A COMPARISON OF TRANSCERVICAL AND LAPAROSCOPIC INTRAUTERINE ARTIFICIAL INSEMINATION TECHNIQUES ON REPRODUCTIVE PERFORMANCE OF EWES

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Introduction

In all domestic species, acceptable pregnancy rates with frozen-thawed semen are only achievable with intrauterine insemination. Unlike the cow and the goat, the cervix of the ewe is a formidable barrier to penetration for transcervical intrauterine insemination not only due to the physical size of the canal and a narrow luminal diameter (even at estrus), but also due to it's caudally facing, eccentric series of four to eight funnel-like rings.

Killen and Caffery (1982) have developed a laparoscopic artificial insemination technique (LAI), and Halbert et al. (1990a) have developed the Guelph System for transcervical artificial insemination (GST-AI) to circumvent the anatomical complexities of the ewe's cervix and provide acceptable pregnancy rates. Conception rates as determined via transrectal or transabdominal ultrasound range from 32% (Windsor et al., 1994) to 64% (Smith et al., 1995), and 48% (Windsor et al., 1994) to 72% (Hill et al., 1998) for GST-AI and LAI, respectively. Relatively few authors reported lambing rates following AI with either method; however, they tend to be at least 5% less than their respective conception rates as determined by ultrasound (Halbert et al., 1990b; Buckrell et al., 1994). The two AI methods have not been compared in the U.S.

The major objectives of this study were to evaluate the effects of GST-AI and LAI on the lambing rates and prolificacy of ewes inseminated with frozen-thawed semen at fixed times following progestagen pessary removal (PPR). The reproductive performance of ewes not conceiving to AI, but conceiving to natural follow-up matings was also evaluated.

Materials and Methods

General. One hundred twenty-one Rambouillet or Rambouillet-Booroola ewes were artificially inseminated with frozen-thawed ram semen either by GST-AI or LAI once on December 9 or 10, 1997 in the Livestock Laboratory on the University of Wisconsin-Madison campus. Ewes were between 1.5 and 5.5 years of age with a mean body weight of 147 lb. and a mean body condition score of 3 (1 = emaciated, 5 = obese). Twenty-eight of the 1.5-yr-old ewes were maiden and were assigned directly to the LAI group; the remainder of ewes (n=93), including the 1.5-yr-old parous ewes, were randomly assigned to either GST-AI or LAI. All parous ewes were at least seven months post-partum.

Estrus was synchronized with vaginal progestagen pessaries (Morlam Sponges, J & M Vet.). After 13 or 14 days, pessaries were removed, and 400 IU of PMSG (Sioux Biochemical, Sioux Center, IA) was administered intramuscularly. Insemination occurred within 50 to 56 hours after progestagen pessary removal (PPR), independent of estrous detection. The times at restraint of the ewe and completion of insemination were recorded. The time of insemination from PPR was calculated by subtracting the mean PPR time from the time of restraint, and all time intervals were rounded to the nearest whole hour. Data from eight and 27 ewes were not recorded for PPR time and for time at completion of insemination, respectively.

Semen handling. Semen was collected with an artificial vagina from three sexually mature Rambouillet rams approximately three months prior to insemination. The collected ejaculates were extended with a sodium citrate and aloe vera based diluent (Rodriguez et al., 1986) and frozen in liquid nitrogen in .125 ml pellets, each containing approximately 110 million live sperm. On each day of the experiment, sample pellets were thawed from each ejaculate for each ram and evaluated for percentage live, motile sperm. Frozen pellets from an ejaculate were used in the experiment only if there were at least 30% live, motile sperm present in the sample pellets from that ejaculate. Just prior to restraint of the ewe, two pellets from a single ram were thawed together and aspirated into a .25 ml straw resulting in an insemination dose of >65 million live, motile sperm per ewe.

Insemination. All ewes were fasted for at least 24 hr prior to insemination. Ewes for LAI were restrained in dorsal recumbency in a 30 degree inclined laparotomy cradle and routinely surgically prepared. One ml of 2% lidocaine hydrocholoride (Phoenix Pharmaceutical, Inc., St. Joseph, MO) was administered subcutaneously at two paramedian sites 10 cm cranial to the udder and 2 cm either side of the ventral midline. One centimeter incisions through the skin were made over the sites of local anesthesia with a no. 10 scalpel blade. A 7 mm laparoscopic trocar in a trocar sleeve, directed caudally, was introduced subcutaneously for 2 cm in the left paramedian incision and then was penetrated into the abdomen. The trocar was removed. The abdomen was sufficiently inflated with carbon dioxide via the trocar sleeve, and the laparoscope was inserted intrabdominally through the trocar sleeve. Through the right paramedian incision, a 5 mm trocar in a trocar sleeve was inserted intrabdominally with the previous technique. After locating the uterus with the laparoscope, the insemination gun (IMV, L'Aigle, France) was substituted for the 5 mm trocar and inserted intrabdominally through the right trocar sleeve. If necessary, the abdominal omentum was cleared away from the scope with the tip of the insemination gun. Each uterine horn was intraluminally inseminated approximately 4 cm cranial to the cornual bifurcation with one-half of the .25 ml straw. The instruments were removed from the abdomen and the skin incisions closed with Michel® wound clips (Miltex Instrument Co., Germany). Surgical instruments were soaked in a cold-sterilization tray with a chlorhexidine diacetate solution (Nolvasan® solution, Fort Dodge Laboratories, Inc., Fort Dodge, IA) between ewes.

Ewes for GST-AI were restrained in dorsal recumbency with a Commodore® turning cradle (Poldenvale, Somerset, UK). A non-spermicidal lubricated vaginal speculum with light source was inserted intravaginally, and the cervical os located by an experienced transcervical inseminator. The largest of the cervical flaps was grasped with Bozeman forceps and retracted caudally and laterally as the speculum was advanced cranially to elongate the cervical canal. The retro-loading transcervical insemination gun (Colorado State University) with Guelph insemination tip (Ovine Reproductive Technologies, Guelph, Ontario) was passed transcervically to achieve intrauterine semen deposition. If intrauterine penetration of the insemination tip was not permitted, semen was deposited as far cranially in the cervix as possible.

Post-insemination. On December 19, 1997, all ewes, in groups of approximately 20, were exposed to one of six intact rams for natural service. Rams were removed from the ewes on January 14, 1998. Breeding dates were not recorded. On February 9, 1998, ewes (n = 119) were transabdominally ultrasounded by an experienced technician with a 3.5 MHz 170° sector transducer (Vetscan-II®, BCF Technologies, Edinburgh, Scotland) to determine pregnancy, gestational age of feti, and number of feti. Pregnancy was determined by the presence of fetal structures and cotyledons. The ultrasound unit was commercially programmed to estimate gestational age using fetal trunk width measurements determined by the technician. Fetal numbers were counted by the technician. Ewes inseminated artificially would have been either

60 or 61 days pregnant, and ewes that failed to conceive to artificial insemination but conceived naturally would have been an average of 42 days pregnant on the day of ultrasound examination.

Results

Artificial insemination. There was a 10 day interval between the day the last ewe lambed to the AI and the day the first ewe lambed to the natural mating. Ewes lambing to AI (n=41) gave birth between May 5 and 12, 1998, and results are summarized in Table 1.

Lambing rate for LAI was much higher ($P < .01$) than for GST-AI (43.9% vs. 20.7%, respectively), and ewes inseminated at <53 hours post-PPR had a lower lambing rate ($P = .07$) than ewes inseminated at >=53 post-PPR (22% vs. 42.7% respectively). Even though there appeared to be large differences in prolificacy between treatments, these differences were not statistically significant.

Table 1. Mean reproductive performance (±SE) of ewes artificially inseminated

Item	Number of ewes lambing / number of ewes present at lambing	Lambing rate to AI, %	Prolificacy, no.
AI method			
GST-AI	8/47	20.7[a]	1.47±.47
LAI	33/74	43.9[b]	1.89±.21
Pessary[¥]			
50-52 hrs	13/50	22.0[c]	1.93±.43
53-56 hrs	24/63	42.7[d]	1.44±.25

[¥] The number of hours after progestagen pessary removal at which insemination took place.
[a,b] Within a column and independent main factor, means lacking a common superscript letter differ ($P < .05$).
[c,d] Within a column and independent main factor, means lacking a common superscript letter differ ($P < .10$).

The relatively poor results from the GST-AI were due to the extremely poor lambing rates of ewes inseminated with the technique on the first day (Dec. 9), because on the second day (Dec. 10), ewes inseminated with by GST-AI had the same lambing rate as ewes inseminated by LAI (Table 2). There is no ready explanation for the different results obtained on the two days of insemination, but the results may suggest some inconsistency of results with the GST-AI technique.

Table 2. Mean lambing rate of artificially inseminated ewes by insemination method and date of insemination

AI method	Date December 9, 1997	December 10, 1997
GST-AI	2.6%[a]	38.9%[b]
LAI	48.9%[b]	38.9%[b]

[a,b] Means lacking a common superscript letter differ ($P < .05$).

Ewes lambing to natural mating. Table 3 summarizes the performance of ewes that did not conceive to artificial insemination. Of the 80 ewes which did not conceive to the AI, 66 lambed to natural matings between May 22 and June 12, 1998.

The effects of LAI and GST-AI on future reproductive performance has been evaluated in terms of histological trauma to the reproductive tract as well as in terms of subsequent fertility to natural matings by other researchers. Campbell et al. (1996) and McKelvey (1994) have shown there is epithelial damage to the lining of the cervix ranging from bruising to puncture of the cervical wall when the GST-AI technique is used. The LAI technique, however, has been shown to result in no fibrinous adhesions nor detectable damage to the reproductive tract when examined 3 to 5 days following insemination (McKelvey et al., 1985). Thus, it is possible that ewes not achieving conception to GST-AI could have decreased fertility to subsequent natural mating due to local inflammation and possibly cervical infection. However, Windsor et al. (1994) did not find significant differences in fertility between GST-AI and LAI at subsequent natural matings (two weeks after artificial insemination) which agrees with the results of this study. Lambing rates to natural mating and days from ram exposure to lambing were similar for ewes exposed to the two AI techniques in this study (Table 3).

However, we are concerned that the application of an AI program may result in an overall decrease in lambing rate compared with natural mating alone. From 1992 to 1996, this flock had lambing rates ranging from 95% to 100% with natural mating alone (Southey, 1996 and unpublished data). In 1997, with AI followed by natural mating, the flock had a lambing rate of 84% (unpublished data), and in 1998 in this study, the overall flock lambing rate was 88.4% (107/121). This represents about a 10% decrease in percentage of ewes lambing when an AI program was used and represents a significant decrease in flock productivity.

Ewes that were inseminated at >=53 hours post-PPR by either method were more likely to lamb ($P < .10$) to subsequent natural matings than ewes that were inseminated at <53 hours post-PPR (92.8% and 70.9%, respectively).

Table 3. Mean reproductive performance (±SE) of ewes not lambing to AI

Item	Number of ewes lambing / number of ewes present at lambing	Lambing rate to natural mating, %	Prolificacy, no.	Days to lambing[1]
AI method				
GST-AI	35/39	88.1	1.81±.16	160.51±1.14
LAI	31/41	75.7	1.71±.17	159.83±1.04
Pessary[¥]				
50-52 hrs	27/37	70.9[a]	1.78±.20	159.92±1.30
53-56 hrs	35/39	92.8[b]	1.73±.19	160.42±1.26

[1] Days from December 19, 1997, the day the intact clean-up rams were introduced to the ewes, to the date of lambing.

[¥] The number of hours after progestagen pessary removal at which insemination took place.

[a,b] Within a column and independent main effect, means lacking a common superscript letter differ (*P* < .10).

Insemination procedure time. The length of insemination procedure time for all ewes is presented in Table 4. A considerably shorter insemination procedure time per ewe (*P* < .001) was required with LAI than with GST-AI (2.98 and 5.25 min, respectively), and it took less time (*P* < .05) to inseminate ewes at <53 hours post-PPR than was necessary for ewes at >=53 hours post-PPR (3.52 and 4.70 min, respectively).

Table 4. Mean insemination procedure time (±SE) for all ewes

Item	Insemination procedure time, min.
AI method	
GST-AI	5.25±.36[a]
LAI	2.98±.29[b]
Pessary[¥]	
50-52 hrs	3.52±.37[a]
53-56 hrs	4.70±.33[b]

[¥] The number of hours after progestagen pessary removal at which insemination took place.

[a,b] Within a column and independent main effect, means lacking a common superscript letter differ (*P* < .05).

Transabdominal ultrasound. Of the 119 ewes ultrasounded, 105 of them lambed (88.3%) to either AI or to natural service. Three ewes were predicted pregnant and did not lamb; all ewes predicted not pregnant (n = 10) did not lamb.

Fetal number predictions were within ±2 feti 100% of the time and within ±1 fetus 95% to 97% of the time for ewes lambing to AI or to natural matings (Figure 1). Ultrasound predicted the number of feti correctly 77% of the time for ewes lambing to AI and 66% of the time for ewes lambing to natural mating. At the time of ultrasound, AI ewes were 60 or 61 days pregnant and naturally mated ewes were approximately 42 days pregnant. Therefore, ultrasound was more accurate in predicting number of feti at 60 to 61 days of pregnancy than at 42 days of pregnancy.

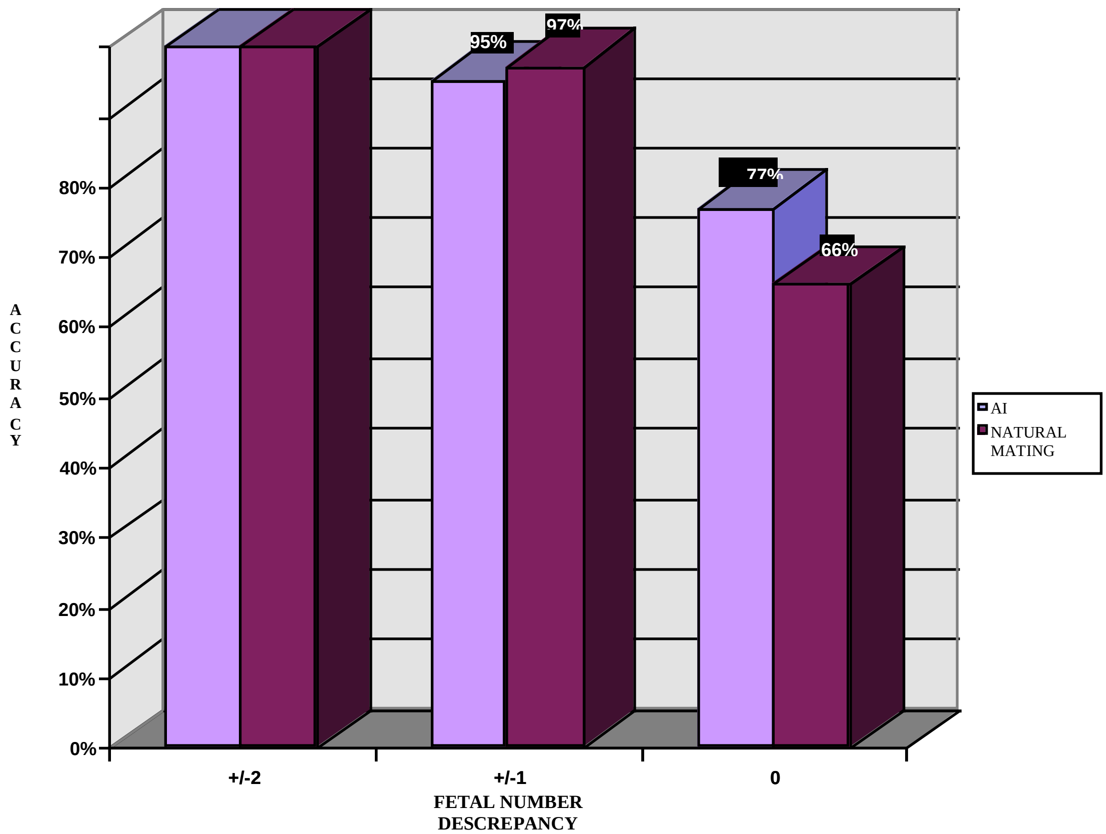

Figure 1. Histograph of the cumulative accuracy of transabdominal ultrasound predicting fetal number in ewes lambing to AI and to natural mating.

Accuracy of predicting fetal numbers tended to be greatest for singles and least for triplets or more (Figure 2). Of the ewes predicted to have three or more feti by ultrasound, only 42.9% or 45.4% actually gave birth to triplets or more, but of ewes predicted to have 1 fetus by ultrasound, 70% to 93.8% gave birth to a single lamb.

Gestational age for ewes lambing to AI was predicted within ±4 days (about one week) 71% of the time and within ±7 days (two weeks) 95% of the time (Figure 3).

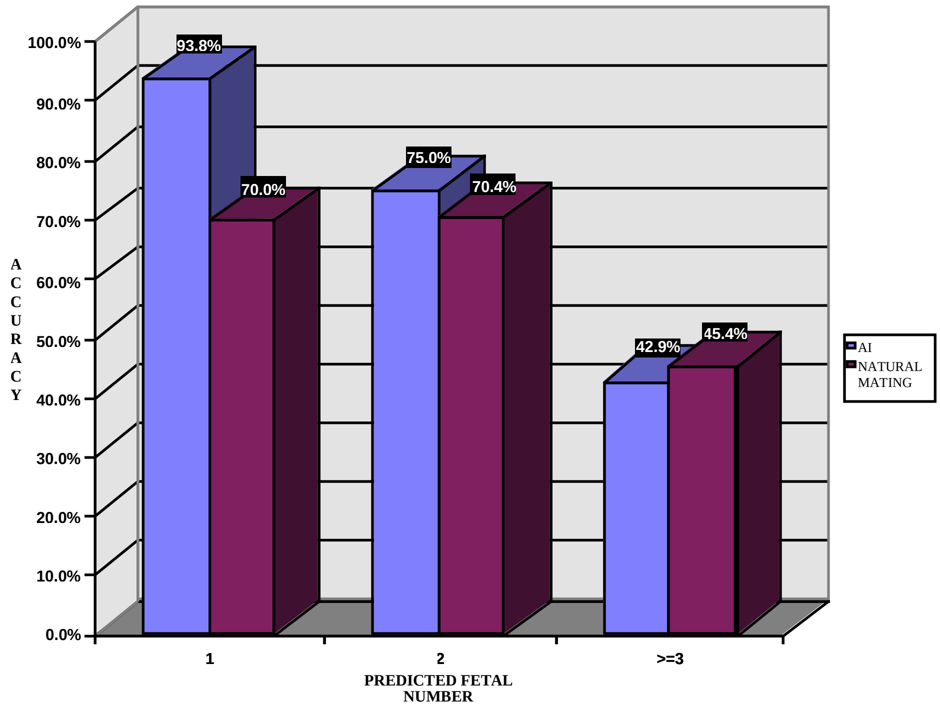

Figure 2. Histograph of accuracy of transabdominal ultrasound in predicting fetal number for ewes lambing to AI or natural mating.

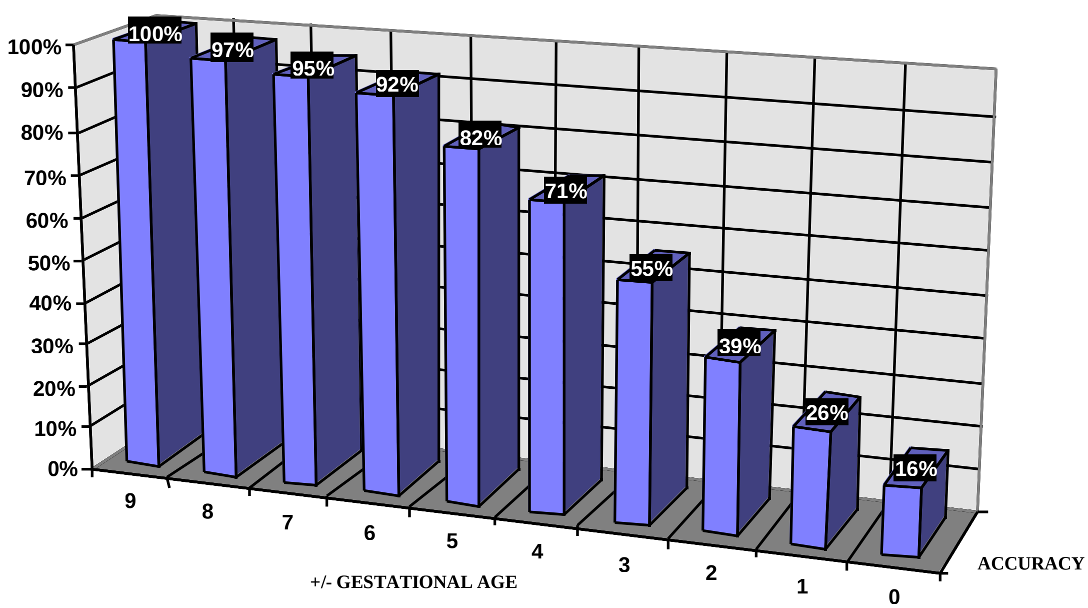

Figure 3. Histograph of the cumulative accuracy of transabdominal ultrasound of predicting gestational age for ewes lambing to AI.

Implications

Laparoscopic insemination is superior to transcervical insemination for pregnancy rates, and the inferiority of the latter is due largely to its inconsistency. Regardless of insemination method used, insemination performed after 52 hours post-progestagen pessary removal resulted in higher lambing rates and higher fertility of ewes not conceiving to AI but conceiving to subsequent natural mating. However, the AI procedures followed by natural mating appeared to result in a lower overall flock reproductive performance than natural mating alone. Transabdominal ultrasound can be employed between 40 and 60 days of gestation to accurately predict pregnancy, gestational age, and fetal number. Knowledge of the reproductive status and fetal number of ewes allows shepherds to effectively manage their sheep flocks in terms of nutrition and labor for increased economic returns.

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