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TRANSCERVICAL VERSUS LAPAROSCOPIC INSEMINATION IN NULLIPAROUS  
AND MULTIPAROUS EWES AFTER ESTRADIOL CYPIONATE TREATMENT

by

Tami L. Gage

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science

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Tami L. Gage

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## ABSTRACT

Transcervical Versus Laparoscopic Insemination in  
Nulliparous and Multiparous Ewes After  
Estradiol Cypionate Treatment

by

Tami L. Gage, Master of Science

Utah State University, 1993

Major Professor: Dr. Thomas D. Bunch  
Department: Animal, Dairy and Veterinary Sciences

The only practical method for artificially breeding ewes with frozen semen is laparoscopic insemination into the lumen of the uterine horn. Like all surgical procedures, however, laparoscopic artificial insemination has limitations. The procedure requires surgical skill and costly equipment. Repeated passage of the laparoscope through the abdominal wall causes adhesions. Depositing frozen/thawed semen at the os cervix results in low conception rates. The inability to pass an insemination pipette through the cervix has prevented artificial insemination in sheep from becoming a standard breeding method as in the cattle industry. This study compared laparoscopic and transcervical methods of

insemination in nulliparous ewes and transcervical insemination in multiparous ewes after estradiol cypionate treatment.

Forty nulliparous commercial-cross Rambouillet ewes were treated with pessaries containing fluorogesterone acetate to synchronize estrus. After 14 days, pessaries were removed and ewes were injected IM with 400 IU of pregnant mare serum gonadotropin (PMSG). Ewes were randomly divided into laparoscopic and transcervical treatment groups, 14 and 26 respectively. Ewes were inseminated with thawed semen ( $75 \times 10^6$  motile spermatozoa). Pregnancy rate with laparoscopic insemination was 85% (confirmed at 55 days with real-time ultrasound). With the transcervical method, the speculum could only be inserted into the vagina of five ewes and the insemination pipette could only be passed through the cervix in two ewes. The combined pregnancy rate for deep cervical and transcervical insemination was 40% at 55 days.

Forty multiparous commercial-cross Rambouillet ewes were synchronized as previously described. Ewes were randomly separated into a control and estradiol cypionate treatment group of 20 animals each. Within each group 10 ewes were inseminated with frozen/thawed Suffolk semen and 10 with Rambouillet semen. Ten ewes within each sire genotype were treated with 1 mg IM of estradiol cypionate 16 hours prior to insemination. Treated ewes were inseminated into the uterine body 90% of the time and non-treated ewes 95%. There was no significant difference in



cervical passage between these groups.

Pregnancy rates for transcervical artificial insemination in the Suffolk genotype were 0.05% and 40% in the Rambouillet (pregnancy confirmed at 55 days by real-time ultrasound). Lambing rates for the Suffolk and Rambouillet groups were zero and 0.05%, respectively.

(48 pages)

## INTRODUCTION

Artificial insemination (AI) is one of the most important techniques to improve livestock genetics. It has been used to a large extent in the dairy and beef cattle industry. Artificial insemination in sheep is not necessarily a new technique, having been practiced for 50 years on a worldwide basis. The impact of artificial insemination in the sheep industry has not been fully realized in its ability to improve production (e.g., carcass quality, wool, and milk). However, in recent years, emphasis on genetic improvement has moved ahead of methods of former years of selection on visual appraisal and commonly the show ring for various popular breeds of sheep. Other advantages of artificial insemination over natural mating suggested by Ruttle (1989) include:

- 1) Use of outstanding sires for rapid genetic gain.
- 2) Access to sires from countries where purchase or import of rams may not be possible.
- 3) Breeding large numbers of females to only one or two select rams to maximize progress.
- 4) Utility in synchronized breeding programs to produce offspring for specific markets or restricted lambing periods.

Laparoscopic insemination into the lumen of the uterine horn is currently the only practical method for artificially breeding ewes with frozen semen. Like

many surgical procedures, however, laparoscopic insemination has limited application. The procedure requires surgical skills, equipment that is costly, and repeated passage of the laparoscope through the abdominal wall, which causes adhesions. Artificially breeding by depositing frozen/thawed semen at the os cervix has resulted in very low conception rates. Freezing ram semen results in approximately a 50% or more loss in viability. Nonsurgical AI requires nearly the entire frozen ejaculate to be deposited at the os cervix to be effective. The poor freezability and the inability to pass an insemination pipette through the cervix have been the major limiting factors in applying artificial insemination in sheep in contrast to what has been done in the cattle industry.

Passing an insemination pipette through the cervix in the cow or goat is the method of choice for AI with frozen semen. This has not been the case for the sheep because of the anatomical differences of the ewe cervix. The cervix of the ewe consists of five to six concentric folds or annular rings. The second and third fold or rings are oblique to the others. Since actual palpation of the ovine reproductive tract is not possible, threading the cervix has been considered almost impossible.

Recently there has been renewed interest in passing the ewe cervix by advancements in AI instrumentation and possibilities of cervical relaxation by hormone treatment. Researchers at the University of Guelph have recently

modified techniques that allow for a high incidence of cervical passage with an AI pipette. A study by Halbert et al. (1990b) resulted in an 82% uterine penetration rate.

The objective of this study was to develop or modify the currently known methods to pass the ewe cervix with an insemination pipette and to compare pregnancy rates to laparoscopic AI using frozen semen.

## LITERATURE REVIEW

Artificial Insemination in sheep has been practiced worldwide for more than 50 years, and until recently, most inseminations were done with fresh semen (Evans, 1988). The development of improved quality of frozen/thawed semen and the aid of intrauterine insemination with a laparoscope have renewed interest in applying AI to the sheep industry.

A vaginal method of insemination, in which the semen is simply "dumped" into the vagina without attempting to locate the cervix, was developed in the U.S.S.R. This method, however, is ineffective with frozen/thawed semen (Maxwell, 1986).

Evans (1988) reported that depositing of semen into the cervix was the most common form of AI. This method is quite acceptable with fresh semen; however, in using frozen/thawed semen, pregnancy rates are too low for commercial application (Hawk, 1983). Evans and Maxwell (1987) reported that use of frozen/thawed semen for cervical insemination yielded lambing rates of 25-40%. This is far below results of 65-75% pregnant when fresh diluted or undiluted semen was used in the same method. Low fertility rates from frozen/thawed semen are due to reduced viability of sperm in the reproductive tract. There are too few viable sperm that pass through the cervix and uterus to

reach the fertilization site.

Laparoscopic AI is the method of choice for AI of frozen semen, and conception rates are comparable to natural service or use of fresh semen (Maxwell, 1986). Since the cervix is a physical barrier for the passage of an AI pipette, intrauterine AI with a laparoscope has been used to overcome this problem. McKelvey et al. (1985) reported fertilization rates of 89% in synchronized ewes using laparoscopy and fresh semen. Rodriguez et al. (1990) reported conception rates of 71% with laparoscopic AI using frozen semen. This method is a surgical approach; however, and has limited use for many sheep producers due to cost of equipment and time involved in the procedure (Evans, 1988).

Gourley and Riese (1990) stressed that the beneficial use of laparoscopic AI is not only the actual insemination process, but also includes other factors such as improved flock health and management, reduces environmental effects, and promotes estrus synchronization that shortens the lambing interval. In addition, the producer usually uses higher quality semen. Each of these factors affects the success of AI.

Gourley and Riese (1990) suggested that laparoscopic AI may effectively be used for out-of-season breeding. They also noted that this method could be a simpler form of ovine embryo transfer and that the method could also be used in

research in such areas as spider lamb syndrome. In this case, semen from spider rams may be frozen and used after the ram is no longer able to breed. They also pointed out there were disadvantages to laparoscopic AI. These include the expense in owning laparoscopic equipment, costs in the trained help for operation, costs associated with drugs for synchronization of ewes, and investments in collecting, processing, and storing of ram semen. According to Gourley and Riese (1990), it is also possible to introduce genetically transmittable defects as well as diseases through semen.

#### Estrous Synchronization

Estrus synchronization is categorized into pharmacological or natural methods (Evans and Maxwell, 1987). For artificial insemination, natural synchrony or breeding ewes as they come into estrus (heat) may be too time consuming and laborious in heat detection (Gourley and Riese, 1990). Evans and Maxwell (1987) stated that natural estrus detection is less expensive than pharmacological methods; however, there is a loss in close synchronization and it is limited to the natural breeding season.

Pharmacological methods of synchronization using progesterone or prostaglandin treatments have proven to be successful (Gourley and Riese, 1990). Progestogens are usually given for 12-14 continuous days, which inhibits

estrus and ovulation. Treatments with pharmacological methods must be at least as long as the life span of the corpus luteum (CL) (Evans and Maxwell, 1987). Progestin treatment blocks GnRH release, which prevents gonadotropin release, and estrus and ovulation do not occur (Bearden and Fuquay, 1984). Progesterone acts as a replacement for the CL on the ewe's ovary (Evans and Maxwell, 1987).

Progestin may be administered as a subcutaneous implant or intravaginal pessary. Intravaginal sponges (pessary) are often treated with either fluorogesterone acetate, a synthetic progestogen, or medroxyprogesterone acetate (Evans and Maxwell, 1987). Vaginal pessaries are not available in all countries, including the United States (Buckrell et al., 1991). Cattle implants are commercially available in the U.S. One half of an ear implant containing Norgestomet 3 mg (Syncro-Mate B, Ceva Laboratories, Overland Park, KS) is inserted subcutaneously. Both the implant and pessary usually remain in place for 14 days. Upon removal of progestogens, ewes are treated with pregnant mare serum gonadotropin (PMSG) to induce ovulation. Increased levels of PMSG are sometimes used for superovulation. Although the effects of PMSG are less important during the natural breeding season, Buckrell et al. (1991) reported better synchrony.

Prostaglandins are available in synthetic form. The recognized trade



names are Cloprostenol (Estrumate, ICI) and Prosolvin (Intervet) (Evans and Maxwell, 1987). Dinoprost tromethamine (Lutalyse, Upjohn) (Gourley and Riese, 1990) is also available and is a natural form of prostaglandin. Prostaglandin is only effective on days 5 through 14 of the ewe's estrus cycle when administered exogenously. Evans and Maxwell (1987) suggested giving two injections at intervals of 14 days apart so that all ewes within a breeding group will respond. Estrus usually occurs 24 to 48 hours after the last injection (Buckrell et al., 1991).

Prostaglandin causes regression of existing CL. Progesterone produced by a CL blocks the pituitary from releasing gonadotropins. Once the progesterone block is removed, the pituitary begins to increase the release of gonadotropins, which stimulates follicle growth. Prostaglandins are effective on ewes during the breeding season (Buckrell et al., 1991). In general, response to treatments is variable and fertility rates are usually lower (Gourley and Riese, 1990; Evans and Maxwell, 1987). Synchronization with pessaries provides the most consistent results.

The duration of the ovine estrus cycle is 14 to 19 days (Hafez, 1987). Some breed differences have been reported by Gourley and Riese (1990); however, the average is 17 days. Ewes remain in estrus for 24 to 36 hours. Ovulation occurs near the end of estrus, which is approximately 24 to 36 hours after the onset of standing heat. The oocyte is released from the ovary and

picked up by the infundibulum. It is fertilized in the ampullary-asthmatic junction. Oocytes must be fertilized within 24 hours in the oviduct, or else losses occur (Hafez, 1987). Sperm cells are viable in the female reproductive tract up to 48 hours (Hafez, 1987); however, the functional life span is only 10 to 12 hours (Maxwell, 1986). Frozen/thawed sperm have a much shorter life span in the uterus and oviduct.

Time of insemination is very important with respect to sperm and oocyte viability. The site and method for which semen is deposited into the reproductive tract affect the time of insemination. Thus, the further up into the reproductive tract semen is deposited, the less time it takes to reach the fertilization site (Rodriguez et al., 1990). Single inseminations are approximately 55 hours after pessary removal (Buckrell et al., 1991). Rodriguez et al. (1990) reported fertilization rates of 71% with insemination at 52 to 56 hours after pessary removal using frozen/thawed semen. Ewes were inseminated with 70 million motile sperm by laparoscopy.

#### Structure and Function of the Ovine Cervix

The structure and function of the ovine reproductive tract differs from that of the goat and cow. The latter species are commonly artificially inseminated with success. More (1984) described the ovine cervix as being 5.5 cm long with a

corkscrew shaped lumen. He found the internal structure had six folds. Fitzpatrick and Dobson (1979) also observed the same structure in her work and referred to the folds as annular rings. Both researchers reported that the second fold on the vaginal side of the reproductive tract was out of alignment from the first and third. Further work by Halbert et al. (1990a) observed that the most consistently eccentric rings were the second and third. Passage of a typical insemination pipette through the eccentric rings is impossible due to the inability to locate the small cervical ring openings. They suggested that a new pipette design and modification of the insemination technique are necessary for cervical passage into the uterus.

Halbert et al. (1990a) further examined the anatomy of the vaginal folds and used the description of Dun (1955) to classify the os cervix into four discreet types:

- 1) the duckbill - two opposing vaginal folds
- 2) the flap - with one vaginal fold
- 3) the rosette - a cluster of vaginal folds
- 4) spiral - a spiral-shaped tissue

Halbert et al. (1990a) stated that passage of an AI instrument through the cervix is possible and identified some of the likely reasons why passage often does not occur, which includes the following:

- 1) Blind areas due to folds in vaginal tissue make identification of the cervical opening difficult.

- 2) The narrow end of the funnel-shaped rings may be directed caudally.
- 3) The second and third rings are not aligned.
- 4) Alignment, number, size, and spacing of rings are variable among different ewes.

With these obstacles in mind, Halbert et al. (1990b) developed a new technique with refined instrumentation. They found that when restraining a ewe in a commodore cradle, the animal would lie relaxed in dorsal recumbency. In this position the ewe does not strain and the position allows for exposure and straightening of the reproductive tract. In a field trial on 89 mature multiparous ewes, 82% were transcervically passed (Halbert et al., 1990b). This procedure involves the introduction of a plexiglass speculum (with a light source) into the vagina. A 10-inch sponge forcep is used to grasp the folds of the os cervix and retract it back slightly. A modified Cassou insemination gun with a bent tip is introduced into the cervical opening and manipulated through the annular rings. Halbert et al. (1990c) later reported pregnancy rates of 50 to 68% with lambing rates of 40 to 55%. They noted that maiden ewe lambs were difficult to penetrate since the speculum was not easily passed in the vagina and, therefore, locating the os cervix was difficult.

Rodriguez et al. (1990) compared lambing rates of commercial multiparous Western range ewes using transcervical versus intrauterine insemination with frozen semen. Ewes on the study were divided into two synchronization groups. One

group received medroxyprogesterone acetate-treated pessaries for 12 days and then, upon removal, were injected with 400 iu of PMSG. The second group were synchronized with MAP pessaries for 7 days. On day 6, 15 mg Lutalyse (PGF<sub>2α</sub>) was given. Sponges were removed on day 7 and ewes received 400 iu of PMSG. Ewes were inseminated one time with a .15 ml dose of frozen/thawed semen containing  $100 \times 10^3$  spermatozoa. Semen was diluted and processed using the method of Rodriguez et al. (1990). Lambing rates for the 7-day synchrony method were 16.6% for the laparoscopy method and 19% for the transcervical method. For the 12-day synchrony method, lambing rates were 43.4% and 10%, respectively.

Coonrod et al. (1986) were able to successfully traverse the cervix to collect embryos. Ewes were placed in dorsal recumbency. A cold light speculum was placed in the vagina. Alligator forceps were used to grasp the os cervix and retract it back into the vaginal canal. The speculum was then removed and replaced with a human vaginal speculum with a tenaculum attached to the papillum of the os cervix. Three catheters were used to pass the cervical canal (e.g., foley catheter, a ball-tipped needle, and a verres needle). A 63% recovery rate of ova was obtained, which indicates that if the right instrument and technique are used, it is possible to by-pass the cervical barrier.

Buckrell et al. (1991) reported cervical passage rates of up to 96% with overall lambing rates of 50% using the transcervical insemination method. Lambing

rates using frozen semen were higher in ewes during normal estrus season versus anestrus season: 68% versus 39%, respectively.

#### Hormonal Effects

The cervix displays its own spontaneous motility separately from that of the uterine body (Villar et al., 1982). This difference in motility is essential for sperm transport to the site of fertilization. Sperm motility is important in gaining passage through the cervix. Muscular contraction from the cervix and the uterine body are essential in attaining complete passage and fertilization. According to Hawk (1983), 5 hours after estrus, 67% of the uterine contractions begin at the cervix and propel spermatozoa inward. At 48 hours after estrus, 75% of the contractions are moving from the uterotubal junction toward the cervix.

Nonsurgical ovine embryo collection with a 65% recovery rate was reported by Barry et al. (1990). In their study, both nulliparous and multiparous ewes (N=15) were treated with progesterone sponges and superovulated with follicle stimulating hormone (FSH). Human chorionic gonadotropin (500 iu) was injected IV at the first signs of estrus. Ewes were bred three times every 12 hours after first signs of standing heat. Six days post estrus, 10 ewes were placed in dorsal recumbency. Two 0.5 mg prostaglandin E<sub>2</sub> tablets (Prostin E<sub>2</sub>, Upjohn, South America) were dissolved in saline then infused into the os cervix. One mg of estradiol cypionate

(ECP) was also injected IM. The PGE<sub>2</sub> treatment was repeated three times, 12 hours apart up to 12 hours prior to embryo removal. Cervices were retracted with tissue forceps and expanded with a human dilator. A three-way catheter for small ruminants was used to pass the cervix and recover embryos. Results of the study showed that the cervix may be successfully "ripened" and that embryos are viable for transfer. Neither PGE<sub>2</sub> nor estradiol had any negative effects on embryo development or survival. Upjohn is the pharmaceutical company that supplies PGE<sub>2</sub>; however, it is not commercially available for use.

Mature ovariectomized ewes weighing 42-53 kg were injected with 1 mg per day of estradiol benzoate (E<sub>2</sub>B) IM for 4 days. The first injection of estradiol benzoate yielded complete inhibition of cervical motility for 7 to 10 hours. Upon the first injection, however, there was a 30 to 70 minute delay in action of cervical motility. After the third and fourth injection a regular action of motility resumed (Villar et al., 1982). This study suggested that it may be possible to inhibit cervical contractions long enough to gain passage and not affect sperm transport or viability.

Estradiol benzoate (10 µg) was administered to mature ovariectomized Rambouillet ewes followed by relaxin 12 hours later in an attempt to dilate the cervix for catheter passage at 12, 24, and 36 hours after relaxin treatment. Dilatory effects were more variable at 36 hours than at 12 or 24. Cervical passage was attempted with two catheter sizes (14 and 16 gauge). Nine of 15 attempts were successful for

the small catheter and 6 of 15 for the large. It was concluded that E<sub>2</sub>B priming before relaxin treatment will increase transcervical passage (Nemec et al., 1988).

Edqvist et al. (1975) reported that the inhibiting effects of estrogen on uterine activity in the rat and ewe may be mediated by the presence of relaxin. Fitzpatrick and Dobson (1979) suggested that there is pharmacological evidence for estradiol 17<sub>β</sub> induced cervical softening when injected into the sheep cervix. According to Bearden and Fuquay (1984), high levels of estrogen may cause the cervical canal to dilate during estrus.

During the normal estrus season, mature parous ewes were administered subcutaneously 20 μg of E<sub>2</sub>B in 2 cc of corn oil at the time of mating. An increase in spermatozoa in the reproductive tract 24 hours after breeding occurred in the ewes treated with 30 μg of estradiol (Hawk and Cooper, 1974; Hawk and Conley, 1975). Results of this study showed that the number of sperm cells throughout the reproductive tract of treated ewes did not differ from those not treated at 2 hours after mating. At 24 hours post breeding there was still no significant difference in sperm transport between the two groups. Thus, the use of an exogenous estradiol product should not affect sperm viability while attempting to increase cervical relaxation.

Dairy cows were injected with PGF<sub>2α</sub>, then treated 40 to 48 hours later with 400 μg of estradiol benzoate. Cattle were then inseminated 12 hours after heat



detection. Estrus was usually detected within 5 days of PGF<sub>2α</sub> injection. Estradiol had no negative effect on pregnancy rate (Dailey et al., 1986). This suggests that it is possible to treat ewes prior to insemination with an estradiol product without affecting pregnancy rates.

## OBJECTIVES

This study evaluates:

1. Transcervical versus laparoscopic insemination in nulliparous ewes.
2. Transcervical insemination in multiparous ewes after estradiol cypionate treatment.

## MATERIALS AND METHODS

### Objective 1: Transcervical Versus Laparoscopic

#### Insemination in Nulliparous Ewes

Forty nulliparous commercial cross Rambouillet ewes were maintained at the USU South Farm on pasture. The breeding study took place during November, 1990. Ewes were implanted October 29 and 30 with intravaginal pessaries containing fluorogesterone acetate (40 mg) to synchronize estrus. Pessaries were removed on the morning of day 14 and ewes were injected (IM) with 400 iu of pregnant mare serum gonadotropin (PMSG). Observation of estrus was checked twice daily on days 14 through 16 using three vasectomized rams.

Ewes were inseminated with frozen Targhee ram semen obtained from rams that were located at the USDA Sheep Research Station, Dubois, ID, following the procedures of Rodriguez et al. (1990). After it was thawed, the semen was drawn into one-half cc straws for insemination. Ewes were inseminated 51 to 55 hours after pessary removal. Seventy-five million motile sperm were used in each inseminate.

Twenty-seven ewes were divided into a control and two treatment groups as follows:

#### Control:

Fourteen control ewes were restrained in dorsal recumbency in a

laparoscopic cradle. Animals were closely shorn on their abdomen at the surgical site and disinfected with an iodine scrub, and then lidocaine was administered (SC) at the incision site. Laparoscopic insemination was performed by Dr. Fernando Rodriguez, USDA Sheep Research Station. Ewes were inseminated with one half of the inseminate into the lumen of each uterine horn. Upon completion of insemination, incision sites were closed with wound clips. Ewes then received 8 cc of combiotic IM and were returned to their contemporaries.

#### Treatment:

Thirteen ewes were inseminated using the Guelph method. Animals were restrained in dorsal recumbency in the Commodore sheep cradle. A clear slotted speculum with a light source was lubricated with KY jelly and then introduced into the vaginal canal. The speculum was manipulated in order to locate the opening of the os cervix. Ten-inch Bozeman forceps were used to grasp the flap of the os cervix and retract it back. This was necessary in order to straighten the reproductive tract and allow for access of the insemination pipette. The modified pipette with a curved tip was introduced and manipulated through the folds of the cervix. While the forceps grasped the os cervix, a constant pressure was maintained, while at the same time the pipette was turned and pushed downward through the cervical canal. Each ring could be counted as it was passed. The second and third rings were the

most difficult to pass. Passage time was usually 2 minutes or less. Semen was deposited into the uterus following cervical passage.

Fourteen days following insemination, ewes were placed with a marked fertile ram. Pregnancy was confirmed at 55 days using real-time ultrasound. Results of pregnancy diagnosis were compared to the lambing incidence per ewe diagnosed pregnant.

## Objective 2: Transcervical Insemination in Multiparous

### Ewes after Estradiol Cypionate Treatment

Forty multiparous commercial cross Rambouillet ewes were maintained at the USU South Farm on pasture. The breeding study took place in September 1991. Ewes were implanted on September 1 with intravaginal pessaries containing fluorogesterone acetate (40 mg) to synchronize estrus. Pessaries were removed the morning of day 14 and ewes were injected with 400 iu IM of pregnant mare serum gonadotropin. Observation of estrus was checked twice daily on days 14 through 16 using three vasectomized rams.

### Control:

Twenty ewes were inseminated transcervically using the Guelph method as described in the first study. Ten ewes were inseminated with semen from a Suffolk

ram and 10 from a Rambouillet ram. Semen was collected from two rams maintained at the USU South Farm and was processed and frozen in pellet form using the procedures of Rodriguez et al. (1990). Seventy-five million motile sperm were frozen in each pellet, with one pellet used per inseminate.

#### Treatment:

Twenty ewes were administered 1 mg IM of estradiol cypionate (ECP) 16 hours prior to insemination. Ewes were inseminated using the Guelph method. Ten ewes were inseminated using semen from a Suffolk ram and 10 from a Rambouillet ram. The same semen was used as in the above control group.

The site of semen deposition was recorded in all breeding groups and scored as one, two, or three: 1 uterine insemination, 2 mid-cervical insemination, and 3 deposited at the os cervix. If cervical penetration was not achieved within 5 minutes, semen was deposited and the site recorded.

Fourteen days following insemination, ewes were placed with a marked fertile ram of the breed in which they were artificially inseminated. The number of ewes returning to estrus was recorded. Pregnancy was confirmed at 55 days with real-time ultrasound. Results of pregnancy diagnosis were compared to incidence of lambing.

## RESULTS

## Objective 1: Transcervical versus Laparoscopic

## Insemination in Nulliparous Ewes

The speculum was inserted into the vagina of 5 out of 24 ewes. The other 19 had a restrictive hymen ridge which would not allow introduction of the speculum without tissue damage. Two of the five ewes were inseminated in the lumen of the uterine body and three within the first two rings of the os cervix. The two ewes transcervically inseminated were marked by vasectomized rams by 48 hours post pessary removal. Two of the three ewes inseminated intracervically were marked by the teaser ram by 48 hours (Table 1).

TABLE 1. INSEMINATION SITE AND PREGNANCY DIAGNOSIS IN TRANSCERVICAL AI OF NULLIPAROUS EWES

Insemination site	(N)	Number showing estrus, marked by teaser ram	Number preg. at 55 days	Number ewes lambing
Intrauterine	2	2	1	0
Intracervical	3	2	1	0

Artificially inseminated ewes that did not breed back at 16 days were pregnancy checked at 55 days with ultrasound. One of the two ewes inseminated in the uterine body was pregnant (50%) and one of three inseminated intracervically was pregnant (33%). Ewes were detected as being pregnant by ultrasound; however, they lambled 17 days later than the project ewes. Thus, they were thought to have been bred by the clean-up ram, although rebreeding was not detected with marked rams.

#### Laparoscopic insemination:

Thirteen ewes were inseminated laparoscopically. One of the 14 was observed pregnant during the insemination process and removed from the project. Eight of the 13 ewes were observed in estrus as marked by a teaser ram. Pregnancy examination by ultrasound at 55 days post AI showed 11 of the remaining 13 ewes pregnant (85%). Seven of the 13 ewes lambled (53%). Two of the 13 ewes bred back to clean-up rams 18 days post pessary removal. Four ewes did not breed back and never lambled. Two of those four ewes were diagnosed pregnant with ultrasound (Table 2).



TABLE 2. ESTRUS RESPONSE AND PREGNANCY RATES IN EWES TRANSCERVICALLY OR LAPAROSCOPICALLY INSEMINATED

Insemination method	Number ewes insemin.		Estrus response	Number ewes pregnant	Number ewes lambing
	Uterine	Cervical			
Transcervical N=5	2	3	4	2 <sup>a</sup>	0
Laparoscopic N=13	13	NA	8	11	7

<sup>a</sup> Ewes were examined with ultrasound for pregnancy at 55 days post insemination.

There was a statistical difference between number of ewes lambing per insemination method (p 1.0).

#### Objective 2: Transcervical Insemination in Multiparous

##### Ewes after Estradiol Cypionate Treatment

Ewes were checked for estrus by marked vasectomized rams. Ewes began showing estrus at approximately 24 hours after pessary removal. By 48 hours, 36 of the 40 ewes (90%) were marked by teaser rams. In the control group, 19 of 20 (95%) ewes were inseminated into the lumen uterine body within 5 minutes. Eighteen of 20 (90%) ewes in the estradiol-treated group were inseminated into the uterine body within 5 minutes (Table 3).

TABLE 3. EWE ESTRUS RESPONSE AND TRANSCERVICAL PASSAGE IN TREATED VERSUS NONTREATED EWES, BY SIRE GROUP

Genotype of sire	Number of ewes	Number ewes showing estrus (%)	Transcervical passage	
			Treat ewes (%)	Non treat (%)
Suffolk	20	19 (95)	8 (80)	10 (100)
Rambouillet	20	17 (85)	10 (100)	9 (90)
Total	40	36 (90)	18 (90)	19 (95)

Pregnancy diagnosis at 55 days post pessary removal confirmed that nine ewes were pregnant (22.5%). There was statistical difference between sire groups in number ewes bred back. One of the nine lambed (11%). One ewe in the Suffolk group confirmed pregnant and aborted twin fetuses (Table 4).

TABLE 4. PREGNANCY AND LAMBING RATES IN TREATED VERSUS NONTREATED EWES

Genotype of sire	Total number	Number ewes bred back 16 d. (%)	Preg. by ultrasound 54 days (%)	Number of ewes lambing
Suffolk	20	15 (75)	1 (.05)	0 <sup>a</sup>
Rambouillet	20	7 (30)	8 (40)	1

<sup>a</sup>Aborted twin fetuses were found at approximately 120 days.

## DISCUSSION

## Objective 1. Transcervical versus Laparoscopic Insemination in Nulliparous Ewes

Transcervical insemination of nulliparous ewes was nearly impossible due to the low passage of the speculum into the vagina (5 of 24). Nulliparous ewes have a highly restrictive hymen ridge. Forcing the speculum into the reproductive tract resulted in tissue damage and hemorrhaging and, therefore, was only attempted on the first few ewes. If the speculum was not easily inserted, no further attempt was made to breed the ewe artificially. Even when the speculum was inserted, nulliparous ewes had tight cervixes and were seldom passed with an AI pipette. Only in two of five ewes with the speculum inserted could the cervix be passed (40%) with an insemination pipette. Frame size, weight, and age did not appear as important factors in transcervical passage. Ewes on the study were physically and reproductively mature (20 months of age), but had never gone through parturition. Transcervical AI seems incumbent on ewes going through at least one pregnancy. The ewes in Objective 1 were randomly selected from the USU commercial flock. Many of the same ewes in the second objective were randomly drafted the following year. When ewes that were used in Objective 1, where the speculum could not be inserted into the vagina, had gone through parturition, they were easily transcervically inseminated into the uterine lumen. A small number (5) of nulliparous

18-month-old ewes from the USU purebred flock were checked for insertion of the speculum; however, they could not be passed. Most of these ewes had a large frame size and with weights greater than 200 pounds. In every case, the speculum could not be passed over the hymen ridge. The process of parturition leaves the cervix softened due to collagen breakdown from the dilation and regression after the birthing process. The annular rings still remain nonaligned; however, they are less restrictive.

Teaser rams marked only 66% of the 18 ewes inseminated. This is much lower compared to the 90% showing estrus in Objective 2. It has been suggested by Gourley and Riese (1990) that it is more desirable to inseminate fertile mature ewes rather than ewe lambs, since the mature ewes often have suboptimal reproductive performance and may vary among breeds. After synchronization, ewes begin showing signs of estrus at approximately 24 hours. Gourley and Riese (1990) reported that there is a breed difference associated with the time of onset of estrus, and ovulation may be delayed in white-face fine wool breeds. Ewes on the study that were not in estrus by 48 hours may have been approaching estrus and ovulated later, as in the white-face fine wool breeds. In this study, using nulliparous, white-face yearling ewes may have attributed to the lowered response to synchronization as compared to mature reproductively functioning multiparous ewes, or ewes of another breed.

Laparoscopic insemination is the method of choice in nulliparous ewes as evidenced in this study. Eighty-five percent of the ewes were diagnosed pregnant by ultrasound. No pregnancies resulted from the transcervical procedure. Some fetal loss did occur in the laparoscopically inseminated group with 58% of the ewes actually lambing. Two ewes that were pregnant from the ultrasound examination had aborted fetuses and never lambed during the season. Fetal loss generally does not occur after 55 days and there is no explanation of why the pregnancy failed. Ewes on this study were on a chopped straw and supplement diet. Ewes carrying multiple fetuses may have been nutritionally stressed, attributing to fetal loss during late pregnancy. Other factors possibly contributing to fetal loss include vibrio or other health-related stress.

#### Objective 2. Transcervical Insemination in Multiparous Ewes after Estradiol Cypionate Treatment.

The ewes on the study responded to synchronization treatment much better than in Objective 1 even though the same procedure was used. Only 4 of the 40 ewes did not show estrus; however, all four were easily transcervically inseminated. Passage of the insemination pipette in 92.5% of the ewes is comparable to the report by Buckrell et al. (1991) in which 96% of the ewes were transcervically inseminated. Earlier work by this group had yielded up to 80% cervical passage. Estradiol cypionate was used as a treatment in this study to improve passage rates;

however, nontreated ewes were inseminated into lumen of the uterine body at a slightly higher incidence than the treated ewes (95% versus 90%, respectively). Practicing the insemination technique was the major factor that improved the percentage of ewes being inseminated transcervically.

During a study at the US Sheep Experiment Station at Dubois, Idaho in March 1991, I was able to pass an AI pipette through the cervical canal of multiparous ewes 87% of the time. The technician from United Breeders in Canada, whom I assisted on the study, had a 73% passage rate. On another occasion, I attempted transcervical insemination on 94 multiparous Suffolk ewes. I passed the pipette in 84% of the ewes. In this study 92.5% were inseminated into lumen of the uterine body, which shows that repetition of the technique improves the passage rates.

Ewes on the study were returned to their respective breeding groups 14 days after insemination. Fertile, harnessed rams remained with the ewes through the remainder of the breeding season (10 weeks). Beginning on day 16 of their cycle after insemination, 15 ewes (75%) returned to estrus in the Suffolk group and 7 (35%) returned to estrus in the Rambouillet group. Because there were twice as many ewes returning to estrus in the Suffolk group, there may have been a difference in post-thaw viability of semen as compared to the return rate in the Rambouillet group. The Rambouillet group had only 35% bred back, indicating increased fertilization when compared to the Suffolk. Only eight ewes tested

pregnant by ultrasound, combined from both Suffolk and Rambouillet mating groups, suggesting that more ewes in both groups actually rebred on the second cycle. One fertile harnessed ram was placed with each of the respective sire groups used on the study. The high number of ewes rebreeding suggests that not all ewes returning to estrus were bred since there was only one ram per 20 ewes, each of which was synchronized on the same day. It is unlikely the ram could have bred that many ewes given the time frame the ewes were in estrus.

Most AI with frozen ovine semen is done with a laparoscope. This method of insemination places the semen mid-way on the curve of the uterine horn. This is usually about 2 inches from the utero tubal junction (Ruttle, 1989). Transcervical AI places the semen posterior to the cervix into the anterior of the lumen of the uterine body. Thus semen is farther from the fertilization site; however, uterine contraction provides motion to propel sperm toward the fertilization site. Uterine contractions at 50 to 56 hours post pessary removal may not be adequate to move spermatozoa through the uterus. Marginal quality frozen/thawed semen will not have the viability in the female reproductive tract to survive as long as fresh semen. Once semen has reached the fertilization site, ova must be present. If semen is deposited too soon, it reaches the utero tubal junction but dies because the oocyte has not yet reached this point and cannot be fertilized. Sperm reach the site of fertilization within a few hours; thus the time of insemination becomes critical to insure that the sperm will

remain viable to fertilize the oocyte.

An increased concentration of semen may be required to improve fertilization rates. The number of spermatozoa used in laparoscopic insemination, where sperm are placed near the fertilization site, may not be adequate for transcervical insemination.



## CONCLUSION

Use of quality semen with a high post thaw survival is necessary to attain improved fertilization rates. Advancements in ram semen cryopreservation have come about in recent years. United Breeders of Canada will not process semen which has less than 60% survival post thaw. Although this standard may seem high compared to 40 to 50% that has been accepted, Guelph's high pregnancy results using transcervical AI reflect the need for quality semen to obtain desirable results. For transcervical AI to become acceptable for use, quality commercially processed semen must be available. Presently, there are very few commercial ram semen processors in this country, and the few in business market frozen semen with less than 60% viability. Results of my study show that laparoscopic versus transcervical AI results in higher pregnancy rates when using frozen/thawed ram semen. I recommend further research to determine the optimal concentration of post thawed, viable spermatozoa and the best time for transcervical insemination. Time of insemination and concentration of spermatozoa for my study were based upon methods described for laparoscopic AI. Since the deposition of spermatozoa is in different locations, and the direction and strength of uterine contraction change about 48 to 50 hours from the onset of estrus, the poor results I obtained from transcervical insemination may be overcome by simple changes in methodology.

I have received numerous inquiries by producers expressing their interest in

transcervical AI. If pregnancy rates can be improved and remain consistent, transcervical AI may well become the method of choice for artificial insemination of sheep.

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## APPENDIX

PROTOCOL FOR FREEZING RAM SEMEN  
IN PELLET FORM USING ALOE VERA GEL-BASED DILUENTS\*

I. Evaluation and Extension of Semen:

- 1) Immediately upon collection, evaluate ejaculate for volume, percent motility, sperm concentration and percentage of normal cells (consider about 10-15% being the maximum allowable for abnormal cells).
- 2) From above information, determine dilution rate and the number of units of extended semen. Sorensen's (Repro-Lab, 1979) formula applies well to make such calculations:

$$\frac{\text{Volume (ml)} \times \text{concentration/ml} \times \% \text{ Motile} \times \% \text{ Normal}}{\text{No. viable cells/insemination}} = \text{Units of extended semen}$$

\*Since cell loss during freezing and storage may be 40-50%, need to double number of cells per unit. This means that ejaculates can be diluted in extension rates ranging from 1:1 to 1:4 (semen:diluter).

II. Formula and Preparation of Semen Extender:

- 1) Extender for Pelleting Ram Semen:

Ingredients	Composition
-------------	-------------

Buffer:

Sodium Citrate Dehydrate (g)	3.0
Distilled Water (ml)	100

Diluent:

Buffer (% by volume)	50
Reconstituted VPI-40:1 (% by volume)	34
Egg Yolk (% by volume)	10
Glycerol (% by volume)	6

\*NOTE: This extender is supplemented with Fructose and Sulfanilamide at 2.5 g and .300 g per 100 ml of diluent.

\* Rodriguez, F., J.N. Stellflug, J.A. Fitzgerald and V.A. LaVoie. 1990. In vitro viability and fertilizing capacity of frozen-thawed ram semen diluted with aloe vera gel and inseminated via laparoscopy. USDA-ARS, Research Progress Report, U.S. Sheep Experiment Station. pp. 70-76.

2) Procedure for the preparation of 100 ml of extender:

- a) Basic buffer. Mix 3 g of Sodium Citrate Dehydrate in 100 ml of Distilled water and stir until dissolved.
- b) Reconstitution of VPI-40:1. Place 39 parts (ml) of Distilled Water (temperature below 30°C) into a beaker. With gentle agitation (avoid as much air entrapment as possible) slowly add 1 part (ml) of VPI-40:1, and stir the mixture for 5 min at room temperature.
- c) Prepare fresh Egg Yolk
- d) Place 50 ml of basic buffer into a flask. Add 2.5 g Fructose and .300 g Sulfanilamide and stir until dissolve. If using a stirrer hot plate, apply some heat (37°C) to this mixture for complete dissolving of sulfanilamide.
- e) Add 34 ml of reconstituted VPI-40:1 as prepared out and stir the mixture for 5 min at room temperature.
- f) Add to this mixture 10 ml of egg yolk and stir the mixture for another 5 min.
- g) Bring this mixture to 100 ml by adding gradually (drop-wise) 6 ml of Glycerol and mix for 5 min.
- h) The pH of this extender ranges from 6.75 to 7.00 and does not need to be adjusted.
- i) Place the extender in a waterbath at 30°C until used.

III. Processing of Semen:

- 1) Processing of semen should be done within the first 10-15 min following collection and evaluation.
- 2) Both the semen and diluent are placed in a waterbath at 30°C and must be at the same temperature when mixed.
- 3) The extent to which a semen sample can be diluted will be based on the calculations of the number of units of extended semen previously determined (see evaluation and extension of semen). Dilution rates (semen:diluter) higher than one to four (5 fold) are not recommended for ram semen.
- 4) Depending on dilution rate, fill a clean pipette with the required amount of diluent and slowly add it to the semen sample (add diluent to semen!!). Mix gently semen and diluent, and re-examine for sperm motility.



- 5) Cool extended semen slowly to 5°C by setting the vial in a beaker of water (30°C) over a period of about 2 h in a refrigerator or cold room.
- 6) After cooling time (approx. 2 h), leave extended semen at the same temperature (5°C) for another 1-h period to equilibrate (i.e., permit glycerol to complete action).

#### IV. Freezing of Semen by Pelleting on Dry Ice:

- 1) For best sperm survival, the freezing steps should be done in a cold room at 4-5°C. Alternatively, keeping the vial with the equilibrated semen in a tray containing ice-water (5°C) should be done.
- 2) After equilibration, on flat surface of a dry ice block form small (1/4 ") depressions. Can dig out with a heated stainless steel device (rod).
- 3) Pipette into each depression approximately 0.15 ml (3 drops if using 15 cm-long Pasteur pipettes) of extended semen. During pipetting of semen on dry ice use always cold pipettes. This can be done by blushing frequently cold (3-5°C) spare diluent up and down.
- 4) Allow pellets to freeze for 2-3 min, and then transfer pellets to labeled plastic goblets, insert goblets into canisters and into the liquid nitrogen tank.
- 5) Thawing of pellets (1-3) is carried out in a dry test tube shaken in a waterbath at 40°C until dissolve. Then transfer thawed semen to a waterbath at 30°C, maintaining semen at this temperature until used.

## TECHNICIAN INFORMATION SHEET

## TRANSCERVICAL ARTIFICIAL INSEMINATION

Date: Sept. 24, 1991Technician: Tami L. GageTime: 11:30 a.m.Producer: USU commercial

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<u>EWE ID</u>	<u>BREED</u>	<u>CODE</u>	<u>RAM ID</u>	<u>COMMENTS</u>
9048	Ramb	1	84	E2
9015	Ramb	1	84	
9081	Ramb	1	84	
9060	Ramb	1	84	E2
9041	Ramb	1	84	E2
9075	Ramb	1	84	E2
9058	Ramb	1	84	
9068	Ramb	1	84	
9000	Ramb	1	84	
9089	Ramb	1	84	E2
9093	Ramb	2	84	
9051	Ramb	1	84	E2
9046	Ramb	1	84	
9056	Ramb	1	84	E2
9002W	Ramb	1	84	E2
9018	Ramb	1	84	E2
9002R	Ramb	1	84	
9039	Ramb	1	84	
9011	Ramb	1	84	
9029	Ramb	1	84	E2

TECHNICIAN INFORMATION SHEET  
TRANSCERVICAL ARTIFICIAL INSEMINATION

Date: Sept. 24, 1991

Technician: Tami L. Gage

Time: 11:30 a.m.

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<u>EWE ID</u>	<u>BREED</u>	<u>CODE</u>	<u>RAM ID</u>	<u>COMMENTS</u>
9003	Ramb	1	27	E2
9027	Ramb	1	27	
9038	Ramb	1	27	
9032	Ramb	2	27	E2
9001	Ramb	1	27	
9082	Ramb	1	27	E2
9072	Ramb	1	27	
9025	Ramb	1	27	E2
9092	Ramb	1	27	E2
9035	Ramb	1	27	E2
9064	Ramb	1	27	E2
9074	Ramb	1	27	E2
9065	Ramb	1	27	E2
9057	Ramb	2	27	E2
9061	Ramb	1	27	
9073	Ramb	1	27	
9094	Ramb	1	27	
9006	Ramb	1	27	
9071	Ramb	1	27	
9024	Ramb	1	27	