Out of Season Artificial Insemination and Embryo Transfer Results in Ewes: A Field Trial

B Price, T Mittleider, S Collins, P Gibbons, and J Gibbons
College of Veterinary Medicine, Lincoln Memorial University, Harrogate, TN

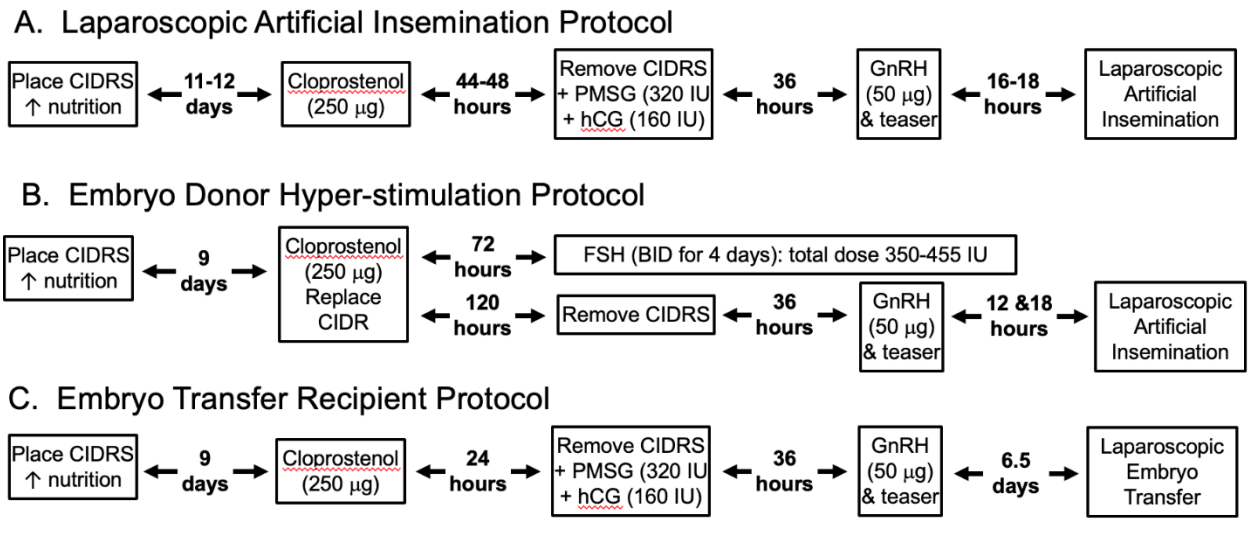
Introduction:

Ewes are seasonally polyestrous short-day breeders with an estrous cycle of approximately 16 – 17 days. In the northern hemisphere ewes have active estrous cycles and are naturally receptive to rams from late September to late December when there is less than 12 hours of daylength. However, progressive sheep breeders often prefer to breed sheep earlier in the year, during periods where there is more than twelve hours of daylength, (July and August) in order to have lambs that are appropriate to target specific show markets. In order to facilitate this out of season breeding and accelerate genetic gain, producers rely on Assisted Reproductive Techniques such as Laparoscopic Artificial Insemination (LAI), ovarian hyper-stimulation, embryo collection from valuable embryo donors, and embryo transfer (ET) into synchronized recipients (1,2,3.) This field trial was conducted during late July through early August in southwest Virginia (latitude 36-38'12" N), during a daylight period of about 14 hours. Pregnancy rates of ewes bred by means of AI were compared to those that underwent ET.

Methods:

A total of 129 ewes were synchronized using a modified Ovsynch protocol (Figure 1). All ewes scheduled for insemination, were bred with frozen-thawed semen, deposited intrauterine via laparoscopic artificial insemination, regardless of whether they displayed signs of estrus. The protocols for ewes presented for artificial insemination (Section A; n=83), hyper-stimulated embryo donors (Section B; n=13), and embryo recipients (Section C; n=33) are shown (Figure 1).

Figure 1. Modified Ovsynch® Protocols for Laparoscopic AI (A), Hyper-Stimulated Embryo Donors (B), and Embryo Recipients (C) During Long Daylenegth Periods.



Embryos were collected on Day 6 post-AI from donor ewes (n=13). Embryos were surgical recovered by exteriorizing the uterine horns and placing a catheter through a small incision in the uterine body. Depending on the number of viable embryos collected and embryo quality from each donor, 1 or 2 embryos were transferred to ET recipients on the same day they were collected.

Results:

Pregnancy status was determined by transrectal ultrasonography at approximately 30 days post-AI or 24 days post ET. Results (analyzed with Chi Square) indicated a trend for significantly higher pregnancy rates (P=0.092) in ET recipients (22/33: 66.7%) compared to ewes that underwent AI (41/83: 49.4%). On average each collection yielded ($\mathrm{Mean} \pm$ SEM) 6.6 ± 1.2 total ova, 4.7 ± 1.1 transferable quality embryos, and 1.9 ± 0.8 unfertilized ova. Embryo donors had an overall fertility rate of 72.1% compared to AI only ewes, suggesting that hyper-stimulated ovaries are minimally impacted by long daylength compared to non-hyper-stimulated ovaries. No statistical significance was observed in pregnancy rates between ET recipients and ewes subjected to LAI relative to whether the ewes displayed signs of estrus or not, nor was there a relationship between embryo quality (single embryo transferred) and pregnancy rate.

Discussion:

Hyper-stimulated ET donors displayed a higher fertility rate than the non-hyper-stimulated ewes under a similar synchronization protocol. This suggests that hyper-stimulated ovaries may not be as sensitive to the stress of out of season, long-day length, breeding as non-hyper-stimulated ovaries. The higher pregnancies rates displayed by the ET recipient ewes also support the hypothesis that the uterus may also be less sensitive to the stress of out of season breeding than the ovaries. However, other publications have shown that out of season hyper-stimulated ovaries may not produce as many ova compared to hyper-stimulation during the natural, short-day period (4). Regardless, this information is critical to producers that seek out of season breeding, indicating that there is greater success when using embryo transfer techniques versus LAI, and suggesting the need for future research to develop more effective reproductive techniques for long photoperiod mating in sheep and goats.

A contributing factor to successful ovarian hyper-stimulation is managing the follicular wave dynamics, especially dominant follicles (5). On average, sheep have about 3 follicular waves per estrous cycle (5), dominated by 1 to 3 follicles that grow large than others. The dominant follicles secrete (at least) Inhibin A and Estradiol, causing the suppression of other smaller follicles (5). Depending upon the timing of emergence of a follicular wave and the endocrine environment, dominant follicles with either become atretic or will ovulate (5). In cattle, removal of the dominant follicle prior to superovulation resulted in less required FSH and an acceptable, consistent, embryo yield (6,7). Eliminating the dominant follicle(s) prior to hyper-stimulation prevents the natural suppression of smaller follicles, allowing endogenous and exogenous FSH to promote the growth of multiple dominant follicles.

Future studies will evaluate the effects of laparoscopic follicular aspiration of dominant ovarian follicles in ewes on hyper- and non-hyper-stimulated follicular waves. Also, a direct comparison of hyper-stimulated and non-hyper-stimulated follicular waves and their endocrine

dynamics will be examined in long versus short photoperiods. The ability to engineer and supplement follicular waves in long and short daylength periods will greatly benefit progressive small ruminant producers in reaching their reproductive, genetic, and financial goals.

References:

1. Casali R, Pinczak A, Cuadro F, Guillen-Muñoz JM, Mezzalira A, Menchaca A. Semen deposition by cervical, transcervical and intrauterine route for fixed-time artificial insemination (FTAI) in the ewe. Theriogenology. 2017 Nov;103:30-35. doi: 10.1016/j.theriogenology.2017.07.021.

2. Cseh S, Faigl V, Amiridis GS. Semen processing and artificial insemination in health management of small ruminants. Anim Reprod Sci. 2012 Feb;130(3-4):187-92. doi: 10.1016/j.anireprosci.2012.01.014.

3. Fonseca JF, Souza-Fabjan JM, Oliveira ME, Leite CR, Nascimento-Penido PM, Brandão FZ, Lehloenya KC. Nonsurgical embryo recovery and transfer in sheep and goats. Theriogenology. 2016 Jul 1;86(1):144-51. doi: 10.1016/j.theriogenology.2016.04.025.

4. Yuan Y, Liu R, Zhang X, Zhang J, Zheng Z, Huang C, Cao G, Liu H, Zhang X. Effects of recipient oocyte source, number of transferred embryos and season on somatic cell nuclear transfer efficiency in sheep. Reprod Domest Anim. 2019 Nov;54(11):1443-1448. doi: 10.1111/rda.13546.

5. Menchaca A, Vilariño M, Crispo M, de Castro T, Rubianes E. New approaches to superovulation and embryo transfer in small ruminants. Reprod Fertil Dev. 2010;22(1):113-8. doi: 10.1071/RD09222.

6. Gibbons J, Anton J. Dominant follicle removal to initiate an engineered follicular wave for hyper-stimulation. Presented at the 2019 American Embryo Transfer Association Annual Conference.

7. Mittleider T, Price B, Gibbons J, Anton J. Dominant follicle removal prior to superovulation. Presented at the 2020 Society of Theriogenology Virtual Conference.