On-Farm Validation of a Liquid Semen Vaginal Artificial Insemination Protocol in Hair Sheep

Abstract
A low-input and simple liquid semen vaginal artificial insemination (AI) protocol developed at Virginia State University was evaluated on four commercial small farms during the peak of the breeding season. Pregnancy rates of 20%-70% were achieved. These results suggest that use of the described AI protocol would provide an effective means of enhancing the genetic potential of herds and maximizing profits for small-scale sheep producers. Cooperative Extension personnel can promote the use of liquid semen vaginal AI as a practical and effective tool for genetic improvement and facilitate its use among interested farmers.

Keywords: artificial insemination, hair sheep, liquid semen, pregnancy

Introduction
Many small and beginning farmers in Virginia are expressing a growing interest in raising small ruminants, especially hair sheep, because they require less labor and feed inputs compared to other livestock. Additionally, the importation of lamb has increased over the last decade (Agricultural Marketing Resource Center, 2012). This situation represents a gap in supply that small-scale/limited-resource farmers in the United States may be able to take advantage of, thereby gaining additional income from an alternative farming venture (Lowry, 2014) or through pooling of resources (Cross, Mills, & O’Conner, 1990). In spite of these circumstances, many small-scale sheep producers lack access to the desirable and affordable breeding stock (Madden, 2010) required to enhance the genetic potential of their herds and maximize profits. There are also health risks associated with purchasing new animals. This issue is even more critical in the wake of growing anthelmintic resistance and concern over drug-resistant parasites being carried from one location to another.

The use of artificial insemination (AI) in small ruminants provides a means for transferring genetic material while eliminating health concerns associated with the movement of live animals between locations. There is a need to make AI more widely available to small ruminant producers, with a significant role for Extension to play in this process (Walker, Vaught, Walker, & Nusz, 2011). Virginia State University (VSU) has developed a simple vaginal AI (shot-in-the-dark) procedure for use in hair sheep that provides acceptable pregnancy rates and has the potential to expand the use of AI in small farm settings and increase the genetic potential.
of many flocks. The vaginal procedure proposed was initially developed using prolific sheep breeds in Norway (Paulenz, Ådnøy, Fossen, Söderquist, & Berg, 2002; Paulenz, Söderquist, Ådnøy, Fossen, & Berg, 2003) and addresses the problem of cervical passage in sheep (Kaabi et al., 2012). In the Norway research, conception rates were 63%, with researchers using liquid semen on 52 farms (Paulenz et al., 2002), and lambing rates ranged from 56% to 83% across 10 farms and 58% to 78% across ewes bred with six rams (Paulenz et al., 2003). At VSU, pregnancy rates of 65% and 85% were initially achieved with extended semen used within 2–4 hr of processing with a single or double insemination, respectively (Wildeus, 2012). Pregnancy rates dropped to 40%–50% when semen was stored at refrigerated temperatures for 12 hr and inseminated once or twice (12 hr apart). When liquid semen vaginal AI was attempted outside the breeding season, pregnancy rates decreased to 15%–25%. As a "proof of concept," we conducted seven small-scale on-farm AI trials over a 3-year period to determine whether this technique could be applied with comparable outcomes under commercial conditions.

**Methods**

We conducted seven AI trials in October/November of 2014, 2015, and 2016, during the peak of seasonal breeding. Participating producers were from four farms within a 3-hr driving distance of VSU's Research Station. These producers had previously indicated an interest in obtaining sheep genetic resources available at our location. Estrus was synchronized in 10 Barbados Blackbelly ewes (Farm A) and five Barbados Blackbelly ewes (Farm B) in 2014; seven Barbados Blackbelly ewes (Farm A), five Barbados Blackbelly ewes (Farm C), and 20 crossbred hair sheep ewes (predominantly Katahdin) (Farm D) in 2015; and 10 Barbados Blackbelly ewes (Farm A) and 30 crossbred hair sheep ewes (predominantly Katahdin) (Farm D) in 2016. Producers synchronized estrus by inserting controlled internal drug-releasing (CIDR) devices, leaving them in for 10 days and removing them 48 hr prior to initial insemination. The morning of each insemination, we collected semen from Barbados Blackbelly and St. Croix rams housed at VSU, using an artificial vagina; we subsequently used the semen from the Barbados Blackbelly rams for insemination of ewes on Farms A, B, and C and semen from the St. Croix rams for insemination of ewes on Farm D. We used semen from two rams on each farm with the exception of Farm D, where semen collected from four rams was used each insemination year.

We extended semen of satisfactory quality (see Table 1) to a final concentration of 250 million/ml in a one-step dilution with a simple ultraheat-treated skim-milk-and-egg-yolk (5%) extender and packaged it into 0.5-ml color-coded straws. Semen straws were then placed in Styrofoam shipping containers with gel cooler packs, allowed to cool to 5°C, and maintained at this temperature during transport to cooperating farms by car. For AI, we used a standard goat insemination pipette with a round tip to deposit semen adjacent to the external cervical os without the use of a speculum. This was achieved by gently inserting the insemination pipette in the vagina at a 45° angle until resistance was felt (10–13 cm). At the initial insemination, cooperating farmers were trained in the technique and later conducted either one or two additional inseminations, at 6 hr (all farms) and 12 hr (Farms A and B, year 1) after the initial insemination. Cooperating farmers were allowed to place their own rams with ewes 5 days after the last insemination to breed ewes not bred by AI on the next cycle. Pregnancy success resulting from AI was determined by conducting transrectal ultrasound 22–23 days after insemination.

**Results**
Ejaculates used to prepare straws for insemination showed considerable variation in volume (0.4–2.5 ml) and concentration (1.2–6.3 billion sperm/ml), but only a limited range regarding estimates of motility, ranging from 70% to 95% (Table 1).

Table 1. 
Ejaculate Characteristics of Rams Used in On-Farm Insemination Trials

<table>
<thead>
<tr>
<th>Year</th>
<th>Farm</th>
<th>Ram¹</th>
<th>Volume (ml)</th>
<th>Concentration (billion sperm/ml)</th>
<th>Motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Farm A</td>
<td>BB #9234</td>
<td>1.1</td>
<td>4.7</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BB #1051</td>
<td>0.8</td>
<td>4.0</td>
<td>90</td>
</tr>
<tr>
<td>2014</td>
<td>Farm B</td>
<td>BB #9244</td>
<td>0.6</td>
<td>3.4</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BB #2032</td>
<td>0.4</td>
<td>3.1</td>
<td>80</td>
</tr>
<tr>
<td>2015</td>
<td>Farm A</td>
<td>BB #2032</td>
<td>1.0</td>
<td>5.2</td>
<td>90</td>
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<tr>
<td></td>
<td></td>
<td>BB #9394</td>
<td>1.0</td>
<td>4.3</td>
<td>90</td>
</tr>
<tr>
<td>2015</td>
<td>Farm C</td>
<td>BB #1051</td>
<td>0.9</td>
<td>3.8</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BB #9244</td>
<td>0.4</td>
<td>1.2</td>
<td>70</td>
</tr>
<tr>
<td>2015</td>
<td>Farm D</td>
<td>SC #1307</td>
<td>1.4</td>
<td>5.0</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC #2059</td>
<td>1.6</td>
<td>5.2</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC #2063</td>
<td>1.5</td>
<td>2.6</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC #2091</td>
<td>2.5</td>
<td>4.1</td>
<td>90</td>
</tr>
<tr>
<td>2016</td>
<td>Farm A</td>
<td>BB #1071</td>
<td>1.8</td>
<td>4.3</td>
<td>80</td>
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<tr>
<td></td>
<td></td>
<td>BB #5170</td>
<td>1.7</td>
<td>6.3</td>
<td>80</td>
</tr>
<tr>
<td>2016</td>
<td>Farm D</td>
<td>SC #1307</td>
<td>1.9</td>
<td>5.1</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>5.6</td>
<td>95</td>
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<td>SC #2063</td>
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<td>SC #2091</td>
<td>1.2</td>
<td>3.5</td>
<td>95</td>
</tr>
</tbody>
</table>

¹Ram breeds used in the study included Barbados Blackbelly (BB) and St. Croix (SC).

In 2014, one ewe from each cooperating farm lost its CIDR device, was not properly synchronized, and thus was excluded from analysis. On Farm B, one ewe had a reproductive tract abnormality that did not allow proper placement of the CIDR device and insertion of the AI gun and thus was also excluded. On Farm A, of nine ewes inseminated with semen from two rams, two became pregnant, for an overall farm pregnancy rate of 22% (Figure 1). On Farm B, three ewes were presented for AI, and two became pregnant, for an overall farm pregnancy rate of 67% (Figure 1).

In 2015, trials were conducted on three farms. On Farm A, seven Blackbelly ewes were inseminated with semen from two rams, resulting in five pregnancies, for a farm pregnancy rate of 71% (Figure 1). Two 7-month-old ewe lambs also were inseminated on Farm A but failed to become pregnant. On Farm C, five Blackbelly ewes were inseminated, resulting in one pregnancy, for a farm pregnancy rate of 20% (Figure 1).
On Farm D, 20 mature crossbred hair sheep ewes, predominantly Katahdin, were inseminated with semen from four St. Croix rams, resulting in nine pregnancies, for an overall pregnancy rate of 45% (Figure 1). On this farm, twelve 6-month-old Dorper crossbred ewe lambs also were inseminated but failed to become pregnant.

In 2016, trials were again conducted on Farms A and D. On Farm A, 10 Blackbelly ewes were inseminated with semen from two rams, resulting in four pregnancies, for an overall farm pregnancy rate of 40% (Figure 1). On Farm D, 30 crossbred hair sheep ewes were inseminated with semen from four St. Croix rams, resulting in 10 pregnancies for a farm pregnancy rate of 30% (Figure 1).

Figure 1.

Pregnancy Rates of Ewes Inseminated Vaginally with Chilled Liquid Semen at Different Farms in Different Years

Semen from a total of 10 different hair sheep rams was used for AI during our project. Although the number of inseminations per sire were limited (4–14), there was considerable sire variation in the AI pregnancy rates, ranging from 0% to 100% (Figure 2). With increasing number of inseminations (greater than 10), pregnancy rates became less variable and were representative of overall pregnancy outcomes for this technique.

Figure 2.

Effect of Sire on Pregnancy Rates of Ewes Inseminated Vaginally with Chilled Liquid Semen
Discussion

Semen quality parameters of individual rams were not related to the wide range of pregnancy rates achieved by the rams (Figure 2). This considerable ram variation in pregnancy outcomes also was observed in earlier trials at our research location (Wildeus, 2014) and in other studies (Donovan, Hanrahan, Kummen, Duffy, & Boland, 2004; Paulenz et al., 2002).

The limited numbers of animals spread across cooperating farms and years, along with the lack of procedural control associated with on-farm research, likely contributed to the range in pregnancy rates observed in our study (20%–70%). However, the on-farm outcomes were comparable to results obtained in our research flock, when timed AI and 12-hr-stored semen were used (39%) (Wildeus, 2012). In other countries, liquid semen AI systems in sheep are more commonly used, and pregnancy rates range from 54% to 64% in Norway (Paulenz et al., 2003), 31% for semen stored for 6 hr (Anel et al., 2005) and 54% for semen stored for 1.5 hr (Palacín et al., 2012) in Spain, and 35% for 12-hr-stored semen (Olivera-Muzantea, Fierroa, López a, & Gil, 2011) in Uruguay. In Ireland, pregnancy rates linearly decreased with storage from 0 hr (60%) to 72 hr (18.3%) (O'Hara et al., 2010). In many of these countries, structures are in place to facilitate liquid semen AI, such as producer co-ops and trained inseminators, and Extension can have a role in setting up similar structures in the United States.

Increasing lamb imports over the last decade (Agricultural Marketing Resource Center, 2012) indicate that there is a gap in supply that small-scale/limited-resource farmers in the United States might take advantage of and thereby gain additional income from sheep production. In addition, a recent consumer study conducted at VSU indicated that consumers of a local food hub would purchase local ground lamb (>96%), would do so at least once per month (35%), and would pay a premium (43%) if it were available (Nartea, Wildeus, Lee, & O’Brien, 2017). However, as stated previously, many small-scale producers lack access to desirable and affordable breeding stock from which to purchase animals that would enhance the genetic potential of their herds and maximize profits. A liquid semen vaginal insemination technique may provide a
practical and economical way for producers to capitalize on current opportunities.

Conclusion and Implications for Extension Personnel

Based on the results of our study, liquid semen vaginal insemination lends itself particularly well to a regional approach of breeders' co-ops within driving distance that can share desirable germplasm managed at selected farms. This scenario will require assistance and training from Extension personnel and access to semen processing equipment.

Use of liquid semen vaginal insemination in hair sheep might be applicable to other locations with support and guidance from Extension practitioners. Recent developments in the U.S. sheep industry, driven primarily from the increase in alternative markets as well as a decline in the profitability of wool compared to lamb, have resulted in the growth of hair sheep production across the United States. The use of AI, as described in our study, would provide an effective means of moving germplasm readily between farms to expand available hair sheep genetics in many states. AI also addresses concerns regarding disease transmission related to the introduction of live animals to closed flocks and welfare of animals associated with transport over long distances.

Acknowledgments

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References


