Effect of different dosages of PG-600 on ovulation and pregnancy rates in ewes during the breeding season

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ABSTRACT: This study compared the reproductive effects of different dosages of PG-600 (Intervet/Merck Animal Health, Madison, NJ) during the breeding season of ewes. PG-600 is a single-dose injectable product labeled for estrous induction in swine, containing equine chorionic gonadotropin (80 IU/mL) and human chorionic gonadotropin (40 IU/mL). PG-600 is routinely used off-label for out-of-season estrous induction in sheep. However, at the most common dose administered to ewes (5 mL), PG-600 is likely to overstimulate the ovaries, resulting in reduced pregnancy rates. Following estrous synchronization with intravaginal progesterone and cloprostenol, Polypay ewes were treated with 5 mL PG-600 (T1; $n = 8$), 1.5 mL PG-600 (T2; $n = 8$), or 5 mL saline (C; $n = 8$) and then mated to rams. Jugular vein samples were collected prior to the PG-600 injection (0 hr) and at 2, 4, 8, 12, and 24 hr after injection. Serum estradiol-17β was determined by chemiluminescence and among groups using repeated measures analysis of covariance. Ovulation and pregnancy rates were determined by transrectal ultrasonography and compared by one-way ANOVA and chi-square, respectively. Estradiol-17β concentrations were greater in T1 compared to T2 and C ($P < 0.001$). Ovulation rate was greater ($P < 0.001$) but pregnancy rate was lower ($P < 0.001$) in the T1 compared to C and T2. These data confirm that a 5 mL dose of PG-600 administered to ewes during the breeding season overstimulates the ovaries, which may then reduce fertilization or embryo survival. Future research will focus on the effects of different dosages of PG-600 on pregnancy rate of ewes during the nonbreeding season.

Key words: corpora lutea, estradiol, estrus induction

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INTRODUCTION

Sheep are seasonal polyestrus breeding during days of shortened daylight and becoming anestrus during exposure to increased hours of daylight. As a result, most ewes have just one lamb crop per year (gestation length is 5 mo). To increase the number of lamb crops to three for every 2 yr or five for every 3 yr, ewes must be bred during the nonbreeding season. This will also create an out-of-season supply of lamb for consumers. However, ovulation and pregnancy rates decrease during the nonbreeding season (Knights et al., 2003; deNicolo et al., 2006).

To stimulate ewes to ovulate during anestrus, they are often treated with follicle-stimulating hormone or equine chorionic gonadotropin (eCG) (Jabbar et al., 1994; Christman et al., 2000; Cline et al., 2001; Windorski et al., 2008; D'Souza et al., 2014). PG-600 (Intervet/Merck Animal Health, Madison, NJ) is a single-dose injectable product

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available for off-label use to U.S. sheep producers, which contains a combination of eCG (80 IU/mL) and with human chorionic gonadotropin (hCG, 40 IU/mL). However, PG-600 has been shown to overstimulate the ovaries, resulting in abnormally large follicles with increased estradiol-17β concentrations at the time of ovulation (Safranski et al., 1992). As a result, fertilization rates were reduced by 42% when compared to ewes bred during the breeding season (Lunstra and Christenson, 1981; Ryan et al., 1991). Therefore, it was hypothesized that using a lower dose of PG-600 should minimize the effects of ovarian overstimulation, resulting in increased pregnancy rates and lamb production. In the current study, the reproductive effects of two dosages of PG-600 were examined during the breeding season.

MATERIALS AND METHODS

Multiparous Polypay ewes were treated with an intravaginal progesterone-releasing device (Eazi-Breed CIDR, Zoetis, Kalamazoo, MI) for 9 d. Two days prior to progesterone withdrawal, cloprostenol (125 µg; Estrumate, Intervet/Merck Animal Health) was administered intramuscularly by injection. At the time of progesterone withdrawal (day 0), ewes were divided into three groups: 1) PG-600 (Intervet/Merck Animal Health, Madison, NJ) high dose (5 mL; $n = 8$), 2) PG-600 low dose (1.5 mL; $n = 8$), and 3) saline control (5 mL; $n = 8$). Ewes were then alternately exposed to each of three different rams over a 4-d period to facilitate natural mating.

Immediately following progesterone removal but prior to giving the PG-600 or saline injection, jugular venous blood samples were collected (0 h) and at 2, 4, 6, 8, 12, and 24 h after PG-600 or saline treatment. Blood samples were allowed to clot overnight at 4 °C before centrifugation at 1,620 × g for 10 min at the same temperature. Sera were separated and stored at −20 °C until analyzed. Serum estradiol-17β concentrations were determined using chemiluminescence (Immulite 1000, Siemens Healthcare Diagnostics, Tarrytown, NY) in a single assay. The intra-assay coefficient of variation was 5.14%. The assay detection limit was 20 pg/mL.

The number of ovulations per animal was determined on days 9 to 11 posttreatment by counting corpora lutea using transrectal ultrasonography (7.5-MHz linear array, MINDRAY model #50L60EAV, Shenzhen, China) and the mean ± SEM ovulation rate was calculated for each group. The height and width of each corpus luteum was recorded and the mean ± SD corpora lutea diameter was calculated for each group. Pregnancy rate was determined using transrectal ultrasonography on day 21 and 28 posttreatment. Number of lambs per ewe (fecundity rate) was recorded for each ewe and the mean ± SD fecundity rate was calculated for each group. Birth and weaning weights were recorded for the lambs from each ewe and the mean ± SEM birth and weaning weights were calculated for each group. In addition, total litter weight was calculated for each ewe and then the mean ± SD litter weight was calculated for each group. All animal procedures were approved by the Oregon State University Institutional Animal Care and Use Committee (#4865).

A repeated measures analysis of covariance was used to analyze estradiol-17β concentrations over time and among groups. A one-way analysis of variance (ANOVA) was used to compare ovulation rate, corpora lutea diameter, fecundity rate, birth weight, litter weight, and weaning weight among treatments. A chi-square was used to compare pregnancy rate among treatment groups. Significance was defined as $P < 0.05$.

RESULTS AND DISCUSSION

In the current study, the ovulation rate was greater in the T1 ewes compared to C and T2 ewes

Table 1. Results of PG-600 administration on reproductive characteristics of ewes during the breeding season

Item	Treatment groups*		
Group	C	T2	T1
Number of ewes per group	8	8	8
Ovulation rate (mean ± SEM)	2.17 ± 0.25[a]	2.29 ± 0.26[a]	3.25 ± 0.47[b]
Pregnancy rate	75%[ab]	87.5%[a]	37.5%[b]
Fecundity rate (mean ± SD)	1.83 ± 0.75[a]	2.14 ± 0.38[a]	2.33 ± 0.58[a]
Birth weight, kg (mean ± SEM)	4.51 ± 0.76[a]	4.43 ± 0.87[a]	4.4 ± 0.74[a]
Litter weight, kg (mean ± SD)	8.3 ± 2.3[a]	9.5 ± 0.9[a]	10.2 ± 1.9[a]
Weaning weight, kg (mean ± SEM)	25.32 ± 4.82[a]	24.81 ± 3.41[a]	25.68 ± 2.76[a]

*Ewes were either treated with 5 mL saline (C), 1.5 mL PG-600 (Intervet/Merck Animal Health, Madison, NJ) (T2), or 5 mL PG-600 (T1).

[a,b]Values with different superscripts are significantly different ($P < 0.001$).

(Table 1; $P < 0.001$). These data were supported by Rutigliano et al. (2014), who found that a ovulation rate increased in a dose-dependent manner in ewes during the breeding season following administration of an analog to human follicle-stimulating hormone. However, Cline et al. (2001) reported that 5 mL PG-600 during the breeding season did not alter ovulation rate in ewes. It is important to mention that in the study by Cline et al. (2001), ewes were examined for ovulation beginning 12 hr after progesterone withdrawal and reexamined every 8 hr until ovulation was observed. It is possible that additional ovulations occurred asynchronously and were therefore not recorded by these investigators. In the current study, ewes were examined for ovulation (based on number of corpora lutea) once daily from 9 to 11 d following progesterone withdrawal. It is important to note that the average corpora lutea diameter was not significantly different per group (T1: 10.9 ± 3.2 mm; T2: 12.4 ± 3.3 mm; C: 11.6 ± 4.5 mm).

Pregnancy rate was lower in the T1 ewes compared to C and T2 (Table 1; $P < 0.001$). This result was supported by data of Lunstra and Christenson (1981) who found that treatment of ewes with eCG during the breeding season resulted in greater pregnancy losses (49%) compared to untreated sheep (25%). This is in contrast to results from Quintero-Elisea et al. (2011) who reported higher pregnancy and fecundity rates in haired ewes treated with 400 IU eCG in tropical latitudes. However, fecundity rate did not differ in the current study among treatment groups (Table 1). In addition, there was no effect of treatment on birth, litter, or weaning weights of lambs consistent with previous research reported by Rekik et al. (2016).

Windorski et al. (2008) speculated that high doses of PG-600 reduced fertility by reducing embryo survival. In addition, Safranski et al. (1992) speculated that high doses of PG-600 reduced fertility by preventing the embryo from attaching to the endometrium. Either theory may be the result of elevated circulating estradiol-17β concentrations causing an increase in endometrial estrogen receptor expression with a consequent detrimental change in cell function. This belief is supported by the research of Arakawa et al. (1989) who reported that administration of eCG to rats increased endometrial estrogen receptor expression. Additional research is needed to determine if PG-600 increases endometrial estrogen receptor expression and altered cell function.

Serum estradiol-17 concentrations over all postinjection time points examined were on average greater ($P < 0.001$) in T1 treated as compared to T2 and C ewes (Figure 1). Most notable was the marked increase in serum estradiol concentration of T1 ewes at 24 h. This finding was supported by Barrett et al. (2004) who reported that serum estradiol concentrations were greater in ewes during the breeding season treated with 500 IU eCG at the time of progesterone withdrawal compared to control ewes during the pre- and early postovulatory period. In the current study, three of the eight ewes treated with 5 mL of PG-600 had estradiol-17β concentrations over 1000 pg/mL in the first 24 hr following treatment. Several studies have previously shown that large unovulated follicles in sheep are associated with increased estradiol-17β concentrations (Christman et al., 2000; Barrett et al., 2004). In the current study, two of the eight ewes treated with 5 mL of PG-600 had follicular cysts. The diameter of the walls of the follicular cysts ranged from 2.0 to 3.5 mm. It was not possible to determine if these were luteinized follicular cysts based upon the follicle wall thickness. However, estradiol-17β concentrations in these two ewes remained below 40 pg/mL.

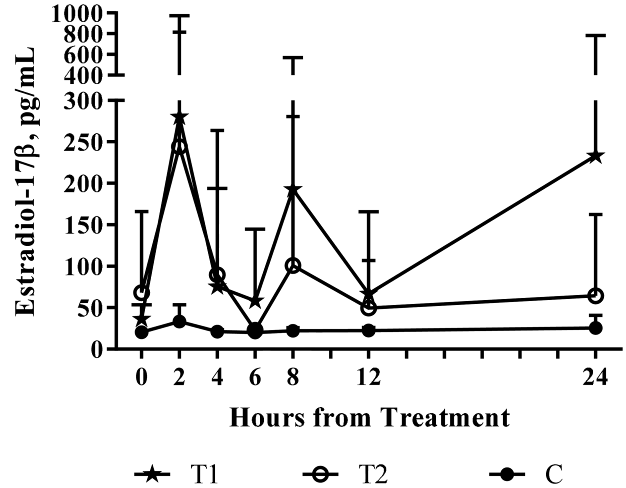

Figure 1. Effect of PG-600 on mean (±SD) serum estrogen concentrations. Serum estradiol-17β concentrations over all postinjection time points examined were on average greater ($P < 0.001$) in 5 mL PG-600 group (T1) compared to control (C) and 1.5 mL PG-600 (T2) ($P < 0.05$). Most notable was the marked increase in serum estradiol concentration of T1 ewes at 24 h.

In conclusion, PG-600 at a dose commonly used by U.S. sheep producers administered during the breeding season increased circulating estradiol-17β concentrations and ovulation rate while reducing pregnancy rate, without affecting fecundity rate or birth and weaning weights. Additional research is planned to investigate the effects of PG-600 at the dosages reported in this study during the nonbreeding season in sheep.

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Conflict of interest statement: None declared.

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