Ewes & Progestogen - 1998 Sheep Day Report

Use of a synthetic progestogen in combination with a superovulatory treatment for induction of synchronized estrus in seasonally anovular ewes.

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Introduction

Production of animals for meat is a multi-billion dollar industry in the U.S. alone. Income from sale of animals, feed consumed by animals, and meat consumed in the U.S. was, conservatively, $235 billion in 1994 (Agricultural Statistics, 1995-96), which does not include the economic impact of the food industry and its employees. Because maintenance of reproductively sound females is the primary expense for livestock producers, reproductive failure remains one of the most limiting and costly factors facing the livestock industry (Trenkle and Willham, 1977; Ferrell and Jenkins, 1985). Thus, improvements in reproductive efficiency would have a major impact on the profitability of animal agriculture. Beyond that, with the continued explosive growth of the world's population, which shows no signs of slowing any time soon, the ability to efficiently produce animal protein, especially from low-quality forages, should be and is a major driving force for agricultural research (NRC, 1989; Mann, 1997). Thus, understanding the mechanisms controlling reproductive efficiency of farm animals has important socioeconomic implications for North Dakota, the nation, and the world.

The **goal** of this research program is to develop an effective procedure for production and storage of fertile eggs from ewes during anestrus (i.e., out-of-season), when they normally are not cycling and their ovaries are quiescent. The **specific objective** of this experiment was to evaluate the use of norgestomet (a synthetic progestogen) in combination with a superovulatory treatment regimen (FSH) for the production of fertile eggs from seasonally anestrus ewes. A future objective of this line of research is to develop improved procedures for cryopreservation and transfer of sheep embryos.

Sheep are seasonal breeders, and normally exhibit ovarian function and estrous cycles during late summer and fall. Conversely, they exhibit anestrus and ovarian quiescence normally during the winter and early spring. Synthetic progestogens have been used successfully for estrus synchronization in the cycling ewe (Hinds et. at. 1961; Tilton et. al., 1966, 1967). Synthetic progestogens also have been shown to stimulate estrus behavior and ovarian activity in seasonally anestrous ewes (Haugen et al., 1997; Safranski et al., 1992; Jabbar et al., 1994). In all of these studies, however, a substantial number of ewes do not respond to progestogen treatment alone during seasonal anestrous.

However, it has been shown that a variety of gonadotropin treatments can augment the ovarian response to progestogen treatment during the anestrous period (Safranski et al., 1992; Jabbar et al., 1994). Over the past 10 years, we have developed an extremely successful technique for inducing multiple ovulations (superovulation) in cyclic ewes (Jablonka-Shariff et al., 1994, 1996). We therefore **hypothesized** that progestogen treatment in combination with our technique for inducing superovulation in ewes would provide an effective means to produce large numbers of fertile eggs from seasonally anestrous ewes.

With successful techniques for induction of out-of-season superovulation, our laboratory will begin to focus on embryo collection, culture, cryopreservation, and transfer procedures for optimizing the use of genetically superior ewes during a time when they are naturally unproductive. This technology should prove to be extremely useful to the sheep industry as a whole.

Materials and Methods

Twenty seasonally anestrous non-lactating ewes of various ages were randomly assigned to progestogen (Synchro-Mate-B [SMB], Merial Limited, Athens, GA) alone (SMB treatment, n=10 ewes) or progestogen in combination with an FSH superovulation regimen (SMB/FSH treatment, n=10 ewes). One-half of an SMB implant (½ of cow implant) was implanted into the left ear of each ewe and left in place for 10 days as described by Jabbar et al. (1994). At the end of SMB treatment, SMB implants were removed through a small incision made in the skin at the distal end of the implant. Beginning on the morning of removal of SMB implants, ewes received twice daily (morning and evening) intramuscular injections of saline (salt water) or FSH (FSH-P, a pituitary extract; Sioux Biochemical, Sioux Center, IA) for three days as follows; Day 1, 5 mg/injection; Day 2, 4 mg/injection; Day 3, 3 mg/injection (total dose = 24 mg).

Rams were introduced to ewes at the time of the first saline or FSH-P injection and remained with the ewes for a total of 25 days. Rams were brisket painted daily to aid in estrous detection. On Days 7-9 after the synchronized estrus, all ewes were subjected to a laparoscopic exam to determine ovulation rates.

Results

Table 1 shows the estrus response of ewes to the SMB or SMB/FSH treatments. Nine of 10 ewes in each treatment group expressed out-of-season estrus in response to the SMB treatment alone. Furthermore, estrus in all ewes was synchronized, with estrus occurring between 1 and 3 days after removal of the SMB implant. However, ewes receiving the superovulation treatment (SMB/FSH) exhibited estrus an average of one day earlier ($P<0.01$) than ewes receiving SMB alone (see Table 1, below).

Table 1. Estrus responses to Synchro-Mate-B and superovulation treatment in seasonally anestrous ewes.

Treatment	No. of ewes	No. ewes synchronized*	Days to estrus after SMB removal	Range in no. days to estrus
SMB	10	9	2.2 ± 0.2	1.5 - 3.0
SMB/FSH	10	9	1.2 ± 0.1	1.0 - 2.0

*Ewes in estrus within 72 hours of SMB removal.

Ovulation rates differed significantly ($P<0.01$) between the two treatments, with those ewes receiving the superovulation treatment (SMB/FSH) exhibiting 8.2 more ovulations than ewes receiving SMB alone (see Table 2). All ewes receiving the superovulation treatment were superovulated, indicating that in this study, the superovulation treatment was 100% effective. The range in ovulation rates was reasonable for use in an embryo transfer program. Only one ewe in the superovulation treatment exhibited only 3 ovulations, whereas all other ewes had 5 or more ovulations (Table 2).

Table 2. Ovarian responses to Synchro-Mate-B and superovulation treatment in seasonally anestrous ewes.

Treatment	No. of ewes	No. ewes superovulated*	Ovulation rate (no. CL)	Range in no. of ovulations
SMB	10	2[a]	1.7 ± 0.4	1-4
SMB/FSH	10	10[b]	9.9 ± 1.5	3-17

*Ewes with more than 2 ovulations.

[a]One ewe exhibited 3 ovulations, one ewe exhibited 4 ovulations

[b]One ewe was not detected in estrus by the ram but exhibited 6 ovulations in response to

SMB/FSH treatment.

Discussion

Superovulation is considered critical for the long-term success of embryo collection and transfer programs because: 1) donor animals are usually genetically superior; and 2) the procedure is costly. Thus, to ensure that embryo transfer is cost-efficient, maximizing the number of embryos obtained from each donor is important. Superovulation protocols currently in use in the animal industry, however, are

less than optimal.

Although most ewes respond to superovulatory regimens, embryo recovery rates vary between approximately 30 to 60% (Jabbour and Evans, 1991; Rexroad and Powell, 1991; Ryan et al., 1991; Evans et al., 1994). In addition, a lower embryo recovery rate is observed when ewes are superovulated and inseminated during the anestrous period (i.e., winter and early spring) compared with the normal breeding season (late summer and fall), perhaps due to the formation of sub-functional corpora lutea from the superovulated follicles (Hunter, 1991; Ryan et al., 1991). Moreover, these superovulatory regimens involve use of combinations of several hormones, including progesterone, gonadotropin-releasing hormone, pregnant mare serum gonadotropin, follicle-stimulating hormone, and(or) prostaglandin F2a (Hunter, 1991; Jabbour and Evans, 1991; Rexroad and Powell, 1991; Ryan et al., 1991; Evans et al., 1994), and thus are difficult to administer and costly.

Data from the present study not only confirms earlier reports that the use of synthetic progestogens are useful in stimulating ewes to ovulate out-of-season, but also demonstrates that the use of a simple, effective superovulation regimen developed by our laboratory for normally cycling ewes is effective for inducing superovulation in seasonally anestrous ewes (Grazul-Bilska et al., 1991; Jablonka-Shariff et al., 1994; Doraiswamy et al., 1997). This superovulation regimen resulted in 13.2 ± 0.9 ovulations in 85 ewes superovulated during the breeding season over five years (Jablonka-Shariff et al., 1994), which is slightly higher than the 9.9 ovulations observed in the present study. The differences observed from our previous work compared to the present study is likely due to the fact ewes in the present study were induced to ovulate out-of-season.

The results of this study will lead to improved methods for obtaining large numbers of high quality embryos in sheep for use in embryo transfer programs and also will provide for increased flexibility and efficiency of reproductive management for sheep producers. Future studies in our program will be carried out to compare the quality of eggs and/or embryos obtained from superovulated cyclic and seasonally anestrous ewes. Eggs from these ewes will be fertilized in vitro, and the rates of fertilization, development, and survival after cryopreservation will be evaluated. In addition, we also will examine the effectiveness of cryopreservation techniques for the long-term storage of sheep embryos. Future directions in our program also will include transfer of embryos after in vitro (in the laboratory) or in vivo (natural) fertilization.

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