

DEVELOPMENT OF TRANS-CERVICAL ARTIFICIAL INSEMINATION IN SHEEP WITH SPECIAL REFERENCE TO ANATOMY OF CERVIX

Sukanya Leethongdee

Received: Jun 19, 2009; Revised: Nov 10, 2009; Accepted: Nov 16, 2009

Abstract

Artificial insemination has an important role in the sheep industry and sheep genetic improvement. The complexity of the sheep cervix limits the development of trans-cervical artificial insemination. The sheep cervix is a long convoluted tubular organ with internal rings. The internal cervical rings form the funnel-like shape of the cervical canal which is the physiological barrier situated inside the cervical canal. The cervical opening is formed by the internal cervical fold which varies with 5 types of cervical opening including duckbill, slit, rose, papilla and flap. The internal ring is arranged into grades 1, 2, and 3 according to the complexity of the internal fold alignment. These complicated structures of the anatomy of the sheep cervix reduce the ability of the passage of the insemination pipette into the uterine body. The study of the cervical relaxation mechanism and the sheep cervix anatomy is important for the development of trans-cervical artificial insemination in sheep. The administration of an exogenous substance induces the cervical relaxation and enhances the possibility of the success of TCAI in sheep.

Keywords: Trans-cervical artificial insemination, frozen-thawed semen, cervix, cervical relaxation, ewe

Introduction

Artificial insemination (AI) of sheep is an advantageous management practice aimed at the genetic improvement at farm level and a programme of genetic selection. Furthermore AI has the potential for a significant impact on the sheep breeding industry. The main role of AI in sheep production is to increase the rate of genetic improvement and AI also contributes to achieving other goals, e.g. allowing extensive use of the best available rams, therefore increasing

selection pressure and the rate of response to selection. With AI superior rams can be identified more easily through progeny testing. Because progeny testing involves large numbers of animals over long periods of time, sires under test may be too old or even dead by the time their progenies have proven valuable. Therefore AI, by speeding up the identification of superior rams at a younger age, results in faster genetic progress. The use of frozen

Faculty of Veterinary and Animal Sciences, Maharakham University Tambon Talaad, Amphur Muang, Maharakham, 44000, Thailand. Tel.: 0-4374-2823; E-mail: sukanya.l@msu.ac.th

semen for AI can also increase the rate of genetic progress by storing semen collected from young rams before their superiority is confirmed by progeny testing, therefore allowing the use of genetically superior semen more widely. AI ensures good paternal control and fertilization of groups of females by males of different genotypes is easily achieved. In addition AI takes advantage of oestrous synchronization with its precise control of ovulation and parturition and furthermore allows the advantage of out of season breeding.

In addition to the value of AI with frozen semen for genetic improvement, AI is associated with other animal health benefits. This technique helps avoid disease transmission and allows the transport of semen when the risk of disease prevents ram movement and AI reduces the risk of spreading sexually transmitted genital infections associated with natural mating. There are some dangers with the widespread use of AI when used extensively with a limited number of sires, mainly from a reduced genetic variation in the population. Moreover, it is possible that hereditary defects and undesirable traits can be rapidly disseminated.

The Anatomy of the Cervix Limits the Trans-Cervical Artificial Insemination (TCAI) in Sheep

The cervix is the most caudal portion of the uterus and its constricted lumen is surrounded by a thick musculo-connective tissue wall (Moré, 1984). The average length of the sheep cervix is variable depending on the age and breed of ewe (Kaabi *et al.*, 2006). The average

length of the cervical canal studied in the Canadian crossbreed ewe is 6.7 ± 1.1 cm and contains 4.9 ± 1.0 funnel-shaped rings (Halbert *et al.*, 1990). The average cervical length studied in Merino, Castellana, Assaf and Chura is 6.86 cm and contains an average of 4.16 cervical rings (Kaabi *et al.*, 2006). The average cervical length of the Indian native breeds (Malpura and Kheri) in ewe lambs and adult ewes are 3.8 ± 0.12 cm and 5.3 ± 0.15 cm, respectively. The average number of rings in the cervixes of ewe lambs and adult ewes are 3.2 ± 0.19 and 3.4 ± 0.22 respectively (Naqvi *et al.*, 2005). This agrees with the report by Kaabi *et al.* (2006), that in younger ewes the cervix is shorter and narrower, but Kaabi *et al.* (2006) noticed that younger ewes have more cervical rings than older ewes, suggesting the morphology of the cervix depends on the age of the ewe. The average cervical length of the Chura breed is less, the cervix narrower and has more rings than the Merino and Castellana breeds (Kaabi *et al.*, 2006). In the older multiparous ewes, the cervix tended to become longer and wider and with loose rings.

In the ewe, the cervical lumen has a convoluted and tortuous structure that looks like a corkscrew consisting of internal cervical rings (Halbert *et al.*, 1990; Naqvi *et al.*, 2005; Kaabi *et al.*, 2006) (Figure 1). The internal folds of the cervix form a funnel-like shape with the narrow opening projecting caudally into the cervical lumen (Halbert *et al.*, 1990; Naqvi *et al.*, 2005). It is common that the second fold is eccentric to the other concentric folds and thus acts as an anatomical and physiological barrier

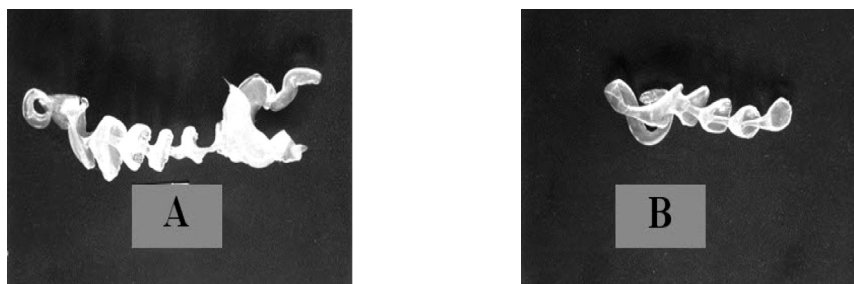


Figure 1. The internal structure of the cervical canal. The silicones casts represent the funnel-like shape of the internal cervical ring (Naqvi *et al.*, 2005)

(Moré, 1984). The cervical lumen is misaligned in 74.43% of ewes with the presence of eccentric folds. The most common eccentricity occurs at the second cervical ring (75%), but the third (14%) and first ring (11%) can also be eccentric (Kaabi *et al.*, 2006). The alignment of cervical rings can be graded by their degree of completeness and inter-digitation (Kershaw *et al.*, 2005) (Figure 2):

Grade 1 the cervix has a series of complete aligned cervical rings lying across the lumen with no inter-digitation of the cervical rings.

Grade 2 the cervix has a mixture of complete folds and incomplete cervical rings that lay

partially across the lumen and inter-digitate with one another, obscuring the central lumen.

Grade 3 the cervix has predominantly incomplete and inter-digitating cervical rings that are not aligned.

The average external diameter of the cervix is 1.36 cm. (Kaabi *et al.*, 2006). The diameter of the cervical lumen is between 1.8 to 6.0 mm. The narrowest point is commonly found at the second, third or fourth rings. The cervix opens caudally into the vagina at the external end of the cervix. Sheep show high variation in the anatomy of the cervical opening. The first and second folds of the

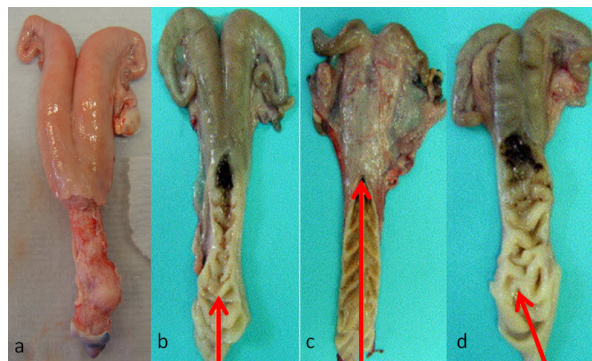


Figure 2. The appearance of sheep cervix undissected, (a) The internal cervical rings, (b) grade 1, (c) grade 2, and (d) grade 3. The red arrows illustrate the direction of the insemination pipette (Kershaw *et al.*, 2005)

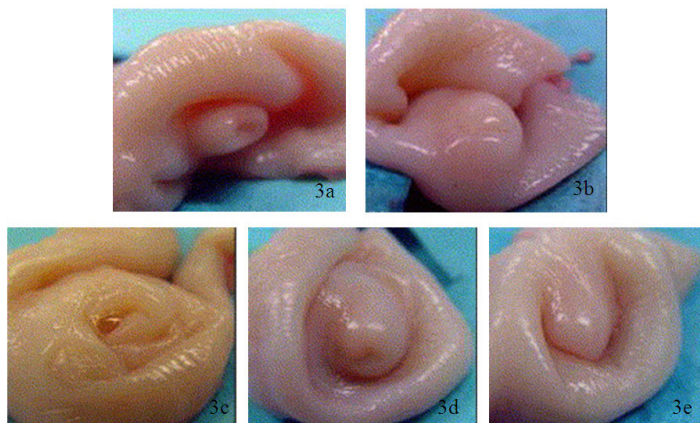


Figure 3. The variation of the sheep cervical opening (3a) duckbill, (3b) slit, (3c) rose, (3d) papilla and (3e) flap (Kershaw *et al.*, 2005)

cervical rings form the shape of the cervical opening which varies with age and probably parity. The ovine external os cervix has been classified on the basis of its morphology (Halbert *et al.*, 1990; Kershaw *et al.*, 2005) (Figure 3).

Duckbill: There are two opposing cervical folds protruding into the vagina to form a slit-like os.

Flap: There is one cervical fold protruding into the vagina that forms a flap that lies over the cervical opening, thus causing difficulty when attempting to locate the cervical os.

Rosette: There are several cervical folds that form a rosette of vaginal folds around the cervical opening.

Slit: There are no cervical folds protruding into the vagina but there is a slit-like opening to the cervix on the anterior wall of the vagina.

Papilla: There is a single cervical fold protruding into the vagina with the external os as its apex.

Spiral: The cervical folds form a spiral which protrudes into the vagina.

The papilla form is more frequent in younger ewes (< 2 year old), and the flap-like form in older ewes (> 3 year old) (Kaabi *et al.*, 2006) which may be the consequence of lambing (Dun, 1995). The rosette type of cervical opening is found commonly in adult ewes and the papilla type of cervical opening is commonly found in younger ewes (Kershaw *et al.*, 2005). The anatomy of the cervical os type does not change with the stage of the oestrous cycle and its appearance is probably determined by genetic factors and by the mechanical consequences of parturition (Kershaw *et al.*, 2005; Kaabi *et al.*, 2006). Because the second fold is consistently out of alignment with the first fold it effectively closes the cervical canal at that point and makes it quite difficult and sometimes impossible to introduce an inseminating pipette into the cervix. Cervical penetrability is positively correlated with the

diameter and the cervical lumen diameter and cervical penetrability is negatively correlated with the number of cervical rings (Kaabi *et al.*, 2006). The complexities of sheep cervical anatomy limit the passage of an inseminating pipette. However, cervical penetrability varies during the oestrous cycle (Kershaw *et al.*, 2005) suggesting that the physical characteristics of the cervix and cervical dilatation are hormone-dependent. The study of the mechanism of cervical dilatation during the peri-ovulatory period is required if we are to develop a practical method for TCAI.

Artificial Insemination Techniques in the Sheep Industry

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 (TCAI). A fourth method for sheep (TCAI) is not widely used. The methods differ in their complexity and success rate. The fertility rates following vaginal, cervical and laparoscopic insemination all vary with the insemination technique (Table 1) used as well as with the farm, age, male, number of insemination per ewe, lambing-insemination interval, technician, flock and management conditions (Anel *et al.*, 2005; Paulenz *et al.*, 2005).

Vaginal Insemination

This method involves depositing semen deep in the vagina without any attempt to locate the cervix. Semen is deposited in the anterior vagina. Vaginal insemination using fresh diluted semen is the simplest and quickest method but requires a large semen dose (150-400 million spermatozoa per insemination) (Figure 4(a)). Vaginal insemination using fresh semen gives an acceptable lambing rate. Unfortunately the transportation and preservation of fresh semen limits its use among sheep farmers. Therefore AI, using frozen-thawed semen is an alternative and accepted option. Vaginal insemination using frozen-thawed semen gives variable lambing rates; 17% (Tervit

Table 1. The fertility rate following the insemination technique compared among the artificial insemination (AI) techniques used recently in sheep industry and the natural insemination technique in sheep and goat

Insemination technique	semen	Animal	Fertility rate			Reference
			Fertility rate	Day 30 reference Rate	Lambing rate	
Natural insemination	Fresh semen	sheep	85%	-	-	Meinecke and Meinecke-Tillmann, 1986
		goat	-	75%	-	Karaca <i>et al.</i> , 2009
		goat	-	88.4%	-	Kumar and Purohit, 2009
Vaginal artificial insemination	Fresh semen	goat	-	-	60%	Roca <i>et al.</i> , 1997
		goat	-	-	80%	Paulenz <i>et al.</i> , 2005
Vaginal artificial insemination	Frozen-thawed semen	goat	30%	-	-	Leboeuf <i>et al.</i> , 2003
*Cervical artificial insemination	Fresh semen	sheep	88.4%	-	48.5%	Khalifa <i>et al.</i> , 2008
	Frozen-thawed semen	sheep	-	11.7% (0-1 cm. deep)	-	Halbert <i>et al.</i> , 1990,
				13.7% (1-2 cm. deep)		Eppleston and Maxwell, 1995,
				22.2% (2-3 cm. deep)		Salamon and Maxwell, 1995
				34.8% (>3 cm. deep)		
		sheep	-	-	38.57%	Khalifa <i>et al.</i> , 2008
Laparoscopic intrauterine artificial insemination	Frozen-thawed semen	goat	89.4%	-	-	Ritar <i>et al.</i> , 1990
		goat	-	52.1-63.6%	-	Meinecke and Meinecke-Tillmann, 1986
			-	41%	-	Ritar <i>et al.</i> , 1990
						Wulster-Radcliffe and Lewis, 2004
		goat	-	58%	-	Holtza <i>et al.</i> , 2008
TCAI		sheep	59.3%	-	-	Sohnrey and Holt, 2005
						Wulster-Radcliffe and Lewis, 2002
						Wulster-Radcliffe and Lewis, 2004

*pregnancy rate is higher when the penetration is deeper.

et al., 1984), 17.6% (Maxwell and Hewitt, 1986), 31.25 % (Anel *et al.*, 2005) and 67.4% (studied in 543 Norwegian crossbred ewes, inseminated with 200 million spermatozoa) (Paulenz *et al.*, 2005).

Cervical insemination with frozen-thawed semen also gives low fertility. Cervical insemination using fresh semen gives a higher pregnancy rate than cervical insemination using frozen-thawed semen (Donovan *et al.*, 2004); 76% compared with 46% (Irish breed) and 36% (Norwegian breed). It is worth noting that in this experiment the ewes were slaughtered at 27-42 days post insemination. The reproductive tract was collected for the determination of the pregnancy rate and the number of corpora lutea was adjusted as the ovulation rate (Donovan

et al., 2004). The use of frozen-thawed semen and cervical insemination gave better fertility than vaginal insemination with frozen-thawed semen. The reports illustrate low fertility rates following vaginal insemination with frozen-thawed semen, such as Maxwell and Hewitt (1986) who reported an 18.4% pregnancy rate detected by ultrasonography at day 40 after cervical insemination with frozen-thawed semen compared with 17.6% after vaginal insemination with frozen-thawed semen (100 million spermatozoa per insemination). Paulenz *et al.* (2005) reported 71% non-return to oestrus and a 67.4% lambing rate following vaginal insemination with frozen-thawed semen that was significantly different from a 75.4% non-return rate and a 72.7% lambing

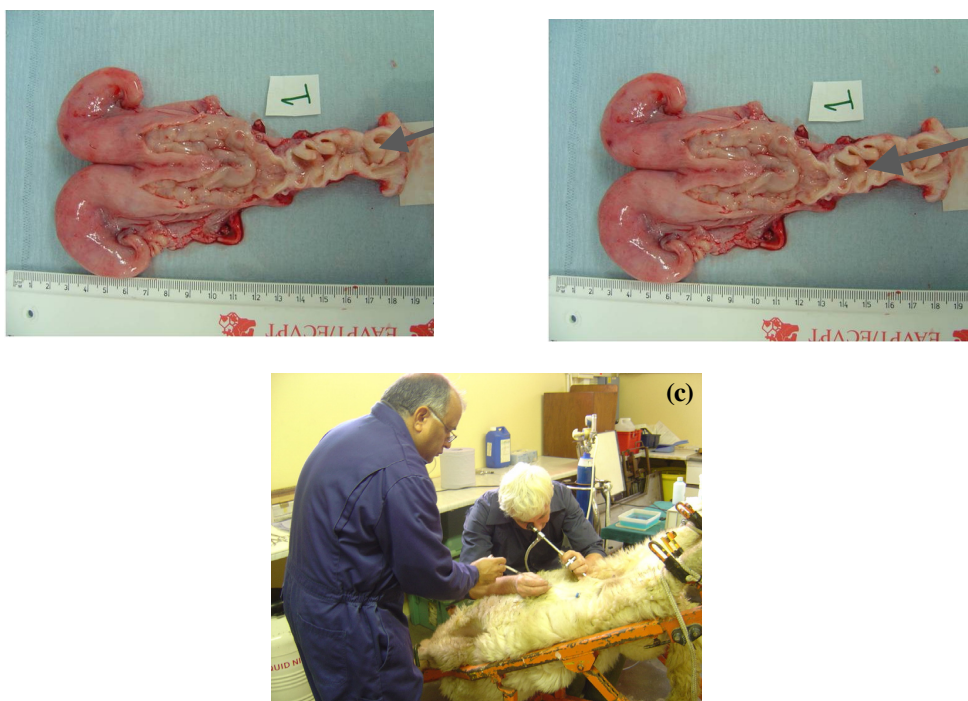


Figure 4. The artificial insemination technique in sheep production

- (a) The vaginal artificial insemination: The red arrow represents the insemination pipette direction
- (b) The cervical artificial insemination: The semen is deposited into the cervical canal. The red arrow represents the insemination pipette direction
- (c) The laparoscopic intrauterine artificial insemination. The semen is deposited directly intrauterine via the laparoscopic operation

rate following vaginal insemination with Frozen-thawed semen (200 million spermatozoa per insemination). The site of insemination influences fertility and cervical insemination has a higher fertility than vaginal insemination when using the frozen-thawed semen.

Intra-cervical Insemination

Intra-cervical insemination using fresh diluted semen is commonly used in AI of sheep (Figure 4(b). When performed properly, cervical insemination with fresh diluted or undiluted semen results in high fertility, whereas the fertility obtained following intra-cervical insemination with frozen-thawed semen is poor. Intra-cervical insemination is performed by insemination at the cervical opening or at the deepest possible intra-cervical site that is easily accessible without attempting to force the inseminating pipette into the cervical canal (Ayad *et al.*, 2004; King *et al.*, 2004). In 31% of ewes the inseminating pipette passes up to 1 cm. into the cervical canal, up to 2 cm in another 31% and up to 3 cm in 30%. In only 8% of ewes did the inseminating pipette pass more than 3 cm beyond the cervical opening (Eppleston and Maxwell, 1995). The depth of penetration is related to breed (Kaabi *et al.*, 2006) and age of ewe (Eppleston and Maxwell, 1993, 1995; Kaabi *et al.*, 2006). In older ewes, the cervix is longer and wider with looser folds, allowing easier passage of an inseminating pipette. The depth of insemination has an effect on fertility and the pregnancy rate detected by ultrasonography at day 40 after insemination and lambing rates increase as the depth of insemination into the cervix increases (Halbert *et al.*, 1990; Eppleston and Maxwell, 1995; Salamon and Maxwell, 1995). The pregnancy rate detected by ultrasonography at day 40 after insemination was 11.7% when frozen-thawed semen was deposited 0-1 cm into the cervix. However as the depth of insemination increased the pregnancy rate increased; 13.7% when frozen-thawed semen was deposited at 1-2 cm, 22.2 % when frozen-thawed semen was deposited at 2-3 cm, and 34.8% when frozen-thawed semen was deposited beyond 3 cm in the cervix. The corresponding lambing rates were 15.5%, 24.1%, 24.2% and 75% respectively

(Eppleston and Maxwell, 1993).

Laparoscopic Intrauterine Artificial Insemination

The complex anatomy of the cervix limits the passage of an inseminating pipette into the cervical canal and causes difficulty with transport of spermatozoa through the cervix. The difficulty of cervical passage can be overcome by direct uterine insemination using laparoscopy (Killeen and Moore, 1970). Semen is deposited directly into the uterus through the uterine wall with the aid of a laparoscope. Sedation and local anesthesia are required. Fertility and pregnancy rates are high with either fresh or frozen-thawed semen. A lower number of spermatozoa can be used, typically 40 to 80 million spermatozoa per insemination (Figure 4(c). Fertilization rates 72 h after laparoscopic insemination (92.5%) with fresh semen are greater than after cervical/trans-cervical insemination (28%) (Sayre and Lewis, 1997). Therefore this technique allows the effective use of frozen-thawed semen. The fertility of frozen-thawed spermatozoa is higher after laparoscopic insemination than after cervical or trans-cervical insemination (Sanchez-Partida *et al.*, 1999). Laparoscopic insemination using frozen-thawed semen has contributed to improved genetic selection in sheep breeding. However it has disadvantages; the main disadvantages are the high cost due to the technical expertise required, the equipment is expensive and easily damaged and it may become unacceptable on animal welfare grounds and legislation. A lower cost and less invasive technique that gives acceptable fertility using frozen-thawed semen is required.

Even though there has been a lot of research attempting to improve these AI results following cervical insemination, there are only two general commercial categories that have been used in sheep AI: 1) using refrigerated semen (15°C) with superficial intra-cervical insemination and 2) using frozen-thawed semen with laparoscopic insemination (Anel *et al.*, 2006). Unfortunately laparoscopic insemination is costly and not welfare friendly. Therefore it is highly desirable to develop intrauterine trans-cervical AI which allows the use of frozen-thawed

semen to be inseminated into the uterus via the vagina and cervix.

Trans-cervical Artificial Insemination (TCAI)

Trans-cervical artificial insemination (TCAI) is a method of insemination where semen is deposited deep in the cervix or even into the uterus via the cervix (Figure 5). This method involves depositing semen as deeply as possible in the cervix. The greater the depth of insemination, the higher the expected pregnancy and lambing rates (Eppleston and Maxwell, 1993; Salamon and Maxwell, 1995). A non-return rate of 58% following deep cervical insemination with Frozen-thawed semen has been reported (Donovan *et al.*, 2004). It is likely that the anatomical complexity of the sheep cervix limits the success of TCAI. Histological examination showed damage to the epithelial lining of the cervical canal following cervical penetration with an unmodified, conventional straight inseminating pipette, either into the uterus or the middle of the cervix.

There are varying degrees of damage to the cervical lining over the length of the cervix canal (Campbell *et al.*, 1996). A TCAI catheter has been developed that is said to prevent cervical trauma during trans-cervical passage (Wulster-Radcliffe and Lewis, 2002). The difficulty of traversing the cervix severely limits

the use of TCAI because it causes cervical trauma and impairment of the transport of spermatozoa. When the new TCAI catheter was used in a comparison with laparoscopic AI using the frozen-thawed semen, the results showed that there was no difference between the techniques for ovum and embryo recovery rates (mean = 87.3%), fertilization rates (59.3%) or day 3 pregnancy rates (mean = 66.6%) respectively. These results indicate that both of TCAI and laparoscopic AI provides a high fertility rate when the fertility rate such as embryo recovery rate, fertilization rate or day 3 pregnancy rate was evaluated at the early period after the insemination. It is worth noting that the pregnancy detection for this experiment was calculated by pregnancy rate: (number of ewes with embryos/total number of ewes) x 100 (Wulster-Radcliffe and Lewis, 2002). Considering this information it suggests that in the future TCAI may be able to replace laparoscopic AI in sheep. However the fertility rate after TCAI with frozen-thawed semen is lower compared with laparoscopic insemination, although both techniques deposit semen in the uterus when the fertility rate was determinate by the data of pregnancy rate at day 30 detected by ultrasonography or lambing rate (Wulster-Radcliffe *et al.*, 2004). It is likely that the manipulation of TCAI procedure may cause the lower

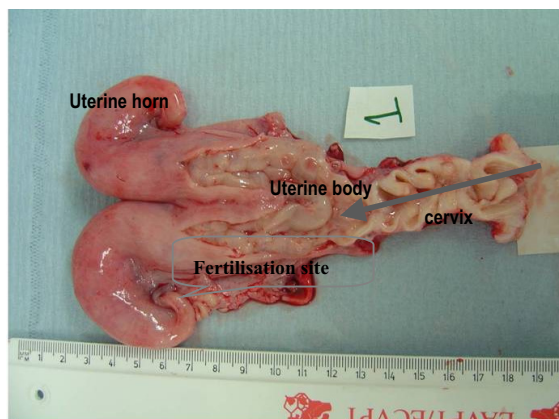


Figure 5. The trans-cervical artificial insemination. The semen is deposited into the uterine body. The insemination pipette is passed into the uterine body through the cervical canal. The red arrow represents the direction of the insemination pipette

fertility rate by damage the embryo or conceptus therefore the fertility rate such as day 30 pregnancy rate or lambing rate following the TCAI is lower than those in laparoscopic. Wulster-Radcliffe *et al.* (2004) reported that TCAI with frozen-thawed semen had a much lower fertility rate than laparoscopic AI; the pregnancy rate at day 30 detected by ultrasonography was 5% versus 46% and at day 50 it was 4% versus 41%, suggesting there are other factors that influence fertility after TCAI, such as ewe breed (Donovan *et al.*, 2004) or stress from animal restraint or season (Langford *et al.*, 1983). The study of alternative methods that overcome the poor fertility following TCAI with frozen-thawed semen is continuing. The use of an exogenous cervical dilators in sheep, such as oxytocin or oestradiol have been investigated (Khalifa *et al.*, 1992; Stellflug *et al.*, 2001) but a much better understanding of sheep cervical physiology and the mechanism of natural cervical dilatation at oestrus is required to facilitate the aim of developing an effective method of TCAI for sheep with frozen-thawed semen.

The Application of Exogenous Substances to Relax the Cervix

Physiological cervical ripening is characterised by a diffuse loosening of the collagenous connective tissue with widely scattered collagen fibrils and an increased amount of extracellular ground substance (Rath *et al.*, 1990, 1993). Therefore cervical dilatation requires a change in collagen within the cervical stroma (fibroblasts and smooth muscle) from the highly organized network of tightly bound collagen fibrils under the influence of high progesterone levels to a much looser arrangement at oestrus (Calder, 1994) that may facilitate cervical passage of an AI pipette. Therefore there have been numerous attempts, using exogenous substances, to dilate the cervix at the oestrus and facilitate trans-cervical AI.

The cervix naturally dilates slightly at oestrus, at a time when progesterone is low and oestradiol and oxytocin are high and effecting uterine contractility. Cervical dilatation

accompanies uterine contractility during labor, at a time when progesterone is declining as well as oestrogen and oxytocin rising (Challis *et al.*, 1983). Oestrus in sheep is a behavioural response to ovarian oestrogen acting on the hypothalamus. Oestradiol reaches its peak and then oestrus starts. Oestradiol is produced from the granulosa cells and secreted into the circulation. Oestradiol and progesterone are gonadal hormones that exert a significant regulatory effect on Gonadotrophin releasing hormone (GnRH) secretion during the preovulatory period in sheep. The Gonadotrophin hormone regulates the secretion of Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) which play the main role during the periovulatory period of the oestrous cycle in sheep. The feedback actions of oestradiol during this period after an initial period of inhibition of the size and frequency of GnRH pulses follows a large and sustained increase of preovulatory GnRH (Evans *et al.*, 1994a, 1994b). The positive feedback actions of ovarian oestradiol directly induce this surge of GnRH. After the progesterone decline and regression of corpus luteum from the previous oestrous cycle, oestradiol secretion from the maturing Graafian follicle increases and induces the preovulatory LH surge (Evans *et al.*, 1994a, 1994b). The use of exogenous oxytocin to increase cervical dilatation at oestrus was investigated (Khalifa *et al.*, 1992). The injection of 200, 400 or 600 IU of oxytocin at 44h and 52h after the removal of a progestagen pessary facilitated passage of a stainless steel rod into the uterus. In addition the combination of oxytocin with 100 or 200 µg of oestradiol-17β (E2) injected 9 days after the removal of a progestagen pessary also allowed passage of a stainless steel rod into the uterus (Khalifa *et al.*, 1992), suggesting that increased secretion of oxytocin and E2 at oestrus reduced the difficulty of passing a pipette through the cervix. However the mechanism of oxytocin-induced cervical dilatation is not known.

Another report revealed that a combination of oxytocin and E2 facilitated trans-cervical embryo transfer in ewes. The ewes received 100 µg of E2 by the intravenous injection 7d after the onset of oestrus and received 400 IU

of oxytocin 12h later (Wulster-Radcliffe *et al.* 1999). This combination of oxytocin and E2 induced the relaxation of the cervix resulting in the increase of the success of the embryo transfer via the trans-cervical route (Wulster-Radcliffe *et al.*, 1999). The application of oxytocin is likely to facilitate cervical dilatation but oxytocin may affect reproductive performance. This report also showed that the combination of oxytocin and E2 did not affect luteal function (Wulster-Radcliffe *et al.*, 1999). The effect of exogenous oxytocin on the cervical dilatation and its effects on reproductive variables were investigated (Stellflug *et al.*, 2001) and exogenous oxytocin tended to reduce the ovulatory interval. Cervical manipulation following oxytocin decreased the fertilization rate, however cervical manipulation alone did not affect the fertilization and lambing rates. Furthermore, exogenous oxytocin decreased the pregnancy-specific protein B and lambing rates in ewes (Stellflug *et al.*, 2001). Thus it seems that exogenous oxytocin is not a practical solution to the problem of TCAI. In another experiment that investigated the effect of exogenous oxytocin on the fertilization rate that followed laparoscopic or TCAI (Sayre and Lewis, 1997), ewes were inseminated laparoscopically or trans-cervically with 200 million spermatozoa per insemination 54 h after removal of progestagen pessaries. Thirty min before AI the ewes were injected with 200 IU of oxytocin. Fertilisation rates 72 h after AI were lower in ewes following TCAI compared with ewes inseminated by laparoscopic AI (Sayre and Lewis, 1997) indicating exogenous oxytocin did not, but that TCAI per se did, reduce fertilization rates (Sayre and Lewis, 1997). The lambing rate (percentage of treated ewes lambing) and litter size (lambs per ewe lambing) following the intrauterine insemination with frozen semen (0.2 ml of 400 million per ml) were tested with and without oxytocin (10 IU given by intramuscular injection) prior to fixed-time insemination (King *et al.*, 2004). Oxytocin permits the deeper cervical penetration in ewes. However, in this experiment complete cervical penetration was successful only in some ewe. The using of oxytocin as a cervical relaxant prior

to the fixed-time insemination caused a decrease of the lambing rate but not the litter size in ewe (King *et al.*, 2004), suggesting oxytocin may cause the reduction of the fertility rate in sheep. It is likely that oxytocin is able to dilate the cervix allowing access to the uterus during conventional cervical insemination however the fertility rate following the utilizing of oxytocin may need an extensively study. This information warrants further investigation.

Conclusions

TCAI is the artificial insemination technique which allows the potential of the use of frozen thawed semen in sheep. The frozen thawed semen is deposited directly intrauterine via the passage of an insemination pipette through the cervical canal. Unfortunately, the anatomy of the cervix of sheep limits the development of TCAI. The sheep cervix is a long tubular fibrous organ. It is composited by the layers of smooth muscle and the connective tissues. The sheep external os cervix has been classified on the basis of its morphology. The internal folds of the cervix form a funnel-like shape with the narrow opening projecting caudally into the cervical lumen which prevents the passage of the insemination pipette through the cervical canal. The application of an exogenous substance to relax the cervix provides the depth of the passage of cervical penetration which leads to the possibility of the TCAI in ewes.

References

- Anel, L., Alvarez, M., Martinez-Pastor, F., Garcia-Macias, V., Anel, E., and de Paz, P. (2006). Improvement strategies in ovine artificial insemination. *Reprod. Domest. Anim.*, 41(12):30-42.
- Anel, L., Kaabi, M., Abroug, B., Alvarez, M., Anel, E., Boixo, J.C., de la Fuente, L.F., and de Paz, P. (2005). Factors influencing the success of vaginal and laparoscopic artificial insemination in churra ewes: a field assay. *Theriogenology*, 63(4):1235-1247.
- Ayad, V.J., Leung, S.T., Parkinson, T.J., and

- 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(3-4):237-258.
- Calder, A.A. (1994). Prostaglandins and biological control of cervical function. *Aust. N. Z. J. Obstet. Gynaecol.*, 34(3):347-351.
- Campbell, J.W., Harvey, T.G., McDonald, M.F., and Sparksman, R.I. (1996). Transcervical insemination in sheep: An anatomical and histological evaluation. *Theriogenology*, 45:1535-1544.
- Challis, J.R., Sprague, C., and Patrick, J.E. (1983). Relationship between diurnal changes in peripheral plasma progesterone, cortisol, and estriol in normal women at 30-31, 34-35, and 38-39 weeks of gestation. *Gynecol. Obstet. Invest.*, 16(1):33-44.
- Donovan, A., Hanrahan, J.P., Kummer, E., Duffy, P., and Boland, M.P. (2004). Fertility in the ewe following cervical insemination with fresh or frozen-thawed semen at a natural or synchronised oestrus. *Anim. Reprod. Sci.*, 84(3-4):359-368.
- Dun, R.B. (1995). The cervix of the ewe its importance in the artificial insemination of sheep. *Aust. Vet. J.*, 31:101-103.
- Eppleston, J. and Maxwell, W.M. (1995). Sources of variation in the reproductive performance of ewes inseminated with frozen-thawed ram semen by laparoscopy. *Theriogenology*, 43(4):777-788.
- Eppleston, J. and Maxwell, W.M.C. (1993). Recent attempts to improve fertility of frozen ram semen inseminated into the cervix. *Wool Techn. Sheep Bree.*, 41:291-302.
- Evans, G., Brooks, J., Struthers, W., McNeilly, A.S. (1994a). Superovulation and embryo recovery in ewes treated with gonadotrophin-releasing hormone agonist and purified follicle-stimulating hormone. *Reprod. Fertil. Dev.*, 6:247-252.
- Evans, N.P., Dahl, G.E., Glover, B.H., Karsch, F.J. (1994b). Central regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion by estradiol during the period leading up to the preovulatory GnRH surge in the ewe. *Endocrinology*, 134:1806-1811.
- Halbert, G.W., Dobson, H., Walton, J.S., and Buckrell, B.C. (1990). The structure of the cervical canal of the ewe. *Theriogenology*, 33(5):977-992.
- Holtza, W., B. Sohnreya, M. Gerlanda and M.-A. (2008). Driancourt, Ovsynch synchronization and fixed-time insemination in goats, *Theriogenology*, 69(7):785-792.
- Kaabi, M., Alvarez, M., Anel, E., Chamorro, C.A., Boixo, J.C., de Paz, P., and Anel, L. (2006). Influence of breed and age on morphometry and depth of inseminating catheter penetration in the ewe cervix: a postmortem study. *Theriogenology*, 66(8):1876-1883.
- Karaca F., Tasal, I., and Alan, M. (2009). Preliminary report on induction of estrus with multiple eCG injections in Colored Mohair goats during the anestrus season *Animal Reproduction Science*, 114(1-3):306-310.
- Kershaw, C.M., Khalid, M., McGowan, M.R., Ingram, K., Leethongdee, S., Wax, G., and Scaramuzzi, R.J. (2005). The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. *Theriogenology*, 64(5):1225-1235.
- Khalifa T.A., Lymberopoulos, A.G., and El-Saidy, B.E. (2008). Testing usability of butylated hydroxytoluene in conservation of goat semen., *Reprod. Domest. Anim.*, 43(5):525-530.
- Khalifa, R.M., Sayre, B.L., and Lewis, G.S. (1992). Exogenous oxytocin dilates the cervix in ewes. *J. Anim. Sci.*, 70(1):38-42.
- Killeen, I.D. and Moore, N.W. (1970). Transport of spermatozoa, and fertilization in the ewe following cervical and late in oestrus insemination early and late in oestrus. *Aust. J. Biol. Sci.*, 23(6):1271-1277.
- King, M.E., McKelvey, W.A., Dingwall, W.S., Matthews, K.P., Gebbie, F.E., Mylne, M.J., Stewart, E., and Robinson, J.J. (2004). Lambing rates and litter sizes following intrauterine or cervical insemination of frozen/thawed semen with or without oxytocin administration. *Theriogenology*, 62(7):1236-1244.
- Kumar, S. and Purohit, G.N. (2009). Effect of a

- single subcutaneous injection of melatonin on estrous response and conception rate in goats. *Small Ruminant Research*, 82(2-3):152-155.
- Langford, G.A., Marcus, G.J., and Batra, T.R. (1983). Seasonal effects of PMSG and number of inseminations on fertility of progestogen-treated sheep. *J. Anim. Sci.*, 57(2):307-312.
- Leboeuf B., Forgerit, Y., Bernelas D., Pougard, J.L., Sent, E., and Driancourt, M.A., (2003). Efficacy of two types of vaginal sponges to control onset of oestrus, time of pre-ovulatory LH peak and kidding rate in goats inseminated with variable numbers of spermatozoa. *Theriogenology*, 60:1371-1378.
- Maxwell, W.M.C. and Hewitt, L.J. (1986). A comparison of vaginal, cervical and intrauterine insemination of sheep. *Journal of Agricultural Science, Cambridge* 106:191-193.
- Meinecke, B. and Meinecke-Tillmann, S. (1986). Fertilization results in superovulated ewes and goats after transmural-intrauterine insemination controlled by laparoscopy, *Tierarztl. Prax.*, 14(1):35-41.
- Moré (1984). Anatomy and histology of the cervix uteri of the ewe: new insights. *Acta. Anat. (Basel)*, 120(3):156-9.
- Naqvi, S.M., Pandey, G.K., Gautam, K.K., Joshi, A., Geethalakshmi, V., and Mittal, J.P. (2005). Evaluation of gross anatomical features of cervix of tropical sheep using cervical silicone moulds. *Anim. Reprod. Sci.*, 85(3-4):337-344.
- Paulenz, H., Soderquist, L., Adnoy, T., Nordstoga, A.B., and Andersen Berg, K. (2005). Effect of vaginal and cervical deposition of semen on the fertility of sheep inseminated with frozen-thawed semen. *Vet. Rec.* 156(12):372-375.
- Rath, W., Osmers, R., Adelman-Grill, B.C., Stuhlsatz, H.W., Szevereny, M., and Kuhn, W. (1993). Biochemical changes in human cervical connective tissue after intracervical application of prostaglandin E2. *Prostaglandins*, 45(4):375-384.
- Rath, W., Osmers, R., Adelman-Grill, B.C., Stuhlsatz, H.W., Tschesche, H., and Szeverini, M. (1990). [Principles of physiologic and drug-induced cervix ripening—recent morphologic and biochemical findings]. *Geburtshilfe Frauenheilkd.*, 50(9):657-664.
- Ritar A.J., Ball, P.D., and O'May, P.J. (1990). Artificial insemination of Cashmere goats: effects on fertility and fecundity of intravaginal treatment, method and time of insemination, semen freezing process, number of motile spermatozoa and age of females, *Reprod. Fertil. Dev.*, 2:377-384.
- Roca J., Carrizosa, J.A., Campos I., Lafuente, A., Vázquez, J.M., and Martínez, E. (1997). Viability and fertility of unwashed Murciano-Granadina goat spermatozoa diluted in Tris-egg yolk extender and stored at 5°C. *Small Ruminant Res.*, 25:147-153.
- Salamon, S. and Maxwell, W.M.C. (1995). Frozen Storage of Ram Semen II. Causes of Low Fertility after Cervical Insemination and Methods of Improvement. *Animal Reproduction Science*, 38:1-36.
- Sanchez-Partida, L.G., Windsor, D.P., Epplston, J., Setchell, B.P. and Maxwell, W.M. (1999). Fertility and its relationship to motility characteristics of spermatozoa in ewes after cervical, transcervical, and intrauterine insemination with frozen-thawed ram semen. *J. Androl.*, 20(2):280-288.
- Sayre, B.L. and Lewis, G.S. (1997). Fertility and ovum fertilization rate after laparoscopic or transcervical intrauterine artificial insemination of oxytocin-treated ewes. *Theriogenology*, 48(2):267-275.
- Sohnrey, B. and Holtz, W. (2005). Technical Note: Transcervical deep cornual insemination of goats, *Journal of Animal Science.*, 83(7):1543-1548.
- Stellflug, J.N., Wulster-Radcliffe, M.C., Hensley, E.L., Cowardin, E.A., Seals, R.C., and Lewis, G.S. (2001). Oxytocin-induced cervical dilation and cervical manipulation in sheep: effects on laparoscopic artificial insemination. *J. Anim. Sci.* 79(3):568-573.
- Tervit, H.R., Goold, P.G., and James, R.W. (1984).

- The insemination of sheep with fresh semen or frozen semen. Proceeding of the new Zealand Society of animal Production, 44:11-13.
- Wulster-Radcliffe, M.C., Costine, B.A., and Lewis, G.S. (1999). Estradiol-17 beta-oxytocin-induced cervical dilation in sheep: application to transcervical embryo transfer. *J. Anim. Sci.* 77(10):2587-2593.
- Wulster-Radcliffe, M.C. and 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(7):1361-1371.
- Wulster-Radcliffe, M.C., Wang, S., and 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(6):990-1002.

