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Progesterone profile and reproductive performance of estrous-induced Alpine goats given hCG five days after breeding

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Abstract

The objective of this study was to investigate the effect of hCG administration five days after breeding on plasma progesterone concentration (P4) and reproductive performance of estrous-induced Alpine goats. A total of 42 lactating does were treated with intravaginal sponges (MAP, 60 mg) for 9 d plus 200 IU eCG and 22.5 μg d-cloprostenol 24 h before sponge removal. After detection of estrus (day of estrus = day 0) and breeding, females were randomly assigned into two treatments (T1 and T2). In T1 (n=19) and T2 (n=18) the animals received intramuscular injection of 1 ml of saline solution (control) or 250 IU hCG, respectively, 5 d after breeding. P4 (ng/ml) was recorded from blood sampled on days 0, 5, 7, 13, 17, 21, 28 and 45 after breeding. Animals were scanned with the aid of transrectal ultra-sound (5 MHz probe) on days 35 and 70 after breeding, for diagnosis of pregnancy. P4 was higher in the hCG-treated than the control animals only at day 45 ($P < 0.05$). No effect of treatment on pregnancy and kidding rates, gestation period, prolificacy and fetal weight was recorded ($P > 0.05$). Results of this study suggest that hCG administration 5 d after breeding may alter the luteal function but not reproductive performance in estrous-induced lactating goats.

Keywords: Goat; hCG; Progesterone; Reproductive Performance.

Introduction

Estrous induction and mating outside the breeding season could have a great impact on the exploration of animals showing reproductive seasonality. Controlled breeding in goats can be efficiently obtained by the use of progesterone or progestagens, combined with gonadotrophins and prostaglandins. Several estrous induction protocols are currently available with varying doses, duration, type and route of administration with progestagens, when applying gonadotrophins and using (or not) prostaglandins. The most commonly protocols used in small stock are progesterone (P4) slow-release intravaginal devices, fluorogesterone acetate-impregnated sponges (FGA; Freitas *et al*., 1996), medroxyprogesterone acetate (MAP; Greyling and van der Nest, 2000), norgestomet subcutaneous implants (Freitas *et al*., 1997), daily intramuscular P4 administration (Patil *et al*., 2000) or daily oral MAP administrations (Goswami *et al*., 1998). All these protocols have presented high percentage of animals in estrus, but the pregnancy rates obtained are lower than those following natural breeding.

Approximately, 30 to 40% of all the fertile breedings in bovine are lost in the first month of gestation (Ayalon, 1978; Diskin and Sreenan, 1980; Roche *et al*., 1981). Although embryo mortalities have been extensively studied on bovine, only a few studies have been carried out in sub-artic areas on goats (Norway). In an epidemiological study, Engeland *et al.* (1997b) reported age, failure in conception, low social status and pregnancy with the number of fetuses ≥ 3 had been associated with embryonic and fetal losses. Protozoan and bacterial infections, as well as nutritional problems have also been associated with embryonic loss in goats (Engeland *et al*., 1996; Buxton, 1998; Engeland *et al*., 1997a; Kumar *et al*., 1997; Hussain *et al*., 1996). High 15-ketodihydro-$PGF_{2\alpha}$ plasma concentrations and corresponding low plasma P4 concentrations have also been reported (Engeland *et al*., 1996; Engeland *et al*., 1997c). Engeland *et al.* (1998) reported that over 11% of the goats studied had embryonic losses after conception. Inadequate plasma P4 concentration is one of the causes quoted for embryonic mortality during the critical period of maternal recognition of pregnancy. According to Rhind *et al.* (1978), plasma P4 concentration during the induced breeding period was only 36% of that observed during the natural breeding season in non-lactating ewes.

Luteal dysfunction, which culminates in inadequate synthesis and secretion of progesterone, is one of the main causes of embryonic death in mammals. The corpus luteum (i.e., adequate progesterone levels) is indispensable for the recognition and maintenance of pregnancy in mammals. In goats, its absence causes the interruption of pregnancy at any phase (Meites *et al*., 1951). It can be speculated that the pregnancy rate obtained in animals whose estrus has been artificially induced may be lower to that obtained at the natural estrus due to the inadequate corpus luteum development and progesterone synthesis and secretion activity. This problem may worsen in high temperature environments and seasons. If this is true, the administration of luteotrophic agents, such as human chorionic gonad-

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otrophin (hCG), could provide a maternal environment more favorable to the establishment and development of the conceptus by increasing the circulating progesterone concentrations, as confirmed in bovine research (Fonseca *et al*., 2001a). Based on the follicular development of the first estrous cycle wave and the development of the corpus luteum in goats, hormonal administration should be conducted on the 5th day of the estrous cycle when the dominant follicle (Ginther and Kot, 1994) and the corpus luteum (Jablonka-Shariff *et al*., 1993) have not fully developed.

The objective of this study was to evaluate the effect of hCG administration 5 d after breeding on the plasma P4 concentration during the first 45 days of pregnancy and the respective reflexes on the reproductive performance of estrous-induced Alpine does.

Materials and Methods

Location

This study was conducted from December to July at the Goat Section of the Department of Animal Science of the Federal University of Viçosa (UFV), in Viçosa, Zona da Mata, Minas Gerais, Brazil, situated at 20º45' S latitude and 42º51' WG longitude. The local average altitude was 692.73 m with CWA (dry winter and humid summer) climate, annual average temperature of 20.9º C and annual rainfall of 1203 mm^3. The local breeding season extend from March to June.

Experimental animals

A total of 42 lactating Alpine goats with body condition score (BCS, ranging between 1 to 5) were evaluated by palpation of the lumbar and sternal region on day of estrus. The mean BCS was 3.9 ± 0.6. Goats were 1.5 to 5 of age and between their first and fourth lactation and more than 70 days postpartum. Animals were kept on collective pens and fed corn silage and concentrate twice a day to provide the production requirements. Water and mineral salt were permanently available. Estrus was induced during December an January (local transition breeding season) by intravaginal sponge with 60 mg MAP (Progespon®, Syntex S.A., Indústria Bioquímica e Farmacêutica, Buenos Aires, Argentina), plus i.m. administration of 200 IU eCG (Novormon® 5.000, Syntex S.A., Indústria Bioquímica e Farmacêutica, Buenos Aires, Argentina) and intra-vulvo-submucosal 22.5 μg d-cloprostenol (Prolise®, ARSA S.R.L., Buenos Aires, Argentina), 24 h before sponge removal. Does were monitored to confirm the onset of estrus twice a day (06:00 and 18:00) by means of a surgically prepared males (teaser by means of penis translocation). The estrous signs observed included: searching for the male; restlessness; vocalization; frequent urination; tailing; contraction, hyperemia and edema of the vulva; vaginal mucous discharge and immobility on mounting (considered as the onset of estrus).

Design of experiment

The does were bred after estrous detection (day 0 = day of estrus) and bred again 24 h later, if does were still in estrus. The does were randomly allocated to two treatments (T1 and T2). In T1 (n=19) and T2 (n=18) groups, the does received 1 ml saline solution (control) or 250 IU hCG (Vetecor®, Laboratórios Calier do Brasil Ltda, São Paulo, Brasil), intramuscularly, 5 d after estrous detection and breeding, respectively.

Plasma progesterone concentration

Blood was sampled on days 0 (estrus), 5, 7, 13, 17, 21, 28 and 45 after breeding from 12 goats (T1=6 and T2=6) to determine plasma P4 concentrations. Blood was always collected in the morning (07:00 h) by jugular puncture in heparinized vacuolated test tubes. After collection, the tubes were kept on ice until centrifugation in a refrigerated centrifuge at 5ºC, at 2500 x g / 15 min. The plasma was then aspired and stored at – 20ºC until analyzed for the P4 concentration. The time between blood collection and plasma aspiration did not exceed two hours. Plasma P4 concentration was determined with the aid of the solid phase radioimmunoassay (RIA), using commercial Kit (Coat-a-count progesterone kit, DPC, Diagnostic Products Co., Los Angeles, CA, USA.), according to the manufacturer's recommendations, in the Laboratory of Animal Reproduction of the Department of Animal Sciences of the UFV. The mean intra-assay coefficient of variation was 9 %.

Pregnancy diagnosis and kidding

All the does were diagnosed with the aid of transrectal ultrasonography using a 5 MHz probe 35 and 70 days after breeding. After kidding, the number and weight of the fetuses and gestation period were recorded.

Variables and statistical analysis

The following parameters were determined:

- Estrous response: number of females in estrus / number of total females X 100;
- Interval to estrus (h): time from sponge removal to first mounting acceptance;
- Duration of estrus (h): time from first to last mounting acceptances;
- Pregnancy rate (%): number of pregnant females / number of mated females X 100;
- Kidding rate (%): number of kidded females / number of pregnant females X 100;
- Gestation period: interval (days) from breeding to

kidding;

- Prolificacy: average number of fetuses born per female kidding;
- Fetal weight (kg): weight of fetuses born.

Statistical analysis comprised one way analysis of variance for testing the differences in variables studied between treatments. Parametric variables were tested with the aid of SNK test processed by the SAEG - Statistical Analysis System (Ribeiro Júnior, 2001). Non-parametric variables were compared using the Chi-square test (Ayres *et al*., 2000). Results were expressed as mean ± S.E.M. and the statistical significance was accepted from $P < 0.05$.

Results

Estrous response

Five animals were culled due to clinical problems. The total percentage of animals in estrus was 95.2 % (40/42) and the interval to estrus and duration of estrus were 22.20 ± 10.37 h and 24.90 ± 4.20 h, respectively.

Plasma progesterone concentration

Plasma P4 concentrations in the hCG– or saline–treated pregnant goats are set out in Fig. 1. The hCG-treated animals showed higher plasma P4 concentration only on day 45 ($P < 0.05$) after breeding.

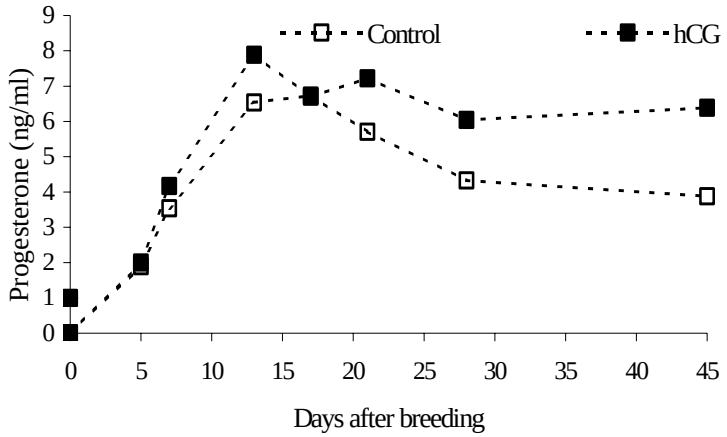

Figure 1. Plasma progesterone concentrations (ng/ml) of pregnant Alpine goats treated with saline (Control) or human chorionic gonadotrophin (hCG) 5 d after breeding.

One non-pregnant hCG-treated doe appeared to have had a prolongation of the luteal phase, reaching P4 peak on day 17. The same animal seemed to have cycled again normally, since it showed high P4 (7 ng/ml) on day 28. One non-pregnant doe from the control group appeared to have returned to the anestrous condition, since it did not show detectable plasma P4 concentrations on days 28 and 45 after breeding.

Pregnancy and kidding

No embryonic mortality was recorded from day 35 to day 70 of pregnancy in both treatments. The pregnancy and kidding rates did not differ between treatments (Fig. 2). One goat treated with hCG died during pregnancy and were subtracted from analysis.

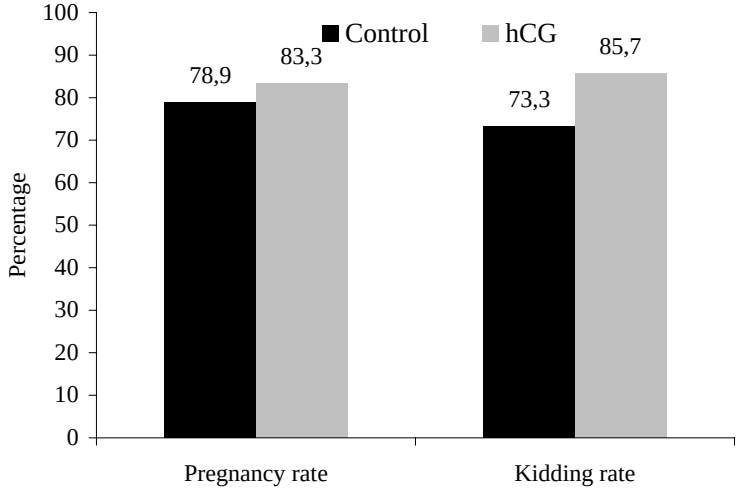

Figure 2. Pregnancy rate (%) and kidding rate (%) of Alpine goats treated with saline (control) or 250 IU hCG 5 d after breeding.

No difference (P > 0.05) between treatments was recorded in the gestation period, prolificacy and weight of fetuses born (Table 1).

Table 1. Average (mean ± SD) gestation period (days), prolificacy (fetuses born) and fetal weight (kg) of Alpine goats treated with saline solution (control) or 250 IU of hCG 5 d after breeding

	Control	hCG	Total
Gestation period	149.5 ± 3.3 (11)	151.8 ± 1.9 (12)	150.7 ± 2.8 (23)
Prolificacy	1.63 ± 0.50 (11)	1.75 ± 0.45 (12)	1.69 ± 0.47(23)
Fetal weight	3.22 ± 0.70 (19)	3.08 ± 0.39 (21)	3.15 ± 0.55 (40)

( ) number of animals.

Discussion

The total estrous response (95.2 %), the mean interval from sponge removal to the onset of estrus (22.20 ± 10.37h) and duration estrus (24.90 ± 4.20 h) reflected the efficiency of the protocol in inducing estrus in this study. Other studies have shown similar efficiencies with protocols using intravaginal sponges containing progestagens plus eCG administration with or without to the implementation of prostaglandins (Baril *et al*, 1993; Greyling and van der Nest, 2000; Fonseca *et al*., 2004).

The possibility to induce accessory corpora lutea formation and increase P4 by means of hCG administration on day 7 post-estrus in female goats was successful reported by Tiwari *et al*. (1998). According to the plasma P4 concentrations recorded in the hCG-treated animals in present study, the hCG luteotrophic activity was shown later after breeding (45 days). Luteotrophic activity was previously reported by Saharrea *et al*. (1998) in superovulated goats treated with saline solution (control), 1000 IU hCG or 50μg GnRH, 84 h after onset of estrus. Based on plasma P4 concentration, goats showed 57.5, 0.0 and 37.0 % of premature luteal regression on day 6 for control, hCG and GnRH groups, respectively.

A decrease in the plasma P4 concentration of pregnant animals after reaching their peak approximately day 13 after breeding is a common phenomenon in goats. In recent study, Regueiro *et al*. (1999) also reported this behavior. The factors leading to this decrease in P4 are still not known. However, more intensive study of this phenomenon could lead to the development of luteotrophic or anti-luteolytic strategies in goats, favoring their reproductive performance, as proposed for bovine (Binelli *et al*., 2001).

In a previous study, hCG administration on the day 5 post-estrus significantly increased the plasma P4 concentration on days 13 and 17 after mating of lactating Alpine goats, during the natural breeding season (Fonseca, 2002). In the present study, the use of eCG in induction of estrus may have masked the hCG effect. The eCG plays a well-known luteotrophic role and, according to Regueiro *et al*. (1999), animals treated with eCG recorded higher plasma P4 concentration than those of the control (non treated) animals.

Although the mean plasma P4 concentration was higher in the hCG-treated does on day 45 after-breeding than in the control animals, this did not cause an increase in pregnancy rate. Under the conditions of this experiment, high plasma P4 concentration at the onset of pregnancy did not seem to have a beneficial effect on the establishment of pregnancy. Kittok *et al*. (1983) increased the pregnancy rates in synchronized ewes, treated on days 11, 12 and 13 after mating, from 29% in the control treatment to 58% by hCG treatment. They also reported that the increase in mean plasma P4 concentration caused by hCG administration might cause an increase in the pregnancy rate. However, pregnancy rate was relatively low in control (29 %) and in this case the increase in P4 level could have beneficiated the establishment and maintenance of the pregnancy. In the present study, increases in P4 concentration did not increase the pregnancy rate, as the control treatment had a relatively high pregnancy rate (79 %). Nephew *et al*. (1994) reported significant increases in plasma P4 concentration in ewes after administration of 100 IU hCG 11.5 days after mating but the pregnancy rate in the hCG-treated animals (91%) did not differ from the pregnancy rate of the control animals (80%). On the other hand, Nishigai *et al*. (2002) reported a significant increase in the pregnancy rate of recipient heifers treated with 1500 IU hCG on the 6th day of the estrous cycle (67.5%), compared to the control group (45%). The authors also reported higher P4 concentration in hCG-treated compared to the control animals on days 40 to 50 of pregnancy. It seems that in the present study, P4 level was not a limiting factor in the establishment and maintenance of pregnancy.

As proposed by Staples and Hansel (1961), a threshold P4 is necessary for the establishment of pregnancy, but from this level, plasma P4 increases may not have any positive effect on the pregnancy rate. In heifers, the hCG administration on the day 5 post-estrus induced the formation of accessory corpora lutea (Fonseca *et al*., 2000) and increased the level of P4 on day 13 post-estrus (Fonseca *et al*., 2001a) but the pregnancy rate was not significantly increased (Fonseca *et al*., 2001b). Therefore, in the present study, significant increase in pregnancy rate-was not induced by hCG because similar progesterone level might have been reached by the does in

both treatment groups.

The overall pregnancy rate recorded in this study (81 %) was superior to that reported earlier in estrous-induced goats. Baril *et al*. (1993) reported a 59 % pregnancy rate in goats treated for 11 d with FGA intra-vaginal sponges (50 mg) and prostaglandin administration (50 μg cloprostenol) and eCG (400 to 600 IU) on day 9. Motlomelo *et al*. (2002) reported a 51 % pregnancy rate in goats treated with MAP sponges (60 mg) for 16 d and eCG administration (300 IU) on the day of sponge withdrawal. Zarkawi *et al*. (1999) reported a pregnancy rate of 65.8 % in Damascus goats with MAP and eCG.

Based on the kidding rate, fetal losses occurred after 70 days of pregnancy when the last ultrasonography was conducted. Although hCG-treated animals had higher plasma P4 concentration at 45 days of pregnancy, no significant benefic effects throughout the pregnancy were evident. According to Engeland *et al*. (1997b, 1998), 11 to 40 % of animals have miscarriages, deliver dead fetuses or are empty after being considered pregnant. These losses are higher in does older than 7 years (33 %), repeat breeders (33 %), with the number of kids equal or more than 3 (28 %), and animals that had experienced previous fetal losses (40 %). Under tropical conditions, embryonic and fetal losses can be even higher, since thermal stress may elevate uterine prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) secretion, influence the luteal function during the initial pregnancy and affect the growth and development of the conceptus (Emesih et al., 1995). Other deleterious effects caused by high ambient temperatures and humidity on reproduction cannot be ignored (Wolfenson et al., 2000). The average ambient temperatures and humidity registered in the present study were 22.7º C (16-32) and 81.3 % (36-96) in January, 22.6º C (15-31) and 82.7 % (42-95) in February and 22.6º C (14-31) and 78.3 % (6-97) in March, respectively. Although the average values are not high, extreme temperature and humidity were registered during the day, what may have compromised pregnancy.

Gestation period was not affected by treatments. No reports are available on hCG effects on the gestation length in goats. In swine, the administration of 1500 IU of hCG on day 12 of the estrous cycle increased the inter-estrous interval. This could have been the effect of the higher P4 concentration, since the authors suggested that a small pseudo-pregnancy can be induced by hCG (Soede *et al*., 2001).

Previous studies reported the embryotrophic effect of hCG in ewes (Nephew *et al*., 1994; Khan *et al*., 2003). In the present study, litter size was not increased by hCG administration in goats. However, Khan *et al*. (2003) reported that administration of 150 IU hCG at mating in the ewe increased the litter size. It was also reported that hCG significantly increased the crown-rump length, amniotic sac width and number of placentomes and embryo weight at 25 days post-mating. Nevertheless, in the same study it was reported similar birth weight between hCG-treated and the control animals. Similar results were found in the present study, where litter size and birth weight were similar for treatments.

In conclusion, the results of this study show that the administration of hCG 5 days after breeding in estrous-induced goats in the transition season did not increase reproductive performance. However, according to the plasma P4 concentration recorded throughout the early pregnancy, more studies should be done with administration of luteotropic agents at other times.

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