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Effects of hCG on progesterone concentrations and fertility in cyclic, lactating Alpine goats

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Abstract

The objective of this study was to evaluate the effect of hCG administration on plasma progesterone concentrations in lactating Alpine goats during the natural breeding season. After detection of estrus, 124 does were randomly assigned to one of two treatments (T1 and T2). In T1 (n = 60) and T2 (n = 64), the animals received 1 ml of saline solution or 250 IU of hCG i.m., respectively, 5 days after the first detection of estrus and breeding. Plasma progesterone concentration (ng/mL) was determined from blood sampled (T1 = 12 and T2 = 13) on Days 0 (day of estrus), 5, 7, 13, 17, 21, 28 and 45 after breeding. Two control goats with short cycles and two nymphomaniac goats (one per treatment) were detected. Plasma progesterone concentration (mean ± SEM) for T1 and T2 females differed on Days 13, 17 ($P < 0.005$) and 21 ($P < 0.075$), being respectively 3.8 ± 0.2 and 6.6 ± 0.7 at Day 13; 3.6 ± 0.1 and 6.5 ± 0.7 at Day 17; 4.3 ± 0.4 and 6.4 ± 0.9 at Day 21. Pregnancy rates on Days 35 and 70 did not differ ($P > 0.05$) between T1 (78.3%) and T2 (84.4%) does. The hCG administration 5 days after breeding increased plasma progesterone concentrations on Days 13 to 21 but did not increase pregnancy rate.

Keywords: hCG, progesterone, fertility, goat.

Introduction

Goat production has experienced a strong expansion around the world in the last decade (Morand-Fehr and Boyazoglu, 1999) because of the rusticity, adaptability, milk production, meat, leather, and fiber products of the goat. Based on these facts, there is a necessity to study the reproductive phenomena of goats to optimize their reproductive and production efficiency.

The knowledge of the animal behavior as well as endocrinology is the first step to control and assist reproduction in any species. In this context, the animal's response to exogenous hormonal challenges may be advantageous, especially when endocrine profiles can be altered. Pregnancy loss, which decreases overall herd fertility, is greatest during early embryonic and fetal development. In sheep, fertility is lower during the first third of the natural breeding season (Hulet *et al*., 1956). The basic cause of this is low luteal activity (Sangha *et al*., 2002) and low progesterone (P4) synthesis and secretion (Niswender and Nett, 1994). Luteal dysfunction is common in the female goat (Sangha *et al*., 2002) and this phenomenon leads to short estrous cycles in function of premature release of prostaglandin F2α (PGF2α), which has been suggested as the main cause of premature luteal regression (Battye *et al*., 1988).

Goats are exclusively dependent on the corpus luteum (CL) for maintenance of pregnancy (Meites *et al*., 1951). Thus, strategies to increase CL life span, number, or its function could enhance fertility. The administration of human chorionic gonadotropin (hCG) can promote ovulation of the first-wave dominant follicle leading to accessory CL formation and increased P4 concentrations in sheep (Farin *et al*., 1988; Nephew *et al*., 1994); however, this has not been investigated in goats. It is known that at Day 5 post-estrus there are follicles in the growing phase (Ginther and Kot, 1994; Menchaca and Rubianes, 2002) and hCG administration on Day 7 post-estrus promoted accessory CL formation on Day 10 (Tiwari *et al.*, 1998). The objective of this study was to investigate the effect of hCG administration 5 days after breeding on plasma P4 concentrations and fertility in lactating Alpine does.

Materials and Methods

Location

This study was conducted from May to August (breeding season extends from February to June) at the Goat Sector of the Department of Animal Science at the Federal University of Viçosa, Brazil (20º45'S latitude and 42º51' WG longitude). The average altitude was 692.73 m with CWA climate, according to the Köppen classification (dry winter and humid summer) with an average annual temperature of 20.9ºC and rainfall of 1203 mm^3.

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Experimental animals

A total of 124, lactating Alpine goats, 2 to 4 years old with a body condition score (BCS, 1 = emaciated to 5 = fat) of 3.2 ± 0.8, on their second or third parity were used. The BCS was evaluated by palpation of the lumbar and sternal region on the day of estrus. The animals were kept in elevated pens and fed corn silage and a concentrated ration twice daily. Water and mineral salt were available ad libitum. Estrus detection was facilitated by the use of a teaser male (lateral penis deviation) three times per day (06:00, 12:00, and 18:00 hours). Signs of estrus were: searching for the male, restlessness, vocalization, frequent urination, tailing, contraction, hyperemia, edema of the vulva, vaginal mucous discharge, and immobility when mounted, which is the characteristic sign of the onset of estrus. Animals were bred during May to June (final third of local breeding season).

Design of experiment

Goats were bred by natural service using one of four proven bucks after estrus detection (day 0 = estrus) and at 12 hour intervals until the end of estrus and were randomly assigned to one of two treatments (T1 and T2). In T1 (n = 60) and T2 (n = 64), animals received 1 ml of saline solution or 250 IU hCG (Vetecor®, Laboratórios Calier do Brasil Ltda, São Paulo, Brasil) i.m., respectively, 5 days after the first detection of estrus and breeding. Bucks were equally distributed across both treatments.

Progesterone

From the total of number of animals (n = 124), 25 goats (T1 = 12 and T2 = 13) were used for progesterone analyses. Blood samples were collected on Days 0, 5, 7, 13, 17, 21, 28, and 45 after breeding to determine plasma P4 concentrations (ng/ml). Blood was collected from the jugular vein into heparinized test tubes. After collection, the tubes were placed into a box with ice until centrifugation in a refrigerated centrifuge at 5ºC and 2500 x g for 15 minutes. Within two hours after collection, plasma was stored at -20ºC until assays were performed. Plasma P4 concentrations were determined by a solid-phase radioimmunoassay technique (Menchaca and Rubianes, 2001) using a commercially-available kit (Coat-a-Count Progesterone Kit®, DPC, Diagnostic Products Co., Los Angeles, CA, USA), according to the manufacturer's recommendations. The mean intra- and interassay coefficients of variation were 9% and 8%, respectively.

Pregnancy detection

All the females were evaluated by transrectal ultrasonography using a 5 MHz (Aloka 500®, Tokyo, Japan) probe at 35 and 70 days after breeding for pregnancy detection.

Statistical analyses

Statistical analyses comprised of one-way analysis of variance testing for differences in plasma P4 concentrations between treatments and using the Student Newman Keuls test (SNK) processed by the SAEG (System for Statistical and Genetic Analysis; Ribeiro Júnior, 2001). Variances were not homogeneous and log-transformation was applied to plasma P4 data on Days 13, 17, and 21 after breeding ($\log_{10}$ [x+1], x = P4 value in ng/ml). The chi-square test was used to test for differences in pregnancy rates (Ribeiro Júnior, 2001). Statistical analysis was performed using a significance level of $P < 0.05$.

Results

Progesterone

Plasma P4 concentrations remained unchanged ($P > 0.05$) from Day 7 to 17 in control animals. However, hCG-treated animals had a 125% increase ($P < 0.005$) in P4 from Day 7 (2.93 ng/ml) to 13 (6.59 ng/ml; Table 1). The hCG-treated animals had higher plasma P4 concentrations on Days 13, 17 ($P < 0.005$; and a tendency at day 21; $P < 0.075$) than control animals (Fig. 1). Two control does had short cycles (second estrus 8 days after mating) and did not become pregnant. These does did not show detectable progesterone concentrations on Day 7 after first estrus and breeding (one day before second estrus). Two nymphomaniac does (one for each group) were excluded from progesterone analysis. These does showed persistent estrus and male behavior. They did not have detectable progesterone at anytime.

Pregnancy

Pregnancy rate was 78.3% (47/60) for control and 84.4% (54/64) for hCG-treated does and did not differ between treatments ($P > 0.05$). One hCG-treated doe showed estrus 15 days after breeding and was bred again; however, 17 days after the first breeding or 2 days after the second breeding. This animal had a plasma P4 concentration compatible with pregnancy (5.5 ng/ml). Two well-defined fetuses with heart beats were detected 35 days after the first or 20 days after the second breeding.

Table 1. Mean ± SEM plasma progesterone concentrations (ng/ml) from Days 0 (estrus = day 0) to 45 after breeding in pregnant, lactating Alpine goats treated with saline (control) or 250 IU hCG 5 days after breeding.

Day after breeding	Control (n = 6)	hCG (n = 11)
0	not detectable	not detectable
5	1.1 ± 0.2^{c}	1.3 ± 0.3^{b}
7	2.6 ± 0.1^{b}	2.9 ± 0.5^{b}
13	$3.8 \pm 0.2^{a,b}$	6.6 ± 0.7^{a}
17	$3.6 \pm 0.1^{a,b}$	6.5 ± 0.7^{a}
21	4.3 ± 0.4^{a}	6.4 ± 0.9^{a}
28	4.2 ± 0.7^{a}	5.1 ± 0.6^{a}
45	4.3 ± 0.6^{a}	6.3 ± 0.9^{a}

[a,b,c] Means with different superscripts within columns differ (P < 0.05).

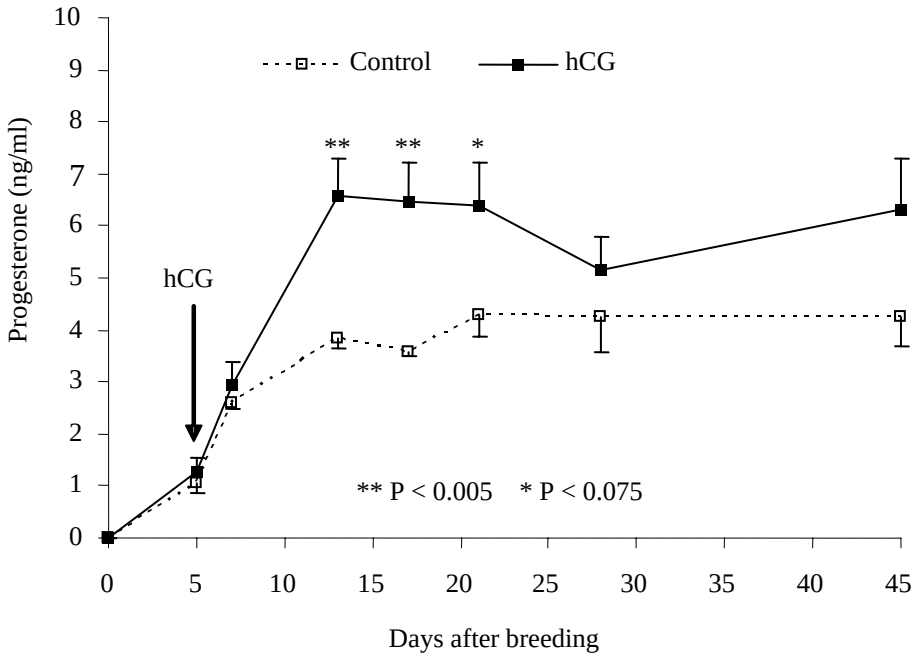

Figure 1. Mean ± SEM plasma progesterone concentrations (ng/ml) from Days 0 (estrus = day 0) to 45 after breeding in pregnant, lactating Alpine goats treated with saline (control) or 250 IU hCG 5 days after breeding.

Discussion

The higher plasma progesterone concentrations after hCG administration in this study were similar to those reported in sheep (Farin *et al*., 1988). The mechanisms by which hCG promotes its effects are diverse. In cattle, hCG enhances progesterone and promotes the ovulation of the first wave dominant follicle leading to the formation of accessory CLs (Fonseca *et al*., 2000) and alterations in luteal morphology (Schmitt *et al*., 1996). Increases in luteal weight, progesterone content, and number of large luteal cells were reported in cattle and sheep (Schmitt *et al*., 1996; Farin *et al*., 1988). In goats, *in vitro* cell agglutination evoked by hCG action directly on granulosa cells of large follicles was reported (Prashad and Guraya, 1987).

There are few reports of the use of hCG in goats. In one study, 250 IU of hCG was able to induce estrus in anoestrous goats when administered 24 hours before removal of a progesterone-impregnated sponge (six days protocol; Fonseca *et al*., 2005a). However, the dose used in the present study (250 IU hCG) was arbitrary and was one-tenth of the effective dose used in cattle (2000 to 3000 IU; Fonseca *et al*., 2001). Thus, other doses should be investigated.

Goat CLs reach their maximum diameter between Days 6 and 8 (Orita *et al*., 2000) or 8 and 14 (de Castro *et al*., 1999) of the estrous cycle. Therefore, in this study, CLs from hCG-treated does could have had a greater growth rate and final weight. According to Orita *et al*. (2000), the maximum diameter of the CL is related to its capacity to synthesize and secrete progesterone. In sheep, Farin *et al*. (1988) administered 300 IU of hCG on Days 5 and 7.5 of the estrous cycle

(Day 0 = estrus) and reported an increase in luteal weight and large luteal cell number and a decrease in the small:large luteal cell ratio. In the same study, these authors reported accessory CLs and increased progesterone on Day 10 with hCG treatment but not with the saline treatment. Although the CL weight and luteal cell numbers were not assessed in the present study, the effect of hCG on these should not be discarded.

In control does, progesterone reached a peak on Day 21 post-estrus, possibly due to the action of embryonic luteotropic factors (Thatcher *et al*., 1997). In hCG-treated does, progesterone reached a peak on Day 13 post-estrus, an evident effect of the luteotropic action of hCG. This effect could have resulted from gonadotropin action on the original CLs, which were not fully developed by Day 5 post-estrus (Orita *et al*., 2000) as reported in cattle (Schmitt *et al*., 1996). Accessory CLs may be formed from accessory ovulations of the dominant follicles from the first follicular wave. These follicles reach maximal diameter and are functional at Day 5 to 6 post-estrus (Ginther and Kot, 1994; Menchaca and Rubianes, 2002) and ovulate under gonadotropin stimulus (Tiwari *et al*., 1998). Similar to results reported in sheep in which a significant increase in plasma progesterone concentrations was noted only 5 days after hCG administration (Day 10 of estrous cycle; Farin *et al*., 1988), this phenomenon was detected on Day 13 in the present study.

In this study, pregnancy rate did not differ ($P > 0.05$) between control (78.3%) and hCG-treated does (84.4%). During the transition season (anestrus to breeding season), the same protocol was used in pluriparous (Fonseca *et al*., 2005b) and nulliparous (Fonseca and Torres, 2005) goats, but no increases in progesterone concentrations during the expected window of maternal recognition of pregnancy and pregnancy rate were observed.

Nymphomaniac behavior has been reported in goats (Gordon, 1997). However, the endocrine basis of this reproductive abnormality is not cited. In the present study, nymphomaniac does from both the control and hCG-treated group did not show detectable progesterone concentrations during the interval from Day 0 to 45 after the first estrus and breeding. Thus, nymphomaniac goats were not associated with luteal cysts as in cattle (Gordon, 1996), and gonadotropin administration could not restore normal reproductive activity as commonly recommended (Pugh, 2002). Furthermore, animals displayed all signs of estrus (Gordon, 1997). Nevertheless, interesting courtship behavior, commonly manifested by bucks, was observed when experimental does were put in the presence of other females in estrus. The persistent estrus and male behavior displayed by does might indicate that nymphomania was associated with follicular cysts, which produce estrogens and possibly androgens too.

Interestingly, one goat displayed signs of estrus and was bred again 15 days after first estrus. In goats, functional CLs are usually found from Day 4 to 16 of the estrous cycle. According to the results of this study, animals from both the control and hCG-treated groups had relatively low plasma progesterone concentrations on Day 5 of the estrous cycle (1.05 ± 0.47 and 1.30 ± 0.72 ng/ml, respectively). Similar values were reported earlier (de Castro *et al*., 1999; Kanuya *et al*., 2000). This means that a doe could not reach a plasma progesterone concentration of 5.47 ng/ml 2 days after estrus (Day 17 after first estrus). Additionally, it was not possible to see a well-formed fetus and with a heart beat at Day 20 after breeding (Fonseca, 2002). Restall *et al*. (1990) reported that 36.5% of pregnant goats showed estrus during pregnancy. Machado (1989) also reported on the occurrence of estrus in pregnant goats and suggested that the environmental changes and the stress of transport could be the cause of this phenomenon. Estrogens produced by intermediary follicle waves (de Castro *et al*., 1999) might also be involved. The present study showed that the pregnant does (confirmed by ultrasonography and plasma progesterone concentrations) can show estrus without a disturbance in pregnancy.

Results of this study showed that hCG administration 5 days after breeding was effective to increase plasma progesterone concentrations during the critical period of pregnancy recognition but did not influence pregnancy rate. Although the number of animals per group used in the present study was adequate for investigation of progesterone profiles, it was not large enough to show possible differences in pregnancy rates. However, results were similar to studies that used other species like sheep and cattle in which pregnancy rates were also not affected. Thus, since the hCG group had a 6% greater pregnancy rate than the control group in the present study, increasing the number of animals in field conditions should be encouraged in hCG treatments.

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