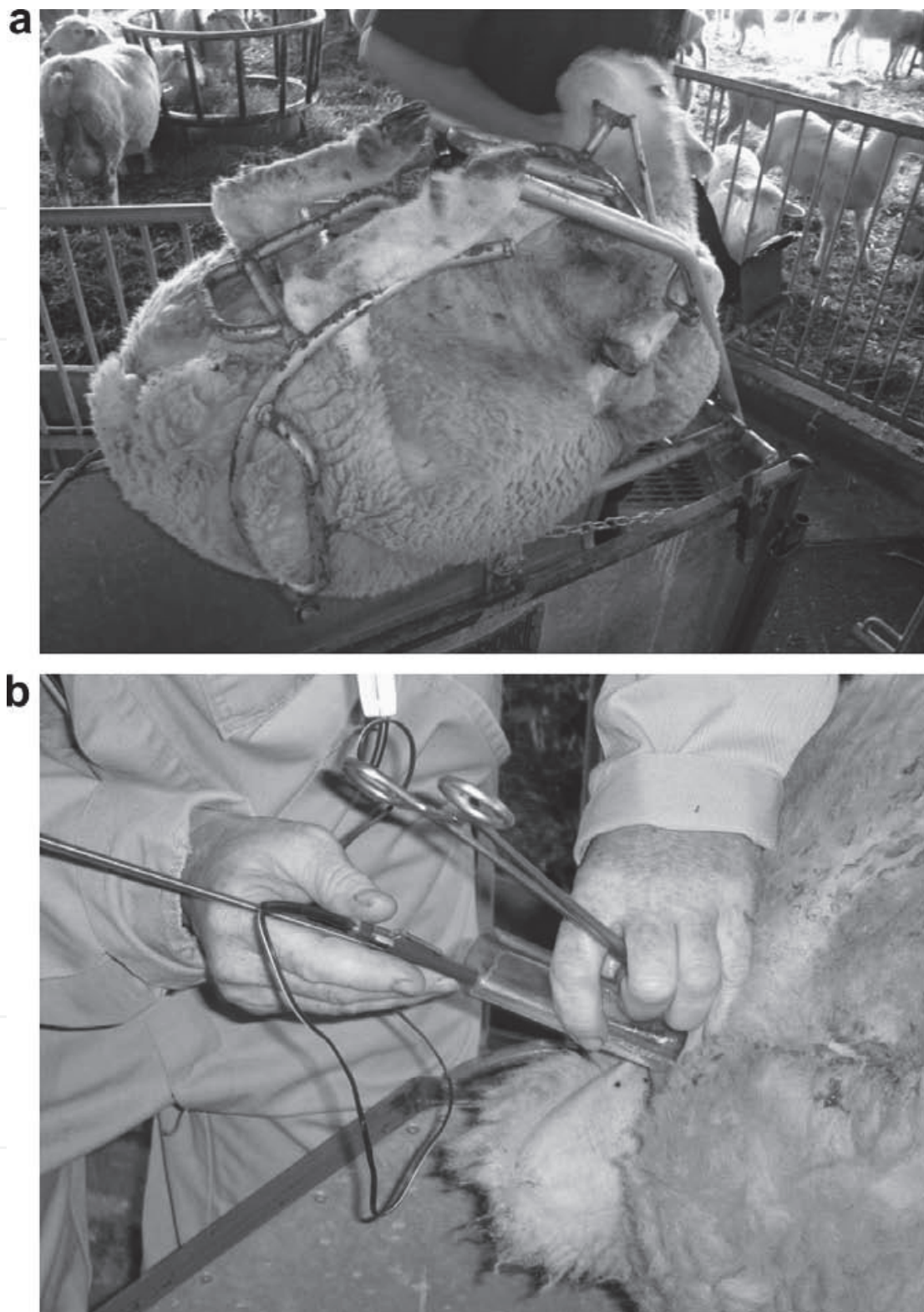


Fig. 2. The Guelph TCAI System: (a) fetal-like positioning of ewes in dorsal recumbency; (b) a plexiglass speculum with light source is inserted into the vagina and forceps are used to grab the cervix near the os and retract it. Subsequently, a bent-tipped and preloaded insemination gun is introduced and manipulated through the cervix to deposit semen. From Candappa et al., 2009.

penetration in low rate in multiparous ewes seems to be possible. The cervical relaxation is due to affect of peri-ovulatory hormones such as oxytocin, estradiol and progesterone on the cervix. In Cows, the potential effect of oxytocin on cervical relaxation, leads to local growth of cyclooxygenase-2 (COX-2) after that, COX-2 causes an increase in the synthesis of prostaglandin E2 (PGE2) (Zhang et al., 2007).

3.1 The Guelph System for TCAI

The Guelph System of AI equipment fetal-like positioning of ewes in dorsal recumbency (Fig. 2). A speculum with light source is inserted into the vagina, and upon apparition of the cervix. A bent-tipped and preloaded insemination gun is lead into the cervix to deposit semen (Wulster- Radcliffe and Lewis, 2002; Candappa et al., 2009).

3.2 Application of TCAI catheter

Some studies were used an insemination catheter with a blunt and angled end, some flexibility, and a diameter that is smaller than the narrowest point of the cervical lumen in the ewe (Halbert et al., 1990; Campbell et al., 1996; Wulster-Radcliffe Lewis, 2002; Kaabi et al., 2006; Candappa et al., 2009).

The catheter used for embryo transfer. Later, he modified for sheep transcervical AI. The catheter is depicted as the actual size (17.5 cm long, 1.47 mm o.d, 1.07 mm i.d.) (Wulster-Radcliffe et al., 1999).

Ewes are held back in a chute used for sheep in a dorsal recumbent position. The perineal area is cleaned with an antiseptic soap and rinsed with water. Excess water and antiseptic are removed with paper towel. A outside layer of obstetrics lubricant is applied to a speculum, and the speculum is inserted into the vagina and pressed against tissue nearby the cervix. The TC-AI catheter is located at the external cervical os and manually control from beginning to end of the cervix. A TC-AI catheter used for cervical manipulation in transcervical AI (Fig 3) is equal to the TC embryo transfer apparatus described previously (Wulster-Radcliffe et al., 1999).



Fig. 3. Modified embryo transfer catheter. A machined brass zero-volume fitting was used to secure the catheter. From Wulster-Radcliffe et al., 1999.

During penetration of a pipette through the cervix the sheep position affects the success of the transition. Pipette passage is more easily with the standing ewe than over-the rail ewe. Rear side of the light (approximately 15 cm) elevated ewe with standing is stated as in a very convenient position (leethongdee, 2010). In this position, uterine penetration is achieved in 82.0 %.

Transcervical AI is applied using specially designed inseminating equipment (Wulster-Radcliffe et al., 2002; Wulster-Radcliffe et al., 2004) and manipulation of the cervix using forceps (Halbert et al., 1990). The number of reports in which position of semen has been achieved is relatively low and there are concerns about the potential trauma involved.

4. Application of Air Pressure with Cervical Artificial Insemination (APCAI)

Cervical artificial insemination (CAI) is a less expensive and invasive method in comparison to the transcervical and intrauterine methods, and has been widely used for the artificial insemination of ewes. The site of deposition of frozen-thawed semen in ewes has a major effect on fertilization rate (Fair et al., 2005).

Whilst pregnancy rates in excess of 60% can be achieved with a single artificial insemination of fresh semen deposited at the external cervical opening , corresponding rates for frozen-thawed semen occasionally exceed 45%, with values less than 17% not rare (O’Meara et al., 2005).

The insemination device in Air Pressure with Cervical Artificial Insemination method (APCAI) is modified from a stainless steel outer pipette sheath of a cattle AI pipette. The air pump (2 liter/min capacity; aerator, portable battery pump) is attached to the blunt rounded end of the pipette by means of a rubber pipe with an internal diameter of 2.6 mm (Fig 4). A speculum was introduced into the vagina so that the external opening of the cervix could be seen in the light of the speculum lamp. Subsequently, 0.1 ml of semen is drawn into the pipette sheath through a plastic syringe, and the pipette sheath is connected to the air pump. The pipette tip is placed at the external opening of the cervix and the air pump is run to spray semen into the cervical canal. For each ewe, a different pipette sheath is used (Aral et al., 2010).

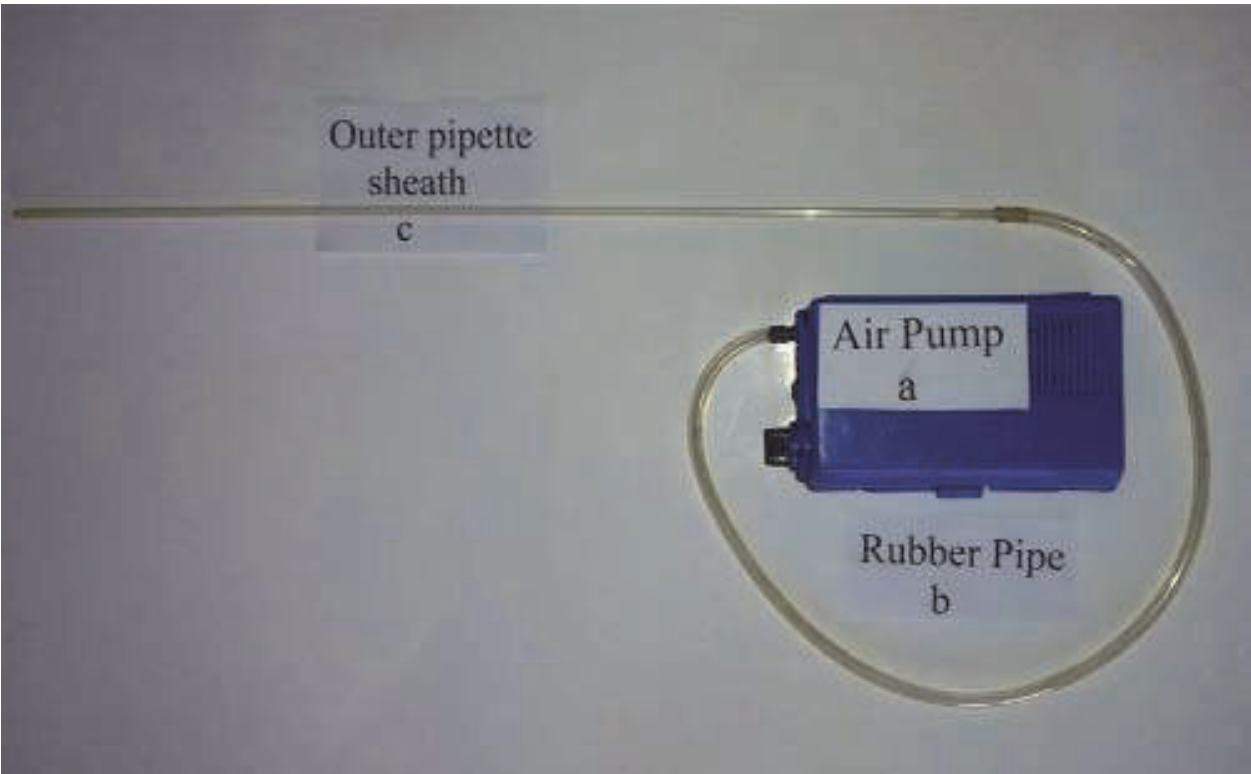


Fig. 4. Artificial insemination equipment of APCA method; (A) Air pump, (B) Rubber pipe, (C) a stainless steel outer pipette sheath used in cattle AI. From Aral et al., 2010.

The pregnancy rate in this method was found to be significantly high the APCAI group than in the CAI group (80.0% versus 46.7%) Table 1.

Parameters Number of ewes		CAI (n=30)	APCAI (n=30)
Pregnancy rate	(n/n) %	(14/30) 46.7 a	(24/30) 80.0 b
Lambing rate	(n/n) %	(14/30) 46.7 c	(21/30) 70.0 d
Prolificacy	(n/n)	(16/14) 1.14	(23/21) 1.09

Table 1. Pregnancy, lambing rate, prolificacy, following different AI methods in Awassi ewes. From Aral et al., 2010.

5. Pharmacologic cervical dilatation

5.1 Prostaglandin E analogues

5.1.1 Cervidil

Cervical ripening involves enzymatic corruption of the connective tissues. It causes a relaxation of its smooth muscle fibers. Cervical ripening encourage with chemical matter. Thus, penetration of the cervix during TCAI may be achieved with effortlessness (Candappa et al., 2009). Cervidil® contains 10 mg of dinoprostone and have a vaginal form. Hormone releases a constant rate of 0.3 mg/h over a 12-h period in a women vagina. For cervical ripening and induction of labour in women is a safe agent (Sánchez-Partida et al., 1999; Lambers et al., 2001). Adapting the use of Cervidil® to sheep was performed by Candappa et al., 2009.

Cervidil® is inserted 12 h prior to insemination in ewes. Cervidil®, containing 10 mg dinoprostone (prostaglandin E2), introduce into the vagina in the immediacy of the cervix. After the 12-h priming with Cervidil, transcervical semen deposition is possible in all ewes (Candappa et al., 2009).

5.1.2 Ovagen and misoprostol

Cervical relaxation and penetration were examined in Ovagen and misoprostol-treated sheep in a previous study. Ovine FSH (2 mg; Ovagen; 1 CP bio (UK) limited, Wiltshire, UK) was administered at a dose of 2 mg dissolved in 0.5 ml of 50 % Gum acacia (Sigma-Aldrich Co.,). A prostaglandin E₁ analogue, Misoprostol (1 mg; Misoprostol; Sigma-Aldrich Co., Dorset England) was administered at a dose of 1 mg dissolved in 0,5 ml of 30 % gelatine (Sigma-Aldrich Co.,). External opening of the cervix treated sheep Ovagen and misoprostol significantly loosened. It is easier penetration of the cervix in these sheep (Leethongdee et al., 2007).

5.2 Hyaluronan

The cervix is relatively relaxed at oestrus. These are both a high-hyaluronan (HA) content of the cervix and the related increase in its water content. When aqueous 0.5 ml of the HA suspension (2 mg low molecular weight (LMW) HA) and (25 mg high molecular weight (HMW) HA) is applied to an intra-cervical, it promotes cervical relaxation in oestrus ewes.

LMW HA has the greatest impact on vascularization, leading to the collection of leukocytes. In addition, it stimulates the biochemical changes in the cervix during softening (Perry et al., 2010).

5.3 Oxytocin

Oxytocin treatment caused relaxation of cervix and uterine catheter through the cervix have demonstrated that reduced the difficulty of the transition (Khalifa et al., 1992; Sayre and Lewis, 1996). The effect of oxytocin as a cervical dilator is different on the reproductive outcome in ewe. Sayre and Lewis (1997) observed no undesirable effect of oxytocin on ovum fertilisation rate. Stellflug et al., (2001) show a negative effect of oxytocin but not of the transcervical insemination procedure. Fertilization rate decreases in the treatment of oxytocin-cervical manipulations. However, the oxytocin treatment does not affect ovulation rate. Atraumatic cervical manipulation, does not affect the time of ovulation, fertilization rate, early embryonic development and rate of lambing. Thus, oxytocin is used to softening the cervix decreases the ease of transition to a transcervical AI instrument, the fertilization rate, pregnancy rate and lambing rate.

For transcervical AI, different (50 to 400 USP units in Table 2 and 3; 10 IU of oxytocin) dose of oxytocin can be given to ewes via intravenously 30 min. before AI to dilate the cervix. When oxytocin is given intramuscularly 15–30 min before insemination with frozen/thawed semen, it produced an impressive reduction (10% versus 42%) in the lambing rate of ewes inseminated cervically. However, oxytocin makes possible intrauterine insemination via the cervix, its undesirable effect on lambing rate may be less significant oxytocin has a small and non-significant damaging outcome on litter size (King et al., 2004; Table 2, 3).

	oxytocin. USP units					
Item	0	200	400	600	SE	Overall oxytocine
Cervical penetration, cm						
Before oxytocin	2.6	2.2d	1.0d	.9d	.3	1.5d
After oxytocin	2.9	5.6e	6.1e	5.1e	.3	5.7e
Time to deepest cervical penetrationf, min	10.0g	6.9h	5.6h	6.2h	.4	6.4
Uterine entries/no. Of ewes	0/15g	15/19h	10/12h	8/12h	-	33/43
(%)	0	79	83	67		77

*Values are means or proportions.
bSE are standard errors from analyses of variance models used to analyze the data.
Values with different superscripts differ (P < .01).

Table 2. Effects of intravenous oxytocin injection 52 hours after removal of progestogenated pessaries from ewes. From Khalifa et al., 1992.

Oxytocin injections dilate the cervix in some ewes. However, oxytocin administration 12 h after 100 or 200 pg of estradiol-17β ((0, 100, or 200 pg in 5 mL of 1:1 saline ethanol) are the majority successful at dilating the cervix and permitting acceptance of a stainless steel rod

into the uterus. The 100- μ g dose of estradiol-17 β seem to be as a valuable dose (Khalifa et al., 1992; Wulster-Radcliffe et al., 1999).

Item	laparoscopic		Cervical		Significance of treatment effects	
	Control	Oxytocin	Control	Oxytocin		
Number of ewes	49	50	100	99		
Number lambing (%)	34(69a)	29(58 c)	42(42 b)	10 (10 d)	a>b**	c>d***
Mean litter size	1.91 a	1.83 c	1.52 b	1.40 d	a>b*	c>dz

Z: P < 0.10.
* P < 0.05.
** P < 0.01.
*** P < 0.001.

Table 3. Data for lambing rates and litter sizes in relation to treatments and week of insemination. From King et al., 2004.

These observations, together with those from the present experiment, suggest that any adverse effect of oxytocin on lamb production when ewes are subjected to laparoscopic intrauterine insemination with frozen/thawed semen is likely to be small. Thus, had the oxytocin in the present experiment. Oxytocin make possible intrauterine insemination via the cervix, its undesirable effect on lambing rate may have been less significant. The cervical manipulation seems to unfavorable an effect on fertility after AI, because the oxytocin treatment is not harmful. It likely to affect the sperm survival in the reproductive tract of sheep, or some issues of sperm capacitation (Stellflug et al., 2001). Perhaps, manipulation of the cervix may affect sperm transport within the reproductive tract, or a stressed cervix may produce a spermicidal compound (Hawk et al., 1981).

5.4 Human interleukin 8 (huIL-8)

Human interleukin 8 (huIL-8) was applied to use in sheep to stimulate the cervical relaxation. This cytokine causes a neutrophil recruitment and an increase in collagenases in the cervix during the peripartum period in mammals. However, its administration is failed to induce cervical dilatation in ewes After estrus synchronization protocol is applied, and a wax suppository (Witepsol as the wax formulation, mm x 3 mm in size) containing either 5 pg huIL-8a (derived from large scale human fibroblast cell culture) with an estimated total release of 0.6 pg huIL-8 is inserted into the anterior vagina near the cervical os (Croy et al., 1999).

5.5 Carazolol

When animals are exposed to stress during artificial insemination, their bodies react by raising the adrenaline amount. The beta 2 adrenoreceptors in the myometrium is affected by adrenalin. After that, uterine contractions occurred by oxytocin is destroyed by it, which in turn produce a long time to get ahead of the genital canal for the spermatozoa. Thus, artificial insemination with aged spermatozoa leads to decrease of fertility in ewes (Kirsan et al., 1998; Gunduz et al., 2010).

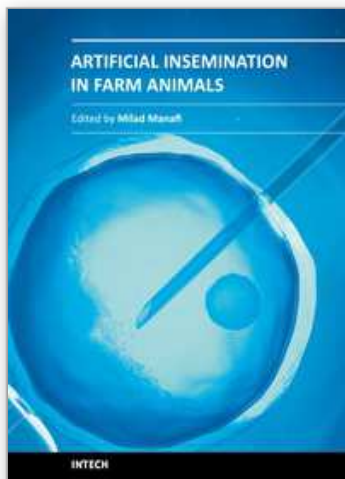
It eliminates the effect of oxytocin, stimulating uterine contractions. Carazolol (Suacron, Divasa, Farmavic, Spain) intramuscularly for each sheep is 0.5 mg administered 30 minutes before insemination. It increases the number of ewes inseminated with deep. In contrast, it does not have a significant effect on the pregnancy rate (85% for control, 95% for carazolol) (Gündüz et al., 2010). The middle and deep penetration rates are high but was found non-significant (for control 82 % and carazolol 85%).

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Artificial insemination is used instead of natural mating for reproduction purposes and its chief priority is that the desirable characteristics of a bull or other male livestock animal can be passed on more quickly and to more progeny than if that animal is mated with females in a natural fashion. This book contains under one cover 16 chapters of concise, up-to-date information on artificial insemination in buffalos, ewes, pigs, swine, sheep, goats, pigs and dogs. Cryopreservation effect on sperm quality and fertility, new method and diagnostic test in semen analysis, management factors affecting fertility after cervical insemination, factors of non-infectious nature affecting the fertility, fatty acids effects on reproductive performance of ruminants, particularities of bovine artificial insemination, sperm preparation techniques and reproductive endocrinology diseases are described. This book will explain the advantages and disadvantages of using AI, the various methodologies used in different species, and how AI can be used to improve reproductive efficiency in farm animals.

How to reference

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