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Original article

Human chorionic gonadotropin affects original (ovulatory) and induced (accessory) corpora lutea, progesterone concentrations, and pregnancy rates in anestrous dairy goats

Juliana Nascimento Duarte Rodrigues[a], José Domingos Guimarães[a], Maria Emilia Franco Oliveira[b,g], Jenniffer Hauschildt Dias[a], Aline Matos Arrais[c], Marco Antonio Paula de Sousa[d], Rosemary Bastos[e], Bahareh Ahmadi[f], Pawel Mieczyslaw Bartlewski[f], Jeferson Ferreira Fonseca[g,*]

[a] Departamento de Medicina Veterinária, Universidade Federal de Viçosa, Av. Peter Henry Rolfs, s/n, CEP 36570-000, Viçosa, MG, Brazil
[b] Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Via de acesso Prof. Paulo Donato Castellane, s/n, CEP 14884-900, Jaboticabal, SP, Brazil
[c] Departamento de Reprodução e Avaliação Animal, Universidade Federal Rural do Rio de Janeiro, Rodovia BR 465, Km7, s/n, CEP 23890-000, Seropédica, RJ, Brazil
[d] Instituto de Medicina Veterinária, Universidade Federal do Pará, Av. dos Universitários, s/n, CEP 68746-360, Castanhal, PA, Brazil
[e] Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, n° 2000, CEP 28013-642, Campos dos Goytacazes, RJ, Brazil
[f] Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
[g] Embrapa Caprinos e Ovinos, Estrada Sobral/Groaíras, km 04, CP 145, CEP 62010-970, Sobral, CE, Brazil

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ABSTRACT

Two experiments were conducted in acyclic Alpine (A) and Saanen (S) goats that received intravaginal sponges containing 60 mg of medroxyprogesterone acetate for 6 days, as well as 200 IU of eCG and 30 μg d-cloprostenol i.m. 24 h before sponge removal. On day 7 (day 0 = onset of synchronized estrus), all goats were randomly divided into two groups: animals treated with 300 IU of hCG i.m. (hCG; Exp.1: n = 8A; Exp.2: n = 75A + S) and untreated controls (Control; Exp.1: n = 8A; Exp. 2: n = 70A + S). In Exp.2, all goats were artificially inseminated. Transrectal ovarian ultrasonography and blood collection were done on days 7, 10, 13, 17, and 21 (Exp.1), and pregnancy detection on day 60 (Exp.2). Estrus and ovulations occurred in five hCG and seven Control animals. Accessory CL (aCL) were detected in all hCG does. The total luteal area of ovulatory corpora lutea (oCL) increased ($P < 0.05$) on day 10 in hCG does and remained greater ($P < 0.05$) than in Control until day 21. Total and high-velocity color Doppler area were greater ($P < 0.05$) for oCL of hCG does on days 13 and 17. Progesterone concentrations were greater ($P < 0.05$) in hCG does from days 13 to 21 and related directly to the total luteal and oCL area for the duration of the study in all does. The pregnancy rate was higher ($P < 0.05$) in hCG than in Control by 22.5 %. Human chorionic gonadotropin given on day 7 of the synchronized estrous cycle positively affected CL function and pregnancy rates in seasonally anovular dairy goats.

1. Introduction

An attainment of a threshold in blood progesterone (P_4) concentration is necessary for the establishment of pregnancy in mammalian species. Inadequate P_4 production causes 30–40 % of embryonic deaths in goats and sheep [1–3], resulting in significantly reduced pregnancy rates in small ruminants. Reduced luteal function occurs mainly in the non-breeding season of small ruminants [4]. Therefore, strategies must be employed to boost luteal function after estrus induction protocols in seasonally anestrous goats. The administration of luteotrophic hormones is a strategy to raise the concentration of P_4 and can be used in the early or late luteal phase. The use in the early phase is based on the induction of accessory corpora lutea (aCL) and initial luteotrophic action on the post-ovulation corpus luteum (oCL), while the late phase has action in increasing ovarian function, conceptus growth, and placental attachment [5].

* Corresponding author at: Embrapa Caprinos e Ovinos, Rodovia MG 133, Km 42, Cep 36155-000, Coronel Pacheco, MG, Brazil.
E-mail address: jeferson.fonseca@embrapa.br (J.F. Fonseca).

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Administration of human chorionic gonadotropin (hCG) during the period when growing antral follicles have sufficient amounts of luteinizing hormone (LH) receptors can induce accessory corpora lutea (aCL) via stimulating follicle rupture and luteogenesis or by luteinization of large antral follicles [6]. Exogenous hCG given on day 5 of the estrous cycle (day 0 = onset of estrous) induces aCL formation and an increase in serum P_4 concentrations in heifers [7] and goats [8,9]. Accessory CL also formed in hair sheep that received hCG on day 7 of the estrous cycle [10]. A significant increase in luteal area and a rise in blood P_4 concentrations were observed; however, neither blood P_4 concentrations nor total luteal tissue changed in Toggenburg goats injected with hCG seven days after the onset of estrus [11], leading the authors to speculate that P_4 mediated the increased pregnancy rates in the hCG-treated goats.

In addition to inducing the formation of aCL, human chorionic gonadtropin also enhances the functionality of the post-ovulation corpora lutea (oCL) [12], because hCG exerts luteotropic effects in LH-responsive small luteal cells [13,14]. Heifers not forming aCL after the administration of hCG five days after the onset of estrus still show a significant increase in circulating P_4 concentrations [12]. However, the influence of hCG on the size and vascularity of post-ovulation CL in small ruminants has yet to be described because all previous ultrasonographic studies of luteal function in hCG-treated ewes [10,15,16] and goats [11,17] only reported the changes in cross-sectional area and blood supply of all detectable luteal structures.

Hence, the main objective of this study was to assess the effects of hCG administrated seven days after the onset of the synchronized estrus on post-ovulation corpus luteum (oCL) and accessory CL (aCL) as well as plasma P_4 concentrations and fertility in artificially inseminated dairy goats during the non-breeding season. We expected that transrectal ovarian ultrasonography (B-mode and color Doppler) would help us delineate the influences of hCG on oCL and aCL in does.

2. Material and methods

All experimental procedures were reviewed and approved by the Ethics Committee of Embrapa Gado de Leite (protocol #3050060218). The present study was conducted from October to November, during the middle portion of the non-breeding season that spans a period from September to November [18].

2.1. Location and experimental animals

Experiment 1 (n = 16 Alpine goats) was conducted in the Embrapa Gado de Leite experimental field station situated in Coronel Pacheco, MG, Brazil (latitude 21°35′S, longitude 43°15′W, and altitude of 435 masl). Experiment 2 (n = 60 Alpine goats and n = 85 Saanen goats) was conducted in a commercial dairy goat farm in Ouro Fino, MG, Brazil (22°16′ S and 46°22′ W, and altitude of 908 masl). In both experiments, goats were reared in an intensive system, fed 50 % of corn silage and 50 % of Napier grass with concentrate supplementation (soybean, corn, and mineral nucleus based mixture; with 16 % of crude protein and 68 % of total digestible nutrients) according to their nutritional needs [[19]]. Mineral salt licks and water were available *ad libitum*. All goats were clinically healthy and free of reproductive disorders. The mean body condition score (BCS: 1 = very thin and 5 = very fat [20]) was 3.0 ± 0.1, and body weight was 56.8 ± 4.2 kg. All goats were in the last third portion of their lactation period, with a mean annual milk yield (305 days of lactation) of 727 kg [21].

2.2. Estrus induction and treatments applied

All goats received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Zoetis, São Paulo, SP, Brazil), which were inserted in the late afternoon (1700–1800 h) and were left in place for six days. One day before sponge removal, all goats received an i.m. injection of 200 IU of equine chorionic gonadotropin (eCG; Novormon 5000®, Zoetis, São Paulo, SP, Brazil) plus 30 μg d-cloprostenol (Prolise®: Tecnopec, São Paulo, Brazil), late in the afternoon (1700–1800 h). In both experiments, estrus was detected twice daily with fertile bucks placed in a pen with the does for 30 min. In Experiment 2, artificial insemination (AI) was performed by the Embrapa® transcervical technique [22] using the flexible-timed approach (FxTAI) [23]. Semen from seven bucks (three Alpine and four Saanen bucks owned by the Brazilian progeny testing corporation CapraGene) was donated by Embrapa Goats and Sheep. Inseminate doses stored frozen in French straws (0.25 mL) contained 100×10^6 viable spermatozoa before freezing, with minimum progressive motility of 45 % and spermatic vigor of 3 (range 0–5) were thawed in a water bath at 35 °C for 30 s. Semen quality of all bucks was subjected to semen soundness evaluation protocols and was assessed during the breeding [24] and non-breeding seasons [23]. The time of AI time was based on the detection of estrus; does with an early, intermediate, or late onset of estrus (relative to the time of MAP sponge removal) were inseminated 24, 18, or 10 h after the onset of estrus, respectively. Following the onset of behavioral estrus (Experiment 1) or FxTAI (Experiment 2), goats were divided by breed, body weight, BCS, age, parity, and time elapsed from intravaginal device removal to estrus into two equinumerous groups: goats that received i.m. injections of 300 IU of hCG (Vetecor®; Hertape-Calier do Brasil Ltda, São Paulo, SP; Brazil) (hCG; n = 83) and animals injected with 1 mL of saline solution (Control; n = 78) on day 7 after the onset of estrus (Fig. 1).

2.3. Ultrasound and luteal evaluation

In Exp. 1, transrectal ovarian ultrasonography (B-mode and color Doppler) was conducted one week before estrous induction to assure seasonal anestrus (CL absence) and on days 7, 10, 13, 17, and 21 (day 0 = onset of behavioral estrus) using a portable ultrasound scanner equipped with a 7.5-MHz transducer (M5 Vet®; Mindray Medical International Limited, Shenzhen, China) (Fig. 1). The transducer was taped to a PVC tube to facilitate external manipulation during the transrectal exam. Does were examined in a standing position. Fecal pellets were removed manually from the rectum, and 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, SP, Brazil) were deposited with a syringe into the rectum and approximately 5 mL were applied to the transducer before each ultrasonographic examination. Corpora lutea forming after initial ovulations (oCL) were observed on day 7, whereas the accessory corpora lutea (aCL) were recorded from day 10 onwards. The diameter, position, and vascularization of all detected luteal structures were sketched on individual ovarian charts. B-mode images were used to measure the luteal area (cm^2) defined as the sum of the cross-sectional regions of all detected luteal structures (oCL and aCL); the areas of central cavities, if present, were subtracted from the total luteal area [11]. The Doppler area (DA) of each corpus luteum was determined using ImageJ® software, with the number of color pixels ultimately converted to cm^2 [25]. The high-velocity DA (HVDA) was then determined using ImageProPlus® analytical software (Media Cybernetics Inc., San Diego, CA, USA); HVDA color pixels (an upper and lower quarter of the Doppler scale bar,

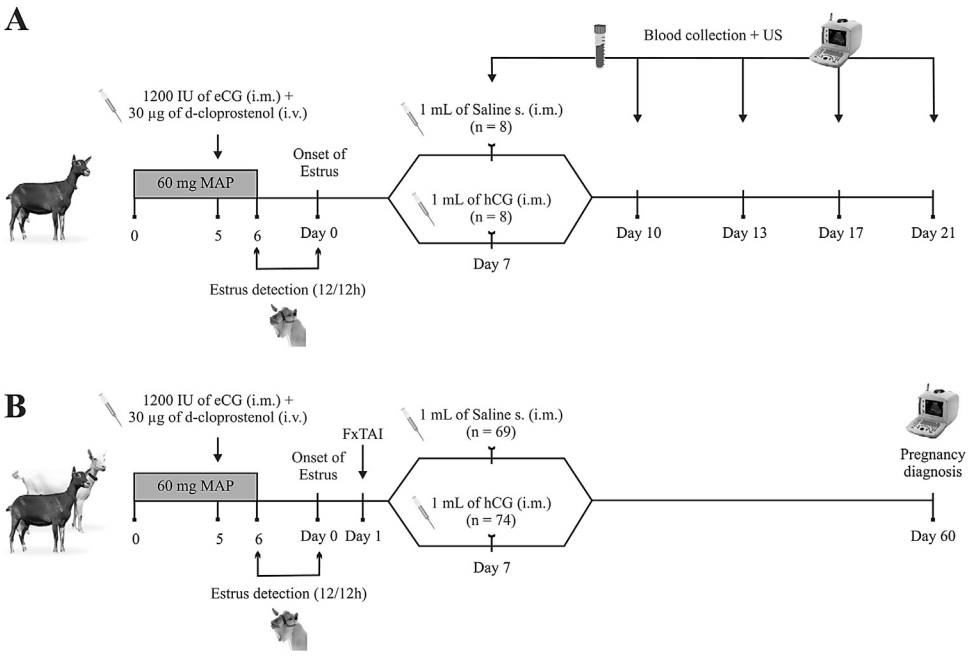

Fig. 1. Experimental design of the study. Evaluation of the effect of administering 300 IU of hCG i.m. on day 7 after the onset of estrus (day 0) in dairy goats during the seasonal anestrus. In Exp. 1 (A), Alpine goats received 1 mL of saline or 300 IU hCG. Females were allocated randomly to the two groups immediately after estrus detection. Jugular blood samples were drawn, and B-mode and color Doppler Alpine and Saanen the goats received 1 mL of saline or 300 IU hCG on day 7, and were artificially inseminated based on the timing of behavioral estrus (flexible time artificial insemination, FxTAI); ultrasonographic pregnancy check was done 60 days later. US: transrectal ovarian ultrasonography; MAP: medroxyprogesterone acetate (progestin)-soaked sponges.

that corresponds to the velocity range of 0.04 m/s to 0.08 m/s) were counted with the "Count/size" tool and converted to cm^2 [26]. In Exp. 2, pregnancy was detected with ultrasonography 60 days after FxTAI, using the same equipment and operated by the same experienced technician as in Exp. 1. All examinations were performed at the constant settings of the ultrasound scanner (75 % color gain, pulse repetition frequency (PRF) of 1.0 kHz, 6 cm of depth, and wall filter (WF) of 75 MHz).

2.4. Blood collection and progesterone measurements

In Exp. 1, pre-prandial jugular blood samples were drawn from all goats into vacutainers containing lithium heparin (anticoagulant) on each day of the ultrasonographic examination between 0600 and 0700 h. The collection tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1500 x *g*. After centrifugation, blood plasma was aspirated and stored at −20 °C in 1.5-mL microtubes until P_4 analysis at a later date. Plasma P_4 concentrations were determined by a solid-phase radioimmunoassay technique using commercial kits (ImmuChem, MP Biomedicals, Santa Ana, CA, USA). Sensitivity and intra-assay coefficients of variation were 0.05 ng/mL and 11 %, respectively [17].

2.5. Statistical analysis

For statistical analyses in Exp. 1, only goats with active CL on day 7 were included (Control = 7 does and hCG = 5 does). Luteal data were analyzed for the entire observation period (days 7–21) except for various characteristics of aCL that were evaluated from days 13 to 21 (period of ultrasonographic detection of aCL). In Exp. 2, values for the following variables were determined: (after the onset of estrus)-estrus response (number of does in estrus/total number of does×100); interval from MAP sponge removal to the onset of estrus (h); interval from MAP sponge removal to FxTAI (h); interval from the onset of estrus to FxTAI (h); (on days 7, 10, 13, 17 and 21)-numbers of oCL and aCL corpora lutea; total luteal area (TA, cm^2); oCL and aCL area (cm^2); color Doppler area of oCL and aCL (oCL DA and aCL DA, cm2); relative DA areas (oCL DA/oCL area x 100 % and aCL DA/aCL area x 100 %); high-velocity DA for oCL and aCL (oCL HVDA and aCL HVDA, cm2); relative HVDA for oCL and aCL (oCL HVDA/oCL DA x 100 % and aCL HVDA/aCL DA x 100 %; plasma P_4 concentrations (ng/mL); (at the time of pregnancy detection)-pregnancy rate (number of pregnant does/number of does artificially inseminated×100); and proportion of inseminated goats with hydrometra (number of does with hydrometra/number of inseminated does×100).

Data analysis was performed using the libraries of the "car," "stats," "geepack," and "emmeans" packages of the R software (version 3.6.3, The R Foundation for Statistical Computing). The Shapiro-Wilk test was used to evaluate the normality of the residual. The Box-Cox transformation of the data was performed whenever necessary. Luteal dynamics data were analyzed by a repeated measurement statement, using generalized estimation equations (GHG) with a logit link to the counting data; the main effects of the treatment group, day and their interaction were included in the statistical model. Different covariance structures were evaluated, and the self-regressive one was selected for presenting the lowest value for Akaike's criterion (AIC). Fisher's exact test was used for nonparametric analyses and the analysis of variance (ANOVA) was used for parametric data. The Tukey test was used to compare the means of the treatments. Correlational analyses utilized simple linear regression. The significance level used for all analyses was 5%. The values are presented as mean ± SEM.

3. Results

In Exp. 1, twelve out of 16 goats (75 %) that showed estrus had ultrasonographically detectable corpora lutea (CL) on day 7 (hCG: n

= 5, and Control: n = 7). Mean CL count on day 7 was similar (P > 0.05) to hCG (1.8 ± 0.1) and Control (2.1 ± 0.1) goats, while on day 13 it was superior (P < 0.05) in hCG (2.1 ± 0.1) than in Control (1.7 ± 0.1) goats. Accessory CL (aCL) were detected in all hCG treated does and in none of the Control animals. In one hCG goat, an aCL was detected on day 10, while in the remaining does aCL were observed from day 13 onwards. Three of the hCG-treated does had aCL. One doe had a single aCL, and two does formed two aCL.

There were significant effects of day and group and significant day x group interaction for total luteal area and ovulatory CL (oCL) area in goats of the present study (Fig. 2 A and B). Total luteal area increased (P < 0.05) in hCG goats from day 7 to day 10 and again from day 10 to day 17 (Fig. 2A), whereas the mean oCL area in this subset of goats increased (P < 0.05) from day 7 to day 10 but then did not change (P > 0.05) until day 21 (Fig. 2B). Both variables were greater (P < 0.05) in hCG compared with Control (in Control group, the total luteal area was equal to oCL area) from day 10 to 21. The mean cross-sectional area of aCL in hCG-treated goats increased (P < 0.05) from day 13 to 21 (Fig. 2C).

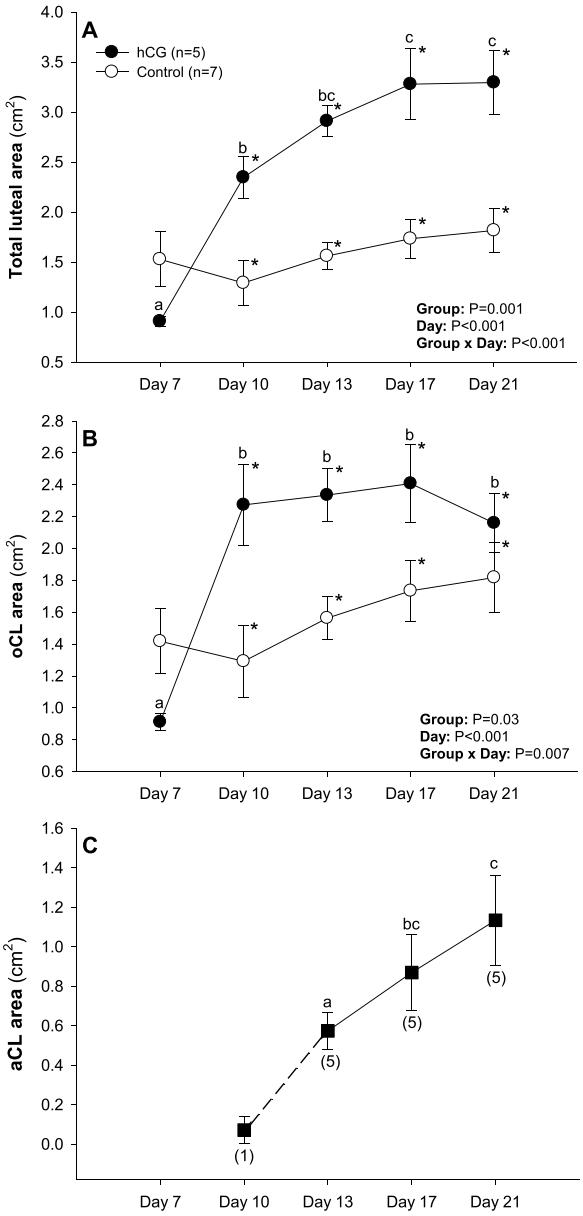

Fig. 2. Mean (±SEM) total luteal area (A) as well as mean cross-sectional areas of ovulatory (oCL) (B) and accessory corpora lutea (aCL) (C) detected ultrasonographically in seasonally anovular Alpine goats with (hCG) or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given seven days after the onset of induced estrus. Numbers of goats with detectable aCL are given in parentheses (C). Mean values denoted by different letters vary over time within each group and asterisks indicate the differences between groups of does. Note: total luteal area = oCL area in Control does.

There was a significant effect of day post-estrus for the color Doppler area (oCL DA) and color Doppler area percentage (oCL DA/oCL area x 100 %) as well as high-velocity DA of ovulatory CL (oCL HVDA; Fig. 3). In addition, there was a significant effect of group x day interaction for oCL DA (Fig. 3A) and oCL HVDA (Fig. 3C), and a significant main effect of the treatment (group) for oCL HVDA (Fig. 3C). Both mean oCL DA and oCL DA/oCL area x 100 % increased (P < 0.05) from day 10 to day 13 and then declined (P < 0.05) from day 17 to day 21 in hCG goats (Figs. 3A and B). Mean oCL DA was greater (P < 0.05) in hCG compared with Control goats on days 13 and 17 (Fig. 3A) and mean oCL DA/oCL area x 100 % was significantly greater in hCG than in Control animals on day 17 (Fig. 3B). Finally, hCG animals exceeded (P < 0.05) their control counterparts in mean oCL HVDA on days 13 and 17, and mean oCL HVDA values increased (P < 0.05) in hCG goats from day 7 to day 13 (Fig. 3C). There were no significant fluctuations in color Doppler characteristics of accessory CL recorded in hCG goats (Fig. 4).

Plasma P_4 concentrations increased (P < 0.05) from day 7 to day 10 in hCG group and from day 10 to day 13 in Control goats (Fig. 5). In control animals, plasma P_4 concentrations declined (P < 0.05) from day 17 to 21. Plasma P_4 concentrations were greater (P < 0.05) in hCG compared with control goats from day 13 to 21. Plasma P_4 concentrations decreased to basal or non-detectable levels on day 21 in one of five hCG goats (20 %) and four of seven control animals (57 %). A diagram of the main morphological and hemodynamic changes observed in the original (oCL) and accessory (aCL, ⁻) corpora lutea, and determined with B-mode and color Doppler transrectal ovarian ultrasonography is presented in Fig. 6. During the entire blood collection period (day 7–21), plasma P_4 concentrations were positively correlated (P < 0.05) with total luteal area, oCL area, oCL DA, and oCL DA/oCL area x 100 in both groups of goats (Table 1).

There was not a breed x group interaction in Exp. 2 (P > 0.05) for any of the variables analyzed. The overall estrus response was 98.6 % (143/145) and mean pregnancy rates were 56.7 % in Alpine and 58.0 % in Saanen goats. The pregnancy rate, however, was 22.5 % higher (P < 0.05) in hCG-treated goats than controls (Table 2). All goats showing hydrometra presented uterine US features with early fetal loss.

4. Discussion

To the best of authors' knowledge, this is the first study documenting different luteotropic effects of hCG on oCL and aCL using B-mode and color Doppler ultrasonographic techniques. In addition, similar to previous studies, the present study confirmed significant effects of hCG on plasma P_4 concentrations [27] and pregnancy rate in goats [11]. Our present results indicate that hCG administration on day 7 after synchronized estrus can effectively be used in the reproductive management of goat herds in an intensive breeding system, increasing the pregnancy rates during the anestrous period.

In Alpine and Saanen goats, estrus can be induced hormonally during seasonal anestrus [18]. High estrus responses in Exp. 1 (100 %) and 2 (98.6 %) confirm the efficacy of the protocol used to induce estrus, which can be employed for both FxTAI [23] and intensive natural mating [11] in seasonally anovular lactating goats.

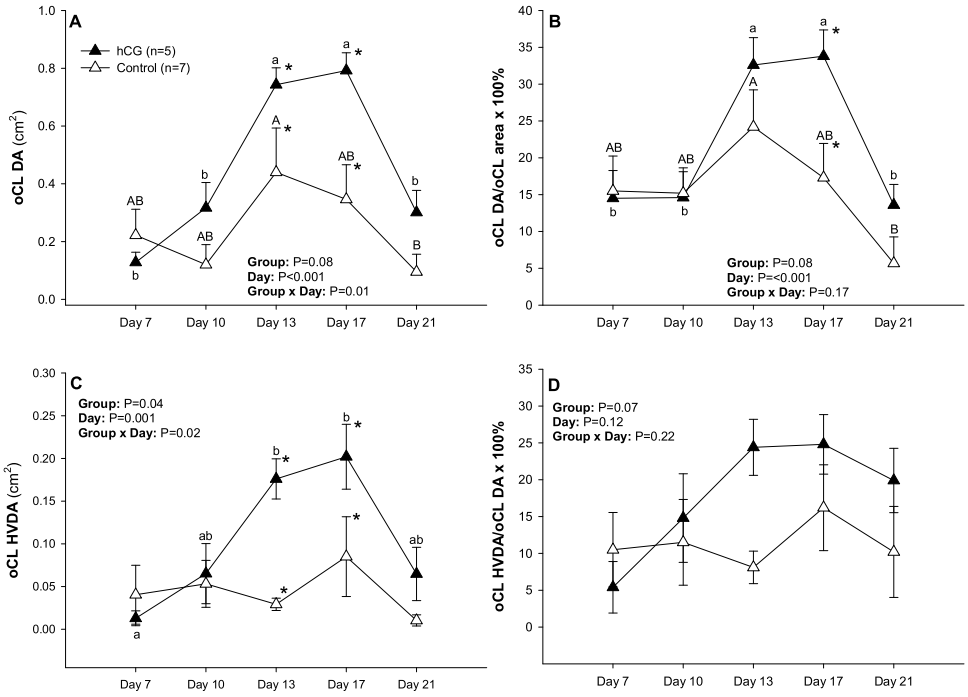

Fig. 3. Summary of the absolute and relative color Doppler area (DA; panels A and B) and high-velocity DA (HVDA) (panels C and D) determined in ovulatory corpora lutea (oCL) in Alpine goats that received a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) seven days after the onset of induced estrus (hCG) and their respective controls (Control). Mean values denoted by different letters vary over time within each group (ab-hCG and AB-Control) and asterisks indicate the differences between the two groups of does.

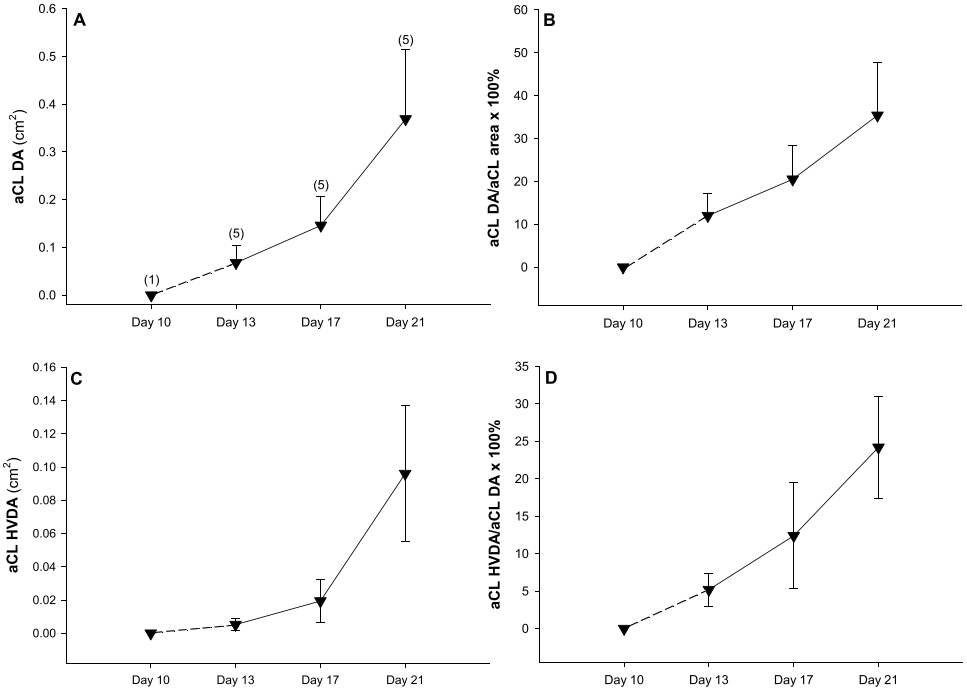

Fig. 4. Summary of the absolute and relative color Doppler area (DA; panels A and B) and high-velocity DA (HVDA) (panels C and D) determined in accessory corpora lutea (aCL) detected in Alpine goats that received a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) seven days after the onset of induced estrus. Numbers of goats with detectable aCL are given in parentheses (A).

However, four out of sixteen Alpine goats (25 %) did not have detectable CL on day 7 (day 0 = onset of estrus). Application of the same estrus induction protocol resulted in an average ovulation rate of 81 % in Toggenburg goats in the non-breeding season [28].

Interestingly, a single dose of hCG given 7 days after the onset of estrus induced CL formation in all ovulating goats in this study. In a previous study using Toggenburg goats during the transitional period (December to January), only 46.5 % of hCG-treated goats

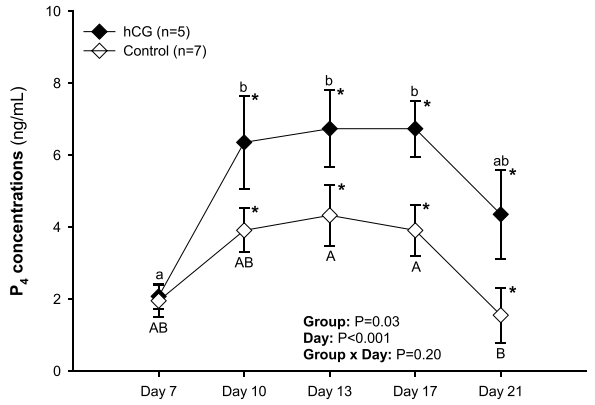

Fig. 5. Mean (±SEM) circulating progesterone (P_4) concentrations in seasonally anovular Alpine goats with (hCG) or without (Control) a single i.m. injection of 300 IU of human chorionic gonadotropin (hCG) given seven days after the onset of induced estrus. Numbers of goats with detectable aCL are given in parentheses (C). Mean values denoted by different letters vary over time within each group and asterisks indicate the differences between the two groups of does.

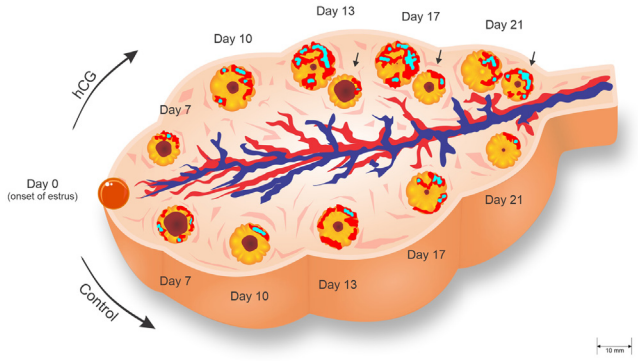

Fig. 6. A diagram of the main morphological and hemodynamic changes observed in the original (oCL) and accessory (aCL, ↓) corpora lutea, and determined with B-mode and color Doppler transrectal ovarian ultrasonography in Alpine goats that received 300 IU of hCG (hCG) or 1.0 mL of saline solution (Control) on day 7 of the synchronized estrous cycle (day 0 = onset of estrus). Both red and blue colors within luteal tissue represent color Doppler area, but blue specifically corresponds to the approximate content of high-velocity Doppler signal.

formed aCL [11]. This difference in the ovarian response to hCG could be due to breed-specific differences in the wave-like pattern of antral follicular development, season, or both of these factors. Two follicular waves per interovulatory interval is a predominant pattern of antral follicular kinetics in cyclic Toggenburg goats [29], but four emerging waves were more commonly in Saanen [30] and Alpine goats [31]. The first wave of the estrous cycle invariably emerges at or around the time of ovulation and the largest (dominant) follicles of the wave attain their maximum diameter approximately 5–6 days later [23,30]. Depending on the periodicity of wave emergence, hCG administered 7 days after the onset of estrus may cause ovulation/luteinization of follicles developing in the first (static phase follicles) and/or second follicular wave after ovulation (growing phase follicles) as long as the follicles present are responsive to LH [32]. However, the days of follicle wave emergence in small ruminants may vary over the year [33] as ovarian follicular responsiveness to gonadotropic stimuli in seasonal breeders is significantly diminished outside of the breeding season [34–37].

The timing of ultrasonographic detection of aCL in does of the present study (days 10–13) agrees with those reported by Côrtes et al. [11] and Fonseca et al. [10] in estrus-synchronized Toggenburg goats and Santa Inês ewes, respectively, which received 300 IU of hCG on day 7 in the non-breeding season. Based on those studies, the average time to induce ovulation and/or luteinization of LH-responsive antral follicles is 108 h (4.5 days) following an application of hCG [38].

In Exp. 1, a sudden decline in plasma P_4 concentrations to a basal or non-detectable level occurred on day 21 in 57 % of control does, whereas the proportion of such does in the hCG group was 20 %. Thus, hCG administration on day 7 apparently did not prevent functional luteal regression in all synchronized goats in the non-breeding season, despite transiently improving oCL functionality and facilitating aCL formation, which ultimately led to an increase in total luteal tissue content and at least a transient rise in plasma P_4 concentration in individual goats. Declining blood P_4 concentrations herald pregnancy failure [11]. In Exp. 2, a lack of ovulation and premature luteolysis likely resulted in 52 % and 30 % of non-pregnant Alpine and Saanen goats, in the control and hCG-treated groups, respectively. In the present study, plasma P_4 concentrations remained elevated for 14 days following hCG administration (day 10 to day 21), which includes the period of maternal recognition of pregnancy in goats [39]. Because such a shift in P_4 secretion did not occur in Toggenburg goats treated with hCG during the transitional period [11], it can be speculated that the mechanisms governing the effects of hCG vary between the reproductive seasons in goats. While during the seasonal anestrus, hCG appears to primarily enhance P_4 secretion of the luteal structures [10,25], during the transitional period its actions may be mainly associated with embryotropic and uterine effects [11,40]. As this is only a speculation based on our earlier

Table 1
Summary of significant correlations between plasma progesterone (P_4) concentrations and quantitative ultrasonographic variables determined in hCG-treated and control Alpine goats on days 7, 10, 13, 17, and 21 (day 0 = onset of estrus and day 7 = day of hCG administration).

Dependent variable (y) vs. independent variable (x)	Coefficient of correlation (r)	P value	Regression equation
hCG			
P_4* vs. total luteal area (cm^2)	0.60	0.002	y=–0.92 + 3.05x
P_4 vs. oCL area (cm^2)	0.75	<0.001	y = 1.10 + 1.64x
P_4 vs. oCL DA (cm^2)	0.63	<0.001	y = 2.55 + 6.00x
P_4 vs. oCL DA (%)	0.42	0.04	y = 3.30 + 0.09x
P_4 vs. oCL HVDA (cm^2)	0.43	0.03	y = 4.07 + 12.40x
Control			
P_4 vs. total luteal area (cm^2) or oCL area (cm^2)	0.69	<0.001	y=–0.94 + 2.66x
P_4 vs. oCL DA (cm^2)	0.76	<0.001	y = 2.03 + 4.84x
P_4 vs. oCL DA (%)	0.65	<0.001	y = 1.23 + 0.12x
P_4 vs. oCL HVDA (cm^2)	0.52	0.005	y = 2.44 + 16.01x
P_4 vs. oCL HVDA/oCL DA x 100%	0.42	0.02	y = 2.41 + 7.34x

* P_4: plasma progesterone concentrations (ng/mL); DA: color Doppler area; HVDA: high-velocity Doppler area; oCL: ovulatory corpora lutea.

Table 2
Reproductive performance of Alpine and Saanen goats that underwent flexible time artificial insemination (FxTAI) after estrus induction in the non-breeding season, with or without 300 IU of hCG given i.m. seven days after the onset of behavioral estrus (Exp. 2).

Variable	Control (n = 69)	hCG (n = 74)
Estrus response (%)		
36 h after device removal (early)	92.8 (64/69)	93.2 (69/74)
48 h after device removal (intermediate)	4.3 (3/69)	6.8 (5/74)
60 h after device removal (late)	2.9 (2/69)	0.0 (0/74)
Total	98.6 (69/70)	98.7 (74/75)
Interval from device removal to the onset of estrus (h)	29.2 ± 1.2	28.5 ± 1.0
Interval from the onset of estrus to FxTAI (h)	25.3 ± 0.8	25.1 ± 0.7
Interval from intravaginal device removal to FxTAI (h)	55.0 ± 1.0	54.6 ± 0.9
Goats with hydrometra (%)	2.9 (2/69)	1.4 (1/74)
Pregnancy rate (%)		
AI 24 h after the onset of estrus	46.4 (32/69)	64.9 (48/74)
AI 18 h after the onset of estrus	0.0 (0/69)	5.4 (4/74)
AI 10 h after the onset of estrus	1.4 (1/69)	0.0 (0/74)
Total	47.8 (33/69)a	70.3 (52/74)b

ab $P < 0.05$.

and present observations, more research is needed to elucidate these mechanisms.

No previous study looking at the effects of hCG on luteal morphology and function in sheep or goats entailed ultrasonographic evaluation of ovulatory and induced CL. In the first experiment, the luteotropic effect of hCG on oCL size was observed as early as 3 days post-treatment (day 10), and it was mediated by LH receptors on luteal cells [41–43] promoting cellular hypertrophy [12]. A nearly 2.5-fold increase in oCL area was noted after day 7 in the hCG-treated group. This stimulatory effect of hCG was sustained up until day 21, resulting in a greater oCL area in hCG-treated compared with control does; no fluctuations in the mean oCL area (or total luteal area) occurred in control animals during the entire observation period. Concurrently, the mean cross-sectional area of aCL nearly doubled from day 13 to day 21 in hCG-treated does.

Present Doppler data and correlational analyses shed a new light on the role of luteal vascularity in sustaining elevated blood P_4 concentrations during early pregnancy in does. While luteal vascularity in untreated animals tended to increase after day 13 (based on the percentage of color Doppler area), the mean Doppler area within oCL of hCG-treated does declined significantly from days 13 to 21. High-velocity blood flow area (indicative of elevated steroidogenic activity of ovarian structures; [26]) increased significantly in oCL of hCG-treated animals. Moreover, the analyses of correlation equations for significant linear relationships between circulating P_4 concentrations and total/color Doppler area of oCL revealed that during days 7–21, the same amounts of luteal tissue and blood vessels were "generating" 1.6 times (2.76/1.72) and 1.2 times (8.55/6.87) more plasma P_4 in hCG-treated than in control does, respectively. Therefore, it may be suggested that hCG treatment not only increased the amount of luteal tissue while suppressing oCL blood perfusion, but it also "improved the efficiency" of luteal cells and blood vessels in synthesizing and releasing luteal P_4 in early pregnant does. The latter appears to be supported by a rise in high-velocity blood flow in the ovulatory CL of hCG-treated goats. This is intriguing, but the specific underlying mechanisms of these effects of hCG remain to be elucidated.

Hydrometra was diagnosed in Exp.2 in three of the inseminated goats (two Control and one hCG doe), which showed early gestational loss ~ 60 days post-AI. Thus, in the present study, fetal loss that occurred despite CL maintenance was probably the cause of hydrometra [44]. Hydrometra is the main cause of reproductive disorders in dairy goats managed in the production systems like that in the present study [44], and the etiology of hydrometra remains complex [45]. Hormonal treatments used to synchronize estrus in cyclic and anovular goats [46] are not a likely cause of hydrometra since this disorder has also been identified in goats with estrus synchronized by the light program [17,47] as well as non-synchronized animals (i.e., without estrus induction) [47].

5. Conclusion

The administration of 300 IU of hCG seven days after the beginning of estrus in anestrous dairy goats resulted in the formation of aCL and had a hypertrophic effect on the existing (ovulatory) CL. Consequently, mean plasma P_4 concentrations were greater in hCG-treated does during the period of maternal recognition of pregnancy. A single dose of hCG increased the pregnancy rate in seasonally anovular estrus-induced dairy goats subjected to FxTAI by 22.5 %. The present study provides novel information on the luteotropic effects of exogenous hCG in gestating goats. It also suggests that the mechanism whereby hCG increases pregnancy rates in dairy goats in the non-breeding season appears to be mainly luteotropic.

CRediT authorship contribution statement

J.N.D. Rodrigues: Methodology, Validation, Investigation, Writing - Original Draft, Writing - review & editing. **J.D. Guimarães:** Writing - review & editing. **J.H. Dias:** Methodology, Investigation. **A.M. Arrais:** Methodology, Investigation. **M.E.F. Oliveira:** Methodology, Writing - review & editing. **M.A.P. Sousa:** Statistical analyses. **R. Bastos:** Methodology, Investigation. **B. Ahmadi:** Formal analysis, Writing - review & editing. **P.M. Bartlewski:** Methodology, Formal analysis, Writing - review & editing. **J.F. Fonseca:** Conceptualization, Methodology, Resources, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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