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INVESTIGATION OF A NOVEL NON-SURGICAL METHOD OF ARTIFICIAL INSEMINATION FOR SHEEP

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ABSTRACT: Transcervical artificial insemination (**AI**) with sheep is not frequently used in the US due to low fertility rates. Consequently, laparoscopic AI has been employed to circumvent this situation. The problem with this technique is that while it provides satisfactory levels of fertility the degree of technical expertise necessary to perform the procedure, the costs associated with purchasing equipment, and/or the costs of hiring an inseminator are prohibitive for many producers. Therefore, the purpose of this study was to investigate a transcervical method of AI that is nonsurgical, simple to perform, and inexpensive. The estrous cycles of 40 ewes were synchronized using CIDRs for 12 days. Upon CIDR removal vasectomized rams (n = 4) fitted with raddle harnesses were turned out with the ewes (one ram per 10 ewes in separate pens) to detect estrus. After ewes were observed in estrus they were removed from the pen and inseminated twice with either fresh (n = 19 ewes) or frozen-thawed semen (n = 21 ewes) 15 and 24 hours after observed estrus using a spiral sow insemination catheter. Least square means for lambing rates for fresh and frozen-thawed treatments were 55% and 9%, respectively ($P < 0.05$). However, mean prolificacy rates did not differ ($P > 0.05$) for fresh (1.3) or frozen-thawed (1.0) treatments. These results demonstrate that this economical, easy to use method of non-surgical AI for sheep is feasible but the results indicate that improvement is needed. Consequently, future studies will be performed with the goal of improving fertility with this technique, particularly when utilizing frozen-thawed semen, which should prove valuable to sheep producers.

Key Words: Artificial insemination, Sheep, Semen.

Introduction

Ram semen can be readily cryopreserved, stored indefinitely, and still produce acceptable levels of fertility (> 50%) provided that laparoscopic insemination is used (Wulster-Radcliffe et al., 2004; King et al., 2004; Fair et al., 2005). When cervical insemination is used the results are more variable and dramatically different with reports of lambing rates from 4 to 75% (Wulster-Radcliffe et al., 2004; King et al., 2004; Fair et al., 2005; Paulenz et al., 2005). Still, non-surgical AI is highly desirable because of its low cost and ease of use.

AI using cryopreserved semen is advantageous to producers because it enables the use of diverse genetics and increases genetic progress. Currently AI is not readily used in the US because of a lack of available frozen ram semen and the difficulty and expense of insemination. The most effective sheep AI method is the laparoscopic (surgical) procedure which is expensive and requires a trained technician. Cervical and vaginal insemination is inexpensive but fertility is generally low because the cervix is not readily breached and as a result the semen is rarely deposited in the uterus (King et al., 2004; Fair et al., 2005; Paulenz et al.,2005). Therefore, a method is needed to enable a producer to non-surgically inseminate sheep.

The goal of this research was to explore a new non-surgical method of sheep AI that is inexpensive, easy to perform and could be conducted by producers.

Materials and Methods

Animal Care. All procedures were approved by the University of Wyoming Institutional Animal Care and Use Committee. Two post-pubertal Rambouillet rams and 40 western white-faced ewes were fed a diet that met all of their nutritional needs and ad libitum water.

Semen Processing. Semen was collected from rams using electroejaculation (Evans and Maxwell, 1987). An aliquot of each ejaculate was diluted in 37ºC Tris buffered saline (Purdy and Graham, 2004) and evaluated using bright field microscopy to ensure that all samples used for fresh or frozen-thawed insemination had an initial minimum of 70% total sperm motility.

Pooled ejaculates from two rams were used for fresh insemination and were diluted in 37ºC Tris buffered saline to 400 x 10^6 sperm/mL and loaded into 0.5 mL French straws (IMV USA, Minneapolis, MN). The samples were maintained at 23ºC until insemination, which was always less than 2 h, and the motility was periodically evaluated to ensure the minimal quality (70% motility).

Ejaculates from the same two rams were used for frozen-thawed insemination. Ejaculates were collected and diluted with Tris-egg yolk-glycerol media (15% egg yolk, 5% glycerol by volume; Sanchez-Partida et al., 1998) to 400 x 10^6 sperm/mL in one step. The samples were then cooled to 5ºC over two hours, held at this temperature for 24 hours, loaded into 0.5 mL French straws, and frozen using a programmable freezer and the following freeze curve: 5ºC to -5ºC at 4ºC/min, -5ºC to -110ºC at 25ºC/min, -110ºC to -140ºC at 35ºC/min. The samples were then plunged into the liquid nitrogen for storage. Samples were thawed by placing the straws in a 37ºC water bath for 30s. Frozen-thawed samples were analyzed for motility using computer automated semen analysis (Hamilton-Thorne Motility Analyzer, Beverly, MA) as described by Purdy (2006).

Estrous Synchronization and Heat Detection. Cycling ewes aged three to seven years were treated with CIDRs for 12 days and assigned to treatment groups which

were stratified across the available ewes based on semen treatment (fresh or frozen-thawed) and ewe age. Once the CIDRs were removed the ewes were placed in pens (10 ewes per pen) and a vasectomized ram fitted with a raddle harness and crayon was introduced into each pen. Ewes were considered to be in estrus when they would stand and allow the vasectomized ram to mount. The time of mounting was noted and the marked ewes were removed from the pen. The ewes were exposed to different rams every two hours.

Artificial Insemination. Ewes were inseminated 15 and 24 hours after observed estrus using a single straw of semen containing 200 x 10^6 sperm at each insemination time. Straws were loaded into an All-2-Mate goat insemination gun, covered with an Apex sheath (Figure 1-A), and then inserted into a spiral tip swine insemination catheter (Figure 1-B; Continental Plastics, Delavan, WI). The catheter was cut prior to loading so that the prepared AI gun could be pushed forward and the tip of the gun would protrude from the opening in the spiral catheter (Figure 1-C). The semen could then be expelled from the gun into the ewe with minimal back-flow into the catheter. Ewes were placed in a squeeze chute and the vagina of the ewe was opened using a 4 inch duck billed speculum. The tip of the spiral catheter was lubricated using a non-spermicidal obstetric lubricant and inserted into the cervix of the ewe. The catheter was threaded through the cervix of the ewes using a slight forward pressure and the semen was deposited once the catheter could be threaded no further. The catheter was then removed by reversing the threading motion. All inseminations were performed by the same inseminator. The fertility (number of ewes lambing) and prolificacy (number of lambs born per ewe lambing) was determined.

Statistics. Differences in fertility and prolificacy were determined using Chi-square analysis and included the effects of semen treatment (fresh or frozen), ewe age, and their interaction (SAS, 1985).

Results

Sperm Motility. As stated previously, all fresh samples had total motility of at least 70%. The motility of the frozen-thawed pooled samples was 32% and 19% for total and progressive motility, respectively.

Fertility and Prolificacy. The age of the ewes was a non-significant source of variation for fertility and prolificacy ($P > 0.05$). Semen treatment was a significant source of variation for fertility (55% and 9% for fresh and frozen-thawed semen, respectively). In contrast, semen treatment was not a significant source of variation for prolificacy (1.3 and 1.0 lambs were born per ewe lambing for the fresh and frozen-thawed semen treatments, respectively). The interaction of ewe age and semen treatment provided a significant source of variation (Table 1).

Discussion

The resulting fertility demonstrates two important points; first, the method was successful as live offspring were produced, and second, based on the fertility rates, modifications are needed to improve fertility with this technique, particularly with frozen-thawed semen. Significant challenges for developing this technique exist. Previous reports mentioned obstacles to achieving fertility using cervical insemination; namely the inability to traverse the ewe's cervix (Kershaw et al., 2005), the lack of inexpensive equipment for AI (Wulster-Radcliffe et a.., 2004), and varying information concerning AI dose and insemination time when using frozen-thawed semen (Salamon and Maxwell, 1995). From our research we have been able to address, to varying degrees, each of these issues.

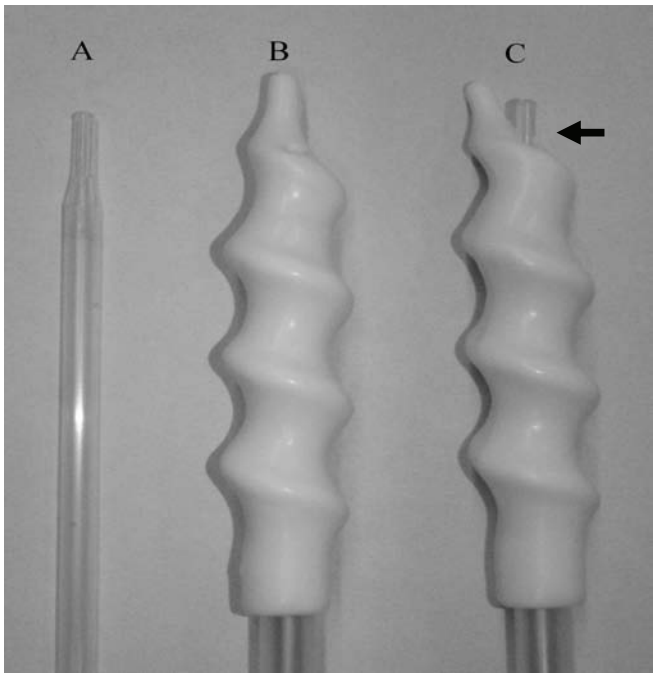

Figure 1. Illustration of the Apex sheath (A), swine spiral catheter (B), and spiral catheter fitted with the AI gun and Apex sheath ready for insemination (C). Note the arrow pointing to the Apex sheath/AI gun extended from the catheter.

Table 1. Fertility rates by ewe age and semen treatment. Treatment 1 = fresh semen and 2 = frozen-thawed semen.

Ewe age	Treatment	Pregnant/bred	% pregnant
3	1	2/2	100^a
4	1	2/5	40^{ab}
5	1	4/4	100^a
6	1	0/5	0^b
7	1	1/3	33^b
3	2	1/3	0^b
4	2	1/4	25^b
5	2	0/6	0^b
6	2	1/5	20^b
7	2	0/2	0^b

Values within a column with a different superscript differ at $P < 0.05$. SEM = 5.5.

Current hypotheses assume that it is not possible to traverse the cervix of a ewe due to its convoluted structure and even if it is possible to enter the cervix the stimulation or trauma would reduce fertility (Wulster-Radcliffe et al.,

2004). The swine catheter was used because we hypothesized its threaded structure would facilitate passage through the cervix without causing trauma. Most other cervical inseminations utilize an insemination device that had a bent tip so that it can potentially be worked into the cervix (King et al., 2004; Wulster-Radcliffe et a.., 2004; Fair et al., 2005) but we hypothesized the insertion depth is limited because the folds can not be pushed aside. In our experiences we learned that we were able to enter the cervix, and in most instances, we believe we were able to enter the uterus based on the depth of the insertion and the eventual decrease in resistance on the catheter when being threaded. This occurred for the majority of the inseminations. In the instance when it did not occur the ewes were not presenting strong signs of estrus; i.e. less engorgement of the vulva and less mucus present. When the ewes were presenting strong signs of estrus inseminations using this method typically took less than 45 seconds. We also observed that the catheters could be threaded quite easily. Only one ewe presented a trace amount of blood on the catheter when inseminated. Similarly, in a separate experiment with Black Welsh Mountain ewes, a lighter breed (~34 kg), the catheters were trimmed to decrease the outer diameter of the device and only two out of 56 ewes presented trace amounts of blood on the catheter which demonstrates the lack of invasiveness of the technique (P. Purdy, unpublished data).

In the initial adaptation of the insemination device in previous years we learned that we could deliver a semen dose although there was considerable backflow of the inseminate in many instances (P. Purdy, unpublished data). Consequently the device was modified to that which was presented here and the amount of the dose that remained in the catheter was greatly reduced. This device is cost effective on a per animal basis ($1.29 per head) when 100 animals are inseminated. This demonstrates that we have identified an inexpensive device that may be a reasonable expense for many producers.

We also believe based on our results and other reports (King et al., 2004; Fair et al., 2005) that the sperm number may need to be adjusted. The two inseminations doses contain 128 x 10^6 motile sperm which is considerable less than that reported by King et al. (2004) where 400 x 10^6 progressively motile frozen-thawed sperm were cervically inseminated and this resulted in a fertility rate of 42%.

In addition, the timing of the AI may have impacted the resulting fertility. Very little is known about the timing of AI when using cervical AI. Many reports suggest using two inseminations 12 and 24 hours post estrus detection (Salamon and Maxwell, 1995) but because we were using frozen-thawed semen we adjusted this to 15 and 24 hours post estrus detection speculating that this would be closer to the time of ovulation and thus the sperm would be in the ewe's reproductive tract for less time. As we were not using drugs to induce ovulation, in the hopes of creating an inexpensive protocol that could be used by producers, we believe that this also contributed to a decrease in fertility as the ovulation times were most likely highly variable. The significant age x semen treatment effect is interesting and could suggest that younger ewes (ages 3 to 5) are potentially better AI candidates due to greater cervical elasticity or a more consistent ovulatory pattern in relation to AI timing.

The results demonstrate that fertility can be achieved with this technique but improvements need to be made in order to reach the levels of the laparoscopic AI. While we are confident the device can successfully deliver an insemination dose more research is needed in regards to amount of sperm per AI dose and AI timing with this technique. Future efforts will focus on adjusting both of these factors with the goal of achieving greater fertility.

Implications

Many benefits can be derived from creating a user friendly technique like the one described here. First, with improvements, this technique could be used by producers to perform AI on their own sheep thereby reducing costs. This would enable a producer to purchase frozen semen from rams with desirable traits and change the genetics of their flock in a short time.

Secondly, once the technique has been improved and greater fertility achieved commercial stud services may be encouraged to collect, cryopreserve, and market genetically desirable rams. Furthermore, we previously demonstrated that ram semen could be cooled and held for up to 48 hours at 5ºC prior to cryopreservation and this did not impact fertility when laparoscopic inseminations were performed indicating the samples were able to maintain their fertilizing ability (Purdy, 2006). Therefore, this approach could also facilitate the sale and use of fresh semen collected at various studs and sent to producers, much the same way the swine industry utilizes highly productive genetics. Thus, this could benefit both the producers, by enabling them to purchase cataloged semen in a manner similar to bull semen, and the commercial enterprises because it would expand their stud service operations.

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