Running Title: hCG and reproduction in cyclic goat

Effects of hCG on progesterone and fertility in cyclic lactating does

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Abstract

The objective of this study was to evaluate the effect of hCG administration on plasma progesterone concentration in Alpine lactating does during the natural breeding season. After estrous identification, 124 does were randomly assigned to 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 intramuscularly, respectively, five days after breeding of 25 goats (T1=12 and T2=13). Plasma progesterone concentration (ng/mL) was determined from blood sampled on days 0 (day of estrus), 5, 7, 13, 17, 21, 28 and 45 after breeding. Two control does with short cycles and two nymphomaniac does (one per treatment) were

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detected. Plasma progesterone concentration (mean ± SD) for T1 and T2 females differed on days 13, 17 (P<0.005) and 21 (P<0.075), being respectively 3.82 ± 0.45 and 6.59 ± 2.10 at day 13; 3.58 ± 0.24 and 6.46 ± 2.10 at day 17; 4.30 ± 1.03 and 6.37 ± 2.39 at day 21. Pregnancy rates detected by trans-abdominal ultrasonography on days 35 and 70 did not differ (P>0.05) between T1 (78.3%) and T2 (84.4%) does. The hCG administration five days after breeding increased plasma progesterone concentrations on days 13 to 21, but it not increased pregnancy rates.

Key words: hCG; progesterone; fertility; goat.

1. Introduction

Goats enterprise have experienced a strong expansion around the world in last decade (Morand-Fehr and Boyazoglu, 1999) because of theirs rusticity, adaptability and great potential to produce milk, meat, leather and fiber. Based on these facts, there is a necessity of regional comprehension and study of the reproductive phenomena to optimize the reproductive and productive efficiency of goats.

The knowledge of the animal behavior as well as its endocrine profile are the first steps to control and assist reproduction in any specie. In this context, the animal response to exogenous hormonal challenges may be so advantageous, mainly when endocrine profiles can be altered. Pregnancy lost is high during the early embryo or fetal development, which decrease overall herd fertility. In sheep, this fertility is lower in the first third of the natural breeding season (Hulet *et al*., 1956). The basic cause of this is the low luteal activity (Sangha *et al*., 2002) with a low progesterone (P4) synthesis and secretion (Niswender and Nett, 1994). The female goat is very susceptive to luteal dysfunction (Sangha *et al*., 2002) leading to short estrous cycles and the premature release of prostaglandin F-2α (PGF-2α) is suggested as the cause of this luteal regression (Battye *et al*., 1988).

Goats are exclusively dependent from corpus luteum for maintenance of pregnancy (Meites *et al*., 1951). Thus, strategies to increase corpus luteum life-span, number or its function could enhance the fertility. The administration of human chorionic gonadotropin (hCG) can promote ovulation of the first wave dominant follicle (FWDF) leading to accessory corpus formation and increase in plasma P4 in sheep (Farin *et al*., 1988; Nephew *et al*., 1994) but remains not reported in goats. Ovarian follicles are in the growing phase at five days post-estrus (Ginther and Kot, 1994; Menchaca and Rubianes, 2002) and hCG administration on day 7 post-estrus promoted accessory corpora lutea formation cheeked on day 10 (Tiwari *et al*, 1998).

The objective of this study was to investigate the effect of hCG administration five days post-breeding on plasma P4 concentration and fertility in lactating Alpine does.

2. Material and methods

2.1. Location

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

2.2. Experimental animals

A total of 124 lactating Alpine goats, 2 to 4 year old, second or third parity with 3.2 ± 0.8 body condition score (BCS, 1= emaciated to 5= fat) were used. The BCS was evaluated by palpation of the lumbar and sternal region on day of estrus. The animals were kept on elevated pens and fed twice a day with corn silage and concentrated ration to meet the required production demand. Water and mineral salt were permanently available. Estrus was monitored three times daily (06:00, 12:00 and 18:00 h) by the use of a teaser (lateral penis deviation). The estrous signs observed were: searching for the male; restlessness; vocalization; frequent urination; tailing; contraction, hyperemia and edema of the vulva; vaginal mucous discharge and immobility on mounting, which is the characteristic signal considered as the onset of estrus. Animals were bred during May to June (final third of local breeding season).

2.3. Design of experiment

Goats were bred detection (day 0= estrus) by natural service with fertile previous tested four bucks after estrous and at 12 h interval until the end of estrus and were randomly assigned into two treatments (T1 and T2). In T1 (n = 60) and T2 (n = 64), the animals received 1 mL saline solution or 250 IU hCG (Vetecor®, Laboratórios Calier do Brasil Ltda, São Paulo, Brasil), respectively, intramuscularly, five days after first estrous detection and breeding. Bucks were equally assigned between treatments.

2.4. Progesterone

Blood was sampled on days 0 (estrus), 5, 7, 13, 17, 21, 28 and 45 after breeding to determine plasma P4 concentration (ng/mL). Blood was collected from jugular vein into heparinized vacuolated test tubes of 25 goats (T1=12 and T2=13). After collection, the tubes were placed into a box with ice until centrifugation in a refrigerated centrifuge at 5 ºC and 2500 x g / 15 min. Within two hours from collection the plasma was stored at – 20 ºC until analysis. Plasma P4 concentration was determined by the solid phase radioimmunoassay technique (Menchaca and Rubianes, 2001), using commercial Kits (Coat-a-count progesterone kit®, DPC, Diagnostic Products Co., Los Angeles, CA, USA.), according to the manufacturer's recommendations. The mean intra and inter-assay coefficient of variation was 9% and 8%, respectively.

2.5. Pregnancy

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

2.6. Statistical analysis

Statistical analysis comprised one way analysis of variance for testing of the differences in plasma P4 concentration between treatments, tested by 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 on plasma P4 concentration on days 13, 17 and 21 after breeding ($\log_{10} [x+1]$, x= P4 value in nanograms). Chi-square test was used to test pregnancy rate differences (Ribeiro Júnior, 2001). Statistical analysis was performed using statistical significance at the 95% confidence interval**.**

3. Results

3.1. Progesterone

The average plasma P4 concentration was stable ($P>0.05$) from day 7 to 17 in control animals. However, hCG-treated animals had a 125% increment ($P<0.005$) 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 concentration on days 13, 17 ($P<0.005$) and 21 ($P<0.075$) than control animals (Fig. 1).

Two control does showed short cycles (second estrus 8 days after mating) and did not become pregnant. These does did not show detectable progesterone levels at 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 showed detectable progesterone at anytime.

3.2. 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 first breeding or 2 days after second breeding, this animal showed plasma P4 concentration compatible with pregnancy (5.47 ng/ml). Two well-defined fetuses with heart beat were detected 35 days after first or 20 days after second breeding.

4. Discussion

The higher plasma progesterone concentration after hCG administration in this study was similar as reported in sheep (Farin *et al*., 1988). The mechanisms by which hCG promotes their effects are diverse. In cattle, hCG enhances progesterone and promotes the ovulation of the first wave dominant follicle leading to the formation of accessory corpora lutea (Fonseca *et al*., 2000) with luteal morphology alteration (Schmitt *et al*., 1996). Increases in luteal weigh, progesterone content and large luteal cells number were reported in cattle (Schmitt *et al*., 1996; Farin *et al*., 1998). In goats, *in vitro* cell agglutination evoked by hCG action directly on granulosa cells of the large follicles was reported (Prashad and Guraya, 1987).

There are few reports of hCG use in goats. In another study, 250 IU hCG were efficient to induce estrus in anoestrous goats whem administered 24 hours before sponje removal (six days protocol; Fonseca *et al*., 2005a). However, the dose used in the presesnt study (250 IU hCG) was arbitrary and the tenth fraction of the effective dose used with this objective in cattle (2000 to 3000 IU; Fonseca *et al*., 2001). Thus, others doses should be investigated.

Goat corpora lutea 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, corpora lutea from hCG-treated does could have reached greater growing rate and final weight. According to Orita *et al*. (2000), the corpus luteum maximum diameter is related to its capacity to synthesize and secrete progesterone. In sheep, Farin *et al*. (1988) administered 300 IU hCG on days 5 and 7.5 of the estrous cycle (day 0 = estrus) and reported increased luteal weight and large luteal cell number and drecresed small:large luteal cell ratio. In the same study these authors reported accessory corpora lutea and increased progesterone day 10 hCG but not in saline treated group. Although the corpora lutea were not measured in the present study, the effect of hCG on this parameter might not be excluded.

In control does, progesterone reached a peak on day 21 post-estrus, possibly by the action of embryo luteotropic factors (Thatcher *et al*., 1997). In hCG-treated does, progesterone reached peak on day 13 post-estrus, an evident effect of the hCG luteotropic action. This effect could be resulted from gonadotropin action on the original corpora lutea, which were not fully developed by day 5 post-estrus (Orita *et al.*, 2000) as reported in sheep (Schmitt *et al.*, 1996). By the way, accessory corpora lutea 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). Similarly to results reported in sheep, which significant increase of the plasma progesterone concentration was noted only five days after hCG administration (day 10 of estrous cycle; Farin *et al.*, 1988), in the present study this phenomenun was detected on day 13.

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 were used in pluriparous (Fonseca *et al.*, 2005b) and nulliparous (Fonseca and Torres, 2005) goats but no increases in progesterone level during the expected maternal recognition of pregnancy window and pregnancy rate were observed.

The fate of short estrous cycle is commonly reported in goats (Smith, 1994; Gordon, 1997), but progesterone profiles of these cycles were not reported. In this study, the two control does showing second estrus 8 days after first estrus did not show detectable levels of progesterone at day 7 (one day before second estrus). It could be a typical case of early luteal regression. It is known that premature release of PGF-2α can cause premature luteal regression (Battye *et al.*, 1988). This release can be elicited by estrogens produced by follicles during early to mid luteal phase (de Castro *et al.*, 1999). We can not affirm that hCG prevented early luteal regression in the present study. However, hCG administered 84 h after the onset of estrus inhibit the luteal regression in superovulated donor goats when compared to saline-treated ones which presented 57%

of luteal regression based on plasma progesterone at day 6 post-estrus (Saharrea *et al*., 1998).

The nymphomaniac behavioral features are reported in goats (Gordon, 1997). However, endocrine basis of this reproductive abnormality are not cited. In this study, nymphomaniac does from both control and hCG-treated group did not show detectable progesterone in the interval from day 0 to day 45 after first estrus and breeding. Thus, nymphomaniac goats were not associated to 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 related females signs of estrus (Gordon, 1997). Nevertheless, interesting courtship behavior, commonly manifested by bucks, were observed when experimental does were taken to the presence of other females in estrus. The persistent estrus and male behavior displayed by does might indicate that nymphomania were associated with follicular cysts, which produce estrogens and possibly androgens too.

Interestingly, one goat displayed signs o estrus and was bred again 15 days after first estrus. In goats, functional corpora lutea are usually found from day 4 to 16 of the estrous cycle. According to results of this study, animals from both, control and hCG group, showed relatively low plasma progesterone concentration at 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). It means that a doe could not reach plasma progesterone concentration of 5.47 ng/ml two days after estrus (day 17 after first estrus). Additionally, it was not possible to see fetus well-formed and with 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 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 be involved. In this study, it was shown that the pregnant doe (confirmed by ultrasonography and plasma progesterone assay) can show estrus without pregnancy disturbance.

Results of this study showed that hCG administration five days after breeding was effective to increase plasma progesterone level during the critical period for pregnancy recognition and but this fact do not influence pregnancy rate.

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References

Battye KM, Fairclough RJ, Cameron AWN, Trouson AO. 1998. Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (*Capra hircus*). *J Reprod Fertil*, 84:425-430.

De Castro T, Rubianes E, Menchaca A, Rivero A. 1999. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. *Theriogenology*, 52:399-411.

Farin CE, Moeller CL, Mayan H, Gamboni F, Sawyer HR, Niswender GD. 1988. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. *Biol Reprod*, 38:413-421.

Fonseca JF, Bruschi JH, Zambrini FN, Demczuk E, Viana JHM, Palhão MP. 2005a. Induction of synchronized estrus in dairy goats with different gonadotrophins. *Anim Reprod*, 2:50-53.

Fonseca JF, Silva Filho JM, Pinto Neto A, Palhares MS, Ruas JRM, Alvim MTT, Belisário H, Pardini WS. 2000. Indução de corpo lúteo acessório em novilhas. *Rev Bras Reprod Anim*, 24:143-147.

Fonseca JF, Silva Filho JM, Pinto Neto A, Palhares MS, Ruas JRM, Alvin MTT, Belisário H, Saliba WP. 2001. Concentração plasmática de P4 em novilhas receptoras submetidas à administração de rbST, GnRH ou hCG no quinto dia do ciclo estral. *Arq Bras Med Vet Zootec*, 53:451-458.

Fonseca JF, Torres CAA, Costa EP, Maffili VV, Carvalho GR, Alves NG, Rubert, MA. 2005b. Progesterone profile and reproductive performance of estrous-induced Alpine goats given hCG five days after breeding. *Anim Reprod*, 2:54-49.

Fonseca JF, Torres CAA. 2005. Administration of hCG 5 days after breeding and reproductive performance in nulliparous dairy goats. *Reprod Dom Anim*, 40:495-499.

Fonseca JF. 2002. Controle e perfil hormonal do ciclo estral e performance reprodutiva de cabras Alpinas e Saanen. PhD Thesis, Departamento de Zootecnia, Universidade Federal de Viçosa, Laboratório de Reprodução Animal, Viçosa-MG, Brasil.

Ginther OJ, Kot K. 1994. Follicular dynamics during the ovulatory season in goats. *Theriogenology*, 42:987-1001.

Gordon I. 1996. Controlled reproduction in cattle and buffaloes. Wallingford (UK): CAB International.

Gordon I. 1997. Controlled reproduction in sheep and goats. CAB International, Cambridge (UK).

Hulet CV, Voigtlander Jr HP, Pope AL, Casida LE. 1956. The nature of early-season infertility in sheep. *J Anim Sci*, 15:607-616.

Kanuya NL, Kessy BM, Nkya R, Mujuni PF. 2000. Plasma progesterone concentrations and fertility of indigenous Small East African goats, bred after treatment with cloprostenol. *Small Rumin Res*, 35:157-161.

Machado TMM. 1989. Dissociação entre cio e ovulação em cabras recém-importadas. In Anais do VIII Congresso Bras. Reprod. Anim. (1) p. 115.

Meites J, Webster HD, Young FW, Thorp Jr. F, Hatch RN. 1951. Effects of corpora lutea removal and replacement with progesterone on pregnancy in goats. *J Anim Sci*, 10:411-416.

Menchaca A, Rubianes E. 2001. Effect of high progesterone concentrations during the early luteal phase on the length of the ovulatory cycle of goats. *Anim Reprod Sci*, 68:69–76.

Menchaca A, Rubianes E. 2002. Relation between progesterone concentrations during the early luteal phase and folicular dynamics in goats. *Theriogenology*, 57:1411-1419.

Morand-Fehr P, Boyazoglu J. 1999. Present state and future outlook of the small ruminant sector. *Small Rumin Res*, 34:175-188.

Nephew KP, Cárdenas H, Mcclure KE, Ott TL, Bazer FW, Pope WF. 1994. Effects of administration of human chorionic gonadotropin or progesterone before maternal recognition of pregnancy on blastocyst development and pregnancy in sheep. *J Anim Sci*, 72:453-458.

Niswender GD, Nett TR. 1994. In: Knobil, E., Neill, J.D. (Eds.), The Physiology of Reproduction, Raven Press Limited, New York, pp. 781-815.

Orita J, Tanaka T, Kamomae H, Kaneda Y. 2000. Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. *J Reprod Devel*, 46:31-37.

Prashad RK, Guraya SS. 1987. Effect of human chorionic gonadotropin and follicular fluid agglutinability on goat ovarian granulosa cells. *Indian J Anim Sci*, 57:1187-1190.

Pugh DG. 2002. Sheep and goat medicine 5 ed. W. B. Saunders Company, Philadelphia (USA).

Restall BJ, Milton JTB, Klong-Yutti P, Kochapakdee S. 1990. Pregnancy diagnosis in Thai native goats. *Theriogenology*, 34:313-317.

Ribeiro Júnior I. 2001. Análises estatísticas no SAEG. Editora UFV, Viçosa (BR).

Saharrea A, Valencia J, Balcázar A, Medja O, Cerbón JL, Caballero V, Zarco L. 1998. Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. *Theriogenology*, 50:1039-1052.

Sangha GK, Sharma RK, Guraya SS. 2002. Biology of corpus luteum in small ruminants. *Small Rumin Res*, 43:53-64.

Schmitt ÉJP, Barros CM, Fields PA, Fields MJ, Diaz T, Kluge JM, Thatcher WW. 1996. A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on day five of the estrous cycle. *J Anim Sci*, 74:1915-1929.

Smith MC. 1994. Goat Medicine. Williams & Williams Press, Media (USA).

Table 1. Plasma progesterone concentration (ng/ml; mean ± SEM) from day 0 (estrus = day 0) to 45 after breeding of pregnant Alpine lactating does treated with saline (control) or 250 IU hCG five days after breeding

Thatcher WW, Binelli M, Burke J, Staples CR, Ambrose JD, Coelho S. 1997. Antiluteolytic signals between the conceptus and endometrium. *Theriogenology*, 47: 131-147.

Thatcher WW, Moreira F, Santos JEP, Mattos RC, Lopes FL, Pancarci SM, Risco CA. 2001. Effects of hormonal treatments on reproductive performance and embryo production. *Theriogenology*, 55:75-89.

Tiwari RP, Ansari MR, Majundar AC. 1998. Ovarian response, embryo recovery and progesterone profile in goats following human chorionic gonadotrophin administration on day 7 of the oestrous cycle prior to superovulatory treatment. *Indian J Anim Sci*, 68:1230-1232.

Table 1. Plasma progesterone concentration (ng/ml; mean ± SEM) from day 0 (estrus = day 0) to 45 after breeding of pregnant Alpine lactating does treated with saline (control) or 250 IU hCG five days after breeding

Days after breeding	Control (n=6)	hCG (n=11)
Day 0	non detectable	non detectable
Day 5	1.05 ± 0.47^{c}	1.30 ± 0.72^{b}
Day 7	2.61 ± 0.34^{b}	2.93 ± 1.31^{b}
Day 13	$3.82 \pm 0.45^{a.b}$	6.59 ± 1.95^{a}
Day 17	$3.58 \pm 0.24^{a.b}$	6.46 ± 2.10^{a}
Day 21	4.30 ± 1.03^{a}	6.37 ± 2.39^{a}
Day 28	4.23 ± 1.62^{a}	5.14 ± 1.81^{a}
Day 45	4.25 ± 1.38^{a}	6.32 ± 2.69^{a}

^{a,b,c} Means with different superscripts within columns differed (SNK, P<0.05).

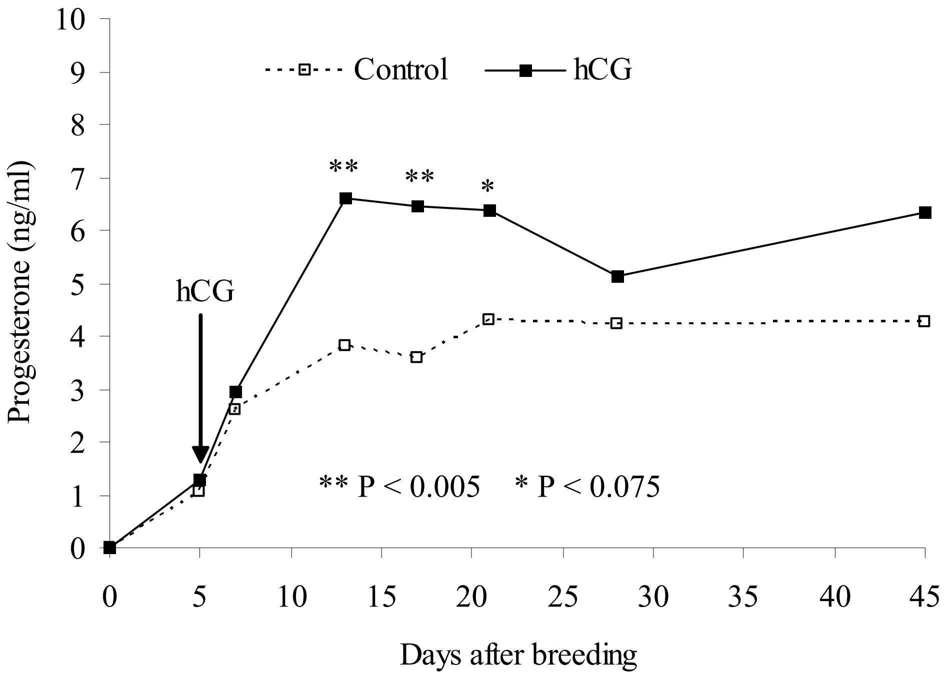

Fig. 1. Plasma progesterone concentration (ng/ml) of pregnant Alpine lactating does from day 0 (day of estrus) to 45 after breeding treated with saline (control) or 250 IU hCG five days after breeding.