# Optimal dose of PG600 when given to progestogen-synchronized ewes during anestrus as affected by day of the year and temperature[1]

**Lucy J. Cross,[†] Robin M. Cross,[‡] and Fredrick Stormshak[‡,2]**

[†]Agna, LLC, Salem, OR 97308; and [‡]Oregon State University, Corvallis, OR 97331

**ABSTRACT:** The objective of this long-term study was to improve the out-of-season breeding rates for ewes by examining effectiveness of dose levels of PG600, a combination of 80-IU/mL pregnant mare serum gonadotropin and 40-IU/mL human chorionic gonadotropin. Each week, during the anestrous season from 1998 through 2016, mature ewes were inserted with 1 of 2 progestogen devices. After 9 to 13 d, at removal of the device, ewes were injected intramuscularly with a specific dose of PG600. A total of 1,402 ewes were treated in this study, with weekly treatment groups ranging from 3 to 12 ewes. Ewes were assigned randomly in equal numbers to 1 of 2 ram pens with one fertile ram in each pen. Rams were fitted with a marking harness. Between 36 and 48 h after removal of the device, rams were switched. Ewes were checked for crayon marks (indicative of estrus) at 36, 48, and 96 h after removal of synchronizing devices. Rams were removed after 1 wk. Pregnancy status was diagnosed at 90 d of gestation using radiography and verified at 120 d or at lambing. To explore the impact of dose level, daylight, and temperature on pregnancy rate, we formulated an ordered multinomial probit model. The model controls statistically for dose level, synchronization device type, device reuse, and natural variation in minimum daily temperature and day of the year (**DOY**). Predicted pregnancy rate and type of birth at 90 d of gestation were significantly affected by DOY and temperature as well as dose level. Both observed and predicted pregnancy rates were higher for PG600 dose levels between 2.00 and 3.50 mL than for those below 2.00 mL or above 3.99 mL. By giving optimal amounts of PG600 to anestrous ewes, producers will have a greater number of pregnant ewes at lambing during the out-of-season lambing time.

**Key words:** anestrus, day of the year, ovine, PG600, pregnancy rate, temperature



Transl. Anim. Sci. 2019.3:433–441
doi: 10.1093/tas/txy092

## INTRODUCTION

To induce ewes in the Northern Hemisphere to lamb more than once per year, it is necessary to breed them when they would commonly be in anestrus. For a high success rate during out-of-season breeding, a producer must manipulate the estrous cycle. This has been done in a variety of ways with varying amounts of success. Ewes can be exposed to vasectomized rams and may show estrus earlier than they normally would. This ram effect has been explored by Cushwa *et al.* (1992). Altering daylight duration as a means of regulating reproduction in sheep has been studied as early as 1952 (Hafez, 1952); however, this can be a difficult environment for the average producer to achieve. Wheaton *et al.* (1990) showed that season

[1]The authors are grateful to J. M. Thompson for expert preliminary trial advice.

[2]Corresponding author: lucysfarm@gmail.com

Accepted August 13, 2018.

and number of days of melatonin treatment may advance the onset of breeding season and affect estrus and conception. Several studies have explored progestin supplementation followed by synthetic prostaglandins (Knights *et al.*, 2001). Melengestrol acetate (**MGA**) in the feed (Safranski *et al.*, 1992), intravaginal sponges (Butler and Maxwell, 1988), GnRH (McLeod *et al.*, 1982), Syncro-Mate-B (**SMB**) (Ainsworth and Wolynetz, 1982), and controlled internal drug–releasing (**CIDR**) devices (Wheaton *et al.*, 1993) have all been used to provoke the onset of estrus. When breeding anestrous ewes, it has been revealed that administering PMSG results in a greater ovulatory response (Butler and Maxwell, 1988). Gordon (1971) demonstrated that a 500 IU PMSG dose may contribute to higher lambing rates and that dose levels of 750 IU and higher may be detrimental to embryo survival. Ryan *et al.* (1991) suggests that embryo survival declines as dose of PMSG increases when given in spring, but not in autumn.

The objective of this study was to determine the optimal dose of PG600 given during the anestrous season that would result in maximal pregnancy rates and whether dose response was affected by variation in day of the year (**DOY**) and temperature.

## MATERIALS AND METHODS

The farm where the study was conducted is licensed by USDA Animal and Plant Health Inspection Service (**APHIS**) Animal Care and follows the Guide for the Care and Use of Agricultural Animals in Research and Teaching. It is located at 45°N and 123°W in Polk County, Oregon. The marine west coast climate is mild with an average high temperature of 17.4 °C and a low temperature of 5.7 °C and annual rainfall of 125 cm. The sheep used in the study were of mixed breed type, primarily crosses of Dorset, Suffolk, Cheviot, Polypay, Romney, and Hampshire. Age ranged between 1.5 and 4 yr; however, most were maiden ewes between 1.5 and 2 yr. In May of each study year, the 1.5 yr ewes averaged 65 kg in weight. Ewes were kept on mixed grass pasture until 90 d of gestation. After 90 d of gestation, they were fed grass hay and alfalfa/oat pellets. If ewes on pasture needed additional feed, they were supplemented with alfalfa pellets. The majority of ewes had a body condition score of 3 on the scale of 1 to 5. Ewes were vaccinated for typical abortion diseases found in the Western states. They were also vaccinated for tetanus and enterotoxemia. The sheep were free of foot rot.

PG600 (Merck Animal Health, Summit, NJ) is a product readily available in the marketplace that is approved for induction of the onset of estrus in swine. Field trials using PG600 to enhance pregnancy rates in sheep were conducted at University of Missouri Agricultural Experiment Station as early as 1990 (Safranski *et al.*, 1992). PG600 consists of 400 IU of PMSG and 200 IU of hCG suspended in 5 mL of saline solution. When administered, PG600 is injected intramuscularly. It is extremely important to producers to use the optimal dose of PG600 not only because of the cost of the PG600, but also to optimize the number of ewes lambing and the number of lambs born.

The weekly group size of 3 to 12 ewes varied throughout the study and was determined by lambing space and management of the farm. In the Northern Hemisphere, the anestrous period is considered from January to August. At weekly intervals throughout anestrus, ewes were inserted with either half a SMB or a CIDR. One half of a SMB capsule, when placed subcutaneously in the ear, provided 3-mg norgestomet, a steroidal progestin (Sanofi Animal Health, Overland Park, KS). This method was used from 1998 through 2004. SMB is no longer available in the marketplace. The Eazi-Breed CIDR (Pfizer Inc., New York, NY) contains 0.3-g progesterone in a silicone intravaginal insert. The CIDR was available for this study beginning in 2001 and used exclusively from 2005 until the study was completed in 2016. Between 2001 and 2004, some ewes received half a SMB and others received a CIDR. Animal care and handling were done by one herdsman over all the years of the study.

At the conclusion of 9 to13 d, the SMB or CIDR was removed and the ewe was given an intramuscular injection of PG600. Dose was assigned to ewes randomly over a total range of 1.50 to 5.00 mL, independent of DOY, temperature, and study year. Dose increment was 0.25 mL between 1.50 and 4.00 mL.

Immediately following the injection of PG600, the breeding group was arbitrarily divided in half and each group penned with one fertile ram fitted with a marking harness. At 36 h, the rams were switched. Ewes were considered to have been bred by the presence of crayon marks at 36, 48, and 96 h. Breeding date of each ewe was recorded. Seven days after removal of the SMB or CIDR, ewes were separated from the rams. A teaser ram was available and provided in the first wk of August for most study years. Between 85 and 99 d of pregnancy, ewes were radiographed and fetuses counted. This count was corroborated at 120 d of gestation or at lambing.

Few large, long-term studies control for the effects of DOY and temperature. As a result, data obtained from these studies may no longer be purely experimental, but also partly observational. Fortunately, a number of statistical methods have been developed to test and remove the impact of nontreatment variables, such as DOY and temperature, from observational data (Aitchison and Silvey, 1957; McKelvey and Zavoina, 1975). Data corrected in this way can be used to test the experimental hypothesis, plus or minus a margin of error, which can be estimated jointly. In addition, interactions between experimental and nonexperimental variables can be explored.

### *Statistical Methods*

Our central hypothesis is that dose level of PG600 affects the probability of each fetus count. We also investigated whether DOY, temperature, and reuse of the devices interact with dose levels and affect fetus count outcomes. To test this, we treat each fetus count $y_i$, for ewe $i$, as an ordered integer corresponding to an increasing production of fetuses,

$$y_i = \begin{cases} 0, & \text{open} \\ 1, & \text{single} \\ 2, & \text{twin} \\ \text{M} & \end{cases} \quad (1)$$

The three treatment variables and three nontreatment variables are shown in Table 1. Of the 1,402 ewes, 57% were inserted with the CIDR device and the balance to the SMB device. Minimum daily environmental temperatures at 11 d prior to breeding averaged 11.3°C and ranged between 1.7 and 23.3 degrees. Day length is captured by the DOY variable and ranges from days 140 to 265, the mean treatment day 199 corresponds to July 19th in a nonleap year. When a SMB device was used multiple times, it is captured by the Synchronization Device Reuse variable, which ranges from 1 to 6 total uses. Because all ewes in the study were of mixed breed types, statistical influence of the dominant observable breed phenotype was tested. No significant breed effects were found at the 95% confidence level, and the variable was excluded from the analysis. Similarly, maiden ewes represented 93% of the study group. The impact of using ewes bred after their first lambing was tested and found to be insignificant and was excluded.

**Table 1.** Summary statistics for treatment and nontreatment variables

| Variable | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|
| PG600[1] Dose, mL | 2.75 | 0.43 | 1.50 | 5.00 |
| CIDR[2], yes=1 | 0.57 | 0.50 | 0 | 1 |
| SMB[3], yes=1 | 0.43 | 0.50 | 0 | 1 |
| Min Temp[4] | 11.31 | 2.84 | 1.70 | 23.33 |
| Day of the year | 199 | 30.43 | 140 | 265 |
| Device Use Count | 1.56 | 1.05 | 1 | 6 |

[1]PG600 (Merck Animal Health, Summit, NJ).

[2]CIDR = controlled internal drug releasing device (Pfizer Inc., New York, NY).

[3]SMB = Syncro-Mate-B device (Sanofi Animal Health, Overland Park, KS).

[4]Min Temp = Minimum Daily Temperature, °C.

A multinomial ordered probit model was used to test and control for influence from nontreatment variables. Statistical modeling was performed and graphics generated in the Stata (2015) and Matlab (2017) programming environments, respectively. Ordered probit was introduced by Aitchison and Silvey (1957) to measure an organism's response level to various chemical treatments and was later extended to multiple variables by McKelvey and Zavoina (1975). It has been used to differentiate genetic from environmental factors in poultry behavior by Mielenz *et al.* (2010) and cattle calving rates by Guerra *et al.* (2006). Our model predicts the probability of ordered fetus counts across a range of doses and temperatures simultaneously, accommodating a larger number of discrete and continuous treatment and nontreatment variables.

The ordered probit model estimates the probability that the actual pregnancy outcome $y_i$ for animal $i$ will be equal to $m$, where $m$ is 1 for a single, 2 for twins, and so on, conditioned on the external dosage and variables,

$$\mathrm{P}(y_i = m | x_i, z_i) = \mathrm{F}(\tau_m - \beta x_i - \gamma z_i) - \mathrm{F}(\tau_{m-1} - \beta x_i - \gamma z_i). \quad (2)$$

Here, $x_i$ represents all linear effects and $z_i$ the interaction terms, F is the normal probability distribution, and $\beta$ and $\gamma$ are model coefficients. Threshold variable $\tau_m$ can be interpreted as the unobservable physiological and environmental state sufficient to support outcome $m$, e.g., twins for $m = 2$, but insufficient to support outcome $m + 1$, e.g., triplets. Thresholds are estimated simultaneously with the other model coefficients and because they have no direct impact on model interpretation, they are omitted from RESULTS section.

Ordered logit is a popular alternative model for estimating biological treatment effects. It is also represented by equation (2) by replacing the

normal probability distribution term $F$ with the logistic probability distribution. Results from the logit model were nearly identical to those from the probit analysis presented here.

A shortcoming of both ordered probit and logit is the restrictive parallel regression assumption, requiring model coefficients $\beta$ and $\gamma$ that are constant across various pregnancy outcomes $m$. A Wald test confirmed that this assumption does not hold for all of our coefficients. Test statistics are often significant, even for relatively small effects, when observation count is large, as it is in this study. Generalized ordered logit is an alternative model that relaxes the parallel regression assumption by estimating a separate coefficient for each outcome, $\beta_m$ and $\gamma_m$, but this model results in a four-fold increase in the total number of coefficients, one for each additional pregnancy type. Unfortunately, the generalized ordered logit model did not converge under the larger number of parameters. As an alternative robustness check, model results were compared with a binary probit model for each pregnancy outcome: open vs. pregnant, single vs. not-single, etc. Some variation in magnitudes and significance levels occurred, consistent with the Wald test finding, but no major departures from reported findings were observed.

Model results can be sensitive to specification. We test and present in RESULTS section two common specifications: Model (a) is the most restrictive, assuming treatment and nontreatment variables are independent of one another and contribute to birth rates at a constant (linear) rate. The second model (b) relaxes these assumptions and tests for the presence of potentially nonlinear interactions between the treatment and nontreatment variables. Because the degree of nonlinearity, if any exists, is not known a priori, we test for up to fifth-order (quintic) nonlinear interactions between PG600 dose and device type, and second-order (quadratic) interactions with minimum temperatures. Higher-order effects were also tested in all treatment and nontreatment variables, but were not found to be significantly different from zero at the 95% level.

## RESULTS

Observed pregnancy rates, before any statistical corrections are applied, are reported in Tables 2–4. Table 2 summarizes pregnancy rates (and number of ewes) for the two device types across a range of dose levels. The PG600 dose range of 2.00–2.49 mL resulted in the highest pregnancy rates of 76% for ewes treated with a CIDR device and 77% for those treated with the SMB device. Pregnancy rates drop noticeably for dose levels below 2.00 mL and above 3.99 mL.

**Table 2.** Pregnancy rates (number of animals) by dosage and device type

| Treatment, mL | CIDR[1] | SMB[2] | Treatment total |
|---|---|---|---|
| 1.50–1.99 | 50% | | 50% |
| | (14) | | (14) |
| 2.00–2.49 | 76% | 77% | 76% |
| | (76) | (13) | (89) |
| 2.50–2.99 | 72% | 60% | 67% |
| | (420) | (258) | (678) |
| 3.00–3.49 | 69% | 68% | 68% |
| | (217) | (279) | (496) |
| 3.50–3.99 | 69% | 53% | 62% |
| | (54) | (36) | (90) |
| 4.00 | 44% | 50% | 47% |
| | (18) | (12) | (30) |
| 5.00 | 100% | 0% | 20% |
| | (1) | (4) | (5) |
| Study Total | 70% | 63% | 67% |
| | (800) | (602) | (1402) |

[1]CIDR = controlled internal drug releasing device (Pfizer Inc., New York, NY).

[2]SMB = Syncro-Mate-B device (Sanofi Animal Health, Overland Park, KS).

Table 3 shows the frequency (and number of ewes) of each type of fetus count by dose range. The total column represents the proportion of the total sample treated by dose level. The overall twinning rate was 33%, with a high of 42% observed in the 2.00- to 2.49-mL dose range. Quadruplets and quintuplets were observed only in the dose range from 2.50 to 4.00 mL.

Table 4 summarizes frequency of pregnancy outcomes (and number of ewes) by month. The total column on the right shows the proportion of the total sample treated each month. The majority of ewes were treated in June through August. Observed pregnancy rates were highest at 70% in June and July and 67% overall. No ewes were treated after early September.

Coefficient estimates from the two ordered logit models and their $z$-statistics are shown in Table 5. The linear model (a) assumes that treatment variables affect pregnancy outcomes linearly and separately. Only SMB Device Reuse variable has a significant impact on pregnancy outcome level, at the 95% confidence level, indicated by the high $z$-statistic value. The negative coefficient −0.27 suggests that each SMB Device Reuse depresses the likelihood of pregnancy by 27%, plus or minus a statistical margin of error. All other treatment and nontreatment variables, including PG600 dose, are

**Table 3.** Pregnancy rates (number of animals) by dosage and pregnancy outcome type

| Treatment, mL | Open | Single | Twin | Triplet | Quad.[1] | Quint.[2] | Treatment total |
|---|---|---|---|---|---|---|---|
| 1.5–1.99 | 50% | 21% | 7% | 21% | | | 1% |
| | (7) | (3) | (1) | (3) | | | (14) |
| 2.00–2.49 | 24% | 21% | 42% | 13% | | | 6% |
| | (21) | (19) | (37) | (12) | | | (89) |
| 2.50–2.99 | 33% | 26% | 34% | 6% | 1% | | 48% |
| | (223) | (174) | (229) | (42) | (10) | | (678) |
| 3.00–3.49 | 32% | 20% | 35% | 11% | 1% | 0.2% | 35% |
| | (158) | (99) | (175) | (56) | (7) | (1) | (496) |
| 3.50–3.99 | 38% | 19% | 27% | 16% | 1% | | 6% |
| | (34) | (17) | (24) | (14) | (1) | | (90) |
| 4.00 | 53% | 20% | 17% | 7% | 3% | | 2% |
| | (16) | (6) | (5) | (2) | (1) | | (30) |
| 5.00 | 80% | 20% | | | | | 0.4% |
| | (4) | (1) | | | | | (5) |
| Study Total | 33% | 23% | 33% | 9% | 1% | 0.1% | 100% |
| | (463) | (319) | (471) | (129) | (19) | (1) | (1402) |

[1]Quadruplet.
[2]Quintuplet.

**Table 4.** Pregnancy outcomes (number of animals) by month

| Month | Open | Single | Twin | Triplet | Quad.[1] | Quint. | Month total |
|---|---|---|---|---|---|---|---|
| May | 32% | 18% | 38% | 12% | | | 4% |
| | (19) | (11) | (23) | (7) | | | (60) |
| June | 30% | 24% | 35% | 9% | 2% | | 27% |
| | (113) | (93) | (134) | (33) | (7) | | (380) |
| July | 30% | 22% | 37% | 9% | 2% | 0.2% | 32% |
| | (133) | (99) | (163) | (38) | (9) | (1) | (443) |
| August | 35% | 24% | 30% | 10% | 1% | | 27% |
| | (130) | (91) | (111) | (38) | (3) | | (373) |
| September | 47% | 17% | 27% | 9% | | | 10% |
| | (68) | (25) | (40) | (13) | | | (146) |
| Study Total | 33% | 23% | 34% | 9% | 1% | 0.1% | 100% |
| | (463) | (319) | (471) | (129) | (19) | (1) | (1402) |

[1]Quadruplet.
[2]Quintuplet.

not significantly different from zero, or a no-impact result.

The second model (b) relaxes the assumption of linear and separable effects and allows nonlinear interactions, including second- through fifth-order effects. All coefficient estimates are significant except CIDR interactions with PG600 dose and minimum temperature, as well as minimum temperature squared, as indicated by the low *z*-statistics. The nature of model (b) nonlinear impacts is illustrated in Figures 1 and 2.

Expected pregnancy probabilities (and standard errors) from the interactive model (b) are reported for each dose level in Table 6, along with their *z*-statistics and 95% confidence intervals. All other nondosage variables are held constant at their mean values. Specifically, minimum temperature is held at 11.31°C and DOY is held at 199.

Predicted pregnancy rates peak at 74% at a 3.50-mL dose rate with a smaller peak of 71% at 2.00 mL. Although a double-peak is interesting, there is no evidence of statistically significant differences between the pregnancy rates within the optimal 2.00- to 3.50-mL dose range, indicated by the confidence intervals. Pregnancy rates are significantly lower, however, for dosage levels outside the 2.00- to 3.50-mL range.

Model results suggest that a 3.50-mL dose is inferior to observed outcomes in Table 2, which showed the highest pregnancy rate of 76% in the 2.00- and 2.49-mL range. There are a number of possible explanations. First, the observed outcome of 76%

**Table 5.** Ordered probit regression results—linear model (a) and interactions model (b)

| | Linear (a) | | Interactions (b) | |
|---|---|---|---|---|
| Variables | Coefficient | *z*-statistic | Coefficient | *z*-statistic |
| Linear variables | | | | |
| PG600[1] | 0.035 | 0.50 | | |
| Min Temp[2] | −0.011 | −1.77 | | |
| Synchronization Device Reuse | −0.27*** | −7.66 | −0.27*** | −7.42 |
| CIDR[3] | −0.13 | −1.94 | | |
| Day of the year | −0.0017 | −1.60 | 0.045*** | 3.10 |
| Non-linear variable | | | | |
| Day of the year (second order)[4] | | | −0.00011*** | −3.23 |
| Dose interactions | | | | |
| PG600 x CIDR | | | 189*** | 2.99 |
| PG600 x SMB[5] | | | 179*** | 2.96 |
| PG600 (second order) x CIDR | | | −129*** | −3.00 |
| PG600 (second order) x SMB | | | −123*** | −3.05 |
| PG600 (third order) x CIDR | | | 43*** | 3.01 |
| PG600 (third order) x SMB | | | 40*** | 3.01 |
| PG600 (fourth order) x CIDR | | | −7.0*** | −3.02 |
| PG600 (fourth order) x SMB | | | −6.3*** | −2.92 |
| PG600 (fifth order) x CIDR | | | 0.44*** | 3.01 |
| PG600 (fifth order) x SMB | | | 0.38*** | 2.79 |
| Temperature interactions | | | | |
| Min Temp x PG600 x CIDR | | | −0.028 | −1.01 |
| Min Temp x PG600 x SMB | | | 0.18*** | 3.15 |
| Min Temp (second order) x PG600 x CIDR | | | 0.00017 | 0.67 |
| Min Temp (second order) x PG600 x SMB | | | −0.0018*** | −3.23 |

***Significant at the 99% confidence level.

[1]PG600 (Merck Animal Health, Summit, NJ).

[2]Min Temp = Minimum Daily Temperature, °C.

[3]CIDR = controlled internal drug releasing device (Pfizer Inc., New York, NY).

[4]Second-order variables are squared ($y^2$), third order are cubic ($y^3$), and so on.

[5]SMB = Syncro-Mate-B device (Sanofi Animal Health, Overland Park, KS).

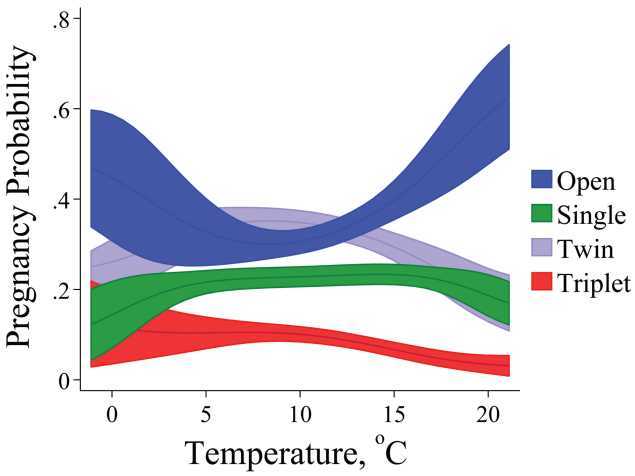


**Figure 1.** Probability of pregnancy types (confidence intervals) across a range of minimum daily temperatures, Celsius.

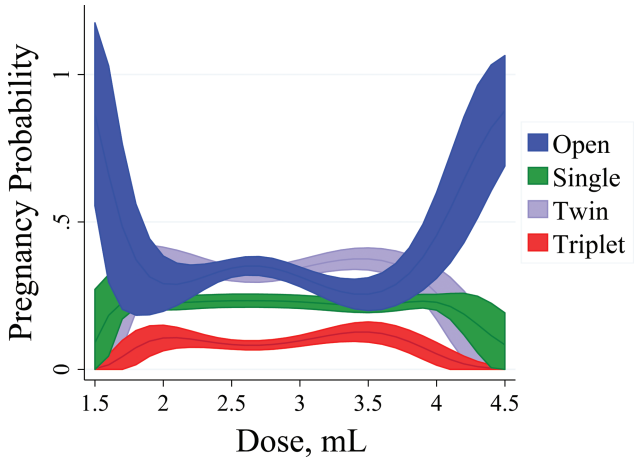


**Figure 2.** Probability of pregnancy types (confidence intervals) across a range of doses, mL.

is contained within the model's confidence interval of 62% to 80% for the 2.00-mL dose. Second, the observed outcomes are not adjusted for DOY and temperature, whereas the predicted rates in Table 4 represent expected outcomes when other variables are held constant at their mean value. Small variations in daylight lengths and temperatures can significantly influence pregnancy rates as illustrated in Figures 1–3.

**Table 6.** Pregnancy probability (SE) at mean daily temperature

| | | | 95% Confidence Interval | |
|---|---|---|---|---|
| Treatment, mL | Pregnancy rate (SE) | *z*-statistic | Lower | Upper |
| 1.50 | 13% (0.16) | 5.49 | −18% | 45% |
| 2.00 | 71% (0.05) | 6.08 | 62% | 80% |
| 2.50 | 65% (0.02) | 21.85 | 63% | 69% |
| 3.00 | 68% (0.02) | 18.53 | 65% | 72% |
| 3.50 | 74% (0.03) | 8.98 | 69% | 80% |
| 4.00 | 54% (0.07) | 6.13 | 40% | 69% |
| 4.50 | 12% (0.10) | 9.19 | −6% | 31% |

Figure 1 plots the expected probability of each pregnancy type (solid lines) across a continuous range of minimum daily temperatures from interactions model (b). The 95% confidence intervals (shaded area) measure outcome variability, which increases at both lower and higher temperatures for all outcomes. The open rate is significantly lower at 8.9°C than for temperatures above 15.5 degrees and below negative 1.1 degrees. These results provide field evidence for the heat-sensitivity finding of Cushman (2013) and support the relationship between cooler temperatures and onset of estrus examined by Dutt and Bush (1955). The twinning rate appears to peak near 8.9°C, whereas the single rate appears to hold steady across the 4 to 16 degree range, and the triplet rate decreases gradually at higher minimum temperatures.

The impact of PG600 dose level on the probability of each pregnancy type (solid line) is shown in Figure 2, along with 95% confidence intervals (shaded area) from model (b). The open rate is significantly lower near 3.50 mL than for doses above 4.00 mL and below 1.50 mL, as well as the small region around 2.75 mL. In contrast, the single rate appears to peak near 2.00 mL, and both triplet and twining rates exhibit a double peak near 3.50 and 2.00 mL. These results suggest that the PG600 dose may be varied substantially to achieve a desired fetus count objective.

Interactions between temperature and PG600 are illustrated in Figure 3, which plots the pregnancy probability along the y-axis (vertical) over a range of PG600 dose levels (x-axis) and temperatures (z-axis), holding all other variables constant at their mean. The plot illustrates again the double peak at doses near 2.00 and 3.50 mL along the optimum temperature level near 8.9°C. This suggests that higher pregnancy rates are possible at temperatures that are lower than the 11.3-degree average observed during the study period by nearly a full standard deviation. The optimal dose level is near 3.50 mL across a wide temperature range, suggesting that there is no need to adjust dose to specific temperatures if pregnancy is the sole objective.

## DISCUSSION

There are several advantages to breeding sheep out-of-season. If a producer has groups of ewes lambing 2 or 3 times during the year, they may provide a more consistent supply of lambs to market. If ewes are synchronized and lamb in a tight time frame, the producer will cut labor costs and expenses each lambing period (Carlson *et al.*, 1989). Producers may choose to lamb every 8 mo or even every 6 mo, thereby increasing profits due to increased numbers of lambs over time (Hulet and Stormshak, 1972).

Past research has shown that pregnancy rates from synchronized ewes vary greatly from year to year (Morrical *et al.*, 1995). Decreasing daylight and low temperatures are both associated with earlier estrus onset and longer duration of first estrus in temperate climates (Dutt and Bush, 1955; Rosa and Bryant, 2003). The current study provides additional evidence for the relationship among DOY, temperature, and specific pregnancy types.

Safranski *et al.* (1992) compared feeding MGA and MGA in combination with PG600 during anestrous. They used 5.00 mL of PG600. The percentage of ewes lambing given only MGA was

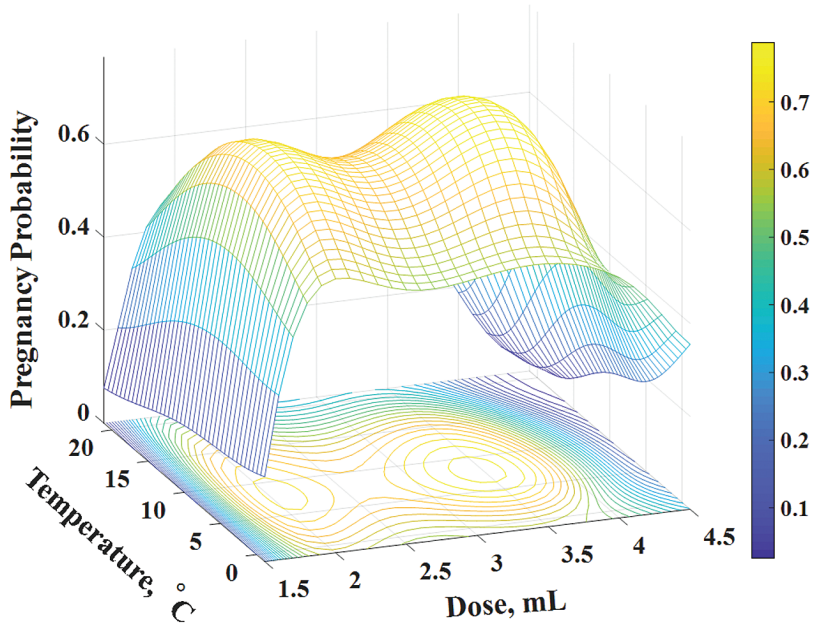


**Figure 3.** Pregnancy probability over a range of PG600 doses and daily minimum temperatures, Celsius.

40.5% and the MGA/PG600 was 41.2%. The ovulation rates when exposed to a ram were 2.30 for MGA/PG600 and only 1.39% when no PG600 was given. Our results suggest that PG600, given at 5.00 mL, while beneficial for ovulation, is too high a dose and ova fail to reach the 90th d of pregnancy.

In another study, Windorski *et al.* (2008) showed similar results at the 5.00-mL dose and stated that "although PG600 increased the number of luteal structures present per ewe, it did not significantly enhance ewe prolificacy." Their study compared the feeding of MGA and MGA/PG600 and used 5.00-mL PG600 during anestrous season. Again, results of the present study suggest that a dose of 5.00-mL PG600 is too high to support embryo/fetus survival.

Ryan *et al.* (1991) proposed that when ewes are given higher doses of gonadotrophins, fertility is reduced due to an increase in the incidence of persistent large follicles and prolonged elevation in concentrations of estrogen. They also reported that in spring the recovery rates of ova and embryos decreased with increasing dose of PMSG. They suggested that with the increase in estrogen, from the large follicles, the premature entry of the embryos into the uterus is detrimental. We show that higher doses of PG600 in spring and summer can lead to decreased overall lambing rates, although each pregnancy type response to dose level is unique.

Embryo mortality and thus lambing rates may be adversely affected during the summer months by heat. The accumulation of heat shock proteins (**HSP**) may counteract damage to oocytes from heat stress. Cushman (2013) summarizes some of the research done with HSP in cattle and states that they may have positive impacts on calving rates in some breeds of cattle. In a human study, Santoro *et al.* (1989) found that two types of prostaglandins suppressed erythroleukemia cells and induced the synthesis of HSP's. The current study does not find that the interaction between dose and temperature is sufficient to vary dose according to temperature, although temperature and dose each have significant impacts on pregnancy rate and type of birth.

During anestrus, SMB and CIDR effectiveness is highest in conjunction with a 2.00- to 3.49-mL PG600 dose. Using more than 3.49-mL PG600 lowers pregnancy rates and increases variability. Given the surprising effectiveness of doses in the low 2.00- to 3.49-mL range, future research on the impacts of even lower doses of PG600 would be of interest. This study suggests that doses far lower than the standard 5.00 mL recommendation will increase lambing rates for out-of-season breeding, thereby increasing profits for sheep producers.

## LITERATURE CITED

Ainsworth, L., and M. S. Wolynetz. 1982. Synchronization of estrus and reproductive performance of ewes treated with synthetic progestogens administered by subcutaneous ear implant or by intravaginal sponge pessary. J. Anim. Sci. 54:1120–1127. doi:10.2527/jas1982.5461120x

Aitchison, J., and S. D. Silvey. 1957. The generalization of the probit model to the case of multiple responses. Biometrika 44:131–140. doi:10.2307/2333245

Butler, L.G., and W. M. C. Maxwell. 1988. PMSG response in ewes of different breeding value for reproductive rate as an aid to selection. Proc. Aust. Soc. Anim. Prod. 17:158–161.

Carlson, K. M., H. A. Pohl, J. M. Marcek, R. K. Muser, and J. E. Wheaton. 1989. Evaluation of progesterone controlled internal drug release dispensers for synchronization of estrus in sheep. Anim. Reprod. Sci. 18:205–218. doi:10.1016/0378-4320(89)90022-5

Cushman, R. A. 2013. Physiology and endocrinology symposium: the current status of heat shock in early embryonic survival and reproductive efficiency. J. Anim. Sci. 91:1141–1142. doi:10.2527/jas.2013-6231

Cushwa, W. T., G. E. Bradford, G. H. Stabenfeldt, Y. M. Berger, and M. R. Dally. 1992. Ram influence on ovarian and sexual activity in anestrous ewes: effects of isolation of ewes from rams before joining and date of ram introduction. J. Anim. Sci. 70:1195–1200. doi:10.2527/1992.7041195x

Dutt, L. H., and R. F. Bush. 1955. The effect of low environmental temperature on initiation of the breeding season and fertility in sheep. J. Anim. Sci. 14:885–896. doi:10.1093/ansci/14.3.885

Gordon, I. 1971. Induction of early breeding in sheep by standard and modified progestogen-PMS treatments. J. Agric. Sci. 76:337–341. doi:10.1017/S0021859600069240

Guerra, J. L., D. E. Franke, and D. C. Blouin. 2006. Genetic parameters for calving rate and calf survival from linear, threshold, and logistic models in a multibreed beef cattle population. J. Anim. Sci. 84:3197–3203. doi:10.2527/jas.2006-007

Hafez, E. S. E. 1952. Studies on the breeding season and reproduction of the ewe. J. Agric. Sci. 42:189–231. doi:10.1017/S0021859600056896

Hulet, C. V., and F. Stormshak. 1972. Some factors affecting response of anestrous ewes to hormone treatment. J. Anim. Sci. 34:1011–1019. doi: 10.2527/jas1972.3461011x

Knights, M., T. Hoehn, P. E. Lewis, and E. K. Inskeep. 2001. Effectiveness of intravaginal progesterone inserts and FSH for inducing synchronized estrus and increasing lambing rate in anestrous ewes. J. Anim. Sci. 79:1120–1131. doi:10.2527/2001.7951120x

Mathworks. 2017. Matlab: Release 17b. Natick, MA: The Mathworks, Inc.

McKelvey, R. D., and W. Zavoina. 1975. A statistical model for the analysis of ordinal level dependent variables. J. Math. Soc. 4:103–120. doi:10.1080/0022250X.1975.9989847

McLeod, B. J., W. Haresign, and G. E. Lamming. 1982. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of gn-RH. J. Reprod. Fertil. 65:215–221. doi:10.1530/jrf.0.0650215

Mielenz, N., J. Spilke, and E. von Borell. 2010. Analysis of ordered categorical data with threshold models exemplified by plumage damage scores from laying hens differing in their genotype and rearing environment. Poult. Sci. 89:2521–2534. doi:10.3382/ps.2010-00819. Available from https://www.extension.iastate.edu/Pages/ansci/sheepreports/asl-1475.pdf

Morrical, D. G., C. R. Youngs, and A. McClain. 1995. The influence of MGA and PG600 on the out of season reproductive performance of ewes. Ani. Sci. Leaflet. R1475. Available from https://www.extension.iastate.edu/Pages/ansci/sheepreports/asl-1475.pdf

Rosa, H. J. D., and M. J. Bryant. 2003. Seasonality of reproduction in sheep. Small Ruminant Res. 48:155–171. doi:10.1016/S0921-4488(03)00038-5

Ryan, J. P., J. R. Hunton, and W. M. Maxwell. 1991. Increased production of sheep embryos following superovulation of merino ewes with a combination of pregnant mare serum gonadotrophin and follicle stimulating hormone. Reprod. Fertil. Dev. 3:551–560. doi:10.1071/RD9910551

Safranski, T. J., W. R. Lamberson, and D. H. Keisler. 1992. Use of melengestrol acetate and gonadotropins to induce fertile estrus in seasonally anestrous ewes. J. Anim. Sci. 70:2935–2941. doi:10.2527/1992.70102935x

Santoro, M. G., E. Garaci, and C. Amici. 1989. Prostaglandins with antiproliferative activity induce the synthesis of a heat shock protein in human cells. Proc. Natl. Acad. Sci. U. S. A. 86:8407–8411. doi:10.1073/pnas.86.21.8407

StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.

Wheaton, J. E., K. M. Carlson, H. F. Windels, and L. J. Johnston. 1993. CIDR: a new progesterone-releasing intravaginal device for induction of estrus and cycle control in sheep and goats. Anim. Reprod. Sci. 33:127–141. doi:10.1016/0378-4320(93)90111-4

Wheaton, J. E., H. A. Pohl, and H. F. Windels. 1990. Effects of melatonin and progesterone administered to ewes in spring and summer. J. Anim. Sci. 68:923–930. doi:10.2527/1990.684923x

Windorski, E. J., C. S. Schauer, A. K. Wurst, E. K. Inskeep, and J. S. Luther. 2008. Effects of melengestrol acetate and P.G. 600 on fertility in rambouillet ewes outside the natural breeding season. Theriogenology 70:227–232. doi:10.1016/j.theriogenology.2008.04.004