Application of Laparoscopic Artificial Insemination Techniques to the North Dakota Sheep Industry

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The small and tortuous nature of the cervical canal in sheep has made surgical or laparoscopic artificial insemination (LAI) the standard technique for AI in the U.S. sheep industry. However, considerable variation still exists when using this technique (conception rates from 10 to 85%). This report details our recent research efforts from NDSU and other institutions, and discusses our future research directions for further improving the LAI technique.

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Introduction

The primary goal of any artificial insemination (AI) program is to create better offspring. Laparoscopic AI is being used in the sheep industry around the world to extend the use of superior rams, and it offers the producer an opportunity to maximize the reproductive potential of his/her sheep. The primary economic benefit to the sheep producer is rapid infusion of valuable genetic traits into the flock.

Four primary factors will contribute to the success of any AI program:

1. Quality of the frozen semen being used;
2. Proper application of an appropriate estrous synchronization protocol;
3. Subsequent physiological "readiness" of the ewe being inseminated;
4. Precision of the laparoscopic AI technique.

Estrous Synchronization

Since LAI is a surgical procedure that requires equipment set-up and ewe preparation, all ewes must come into heat or estrus at a uniform point in time to make the procedure feasible. Synchronization of estrus involves inserting a sponge or CIDR device impregnated with progestogens. Both devices are left in place for 12-14 days and the progestogen is slowly absorbed into the ewe's blood stream. The progestogen inhibits follicle development on the ewe's ovary and prevents her from coming into heat or estrus. Once the device is removed, the progestogen level in her circulation will drop off and follicles will develop. At the time of removal, pregnant mare's serum gonadotropin (PMSG) or PG600 (a product which contains PMSG) is given to ensure follicle development and ovulation. Normally, 400 international units (IU) of PMSG are given, but if the procedure is being performed during the natural breeding season, less can be administered. If too much PMSG is given to the ewe, her ovulation rate will be too high and this may result in an excessive number of lambs born. Insemination should take place 50 to 60 hours after progestogen withdrawal.

Laparoscopic Artificial Insemination

All ewes are fasted and restricted access to water for 16 hours before the procedure. Ewes are injected with anesthesia 15 minutes before the procedure is performed. The ewe is placed on her back in a laparoscopy cradle. The abdominal region is surgically prepared by shearing the wool and disinfecting the skin. Using the cradle, the rear legs of the ewe are lifted to an approximate 45 degree angle (Figure 1). Two very small incisions are made in the skin of the abdomen to facilitate puncturing the abdominal wall with trocars. Once the abdominal wall is punctured, a laparoscope is placed through one of the cannulas into the abdominal cavity. The laparoscope contains a fiberoptic light, which permits the operator to view the ewe's reproductive tract. Carbon dioxide is used in the abdominal cavity to help visualize the uterus and seperate it from the abdominal wall. The less the reproductive tract

is manipulated, the better the conception rate. Once the uterus is in the correct position, an insemination pipette containing the semen is inserted. The operator then punctures the uterine horn and the semen is injected directly into the lumen of the uterus, and the same procedure is repeated on the other uterine horn. A minimum of 25 million motile sperm (100 µl volume) are deposited in each uterine horn. Once both horns have been inseminated, the cannulas are removed and a topical antibacterial spray is applied on the two small incisions. The entire procedure takes only 5 to 10 minutes. The incidence of infection because of this minor surgery is extremely low.

Figure 1. A ewe placed in the cradle and prepared for LAI.

Current Research Efforts at NDSU

As discussed above, the best method for synchronizing estrus or heat in a group of ewes involves treatment with intravaginal progestogens. Sponges generally contain synthetic sources of progesterone (medroxyprogesterone acetate), whereas CIDR devices contain natural sources of progesterone.

Neither of these products is commercially available in the U.S., although FDA approval of CIDR devices for use in sheep and goats is currently pending.

We have recently evaluated estrous response and pregnancy rate following laparoscopic AI in 129 ewes treated with Sponges or CIDR devices (Luther et al., 2007). The percentage of ewes displaying estrus or 'heat' after treatment with Sponges (88%) and CIDR devices (95%) did not differ. In addition, a high percentage of ewes became pregnant to laparoscopic AI in both groups (Figure 2). This study suggests that approval of CIDR devices by the FDA will provide us with a useful tool for estrous synchronization and laparoscopic AI in sheep.

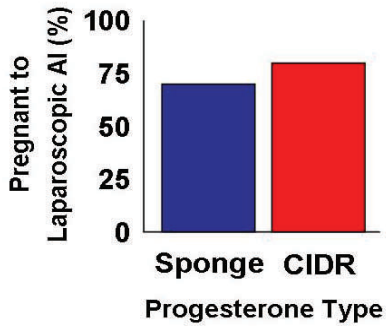

Figure 2. Similar pregnancy rates were achieved following synchronization with sponges or CIDRs.

Pregnant mare's serum gonadotropin (PMSG) is a product that is commonly injected at removal of a CIDR device or Sponge. PMSG helps to ensure that the release of an egg for fertilization or 'ovulation' occurs. We have recently shown that pregnancy rates are greater to laparoscopic AI when ewes are injected with PMSG (Figure 3, Luther et al., 2007).

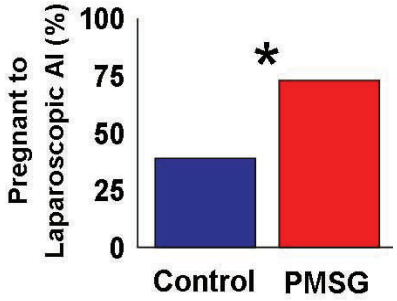

Figure 3. Injection of 400 IU of PMSG at progestogen removal increased pregnancy rates to LAI (Luther et al., 2007).

However, similar to the progesterone products, PMSG is not commercially available in the U.S. The latter has prompted us to investigate the alternative use of PG600. PG600 is a product that is commonly used in pigs and it contains both PMSG and hCG. Preliminary data, collected from only 35 ewes would suggest that relatively high pregnancy rates to laparoscopic AI are achieved when PMSG or PG600 is injected at removal of a CIDR device (Figure 4, Luther et al., unpublished).

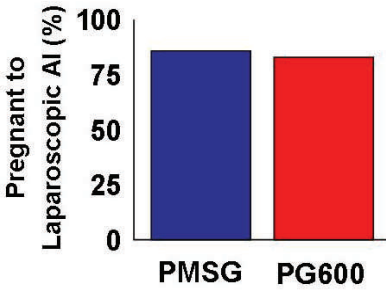

Figure 4. Injection of PMSG or PG600 at progestogen removal results in similar pregnancy rates to LAI (Luther et al., 2007)

These data support the use of a standard estrous synchronization protocol for laparoscopic AI utilizing products that will be, or currently are commercially available in the U.S. (Figure 5).

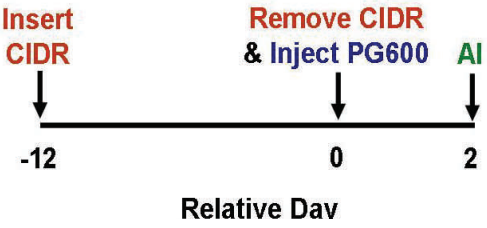

Figure 5. Standard estrous synchronization protocol developed for LAI in sheep.

Future Directions

Although we have achieved reasonable success with LAI in sheep. Additional research is still required to improve the technique. The results reported herein were gathered using sheep housed at NDSU in Fargo. In a

cooperating project between NDSU and sheep producers throughout the state we have achieved variable conception rates (10 to 85%). Thus far this project has found that:

- Considerable variability in conception rates occurs between farms;
- Season (AI in the Fall versus Spring) can greatly affects conception rates;
- Some variability may be explained by differences in semen quality.

Future studies will continue to evaluate the use of commercially available PG600 in place PMSG. The timing of ovulation relative to injection of these products is currently not known. Transrectal ultrasonography and repeated blood sampling techniques will be used to characterize physiological and hormonal events taking place after CIDR removal. These future studies are necessary for reducing between flock variability, and promoting long-term application in the ND sheep industry.