Equine Chorionic Gonadotropin Treatment 11 Days After Artificial Insemination Reduces Pregnancy and Lambing Rates in Creole Ewes

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Abstract | This study evaluated the effects of equine chorionic gonadotropin (eCG), 11 days after artificial insemination (AI) on pregnancy and lambing rates in Creole ewes. After laparoscopic insemination, ewes were randomly assigned to either eCG (nulliparous, n=14 and multiparous, n=16) or no eCG (nulliparous, n=13 and multiparous, n=15) treatment. On day 11 (day zero=AI) 330 IU of eCG was administered IM to eCG treatment ewes. Blood samples were taken on day 11 and 15 to determine the estradiol and progesterone concentrations. The presence or absence of large follicle and large corpus luteum were evaluated on day 15, pregnancy on day 35 by ultrasound, then the lambing rate. In ewes, treatment with eCG increased the number of large follicles (eCG, 11/30 vs. No eCG, 4/28; p=0.041), turgid uteruses (eCG, 13/30 vs. No eCG, 1/28; p=0.005) and large corpora lutea (eCG, 15/30 vs No eCG, 6/28; p=0.013). There were higher concentrations of estradiol-17 β (10.43±0.42 vs. 6.24±0.25 pg mL−1; p=0.0001) and serum progesterone (8.22±0.21 vs. 5.28±0.19 ng mL−1; p=0.0001) in eCG treated ewes. However, eCG reduced the pregnancy rate (eCG, 13/30 vs. no eCG, 23/28; p=0.003) and the lambing rate (eCG, 9/30 vs. no eCG, 20/28; p=0.002). In conclusion, treatment with eCG 11 days after AI reduced pregnancy and lambing rates in Creole ewes.

Keywords | Birth rate, eCG, Gestation rate, Ovine, Sheep

INTRODUCTION

In sheep production, the main objective is to increase pregnancy rate to improve reproductive efficiency. Approximately 20 to 50% of embryonic losses in ewes occurs during the early embryonic period (García-Pintos and Menchaca, 2018; Juengel et al., 2020), possibly due to poor luteal function and low progesterone production (de Brun et al., 2016). Both these factors are crucial for maternal recognition of pregnancy in ewes, a critical step for establishment and maintenance of pregnancy (O’Connell et al., 2016; Van-Lier et al., 2017; Kamil, 2019). Conventionally eCG is administered to ewes upon removal of progesterone impregnated vaginal sponges, which are kept in place for 6 to 14 days to synchronize heat and superovulation (Atalla, 2018; Samartzi et al., 2020). Recently, eCG administration after AI to improve pregnancy has been experimented on in ewes. However, in ewes there is little evidence that eCG administration after AI in fact improves pregnancy rates (García-Pintos and Menchaca, 2016, 2018). Although, there is more evidence of pregnancy improvement in cattle (Bartolome et al.,...
Luteal function and progesterone production are essential for embryonic establishment during the early developmental period. There is a relationship between increased serum progesterone concentration, larger corpus luteum size, and better luteal function (Hashem and Sallam, 2020). When luteal function improves, the progesterone concentration increases in ewes (Vergani et al., 2020). In goats, progesterone production minimizes pregnancy loss without affecting embryonic viability (Hashem et al., 2015). The administration of eCG on day 10 after ovulation improves luteal function, increases progesterone levels in ewes (García-Pintos and Menchaca, 2016), and probably helps the establishment of pregnancy during the first month of gestation (García-Pintos and Menchaca, 2018). The eCG as a luteotrophic agent, post-ovulation, could be an alternative to improve pregnancy rates in anestrous beef cows (Núñez-Olivera et al., 2018). These evidences shows a positive effect of eCG on increasing progesterone concentrations; however, studies using eCG after insemination are minimal and the effects on pregnancy in ewes are still unclear.

One of the strategies to reduce early pregnancy losses in ewes would be to improve the function of the corpus luteum to stimulate the production of sufficient progesterone. eCG shows biological activities similar to those produced by luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Murphy, 2012). These pituitary hormones, mainly LH, are essential for the growth and maintenance of the corpus luteum (Wiltbank et al., 2012). The eCG modulates the gene expression of the corpus luteum and participates in the synthesis of lipids and progesterone (Fátima et al., 2012b). The positive impact of eCG on pregnancy is likely due to the ability of eCG to induce changes at the molecular, cellular and tissue levels related to the corpus luteum (Fátima et al., 2012a) which promotes the production of progesterone (Núñez-Olivera et al., 2018). When eCG is administered after ovulation, it improves the competent corpus luteum in beef heifers (Núñez-Olivera et al., 2020), increases progesterone levels in ewes (García-Pintos and Menchaca, 2016) with different pregnancy rates between cross breed and Merino ewes (García-Pintos and Menchaca 2018). These initial results require further investigation in different conditions and genetic groups of ewes to see the effects of eCG after insemination.

The objective of the present study was to evaluate the administration of eCG 11 days after insemination on pregnancy and parturition rates in nulliparous and multiparous Creole ewes.
MSD, Animal Health, Mexico) 330 IU was administered IM to ewes in the eCG treatment group, the other ewes received sodium chloride as a placebo, 11 days after insemination. Blood samples were taken on days 11 and 15, to determine serum concentrations of estradiol and progesterone. On day 15, large follicle (large ≥ 5 mm, small ≤4 mm in diameter; adapted from Menchaca and Rubianes (Menchaca and Rubianes, 2004) and large corpus luteum (large ≥ 10 mm, small ≤ 9 mm in diameter; adapted from Allam et al. (2015) were evaluated by ultrasound; large follicle or large corpus luteum were classified into two categories as present or absent according to reference sizes. Also, on day 15, turgid uterus was subjectively evaluated using the laparoscopic lens, adapting to the description of Graff (2010) and Juengel et al. (2016), it was considered turgid uterus when it presented the maximum or firm tone with a color tending to pink; on the contrary, if the uterus was flaccid with minimal tone, it was considered a non-turgid uterus. At day 35, pregnancy was diagnosed through transrectal ultrasonography (ProSound 2, Aloka, 7.5 MHz linear transducer, Aloka) (Figure 1). Finally, the lambing was recorded.

Figure 1: Schematic diagram of equine chorionic gonadotropin (eCG) treatment after insemination in Creole ewes. Ch=20 mg chloronolone in intravaginal sponge; AI=artificial insemination; eCG=application of 330 IU of equine chorionic gonadotropin to the group of ewes that received eCG; BS=blood sample; P4=progesterone concentration; E2=estradiol concentration; LF=large follicles evaluation; LCL=large corpus luteum evaluation; TU=turgid uterus evaluation; PD=pregnancy diagnosis.

The reproductive performance of ewes was evaluated by calculating the following variables: pregnancy rate=(ewes pregnant on day 35/total ewes inseminated) x 100; lambing rate=(number of ewes lambing/total ewes inseminated) x 100.

SERUM DETERMINATIONS OF PROGESTERONE AND ESTRADIOL
Blood was collected from 40 ewes total, 10 in each group: eCG nulliparous, eCG multiparous, no eCG nulliparous, and no eCG multiparous. Blood was collected by jugular venipuncture in vacutainer tubes of 10 mL, on days 11 and 15 after AI. Blood was immediately centrifuged at 1500 x g for 20 min and serum was stored in cryovials at -20 °C, until analysis. The serum progesterone concentration was evaluated by solid phase radioimmunoassay (Coat-A-Count, Siemens, CA, USA) with a test sensitivity of 0.02 ng mL⁻¹ and coefficient of variation of 2.80%. The estradiol concentration was determined by an enzyme immunoassay, with a polyclonal antibody (R4972; C. Munro, University of California, Davis, USA) and an estradiol conjugate (17B-3 hemisuccinate-HRP), with a test sensitivity of 0.40 pg mL⁻¹ and a coefficient of variation of 5.57%.

STATISTICAL ANALYSIS
Statistical analyses were performed with the SAS 9.3 statistical package (SAS, 2011). For continuous response variables: Progesterone concentration and estradiol concentration; the statistical model included the effects of treatment (eCG, No eCG), maternal physiological status (nulliparous, multiparous), and their interactions. Before analysis, normality and homogeneity of variances were checked by the Shapiro-Wilk and Levene tests, then analyzed by two-way analysis of variance. Means were compared among groups using Bonferroni test. For categorical response variables: presence of large follicle, a turgid uterus, a large corpus luteum, pregnancy rate and lambing rate; the statistical model considered the effects of treatment, maternal physiological status, and their interaction; these were removed from the model if they were non-significant. The data were analyzed with logistic regression; the stepwise method was used to generate the model. In addition, the Hosmer and Lemeshow tests were performed for the goodness of fit of the model. Differences were considered significant when p value was less than 0.05.

RESULTS AND DISCUSSION

LARGE FOLLICLE, TURGD UTERUS AND LARGE CORPUS LUTEUM
The effects of treatment with eCG 11 days after AI in Creole ewes are shown in Table 1. The eCG affected the number of large follicles (p=0.041). A higher percentage of ewes that received eCG had large follicles (11/30) than those who did not receive eCG (4/28) [odds ratio (OR)=3.88; 95% confidence interval (CI)=1.06 to 14.28]. The eCG treatment also influenced the percentage of ewes with a turgid uterus (p=0.005). More ewes treated with eCG had a turgid uterus (13/30) than those who did not receive eCG treatment (1/28) [OR=20.65; 95% CI=2.47 to 172.46].

The large corpus luteum were influenced by eCG treatment (p=0.013). A higher percentage of ewes that received eCG had large corpus luteum (15/30) than those who did not receive eCG (6/28) [OR=4.59; 95% CI=1.38 to 15.28]. Maternal physiological status did not affect the presence of a large corpus luteum (p=0.185), a turgid uterus (p=0.745).
or large follicles (p=0.686). The interaction between maternal physiological status and eCG treatment for large follicles, turgid uterus and large corpus luteum were not significant.

**Pregnancy rate and lambing rate**

The effect of eCG administration 11 days after AI on pregnancy at 35 days and lambing are shown in Table 2. Ewes that received eCG had a lower (p=0.003) pregnancy rate (13/30) than those did not receive eCG (23/28) [OR=0.17; 95% CI=0.05 to 0.58]. Non-significant differences (p=0.305) were observed in pregnancy rates between nulliparous and multiparous ewes.

**Table 1: Percentage of large follicles, turgid uterus and large corpus luteum in Creole ewes treated with equine chorionic gonadotropin (eCG), 11 days after insemination.**

<table>
<thead>
<tr>
<th></th>
<th>Large follicles*</th>
<th>Turgid uterus*</th>
<th>Large corpus luteum*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>29.6</td>
<td>(8/27)</td>
<td>25.9</td>
</tr>
<tr>
<td>Multiparous</td>
<td>22.6</td>
<td>(7/31)</td>
<td>22.6</td>
</tr>
<tr>
<td>p value</td>
<td>0.686</td>
<td></td>
<td>0.745</td>
</tr>
</tbody>
</table>

Within a column, different superscripts differ significantly (p ≤ 0.05). * Evaluated at four days (15 day) after eCG treatment (large follicles: ≥ 5 mm in diameter, large corpus luteum: ≥ 10 mm in diameter, turgid uterus present or absent, respectively).

**Table 2: Percentage of pregnancy and lambing rates in Creole ewes treated with equine chorionic gonadotropin (eCG), 11 days after insemination.**

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy rate</th>
<th>Lambing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Maternal physiological status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>55.6</td>
<td>(15/27)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>67.7</td>
<td>(21/31)</td>
</tr>
<tr>
<td>p value</td>
<td>0.305</td>
<td></td>
</tr>
</tbody>
</table>

Within a column, different superscripts differ significantly (p ≤ 0.05). Day zero=day of artificial insemination.

Lambing rate was influenced by eCG treatment (p=0.002), ewes that received eCG presented lower lambing rate (9/30) than those did not receive eCG (20/28) [OR=0.13; 95% CI=0.03 to 0.48]. The multiparous ewes had a higher (p=0.042) lambing rate (19/31) than nulliparous ewes (9/30) [OR=4.13; 95% CI=1.60 to 16.17]. The interaction was not significant between maternal physiological status and treatment for lambing rates.

**Estradiol and progesterone concentrations**

The results of eCG treatment on serum estradiol and progesterone concentrations are shown in Table 3. No interaction was found between maternal physiological state and eCG treatment in serum estradiol concentration (p=0.314) on day 15. Between nulliparous and multiparous ewes, serum estrogen concentrations were similar on day 11 (p=0.412) and day 15 (p=0.365). On day 15, a higher serum concentration of 17ß estradiol (p=0.001) was obtained in sheep treated with eCG compared to untreated (10.43±0.42 vs. 6.24±0.25 pg mL⁻¹). Likewise, there was no interaction between maternal physiological state and eCG treatment on serum progesterone concentration (p=0.125). Maternal physiological status did not affect serum progesterone concentration on day 11 (p=0.324) or on day 15 (p=0.434). On day 15, a higher (p=0.001) serum progesterone concentration was found in the group of eCG treated ewes compared to those did not receive eCG (8.22±0.21 vs. 5.28±0.19 ng mL⁻¹).

**Table 3: Serum estradiol and progesterone concentrations (Mean±SEM) in Creole ewes treated with equine chorionic gonadotropin (eCG), 11 days after insemination.**

<table>
<thead>
<tr>
<th></th>
<th>Estradiol-17ß (pg mL⁻¹)</th>
<th>Progesterone (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 11</td>
<td>Day 15</td>
</tr>
<tr>
<td>Maternal physiological status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>5.71±0.24</td>
<td>8.56±0.65</td>
</tr>
<tr>
<td>Multiparous</td>
<td>5.99±0.22</td>
<td>8.11±0.52</td>
</tr>
<tr>
<td>p value</td>
<td>0.412</td>
<td>0.365</td>
</tr>
</tbody>
</table>

Within a column, different superscripts differ significantly (p ≤ 0.05). Day zero=day of artificial insemination.

The hypothesis of this study was that the administration of eCG 11 days after insemination would increase the number of large corpora lutea, the concentration of serum progesterone and improve the pregnancy and lambing rates in Creole ewes. Treatment with eCG modulates the expression of several genes in the corpus luteum that are involved in lipid biosynthesis and progesterone production.
In the present study, the administration of eCG 11 days after insemination resulted in higher numbers of large corpora lutea and a higher concentration of serum progesterone in eCG-treated ewes compared to untreated ones 4 days after administration. These results are supported by García-Pintos and Menchaca (2016) who, when administering eCG on day 10 after insemination of the ewes, found increases in the size of the corpus luteum and progesterone levels. Also the administering eCG on day 12 after mating of the ewes, increased the serum concentrations of progesterone compared to those treated with GnRH (Allam et al., 2015). Treatment with eCG on day 14 after artificial insemination at a fixed time increases the area of the corpus luteum, improving serum concentrations of progesterone in anestrous beef cows (Núñez-Olivera et al., 2018). In rabbits, the administration of eCG 7 days after insemination increased the number of corpora lutea and the serum progesterone concentration (Hashem and Aboul-ezz, 2018). This shows that increases in serum progesterone concentration are related to increases in the number of corpus luteum and improvements in luteal function (Hashem and Sallam, 2020). The greater number of large corpora lutea found in the present study is probably attributed to eCG activating luteotropic mechanisms. Consequently, the progesterone concentration would increase. The luteotropic mechanisms of the effect of eCG after insemination in cattle are still unclear, and therefore warrant further investigation (Núñez-Olivera et al., 2018).

The administration of eCG 11 days after insemination decreased the pregnancy rate at 35 days and the lambing rate in Creole ewes in the present experiment. In other studies, the administration of eCG after insemination did not affect the pregnancy rate in ewes. When administering eCG after insemination, they found different pregnancy rates at 30 days, 35.0% (eCG 5 days), 33.3% (eCG 10 days) in crossbred ewes; 50.5% (eCG 5 days) in Merino ewes (García-Pintos and Menchaca, 2018); 46.4% with eCG 12 days post-mating in Harri ewes (Allam et al., 2015). Although these pregnancy rates are similar to the 43.3% pregnancy rate found in the eCG group, where pregnancy was reduced in the present study. In other species, the administration of eCG after insemination improves the pregnancy rate in bovines (Bartolome et al., 2012; Wecker et al., 2012; Núñez-Olivera et al., 2018) and in rabbits (Hashem and Aboul-ezz, 2018). Previous studies showed that the increase in the concentration of circulating progesterone, during the first week after ovulation, effectively stimulates the growth of the embryo and the secretion of IFN-τ, thus favoring the maintenance of pregnancy, leading to higher rates of pregnancy (O’Hara et al., 2016; Núñez-Olivera et al., 2018; Lonergan and Sánchez, 2020). On the other hand, the administration of eCG after insemination, resulted in a lower pregnancy rate in crossbred ewes than in Merino, these received different driving conditions; therefore, other factors, such as genetics and environment, could play an important role (García-Pintos and Menchaca, 2018). In the present study, there was a greater concentration of serum progesterone in the group of ewes that received eCG, however this increase was insufficient to maintain early gestation and improve the lambing rate. The positive effect of the administration of eCG after insemination on the pregnancy rate in ewes appears to be different than in cattle, likely due to the dose of eCG used, which would require adjustment; the genetic group and the management conditions also seem to influence the gestation rate.

In the present study, the reduction in the pregnancy rate and the lambing rate would have a sequential relationship with the number of large follicles, high estradiol concentration, and the number of turgid uteri found in greater quantity in the group of ewes that received eCG after insemination. The administration of eCG on day 5 or day 10 after insemination promotes the growth of large follicles, which can cause increased estradiol production with negative effects on embryo survival and/or luteal function in ewes (García-Pintos and Menchaca, 2016). In cows, the administration of eCG after insemination increased the number of large follicles (Wecker et al., 2012) and increased the serum concentrations of estradiol-17 β (Núñez-Olivera et al., 2018). A positive relationship exists between the eCG dose and the number of dominant follicles; therefore, the serum concentrations of estradiol increase in a dose-dependent manner (O’Hara et al., 2016). The increase in follicular size is attributed to the fact that eCG has an affinity for the LH and FSH receptors located in the follicles (Murphy, 2012). This eCG performs functions similar to FSH and LH (Beracochea et al., 2020). In the present study, the formation of a greater number of follicles would have caused an increase in estradiol concentration; consequently, an increase in the number of turgid uteri. During estrus, the concentration of estrogens increases as the follicular size increases (Mahmoud and Senosy, 2019; Figueira et al., 2020; Hemati et al., 2020); and the expression of estrogen receptors increases in the endometrium of ewes
In conclusion, treatment with eCG, 11 days after artificial insemination in Creole ewes, reduced pregnancy and lambing rates. The amount of large follicles, turgid uteruses, and serum estradiol concentrations were increased on day 15 after AI in eCG treated ewes. Additional research is needed to analyze breeding performance of Creole ewes using eCG after AI.

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AUTHOR’S CONTRIBUTION

USQG and RRS conceived, designed, executed, analyzed the data and wrote the manuscript. SAAM executed the experiment. JLB interpretation of results, and writing of the manuscript.

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