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Effects of Human Chorionic Gonadotropin on Serum Progesterone Concentration During the First Weeks After Mating, Components of Pre-implantation Complete Blood Counts, and Number of Offspring at Parturition in Ewes

D. T. Yates[1], L. J. Yates, A. R. Otis, C. A. Warner, R. A. Halalsheh, D. M. Hallford, and T. T. Ross

[1] Corresponding author: Dustin Yates, Address: 4101 N. Campbell Dr., Tucson, AZ 85719, Telephone: 1(940)203-1208, Fax: 1(520)626-1283, email: dty4888@yahoo.com
Department of Animal and Range Sciences, New Mexico State University, Box 20003, MSC 3-I, Las Cruces, NM 88003

Summary

Human chorionic gonadotropin (hCG) may boost progesterone production and attenuate maternal-immune response against concepti in ewes, increasing prenatal survival. The present study examined effects of repeated hCG administration after mating on serum progesterone concentration and complete blood counts (CBC) during early gestation, as well as offspring numbers at parturition. Fifty-six ewes were synchronized and mated, then administered saline (CON) or 100 IU hCG on d 4, d 7, d 10, and d 13 after estrus (d 0). Ewes not conceiving were mated again at subsequent estrus. Progesterone was measured on d 4 to d 15 and CBC on d 7 and d 11. In ewes pregnant at treatment, serum progesterone was greater ($P < 0.050$) in hCG-treated ewes than CON from d 7 through d 15 (final sampling day), while in ewes conceiving at subsequent estrus (after treatment), progesterone was greater ($P < 0.050$) in hCG-treated ewes on d 11 through 15 only. On d 7, total white blood cells (WBC) and lymphocytes (LYM) were greater ($P < 0.050$), mean corpuscular volume ($P = 0.067$) tended to be greater, and eosinophil fraction of WBC tended to be less ($P = 0.068$) in hCG-treated ewes. On d 11, red blood cells and hemoglobin were reduced ($P < 0.050$) and hematocrit tended to be reduced ($P = 0.055$) in hCG-treated ewes. Additionally, neutrophil fraction of WBC was greater ($P < 0.050$) in pregnant ewes on d 7, total LYM were less ($P < 0.050$) in pregnant ewes on d 11, and LYM fraction of WBC was less ($P < 0.050$) in pregnant ewes on d 7 and d 11 than in non-pregnant ewes, independent of treatment. No difference ($P > 0.050$) was found between treatments for number of ewes pregnant from mating at estrus just before treatment, the first estrus after treatment, the second estrus after treatment, or the number of ewes not pregnant. Frequency of single or multiple lambs at parturition did not differ ($P > 0.05$) due to treatment in ewes pregnant from mating at any estrus. Repeated administration of hCG during the first two weeks after estrus increased serum progesterone concentration in pregnant and non-pregnant ewes, influenced components of CBC, but did not appear to influence lambing rate or number of offspring at parturition when administered at these doses.

Key words: Ewes, Complete Blood Counts, Human Chorionic Gonadotropin, Progesterone

Introduction

Progesterone insufficiency during embryonic implantation (McLaren, 1973), maternal-immune response toward the conceptus (Howell et al., 1994), or a combination of these and other factors may contribute to prenatal loss in many domestic-livestock species. Progesterone production is influenced by luteal size and maturation (Hunter and Southee, 1989). Thus, exogenous luteotropins, such as human chorionic gonadotropin (hCG), may increase progesterone concentration and reduce early-stage fetal loss in livestock, when administered early in pregnancy (Kelly et al., 1988; Willard et al., 2003; Szmidt et al., 2008). In addition to increasing progesterone, hCG may reduce maternal immune response against developing offspring, as demonstrated in mice (Fabris et al., 1977; Khil et al., 2007). The immunosuppressive ability of hCG appears to supersede that of increased progesterone alone (Fabris et al., 1977), however, the later may act as a partial intermediate of immunosuppressive function (Ramadan et al., 1997; Wulster-Radcliffe et al., 2003). As substantial prenatal loss can begin as early as d 8 of pregnancy in many livestock species (Nancarrow, 1994), hCG may be most effective when administered within the first two weeks after mating. Single injections of hCG have been reported to increase lambing rates and fetal size (Cam and Kuran, 2004), possibly by promoting increased-uterine secretions during early gestation (Nephew et al., 1994). However, effects of repeated hCG administration are less understood. The objective of this study was to examine effects of repeated injections of hCG on serum-progesterone concentrations in sheep during the first week after mating, on components of complete blood counts (CBC) during the period before implantation, and on number of offspring at parturition in ewes.

Materials and Methods

Animal preparation

All procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Before use, ewes were weighed (64.0 kg ± 1.2 kg) and examined for health, and a breeding soundness exam was performed on all rams. Animals had free access to water and shelter and were fed chopped alfalfa hay (approximately 2 kg/ewe) once daily at 0600 for the duration of the study. Experimental procedures were conducted at New Mexico State University, Las Cruces, N.M. (32° 19' 11" N, 106° 45' 55" W; elevation 1,219 m), and breeding began on September 5, 2008. All ewes were of mixed Suffolk x Hampshire breeding and were produced at New Mexico State University. Ewes ranged from 2 yr to 6 yr of age at breeding and were of average body condition.

Experimental procedure

Thirty-six mature, multiparous ewes and 18 nulliparous long-yearlings were used. On d -15 (d 0 = estrus), all ewes received a progesterone-impregnated intravaginal insert (EAZI-BREED CIDR, 0.3 g progesterone; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. Inserts were removed on d -1 and ewes were joined with fertile rams during a 60-d breeding period. Ewes were stratified by age and treatment designation before being divided randomly into four breeding groups. Beginning on d 0, each group was held in an isolated pen for 48 h with one of four mature, fertile rams fitted with marking harnesses. After the 48-h mating period, ewes were pooled in a common pen and two rams were reintroduced (with marking harnesses) for 60 d. Treatments were randomly assigned after stratification by age. Thirty ewes received repeated injections of hCG (hCG; 100 IU in 1 mL physiological saline, i.m.; ProSpec-Tanny TechnoGene, Ltd, Rehovot, Israel, CAS: HOR-250) and the remaining ewes received repeated saline placebo injections (CON; 1 mL physiological saline, i.m.). Injections were administered just after blood collection on d 4, d 7, d 10, and d 13. Serum samples were collected (jugular venipuncture) into sterile vacuum tubes once daily at 0700 on d 3 to d 15. Whole-blood samples were also collected at 0700 on d 7 and d 11 (EDTA-containing vacuum tubes). Immediately after sampling, whole-blood samples were packaged on ice and shipped overnight to the Veterinary Diagnostics Services, Albuquerque, N.M., for CBC analysis, which included: white blood cell (WBC) number, red blood cell (RBC) number, hemoglobin (Hgb) concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet number, and absolute number (and fraction of total WBC) of neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS). On d 15, all ewes received a cautionary dose of liquamycin (5 mL, s.c.; LA-200, Pfizer, Inc., New York, N.Y.), and were returned to the general flock for the remainder of gestation. Numbers of offspring were recorded at parturition. Each ewe was observed as having a single lamb, multiple lambs, or no lambs. Date of parturition was used to determine approximate date of breeding. At parturition, ewes were determined to have given birth to lambs conceived on the estrus just before treatment, the first estrus after treatment, or the second estrus after treatment.

Serum progesterone radioimmunoassay

After collection, blood samples for progesterone analysis were kept at room temperature for 30 to 60 min and then centrifuged (1,500 x g at 4° C for 15 min) to separate serum. After centrifugation, serum was stored in plastic vials at -80° C until assayed. Radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, Calif.; Schneider and Hallford, 1996) was used to quantify progesterone concentration in all serum samples (mean intra-assay CV = 6.5 percent over five assays; inter-assay CV = 5.9 percent).

Statistical analysis

Progesterone data were analyzed as a split-plot design using the mixed procedure of SAS (SAS Inst. Inc., Cary, N.C.) with repeated measure function. Treatment, pregnancy status, and treatment x pregnancy status were included in the main plot and day and associated interactions were in the sub plot. Blood CBC components were subjected to analysis of variance appropriate for a completely randomized design (glm procedure of SAS), and effects of treatment, pregnancy status, and treatment by pregnancy status were examined within sampling day. Pregnancy rates were sub-

jected to Chi square analysis using the frequency procedure of SAS. Fisher's exact test was used for frequency analysis in which more than 25 percent of cells contained expected frequencies of less than five. Number-of-offspring-per-ewe lambing was analyzed for treatment effects by cycle with the glm procedure of SAS. Length of estrous cycle in ewes conceiving on the first or second cycle after treatment was also analyzed by the glm procedure.

Results and Discussion

Serum progesterone concentration

No treatment x pregnancy status (at the time of treatment) x day interaction was observed ($P > 0.050$) for serum progesterone concentration. However, interactions were observed between treatment and pregnancy status ($P = 0.059$) and between day and pregnancy status ($P < 0.001$). Therefore, ewes pregnant at the time of treatment and those not pregnant were analyzed separately for serum-progesterone concentration. A subsequent treatment x day interaction was observed ($P < 0.050$) in both pregnant and non-pregnant groups, thus, serum-progesterone concentrations were examined for treatment effects within each day. In ewes pregnant at the time of treatment, serum-progesterone concentrations (Figure 1) were greater ($P < 0.050$) in hCG-treated ewes than in CON ewes beginning on d 7 and remained greater through the duration of the sampling period, but did not differ ($P > 0.050$) between treatment groups before d 7. In ewes not pregnant at the time of treatment (Figure 2), serum-progesterone concentration was greater ($P < 0.050$) in hCG-treated ewes beginning only on d 11, but still remained greater through the end of the sampling period. Additionally, serum-progesterone concentrations tended to be greater ($P = 0.108$) on d 9, but did not differ ($P > 0.050$) between treatments on d 4 through d 8 or d 10. Effect due to day was observed ($P < 0.001$) within both treatments in both pregnant and non-pregnant ewes, as expected, with curves following natural, temporal patterns.

Complete blood count components

No interactions were observed (P > 0.050) for treatment x pregnancy status

Figure 1. Serum progesterone concentration in pregnant ewes administered hCG or saline on days 4, 7, 10, and 13 after introduction of ram (treatment by day, $P < 0.001$; * denotes treatment differences, $P < 0.05$).

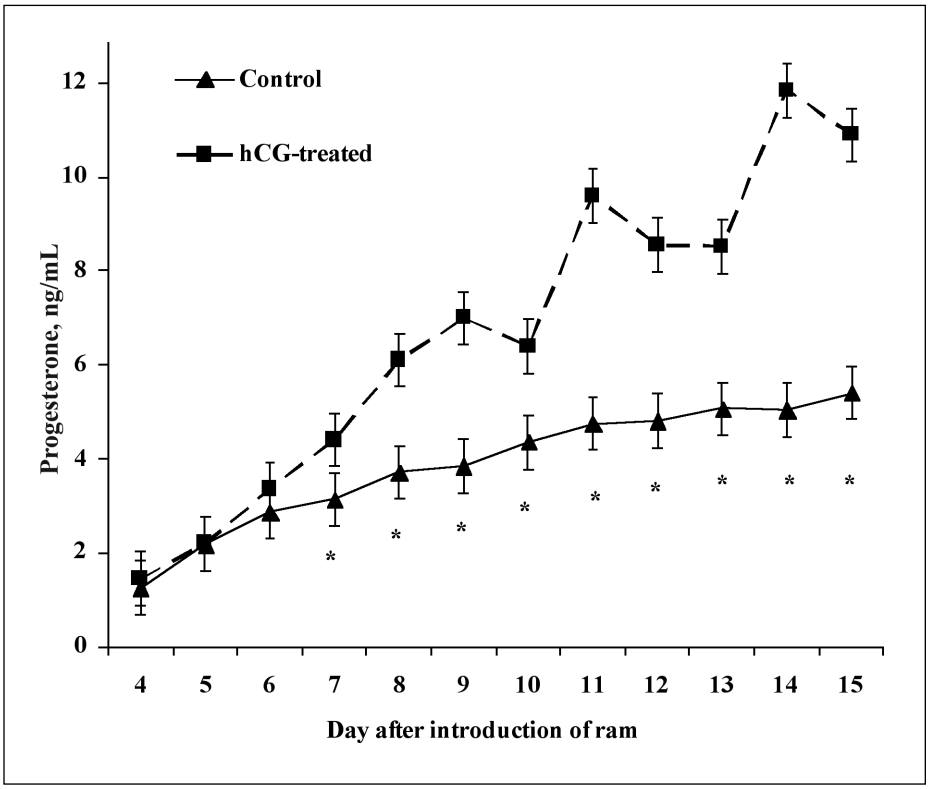

Figure 2. Serum progesterone concentration in non-pregnant ewes administered hCG or saline on days 4, 7, 10, and 13 after introduction of ram (treatment by day, $P = 0.002$; * denotes treatment differences, $P < 0.05$).

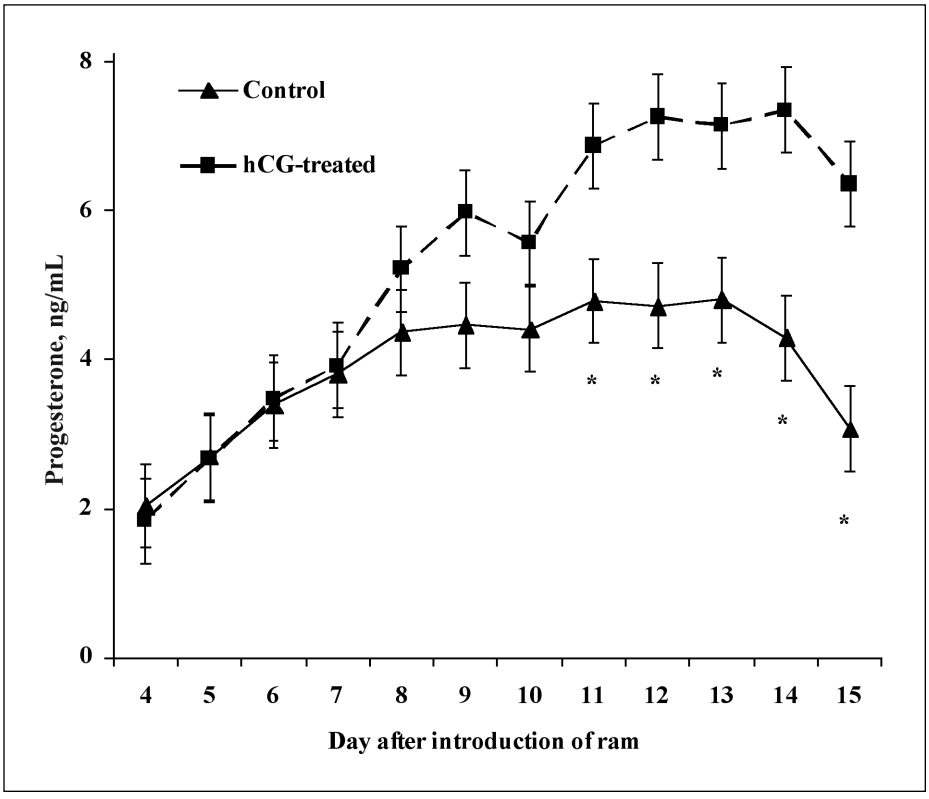

Table 1. Components of whole blood on day 7 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
White blood cells[2]	12.20	23.34	2.85	0.007
Red blood cells[3]	10.96	10.26	0.36	0.113
Hemoglobin[4]	11.96	11.58	0.43	0.468
Hematocrit[5]	33.00	32.11	1.25	0.555
MCV[6]	30.22	31.44	0.53	0.067
MCHC[7]	36.13	35.98	0.19	0.520
Platelets[2]	240.71	187.86	41.51	0.335

[1] Standard error (n = 15).
[2] Count * 10^3.
[3] Count * 10^6.
[4] g/dL.
[5] Percentage of total white blood cells.
[6] Mean corpuscular volume, fL.
[7] Mean corpuscular hemoglobin concentration, g/dL.

Table 2. Immune-cell measurements in whole blood on day 7 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
Neutrophils[2]	33.33	37.33	4.75	0.515
Lymphocytes[2]	55.44	54.89	4.92	0.927
Monocytes[2]	5.78	4.78	1.29	0.574
Eosinophils[2]	5.22	2.33	1.32	0.068
Basophils[2]	0.22	0.22	0.15	0.990
Absolute Neutrophils[3]	4.12	8.24	2.13	0.096
Absolute Lymphocytes[3]	6.69	13.63	1.49	0.002
Absolute Monocytes[3]	0.69	1.00	0.39	0.492
Absolute Eosinophils[3]	0.67	0.40	0.18	0.196
Absolute Basophils[3]	0.03	0.07	0.05	0.483

[1] Standard error (n = 15).
[2] Percentage of total white blood cells.
[3] Count * 10^3.

on d 7 or d 11 for any component of CBC, thus all CBC data are presented as main effects. On d 7 after estrus, numbers of WBC (Table 1) and LYM (Table 2) were greater ($P < 0.050$) in hCG-treated ewes than in CON. Additionally, MCV (P = 0.067) tended to be greater in hCG-treated ewes, and EOS fraction of WBC tended to be reduced ($P = 0.067$) in hCG-treated ewes compared to CON ewes. No differences ($P > 0.050$) were observed between treatments on d 7 for RBC, Hgb, or Hct; however, on d 11 (Table 3) RBC, Hgb, and Hct were reduced ($P < 0.050$) in ewes receiving hCG compared to controls. Additionally, WBC, LYM, MCV, and EOS fraction of total WBC (Table 4), which differed between treatments on d 7 ($P < 0.050$), did not differ at d 11 ($P > 0.050$). Despite treatment differences in total WBC at d 7, total counts of NEU, MON, EOS, and BAS did not differ ($P > 0.050$) between treatments on d 7 or d 11. Likewise, fraction of total WBC comprised by NEU, MON, LYM, and BAS did not differ ($P > 0.050$) between treatments on either day. The NEU fraction of WBC was greater ($P < 0.050$) on d 7 in ewes pregnant at the time of treatment, total LYM were less ($P < 0.050$) at d 11 in ewes pregnant at the time of treatment, and LYM fraction of WBC was less ($P < 0.050$) at d 7 and d 11 in ewes pregnant at the time of treatment compared to those not pregnant at this time. No effect ($P > 0.136$) due to age was observed on any immune component of CBC.

Lambing Results

No difference ($P = 0.546$) was detected between treatments in the number of ewes conceiving at the estrus immediately before treatment, at the first estrus after treatment, at the second estrus after treatment, or the number of ewes failing to deliver lambs (Table 5). Thirteen ewes in each treatment group conceived at the estrus before treatment, while four CON and seven hCG-treated ewes conceived at the first estrus after treatment, and four CON and three hCG-treated ewes conceived at the second estrus after treatment. Only two CON ewes failed to produce a lamb, while six hCG-treated ewes failed to produce offspring. At parturition, frequencies of single-lamb or multiple-lamb births (Table 6) were not different ($P = 0.420$) between treatments for ewes bred at any estrus. Number-of-live-lambs-per-ewe lambing was not different ($P > 0.174$) between treatments in ewes conceiving on any cycle. Additionally, length of estrous cycle did not differ ($P > 0.152$) between treatments in ewes conceiving on the first or second cycle after treatment.

Discussion

Data from this study indicate that repeated hCG administration during early pregnancy can increase circulating levels of progesterone in ewes. Findings support previous demonstrations in both pregnant (Nephew et al., 1994) and non-pregnant ewes (Gómez-Brunet et al., 2007). When hCG was administered to ewes on d 11.5 of pregnancy, Nephew et al. (1994) observed an immediate increase in progesterone concentration that lasted approximately 72 h. Although progesterone response in the current study was delayed 3 d from the initial injection in pregnant ewes, injections spaced 72 h apart succeeded in maintaining increased serum progesterone throughout the remainder of the sampling period. In non-pregnant ewes, increased serum progesterone concentration was delayed for almost one wk after initial injection. This observation was

Table 3. Components of whole blood on day 11 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
White blood cells[2]	15.41	19.32	2.37	0.225
Red blood cells[3]	11.01	9.77	0.30	0.005
Hemoglobin[4]	11.99	10.80	0.36	0.016
Hematocrit[5]	34.22	31.22	1.24	0.055
MCV[6]	31.00	34.86	0.58	0.176
MCHC[7]	35.16	34.86	0.40	0.502
Platelets[2]	205.80	243.80	31.40	0.374

[1] Standard error (n = 15).
[2] Count * 10^3.
[3] Count * 10^6.
[4] g/dL.
[5] Percentage of total white blood cells.
[6] Mean corpuscular volume, fL.
[7] Mean corpuscular hemoglobin concentration, g/dL.

Table 4. Immune-cell measurements in whole blood on day 11 after mating in ewes injected with hCG or saline on days 4, 7, 10, and 13 after estrus detection and mating.

Item	Control	hCG	SE[1]	*P*-value
Neutrophils[2]	32.89	28.22	3.23	0.279
Lymphocytes[2]	59.78	66.33	3.27	0.150
Monocytes[2]	3.56	2.11	0.87	0.197
Eosinophils[2]	3.78	3.22	1.12	0.710
Basophils[2]	0.00	0.11	0.06	0.330
Absolute Neutrophils[3]	5.22	5.04	1.05	0.885
Absolute Lymphocytes[3]	9.10	13.23	2.06	0.091
Absolute Monocytes[3]	0.58	0.43	0.15	0.493
Absolute Eosinophils3	0.51	0.3	0.13	0.899
Absolute Basophils[3]	0.00	0.03	0.02	0.330

[1] Standard error (n = 15).
[2] Percentage of total white blood cells.
[3] Count * 10^3.

Table 5. Number of ewes conceiving at the estrus just before treatment, the first estrus after treatment, the second estrus after treatment, or not pregnant after introduction of rams and administration (i.v.) of hCG or saline on days 4, 7, 10, and 13 after estrus detection of 1st cycle.[1,2]

Cycle	Control	hCG
Estrus before treatment[3]	13	13
1st estrus after treatment	4	7
2nd estrus after treatment	4	3
Not pregnant[4]	2	6

[1] Frequencies did not differ ($P = 0.546$).
2 Cycle was determined by lambing date.
3 Treatment was applied on day 4, 7, 10, and 13 of the 1st cycle only.
4 Ewes were not pregnant at day 75 ultrasound and were removed from the flock. In addition two mature ewes lost eartags between unltrasounding and lambing and were not included in lambing data.

similar to earlier work in which hCG given to non-pregnant ewes on d 0 did not increase progesterone concentration until d 8 (Gómez-Brunet et al., 2007). Although hCG half-life in circulation is approximately 22 h (Schmitt et al., 1996), hCG injections administered 72 h apart in the current study maintained increased progesterone from the day of initial observation through the end of the collection period in both pregnant and non-pregnant ewes, suggesting no desensitization to hCG during this period. This supports similar data involving repeated administration of hCG in cattle (Helmer and Britt, 1987).

Data from d 7 CBC contrast previous reports of an inhibitory effect of hCG on LYM activity (French and Northey, 1983; Khil et al., 2007), as LYM numbers were actually greater in hCG-treated ewes in the current study. However, LYM numbers in ewes receiving hCG were not different from control numbers at d 11, indicating changing dynamics of the influence of hCG on immune response. Additionally, hCG appeared to have no effect on circulating MON, either in total numbers or as a percentage of WBC at d 7 or d 11; a contrast to previous findings in mice (Khil et al., 2007). Indeed, increases in total WBC and LYM numbers at d 7 and a lack of difference in any measured immune component at d 11, does not suggest systemic-immune depression by hCG directly (Khil et al., 2007) or through increased progesterone (Ramadan et al., 1997; Wulster-Radcliffe et al., 2003). Data from the current study do, however, support previous observations of a differential effect of pregnancy on activity of LYM and other immune components (Fabris et al., 1977), as both total LYM and LYM fraction of total WBC were reduced in pregnant ewes compared to non-pregnant ewes in the current study, independent of treatment.

Decreased RBC, Hgb, and Hct recorded at d 11 in hCG-treated ewes contrast to findings in human males treated with hCG, which revealed increased levels of these blood components (Tsujimura et al., 2005). Additionally, Plotka et al. (1988) observed a positive correlation between progesterone concentrations and RBC, Hgb, and Hct levels in wild horses, while in the current study, progesterone concentrations in hCG-treated ewes were

Table 6. Number of ewes with single or multiple lambs at parturition in animals conceiving at the estrus just before treatment, the first estrus after treatment, or the second estrus after treatment and introduction of rams, and number of lambs born per ewe lambing. Ewes were administered (i.v.) hCG or saline on days 4, 7, 10, and 13 after estrus detection of 1st cycle only.[1]

		Treatment[3]		
Estrus[2]	Lambs at term	Control	hCG	*P* - value[4]
Before treatment	Single	6 (6)	4 (4)	0.420
	Multiple	7 (14)	9 (20)	
	Lambs/ewe	1.5 ± 0.16	1.8 ± 0.16	0.174
1st after treatment	Single	0 (0)	1 (1)	0.63
	Multiple	4 (10)	6 (14)	
	Lambs/ewe	2.5 ± 0.28	2.1 ± 0.21	0.321
2nd after treatment	Single	0 (0)	0 (0)	1.000
	Multiple	4 (9)	3 (7)	
	Lambs/ewe	2.3 ± 0.28	2.3 ± 0.33	0.849

[1] Cycle was determined by lambing date.
[2] Treatment was applied on day 4, 7, 10, and 3 of the 1st cycle only.
[3] Total number of lambs per group shown in parenthesis.
[4] Fisher's exact test was used to calculate *P* - value when more than 25 % of cells contained expected values less than 5.

greater than CON ewes at the time that decreased RBC, Hgb, and Hct were observed. The link between hCG administration and RBC, Hgb, and Hct levels is poorly understood, but does appear to be temporal.

Greater frequencies of multiple fetuses in ewes treated with hCG were previously reported (Nephew et al., 1994; Zamiri and Hosseini, 1998; Cam and Kuran, 2004), however, comparable birth frequencies in the current study disagree with these accounts and instead support other findings in which hCG did not improve prolificacy (Gómez-Brunet et al., 2007). Additionally, hCG did not appear to affect prolificacy or pregnancy rate in ewes conceiving on the estrus immediately following treatment, a contrast to previous findings of increased pregnancy rate in heifers treated with hCG before breeding (Breuel et al., 1989). Data from the current study indicate that hCG administration did not improve pregnancy rate, lambs per ewe, or total lambs at parturition, in contrast to results by Kleemann et al. (1991), who reported increasing progesterone concentration resulted in increased embryo survival.

Conclusions

Human chorionic gonadotropin was shown to increase serum-progesterone concentration in both pregnant and non-pregnant ewes. This effect was delayed after initial injection in both groups, but the delay was greater in non-pregnant ewes. Ewes did not appear to become desensitized to hCG despite repeated administration. White blood cells, lymphocytes, mean corpuscular volume, and eosinophil percentage of white blood cells were increased and neutrophils were decreased by hCG at day 7 after estrus, but all returned to control levels by day 11. Red blood cells, hemoglobin, and hematocrit levels were not affected by hCG treatment at day 7, but were decreased at day 11 despite increased progesterone concentrations. Progesterone and specific immune components were altered by administration of hCG, but these changes did not seem to translate to superior lambing data at parturition. This study assumed a certain amount of natural embryonic loss in controls ewes based on previous research, and that this loss would be alleviated by administration of hCG. However, if natural loss did not occur or was overestimated in the ewes used as controls for this study, lambing data could not have been different due to treatment. Future studies must include an account of ovulation rates in order to establish the exact amount of natural embryonic loss, if any.

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