

## Ovulation, pregnancy, and lambing rates during nonbreeding season with or without exogenous gonadotropin stimulation

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### Abstract

Our objective was to evaluate reproductive effects of varying gonadotropin dosages on anestrus ewes pretreated with progesterone and then exposed to a novel ram. Hypothesis was that a lower dosage of P.G. 600<sup>®</sup> (equine chorionic gonadotropin 80 IU/ml and human chorionic gonadotropin 40 IU/ml) induces estrus in anestrus ewes. Twenty-four anestrus ewes were treated with intravaginal progesterone-releasing devices for 9 days and given prostaglandin F<sub>2α</sub> 2 days prior to device withdrawal. On the day of progesterone withdrawal (day 0), ewes were given 5 ml of P.G. 600<sup>®</sup> (T1; n = 8), 1.5 ml of P.G. 600<sup>®</sup> (T2; n = 8) or 5 ml saline (control group, C; n = 8). Three rams were rotated every 4 hours through each group of ewes for 4 days. Venous blood samples were collected on day 0 prior to treatment (0 hour) and at 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 336 hours. Serum estradiol-17<sub>β</sub> and progesterone concentrations were determined by chemiluminescence. Ovulation and pregnancy rates were determined using transrectal ultrasonography on days 9 - 11 and on days 21 and 28. Serum progesterone concentrations increased ( $p < 0.00001$ ) in T1 compared to T2 and C groups. Serum estradiol concentrations, ovulation, pregnancy, and fecundity rates, and weaning weights were not significantly different among groups. We concluded that gonadotropin treatment neither enhanced nor diminished reproductive productivity.

**Keywords:** CIDR, corpora lutea, estrus induction, anestrus, progesterone

### Introduction

Sheep are seasonally polyestrous, cycling during shortened daylight.<sup>1</sup> Breeding management practices that adhere to the ewe's natural breeding season limit ewe productivity (average of 1 lamb crop/ewe/year). To increase ewe's productivity to 3 lamb crops for every 2 years or 5 lamb crops for every 3 years, ewes must be bred outside of the natural breeding season.<sup>2</sup> As hours of daylight increase, decreased secretory pattern of melatonin results in dopamine increase in A15 dopamine neurons in the retrochiasmatic area in the hypothalamus<sup>3</sup> that gradually inhibits kisspeptin from the arcuate nucleus, resulting in inhibition of gonadotropin releasing hormone (GnRH) secretion.<sup>4</sup> Reduced secretion of GnRH results in lower luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion until ovarian follicular activity becomes static. With static follicular activity, estradiol-17<sub>β</sub> concentrations remain low, activating the estrogen responsive inhibitory neural system in the brain. Increasing brain sensitivity to estrogen makes estradiol the primary inhibitor for GnRH and LH pulse frequency.<sup>5,6</sup>

Introduction of a novel ram to anestrus ewes increases LH secretion<sup>7,8</sup> that induces ovulation in approximately half of anestrus ewes without any other treatment.<sup>9,10</sup> The mechanism of this phenomenon is not clear, but it is believed to be mediated through pheromones from the ram's sudoriferous gland. Pheromones act through ewe's olfactory bulb on the ventromedial nucleus and the preoptic area of the hypothalamus, leading to increased kisspeptin release<sup>11</sup> that increases GnRH and LH pulse frequency.<sup>12</sup> In ruminants, prior exposure to progesterone is necessary for both expression of estrous behavior and normal luteal lifespan.<sup>13</sup> Therefore, using progesterone priming prior to novel ram exposure and breeding should result in a fertile estrus with ovulation and normal luteal function. This strategy has resulted in pregnancy in approximately 50% of ewes.<sup>14</sup>

Gonadotrophin treatment increases percentage of ewes ovulating following progesterone pretreatment and ram exposure. Multiple injections of porcine FSH increase rates of ovulation<sup>15</sup> and pregnancy in ewes bred outside the breeding season.<sup>16</sup> However, gonadotropin treatment decreases embryo

viability, based on embryo collection studies.<sup>17</sup> Similar to multiple injections of FSH, a single injection of equine chorionic gonadotropin (eCG) increases ovulation rate,<sup>18,19</sup> but appears to decrease embryo viability.<sup>20</sup> It is noteworthy that anestrus ewes treated with progesterone ovulate when they are also treated with gonadotropins or exposed to rams, but not after progesterone treatment alone.<sup>21</sup>

P.G. 600<sup>®</sup> (Intervet/Merck Animal Health, Madison, NJ) is approved for estrus induction in swine. It contains a combination of equine chorionic gonadotropin (eCG; 80 IU/ml) and human chorionic gonadotropin (hCG; 40 IU/ml). In United States, P.G. 600<sup>®</sup> is commonly used off-label to induce estrus and ovulation in anestrus ewes.<sup>22-25</sup> Ewes are commonly given 5 ml P.G. 600<sup>®</sup>, containing 400 IU eCG and 200 IU hCG.<sup>22,24</sup> However, this amount of gonadotropins to ewes during breeding season has overstimulated ovaries and reduced fertilization rates, as well as increased estradiol-17 $\beta$  concentrations.<sup>26</sup> Therefore, we hypothesized that a lower dosage of P.G. 600<sup>®</sup> induces estrus in anestrus ewes without overstimulating ovaries. Objective of this study was to evaluate reproductive effects of varying gonadotropin dosages in anestrus ewes pretreated with progesterone and then exposed to a novel ram.

## Materials and methods

Twenty-four multiparous Polypay ewes, 2 - 6 years old, were used for this experiment. The fecundity rate for these ewes prior to this experiment was  $1.7 \pm 0.4$ . Animal use was approved by the Oregon State University Institutional Animal Care and Use Committee (ACUP #4865). Estrus induction regimen used for this experiment was the same protocol that was used in the previous experiment conducted during breeding season.<sup>26</sup> Briefly, during late spring (May in the Northern Hemisphere), an intravaginal progesterone-releasing device (0.3 g progesterone; Eazi-Breed<sup>™</sup> CIDR, Zoetis, Kalamazoo, MI) was inserted and removed after 9 days. Cloprostenol (125  $\mu$ g; Estrumate<sup>®</sup>, Intervet/Merck Animal Health, Madison, NJ) was injected intramuscularly 2 days prior to progesterone withdrawal. At progesterone withdrawal (day 0), ewes were treated with P.G. 600<sup>®</sup> (Intervet/Merck Animal Health, Madison, NJ) or saline. Treatment group 1 (T1) received 5 ml (400 IU eCG and 200 IU hCG) intramuscularly (n = 8) and treatment group 2 (T2) received 1.5 ml (120 IU eCG and 60 IU hCG) intramuscularly (n = 8). Control group (C) received an injection of 5 ml saline, equivalent volume of the higher dose of P.G. 600<sup>®</sup> (n = 8). Rams used were novel to ewes prior to their first exposure. Three rams were used and each remained for 4 hours in a given group of ewes over 4 days. Rams were rotated between ewe groups to eliminate any effect of ram preference on pregnancy rates. However, it is understood that this experimental design of rotating rams every 4 hours is not a practical management tool for sheep producers.

Prior to treatment and immediately following progesterone withdrawal, jugular venous blood samples were collected (day 0). Additionally, blood samples were collected at 2, 4, 6, 8, 12, 24 (day 1), 48 (day 2), 72 (day 3), 96 (day 4), 120 (day 5), 144 (day 6), 168 (day 7), and 336 (day 14) hours after treatment. Blood samples were centrifuged at  $1,620 \times g$  after overnight storage at 4°C. Sera were separated and stored at -20°C until analyzed for progesterone and estradiol-17 $\beta$ . Serum progesterone and estradiol-17 $\beta$  concentrations were determined using chemiluminescence (IMMULITE<sup>®</sup>1000, Siemens Healthcare Diagnostics, Tarrytown, NY) in a single assay for each steroid. Progesterone and estradiol-17 $\beta$  intra-assay coefficient of variation was 4.6 and 10.9%, respectively. Assay detection limits for progesterone and estradiol-17 $\beta$  were 0.2 ng/ml and 20 pg/ml, respectively.

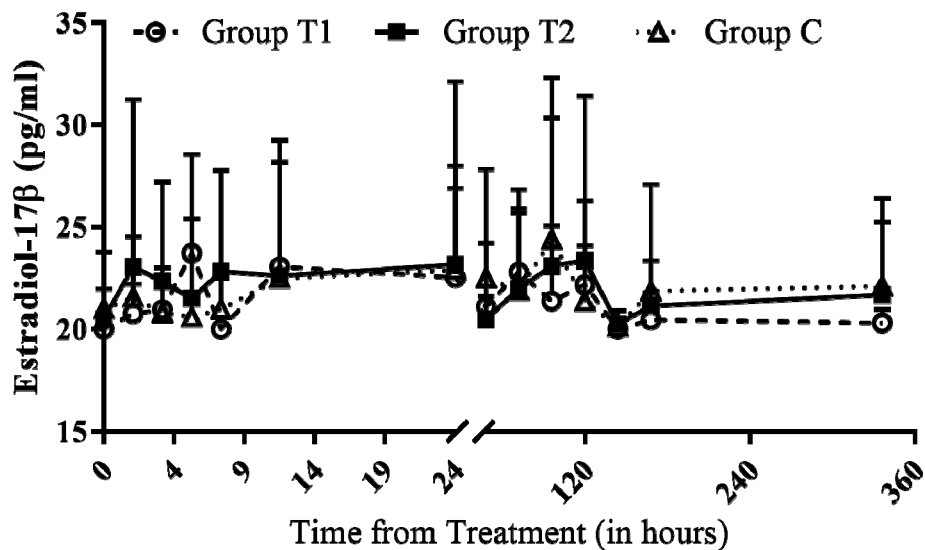
Transrectal ultrasonography (7.5-MHz linear array, MINDRAY model #50L60EAV, Shenzhen, China) was used on days 9 - 11 after treatment, to determine number of ovulations per ewe by counting corpus luteum (CL) present on each ovary, as described.<sup>27</sup> Mean  $\pm$  standard deviation (SD) ovulation rate was calculated for each group. In addition, height and width of each CL was recorded and the mean  $\pm$  SD corpora lutea diameter were calculated for each group. Transrectal ultrasonography was used on days 21 and 28 after treatment injection to image both uterine horns to determine pregnancy status. Pregnancy rate was calculated for each group. Following lambing, number of lambs per ewe (fecundity rate) and birth weights were recorded and mean  $\pm$  SD fecundity rate and birth weight was calculated for each group. In

addition, total litter weight was calculated for each ewe and then the mean  $\pm$  SD litter weight calculated for each group. At weaning, weight was record and mean  $\pm$  SD weaning weight was calculated for each group.

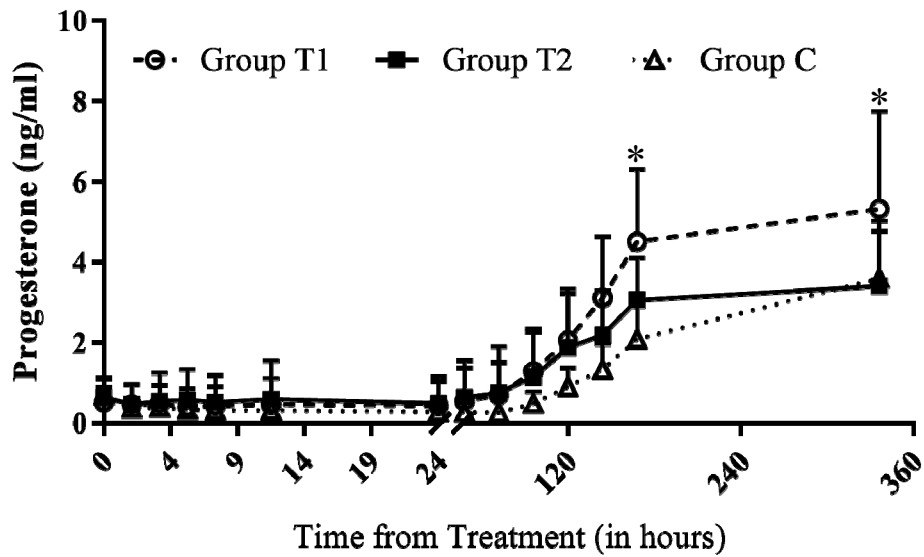
A repeated measure analysis of covariance (ANCOVA) was used to analyze estradiol-17 $\beta$  and progesterone concentrations over time and among treatment groups. A one-way analysis of variance (ANOVA) was used to analyze data on ovulation rate, corpora lutea diameter, fecundity rate, birth weight, litter weight, and weaning weight among treatment groups. A Chi-square test was used to compare pregnancy rates among treatment groups. Significance was defined as  $p < 0.05$ .

## Results

There was no effect of treatment on serum estradiol-17 $\beta$  concentrations (Figure 1). However, serum progesterone concentration was greater in T1 compared to T2 and C ewes at 168 and 360 hours ( $p < 0.00001$ ; Figure 2). Ovulation rate was not significantly different among groups (Table). However, CL diameters were significantly greater in T1 ( $16.1 \pm 3.6$  mm) compared to T2 ( $14.2 \pm 3.5$  mm), but not compared to C ( $14.9 \pm 3.4$  mm). Fecundity rate, birth weight, litter weight, and weaning weight did not significantly differ among treatments (Table). Pregnancy rate was numerically lower in T1 (50%) and T2 (62.5%) compared to C (87.5%) ( $p = 0.1056$ ).



**Figure 1.** Ewes treated with 5 ml P.G. 600<sup>®</sup> (Group T1; n = 8), 1.5 ml P.G. 600<sup>®</sup> (Group T2; n = 8) or 5 ml saline (Group C; n = 8), were sampled hourly from the treatment injection time (0 hour) and at (2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 336 hours) post treatment. There were no effects of treatment on estradiol-17 $\beta$  concentrations over time.



**Figure 2.** Ewes treated with 5 ml P.G. 600® (Group T1; n = 8), 1.5 ml P.G. 600® (Group T2; n = 8) or 5 ml saline (Group C; n = 8), were sampled hourly from the treatment injection time (0 hour) and at (2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 336 hours) post treatment. Serum progesterone concentrations were higher in T1 compared to T2 and C ewes at 168 and 336 hours (\*p < 0.00001).

**Table.** Results of P.G. 600® administration on reproductive characteristics of ewes during the non-breeding season. Ewes were treated with 5 ml P.G. 600® (Group T1; n = 8), 1.5 ml P.G. 600® (Group T2; n = 8) or 5 ml saline (Group C; n = 8). No significant differences among groups in all parameters.

Item Group	Treatment Groups		
	T1	T2	C
Ovulation rate (mean ± SD)	2.23 ± 0.47	2.0 ± 0.26	2.0 ± 0.24
Pregnancy rate	50 %	62.5 %	87.5 %
Fecundity rate (mean ± SD)	2.0 ± 0	2.25 ± 1.25	1.85 ± 0.37
Birth weight, kg (mean ± SD)	4.0 ± 0.53	3.85 ± 1.02	3.96 ± 0.94
Litter weight, kg (mean ± SD)	7.49 ± 1.02	8.66 ± 3.92	7.36 ± 1.56
Weaning weight, kg (mean ± SD)	20.45 ± 3.1	19.92 ± 5.04	21.2 ± 5.45

## Discussion

Ovulation rate in anestrus ewes in our study did not differ between treatment groups receiving progesterone and gonadotropins or progesterone alone, consistent with a previous study.<sup>28</sup> That ovulation rate in anestrus ewes was not affected by gonadotropin dose was similar to an earlier report.<sup>15</sup> It is important to note that in both studies,<sup>15,28</sup> multiple injections of FSH were used instead of P.G. 600® and FSH was administered until 24 or 36 hours prior to progesterone removal instead of concurrent with progesterone removal (as in present study).

Pregnancy rate was not significantly different among groups (control = 87.5%, 1.5 ml P.G. 600<sup>®</sup> = 62.5%, and 5 ml P.G. 600<sup>®</sup> = 50%). Similar findings were reported earlier.<sup>22,29</sup> Anestrous ewes treated with melengestrol acetate (MGA) and 5 ml of P.G. 600<sup>®</sup> had pregnancy rates not different from MGA treatment alone. Furthermore, treatment of anestrous ewes with intravaginal progesterone followed by 3 ml P.G. 600<sup>®</sup> resulted in pregnancy rates that were not different from treatment with intravaginal progesterone alone.<sup>25</sup> However, a higher dose of P.G. 600<sup>®</sup> (> 3.49 ml) decreased lambing rate,<sup>30</sup> a finding that served as the basis for our hypothesis.

P.G. 600<sup>®</sup> (400 IU of eCG and 200 IU of hCG) increased serum progesterone concentrations ( $p < 0.00001$ ) compared to controls (Figure 2), similar to an earlier report<sup>31</sup> wherein ewes were treated with 300 IU eCG after progesterone withdrawal. However, the finding that higher progesterone concentration during early gestation increased embryo survival<sup>32</sup> was not supported by our findings. Increases in serum progesterone concentration were likely due to increased CL diameters in the 5 ml P.G. 600<sup>®</sup> treatment group.<sup>33-35</sup> Similar to earlier findings,<sup>31</sup> fecundity rate, birth weight, litter weight, and weaning weight of lambs did not significantly differ among treatment groups (Table 1).

In conclusion, during nonbreeding season, administration of P.G. 600<sup>®</sup> did not affect ovulation rate, fecundity rate, lambing rate, birth, or weaning weights but did increase circulating progesterone concentrations (and CL diameter at 5 ml dosage). Furthermore, there was a trend for the higher dose of P.G. 600<sup>®</sup> to be detrimental to pregnancy rate in the Polypay breed.

### Acknowledgment

Authors thank the Oregon Sheep Commission for funding this research and the Ministry of Higher Education and Scientific Research in Iraq for graduate student financial assistance.

### Conflict of interest

Authors disclose that there was no actual or potential conflict of interest in conducting this research and with their ability to objectively present/review the research or data.

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