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Administration of a single dose of 300 IU of human chorionic gonadotropin seven days after the onset of estrus improves pregnancy rate in dairy goats by an unknown mechanism

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ABSTRACT

This study examined the effects of exogenous hCG administration on ovarian function and pregnancy rates in estrous-induced dairy goats during the transition into the breeding season. Eighty-six Toggenburg does received 60 mg of medroxyprogesterone acetate intravaginal sponge for 6 d plus 200 IU of equine chorionic gonadotropin and 30 μg of d-cloprostenol i.m. 24 h before sponge removal, and were then bred for 96 h. Seven days (D7) after first mating the does received either 1 mL of saline (the control group, n = 43) or 300 IU of hCG (the hCG-treated group, n = 43) i.m. Transrectal ovarian ultrasonography (B-mode and color Doppler) was performed on D7, D13, D17, and D21 and ultrasonographic pregnancy detection on D30. Pregnancy rate was higher ($P < 0.05$) in hCG-treated goats (90.7%; 39/43) than that in control animals (74.4%; 32/43). Accessory luteal structures (ALSs) were detected in 46.5% (20/43) of hCG-treated does. All hCG-treated does that had ALSs and 82.6% of goats without ALS post-treatment remained pregnant. The total luteal area increased ($P < 0.05$) from D7 to D13 in pregnant animals of both groups, whereas mean vascular area declined ($P < 0.05$) by D21 in all nonpregnant does. Serum progesterone concentrations increased ($P < 0.05$) on D21 in pregnant goats of both groups, but they were related to changes in luteal tissue content only in control does throughout the present study. Mean daily numbers of small- and medium-sized antral follicles decreased ($P < 0.05$) only in pregnant animals of both groups with a decline in medium follicle numbers occurring earlier in hCG-treated (D13) compared with control does (D17). To summarize, a single dose of hCG given on D7 after estrus was followed by a decrease in the number of medium-sized antral follicles in gestating hCG-treated does, induced the formation of ALSs in ~47% of all hCG-treated does, and significantly increased the pregnancy rate in estrous-induced Toggenburg goats in the transition to the breeding season.

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1. Introduction

Small ruminants are of a great economic importance in many countries throughout the world [1]. There are approximately 10 million goats currently raised in Brazil [2]; the Northeast region of the country has ~93% of all herds [3], but in the Southeast region, goat production is more specialized and goat dairy products have gained widespread popularity [4]. Profitability of any livestock operation depends largely on the reproductive efficiency of animals. Goats are seasonally polyestrous animals, becoming sexually active in response to the shortening day length. In the southeastern part of Brazil, the breeding season in goats spans the period from March to August, the seasonal anestrus from September to November, and the gradual return to the cyclic ovarian activity, or the transition to the breeding season, begins in December [5]. The reproductive management of goats must therefore entail the use of strategies (eg, hormonal protocols using exogenous gonadotropins; [6]) to induce estrus and ovulation outside of the normal breeding season.

Several other factors can impinge on the fertility of breeding goats. Corpus luteum (CL) and its progesterone (P_4) secretion are essential for pregnancy maintenance [7], and an abnormal function of CL has been identified as one of the main causes of gestational loss during the preimplantation period [8]. In fact, inadequate CL function is the primary cause of infertility in goats; it was confirmed by several earlier laparoscopic [9,10] and ultrasonographic [11] studies, as well as by measurements of circulating progesterone concentrations before Day 7 of the estrous cycle [10,11]. The incidence of CL inadequacy in sheep and goats can be drastically reduced or even eliminated with progestin priming [12]. However, even in animals pretreated with exogenous progestins, CL secretory ability can remain hampered, especially in females that bred outside of the normal breeding season. In estrous-induced animals, early pregnancy loss due to inadequate CL formation and secretory ability can reach up to 60% [6]. High doses of exogenous P_4 commonly used in estrous induction protocols alter the synthesis of 17β-estradiol and P_4 concentrations in systemic circulation and tubular genitalia, leading to an impaired luteal function and lower fertility [13]. Moreover, circulating P_4 concentrations in ewes are lower after the induced estrous cycles in the nonbreeding season and during the transitional periods into and out of anestrus compared with the middle portion of the breeding season [14–17].

During early pregnancy in ruminants, luteal P_4 plays an important paracrine function in the uterine epithelium and conceptus trophectoderm, influencing the synthesis and action of interferon-tau (IFNT) and prostaglandins as well as expression of oxytocin receptors and of an array of other IFN-stimulated genes [8]. This leads to the changes in transcriptomic profiles that are critical for the proper progression of the maternal recognition of pregnancy, implantation as well as the elongation and survival of the conceptus [18–21]. Several strategies can be used to increase serum P_4 concentrations in gestating animals including, but not limited to, i. manipulation of terminal follicular development to increase the size of preovulatory antral follicles and thus of the resultant CL, ii. supplementation with exogenous P_4, iii. direct stimulation of CL development with luteotropic agents, and iv. the induction of accessory luteal structures (ALSs) with GnRH or hCG [8]. It was demonstrated that accessory CL formation increases plasma P_4 concentrations [22] and pregnancy rates in cattle artificialized inseminated [23] but not in recipient heifers [24]. Similar positive effects (ie, induction of ALSs and increase in P_4 concentration) were reported in sheep [25–27], but the available literature is incipient and controversial about the suitability of this approach in goats [28].

In cyclic goats, the dominant follicles of the first 2 follicular waves of the interovulatory period reach their maximum diameters between D5 and D7 after estrus [29–31]. In estrous-induced goats, the maximum diameter of the dominant follicles of the first follicular waves ($\cong$ 7 mm diameter) occurred between D7 and D8 after estrus, irrespective to the number of follicular waves of the cycle [31]. Therefore, the administration of hCG on day 7 after estrus can promote the luteinization or rupture of mature healthy and growing antral follicles, resulting in the formation of ALSs, enhancement of the growth rate and functionality of the original (postovulatory) CL, and overall increase in P_4 concentrations. All these processes may minimize the embryo loss and positively affect pregnancy rates in goats. Thus, the main goal of the present study was to evaluate the effects of exogenous hCG given on D7 on the ovarian activity and pregnancy rate in estrous-induced dairy goats in the transition into the breeding season.

2. Materials and methods

2.1. Ethics and animal care

The animal care committee of the Universidade Federal Fluminense approved the study design and all experimental procedures performed on live animals (project number 1021/2017). The experiment was in complete compliance with the guidelines of the Brazilian Society of Laboratory Animal Science, which regulates conditions for experiments involving animals.

2.2. Location and general animal management

This study was carried out in a dairy goat farm located in the Minas Gerais State, Brazil (latitude 21°35′S and longitude 43°15′W) during the transition into the breeding season (December to January). In accordance with the Köppen classification, the climate of this region is type Cwa, characterized by a dry winter and hot summer [32]. To verify the ovarian status of the goats of the present study, ultrasound (US) examinations were performed 2 and 1 wk before and on the day of progestin sponge insertion; the percentage of animals with detectable CL on each day was 7.0%, 1.2%, and 12.8%, respectively.

The goats were managed in an intensive production system, confined in group pens, and were fed corn silage. A balanced concentrate supplement was provided in accordance with their metabolic demand. Mineralized salt licks (Caprinofós Tortuga, São Paulo, Brazil) and drinking water were available ad libitum.

2.3. Animals, treatments, and estrus detection

The experiment was carried out in a completely randomized block design, with 3 blocks starting 1 wk apart. Eighty-six Toggenburg does with a mean body weight of 47.6 ± 9.3 kg and BCS of 2.8 ± 0.4 were used in this study (1 to 5 scale, [33]). All does received an intravaginal sponge containing 60 mg of medroxyprogesterone acetate (Progespon; Syntex S.A., Biochemical and Pharmaceutical Industry, Buenos Aires, Argentina), 200 IU of equine chorionic gonadotropin (Folligon; Intervet International B.V., Boxmeer, Netherlands), and 30 μg of d-cloprostenol (Prolise; Tecnopec LTDA, São Paulo, Brazil). Estrous detection and breeding were performed twice daily with intact Toggenburg bucks (male to female ratio of 1:3) previously subjected to the breeding soundness evaluation. Each male was placed in an enclosure with one female at a time to observe the signs of behavioral estrus or sexual receptivity such as actively searching the male, restlessness, vocalization, tail flicking, contraction, hyperemia and vulvar edema, vaginal discharge of mucus, and immobility during the buck's mount. On D6.5 after the onset of estrus, all females had at least 1 CL from estrous induction protocol, and they were allotted in accordance with their parity order (13 nulliparous and 30 multiparous does for each group), body weight (48.1 ± 1.5 vs 47.1 ± 1.4 kg), BCS (2.9 ± 0.1 vs 2.8 ± 0.1), and CL count (1.7 ± 0.1 vs 1.6 ± 0.1) to 1 of the 2 equinumerous groups receiving, respectively, either 1 mL of saline (the control group; n = 43) or 300 IU of hCG (Vetecor 5000; Hertape Calier, Spain; the hCG-treated group, n = 43) i.m on D7 (Fig. 1).

2.4. Ultrasonographic examinations

The same experienced technician performed all ultrasonographic examinations and during the morning hours (8 to 10 AM) using the portable US scanner (Mindray M5Vet, Shenzhen, China) equipped with a linear-array 7.5-MHz transducer. The transducer was taped to a PVC tube so that it could be externally held and manipulated during the transrectal examination. The does were examined in a standing position, fecal pellets were removed manually, and additional 10 mL of carboxymethylcellulose gel (Carbogel UTL; Carbogel Indústria e Comércio LTDA, São Paulo, Brazil) was deposited into the rectum with a syringe.

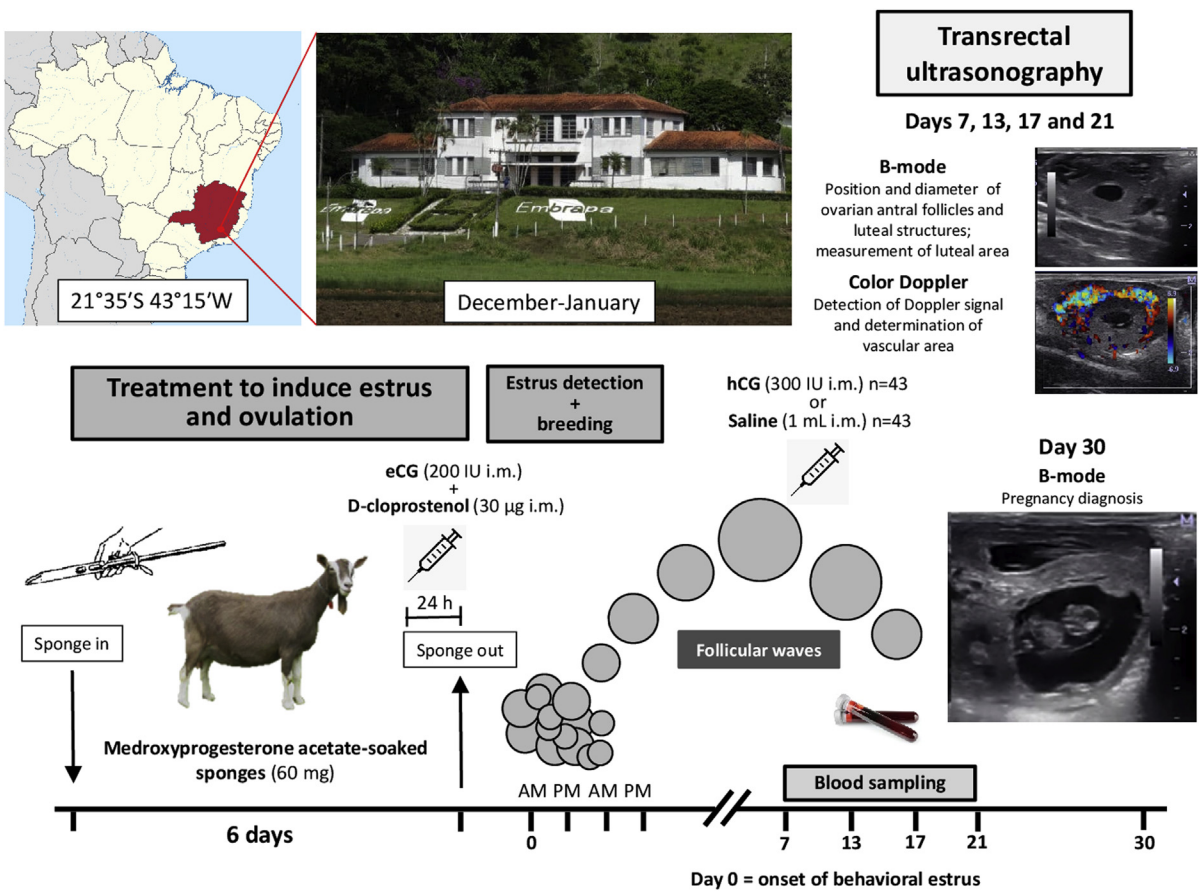

Fig. 1. Experimental design of the study. Evaluation of the effect of administering 300 IU of hCG i.m., on day 7 after the onset of estrus (day 0) in 86 Toggenburg goats during the transition into the breeding season. The goats allocated to the control group received 1 mL of saline. Females were allocated to each group as they presented estrus and were mated by natural breeding. On days 7, 13, 17, and 21, mode B and Doppler ultrasound were performed to evaluate ovarian structures. At the same time, blood was collected from 25 goats to assess the serum progesterone profile. On day 30, the animals underwent ultrasonographic pregnancy detection.

Approximately, 5 mL of lubricating carboxymethylcellulose gel was applied onto the transducer before each ultrasonographic examination. The procedures used to locate the ovaries were the same as those recommended by Souza et al [34]; the transducer was inserted into the rectum until the urinary bladder and uterus could be detected and then the images of both ovaries were obtained by rotating the transducer clockwise and counter clockwise.

On D7, D13, D17, and D21 after the onset of estrus, B-mode and color Doppler ultrasonograms of both ovaries were recorded for subsequent analyses (Fig. 2). The diameter, position, and general characteristics of the detected ovarian structures were recorded on individual ovarian charts. B-mode images were used to measure the total luteal area (TLA) (cm^2) defined as the sum of cross-sectional areas of all detected luteal structures (ie, ovulatory CL and ALSs); for cavitated luteal structures, the area of a central cavity was subtracted. Color Doppler images of the luteal structures were recorded in the frame encapsulating the largest cross-sectional area of each luteal gland for determination of the color Doppler signal and the percentage of vascular area. The color Doppler area representing luteal blood flow was computed using Image-Pro Plus analytical software (Media Cybernetics Inc, San Diego, CA) for each CL and ALS, as previously described by Oliveira et al [35] for sheep ovarian structures. A vascular area percentage (color Doppler area/TLA × 100%) was obtained for each day and for each goat. The Doppler settings were 75% color gain, pulse repetition frequency of 1.0 kHz, 6 cm of depth, and wall filter of 75 MHz [36]. Pregnancy was confirmed ultrasonographically in all goats studied on D30 by detecting embryonic vesicles and heartbeat.

2.5. Circulating P_4 concentrations

In the first subset of experimental animals, jugular blood samples were drawn from 25 goats (n = 12 controls and n = 13 hCG-treated; with a mean body weight of 41.9 ± 1.3 kg and BCS of 2.8 ± 0.1) into vacutainers without anticoagulant, during the preprandial period on D7, D13, D17, and D21 after estrus. The tubes were immediately placed on ice, transported to the laboratory, and centrifuged in a refrigerated centrifuge for 15 min at 1,500× *g*. After centrifugation, blood serum was aspirated and stored at −20°C in 1.5-mL microtubes until the day of laboratory analysis. Circulating P_4 concentrations were determined with a commercial solid-phase RIA kit (MP Biomedicals, LLC, Diagnostics Division, Orangeburg, NY, USA) in accordance with the manufacturer's instructions. The range of standards was 0.15–100 ng/mL. The assay sensitivity and intra-assay coefficient of variation were 0.05 ng/mL and <10%, respectively.

2.6. Variables and statistical analyses

The following end points were determined and analyzed statistically in the present experiment: numbers/proportions of goats in estrus, numbers/proportions of pregnant goats, numbers/proportions of goats with ALSs after hCG treatment, number of postovulatory CL per goat (defined as CL identified ultrasonographically on D7, numbers and time of detection of ALSs (identified ultrasonographically on D13–D21), mean total area (cm^2) of detected luteal structures, daily serum P_4 concentrations determined on D7–D21, and percentage of the color

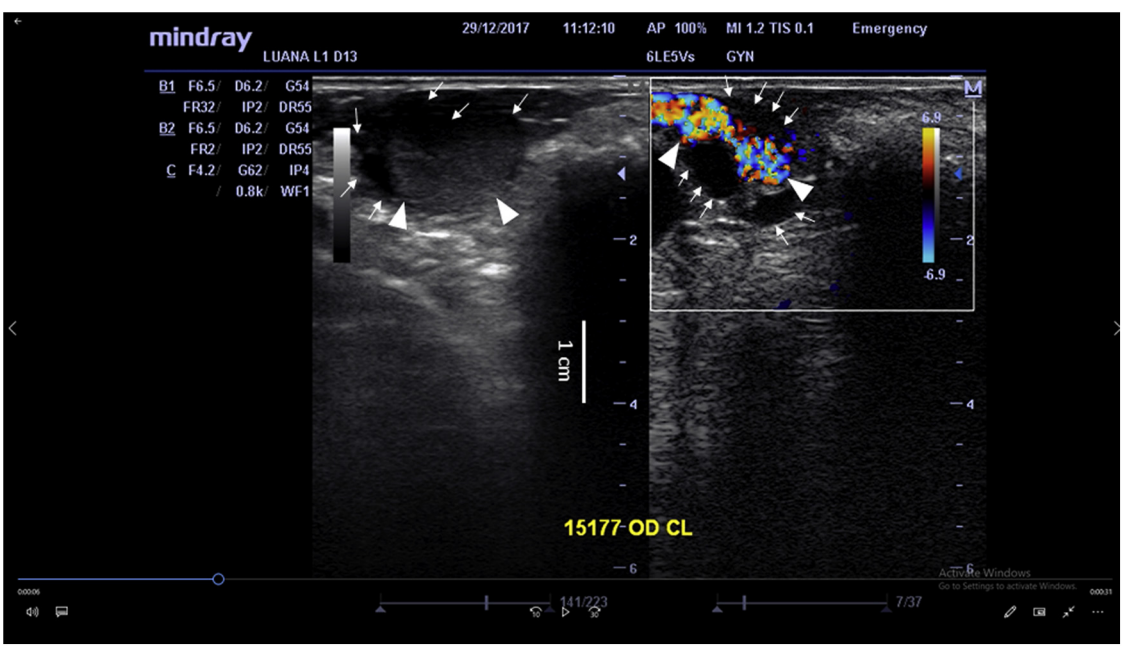

Fig. 2. A photographic reproduction of an ovarian scan from a Toggenburg goat that underwent ultrasonographic examinations during the middle portion of the induced estrous cycle. A panel on the left side depicts B-mode visualization of luteal structures (arrowheads) and individual ovarian antral follicles (arrows) of varying sizes (a scale bar drawn near the middle of the scan corresponds to 1 cm), and an insert in the upper right corner illustrates detection of the Doppler signal within luteal glands.

Doppler (vascular) area. All data are expressed as mean ± SEM unless otherwise indicated. All statistical analyses were performed using the SigmaPlot statistical software (Systat Software Inc, San Jose, CA), and differences among mean values were considered significant when $P \leq 0.05$. The proportions were analyzed by the χ^2 test (Brandt-Snedecor formula; [37]) or Fisher's exact test (depending on variable frequencies). Luteal characteristics and antral follicle numbers were analyzed by three-way ANOVA. Data were checked for normality and homoscedasticity by Shapiro-Wilk and Cochran tests, respectively. Whenever necessary, the data were transformed logarithmically ($\log_n$) before ANOVA. When the main effects of group (control vs hCG-treated), pregnancy status (pregnant vs nonpregnant), day of observation (D7, D13, D17, and D21), or their interactions were significant, the differences between individual means were computed by the Holm-Sidak method. Retrospective analyses of the present data revealed that serum P_4 concentrations were measured only in 4 nonpregnant goats, which precluded further statistical comparisons among pregnant and nonpregnant does randomly allocated to both experimental groups; therefore, circulating P_4 concentrations were analyzed by two-way ANOVA to determine the effect of group, day, and interactions.

Preliminary inspection of our results revealed that ALSs occurred only in 46.5% hCG-treated animals. Therefore, additional statistical comparisons were made between hCG-treated does with or without ALSs post-treatment. Specifically, mean numbers of antral follicles in different size categories, serum P_4 concentrations, and CL characteristics on D7 were compared between the 2 subsets on animals with the Student *t*-test, and the Pearson product Moment tests (correlational analyses) were carried out for luteal characteristics and serum P_4 concentrations.

3. Results

3.1. Pregnancy rates, ultrasonographic evaluation of luteal structures, and serum P_4 concentrations

The proportions of pregnant does, based on an ultrasonographic examination performed on D30, were 32 of 43 (74.4%) and 39 of 43 (90.7%) for the control and hCG-treated animals, respectively ($P < 0.05$). The number of luteal structures (ovulation rate) detected ultrasonographically on D7 did not differ ($P > 0.05$) between the two subsets of does studied (control: 1.7 ± 0.1 and hCG-treated: 1.6 ± 0.1). The ALSs were detected in 20 of 43 (46.5%) does that received hCG injection but in none of the control does between D7 and D21. Accessory luteal structures were first recorded on D13 in 9 does, on D17 in 7 does, on D21 in 3 does, and in 1 hCG-treated animal, ALSs were first detected on D13 and D17. Nineteen hCG-treated does had 1 and 1 goat formed 2 ALSs. This led to a final number of all luteal structures (ovulatory CL + ALSs) on D21 being greater ($P < 0.05$) than CL numbers recorded on D7 in the hCG-treated group of does (2.1 ± 0.1 compared with 1.6 ± 0.1, respectively).

All 20 hCG-treated dairy goats that had ALSs remained pregnant, whereas 19 of 23 (82.6%) does without detectable ALSs after hCG injections were gestating ($P < 0.05$). The percentage of pregnant does in the control group (34/43%; 74.4%) did not vary ($P > 0.05$) from that in hCG-treated goats without ALSs, but it was less ($P < 0.05$) compared with the 100% of pregnant ewes with ALSs after hCG treatment on D7.

General results of statistical comparisons for luteal tissue characteristics determined in this study are summarized in Table 1, and significant differences among different subsets of animals are illustrated in Fig. 3. There was a significant main effect of day, pregnancy status (pregnant vs nonpregnant), and of pregnancy status × day interaction for the TLA and mean vascular area (MVA) of luteal structures. In control does that became pregnant, TLA increased ($P < 0.05$) from D7 to D13, whereas in nonpregnant controls, it declined ($P < 0.05$) from D13 to D21 (Fig. 3A). Mean TLA values were greater ($P < 0.05$) in pregnant than in nonpregnant control does from D13 to D21 (Fig. 3A). In a subset of hCG-treated animals, a rise ($P < 0.05$) in mean TLA occurred in pregnant does between D7 and D13, and TLA was significantly greater in pregnant than that in nonpregnant does on D21 (Fig. 3A). The percentage of vascular tissue detected within all types of luteal structures using color Doppler imaging (MVA) declined ($P < 0.05$) in nonpregnant animals of both groups from D13 to D21

Table 1
Summary of *P* values from three-way ANOVA tables for luteal characteristics and ovarian antral follicular population determined ultrasonographically in estrous-induced Toggenburg goats with or without a single injection of 300 IU of hCG given 7 d after the onset of estrus (D7).

Main effects and interactions	Total luteal area (cm^2)	Mean vascular area (%)	Max. follicle diameter (mm)	Small follicles (<3 mm)	Medium-sized follicles (3 to 6 mm)	Large follicles (≥6 mm)
Treatment group (control vs hCG-treated)	0.07	0.45	0.10	0.64	0.01	0.93
Pregnancy status (pregnant vs nonpregnant)	<0.001	<0.001	0.07	0.02	0.38	0.13
Day (D7, D13, D17, D21)	0.005	<0.001	0.05	<0.001	0.004	0.32
Treatment group × pregnancy status	0.20	0.59	0.27	0.77	0.81	0.13
Treatment group × day	0.86	0.36	0.04	0.87	0.05	0.26
Pregnancy status × day	0.002	<0.001	0.23	0.91	0.57	0.28
Treatment group × pregnancy status × day	0.78	0.48	0.62	0.94	0.46	0.54

Transrectal ovarian ultrasonography was performed on D7, D13, D17, and D21, and pregnancy diagnosis on D30.

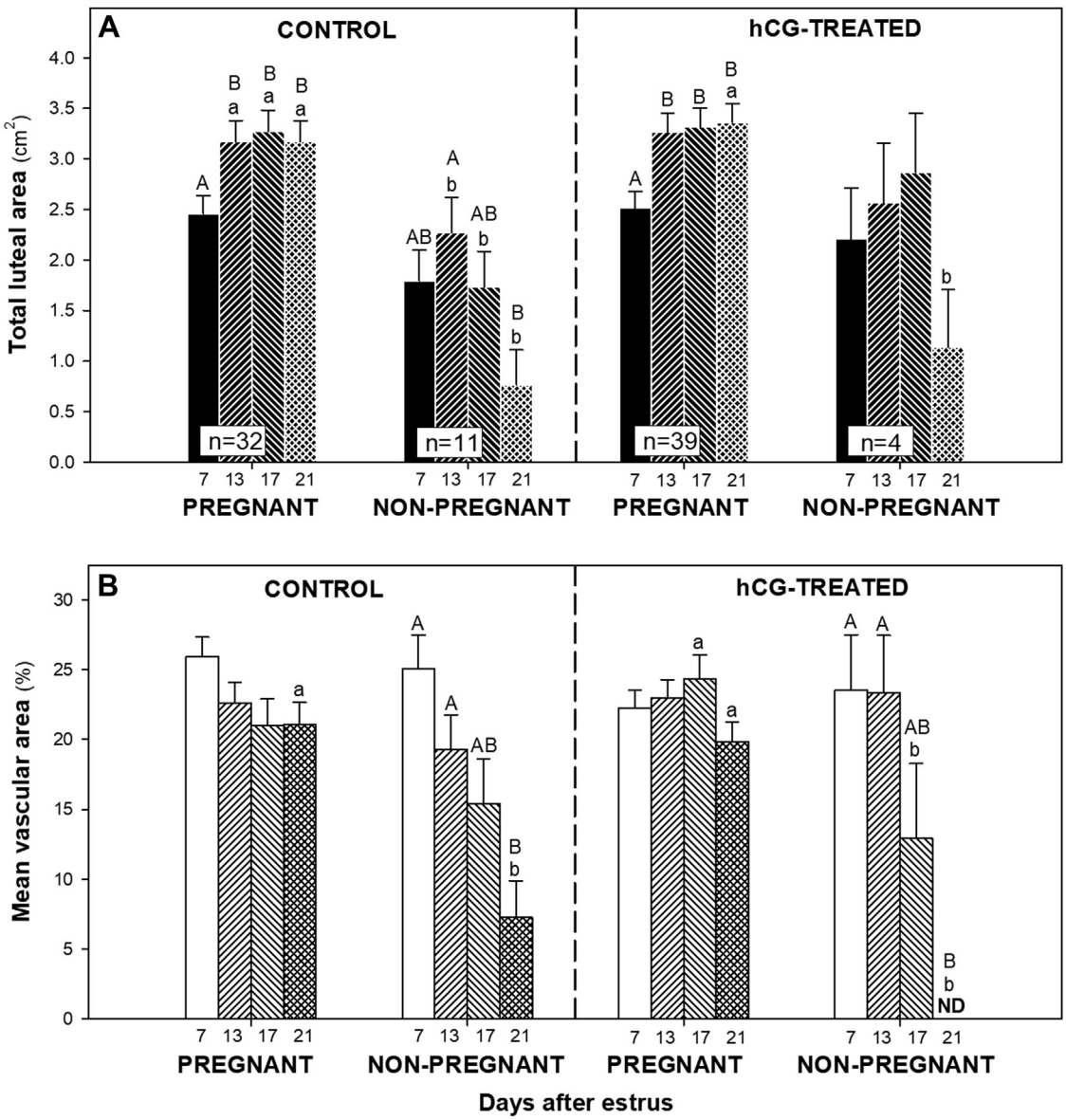

Fig. 3. Mean (±SEM) values for ultrasonographically determined total luteal area (A) and vascular (Doppler) area (B) in Toggenburg goats with or without a single injection of hCG given 7 d after the onset of estrus (D7; n = 43/group). Ultrasonographic observations were conducted on D7, 13, 17, and 21, and ultrasonographic pregnancy detection was carried out on D30. Significant differences between mean values are denoted by different letters: AB between days within each subset of animals; ab between pregnant and nonpregnant does on the same day within the groups.

(Fig. 3B). In control does, MVA was greater in pregnant than that in nonpregnant does on D21, whereas in the hCG-treatment group, pregnant animals exceeded ($P < 0.05$) their nonpregnant counterparts on D17 and D21 (Fig. 3B). Notably, Doppler signal was not detectable in nonpregnant hCG-treated goats on D21.

In the control group, a sudden decline in circulating P_4 concentrations to a basal or nondetectable level occurred in 5 of 12 (41.7%) before D21, whereas the proportion of such does in the treatment group was 7.7% (1/13; $P < 0.05$). Progesterone concentrations were below the assay detection limit in a vast majority on nonpregnant does and in all nonpregnant hCG-treated animals on D21. Circulating P_4 concentrations increased ($P < 0.05$) from D17 to D21 in pregnant does of both groups (Fig. 4). In addition, peripheral P_4 concentrations were lower ($P < 0.05$) on D17 than those on D7 in pregnant controls (Fig. 4).

3.2. Ovarian antral follicles

There was a significant main effect of day for the maximum follicle diameter and daily numbers of small- and medium-sized detected ultrasonographically in the does of the present study (Table 1). In addition, the treatment group × day interaction was significant for maximum follicle diameter and medium follicle numbers, pregnancy

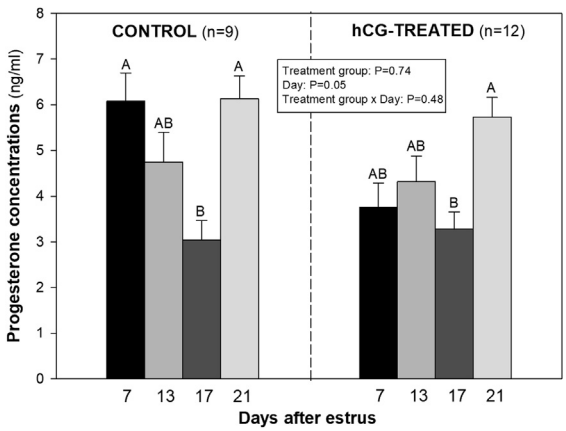

Fig. 4. Systemic concentrations of progesterone (mean ± SEM) determined in pregnant Toggenburg goats (n = 21) with or without a single injection of hCG given 7 d after the onset of estrus (D7). Blood samples were drawn on D7, D13, D17, and D21. AB—different letters indicate significant differences between days within each group.

status was significant for small follicle numbers, and a significant effect of the treatment group was seen for the number of medium-sized antral follicles (Table 1). The maximum follicle diameter increased ($P < 0.05$) from D7 to D13 in hCG-treated does that became pregnant (Fig. 5A). Mean numbers of small antral follicles declined ($P < 0.05$) between D7 and D13 in gestating does of both groups (Fig. 5B). Finally, there was a decline ($P < 0.05$) in the number of medium-sized antral follicles from D7 to D17 in nonpregnant control does and a decline ($P < 0.05$) in the number of such follicles from D7 to D13 in hCG-treated animals that remained pregnant (Fig. 5C).

3.3. Differences between hCG-treated goats with or without ALSs (D7) and correlational analyses

None of the follicular and luteal variables on D7 differed between hCG-treated does with or without ALSs after injection (Table 2). Based on US Doppler, CL feature, and circulating P_4 concentration (>1 ng/mL) of goats on D7, no abnormal CL (short-life CL) was detected. When luteal and endocrine data for pregnant does were analyzed for quantitative correlations, TLA was positively correlated with circulating P_4 concentrations on all days of the study in control animals but only on D7 in the hCG-treated group (Table 3); there were no significant correlations between the TLA or MVA and serum P_4 concentrations.

4. Discussion

This study examined the effects of 300 IU of hCG administered on D7 after estrus on luteal function, antral follicle populations, and pregnancy rates in seasonally anestrous dairy goats. In the goats of the present study, an i.m. injection of hCG resulted in a significant increase in the pregnancy rate by approximately 16% compared with untreated controls. In earlier studies, the administration of 250 IU of hCG on D5 had no apparent effects on the pregnancy rate [28,38,39]. This difference between our present and previous experiments can be due to a dose or the timing of an injection of hCG. In addition, even though abnormally short estrous cycles occur in estrous-induced goats outside of the breeding season, such cycles were not observed in the present study; this can be because our experiment took place during the transition of goats into the breeding activity.

The diameter of the largest follicle reported in the present study was 6.3 mm on D7 after estrus, and this follicle was possibly in growing phase as proposed for sheep [40] and healthy to answer to the gonadotropin stimuli given by hCG. We expected to find larger follicles with 6 to 7 mm diameter on D7 of estrous cycle. The largest follicles from first and second follicular waves grew 0.7 and 0.5 mm daily [31], whereas ovulatory follicle growth was 0.8 mm daily [41]. Thus, considering 0.5 to 0.8 mm daily growth rate (~0.65 mm daily), 3 mm diameter of emerging follicles on D2 after estrus [41], and hCG administration on D7 of estrous cycle or 5 to 6 d after wave emergence (~5.5 d), the largest follicles were of expected size and possibly able to respond to hCG treatment.

Accessory luteal structures were detected ultrasonographically in almost half (46.5%) of animals treated with hCG. The proportion of pregnant hCG-treated does with ALSs was significantly greater than that of does from the control group and with hCG-treated does without ALSs, clearly indicating that the induction of ALSs was instrumental for boosting the pregnancy rates in seasonally anestrous dairy goats studied. The reason for a lack of ALS formation in all hCG-treated does is difficult to explain but could be attributed to goats with pattern of 2 ovarian follicular waves that represented 50.0% of goats previously reported [42] carrying follicles in nonadequate status for answering to hCG challenge. This is all the more important given the fact that further increase in the number of goats with ALSs would have possibly resulted in even higher, nearing 100% pregnancy rates after the treatment with hCG on D7. The largest follicles of the first 2 successive waves in cyclic goats reach their maximum diameter on D5 [30] and D7 [30,31] of the estrous cycle. However, slower rates of antral follicular growth/turnover and prolonged interwave intervals were observed in the nonbreeding season due likely to lower LH secretion and diminished ovarian sensitivity to gonadotropic stimulation [31,43]. Especially, the latter factor may be a primary reason for the absence of ovulation, follicular luteinization or both in response to exogenous GnRH [44] or gonadotropins as frequently seen in seasonally anovular sheep. None of the ovarian variables recorded on D7, including antral follicle numbers and maximum diameter, differed between the goats with or without ALSs after hCG treatment in this study.

It was anticipated that the use of hCG would result in an increase in the total luteal content [27,45,46]. Surprisingly, the TLA increased from D7 to D21 in pregnant goats of both groups, whereas in nonpregnant animals, the TLA did not vary from D7 to D17 and then declined abruptly on D21, coincident with the onset of luteal regression. Significant differences between pregnant and nonpregnant does in the TLA could be observed on D13 to D21 in control animals but only on D21 in the hCG-treated group, and so while

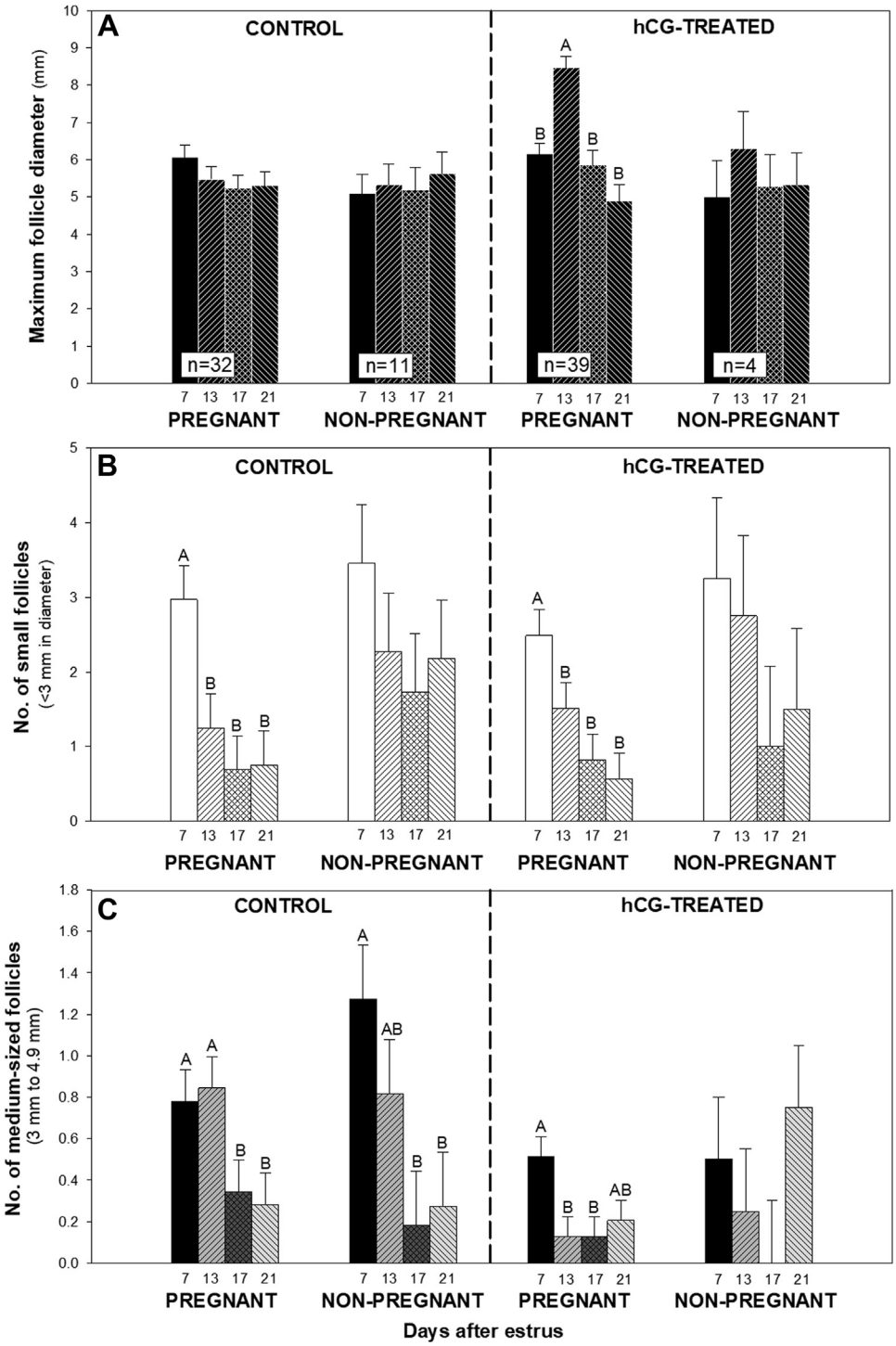

Fig. 5. Maximum follicle diameter (A) and mean daily numbers of small- (B) and medium-sized ovarian antral follicle (C; mean ± SEM) values determined ultrasonographically in Toggenburg goats with or without a single injection of hCG given 7 d after the onset of estrus (D7; n = 43/group). Transrectal ovarian ultrasonography was performed on D7, D13, D17, and D21. Significant differences between mean values are denoted by different letters: AB between days within each subset on animals; ab between pregnant and nonpregnant does on the same day within the groups.

Table 2
Summary of ovarian follicular and luteal characteristics recorded on D7 after estrus on hCG-treated Toggenburg anestrous does with or without accessory luteal structures (ALSs) forming post-treatment.

Variables	ALS bearing (n = 20)	Non-ALS bearing (n = 23)
Maximum follicle diameter (mm)	6.3 ± 0.2	5.8 ± 0.4
No. of small follicles	2.0 ± 0.6	3.0 ± 0.5
No. of medium-sized follicles	0.4 ± 0.2	0.6 ± 0.2
No. of large follicles	1.3 ± 0.2	1.6 ± 0.3
Total luteal area (cm^2)	2.5 ± 0.2	2.5 ± 0.2
Mean vascular area (%)	21.2 ± 1.5	23.4 ± 1.8
Progesterone concentrations (ng/mL)	5.6 ± 1.5 (n = 5)	5.2 ± 0.8 (n = 7)

ALSs were detected ultrasonographically on D13, D17, and D21.

progressing structural luteolysis was associated with a lack of pregnancy in control goats, a similar mechanism was not apparent in hCG-treated animals.

A shift in TLA from D7 to D21 in pregnant goats was not accompanied by a significant change in luteal tissue vascularity, but the percentage of vascular area declined by D21 in nonpregnant does of both groups. Therefore, luteal vascularity does not seem to contribute to the maintenance of early pregnancy in goats, but rather it parallels luteal demise in nonpregnant animals. Significant difference in MVA of the luteal tissue between pregnant and nonpregnant animals was restricted to D21 in control animals and D17–D21 in hCG-treated goats, further confirming that luteal blood flow is not regulated during the maternal recognition of pregnancy that occurs in goats between D15 and D17 [47].

Serum P_4 concentrations did not vary between pregnant goats with or without hCG injections. Previous studies in cyclic goats have shown that TLA is positively correlated with circulating P_4 concretions during the periods of luteogenesis and luteal regression, but an increase in the luteal content in goats with multiple ovulations is not followed by a proportional increase in serum P_4 levels [27,48]. In the pregnant does of the present study, circulating P_4 concentrations were positively related to the TLA throughout the entire observation period, but such a relationship in the treatment group was restricted to D7 (ie, before hCG administration). A lack of correlations between TLA and serum P_4 concentrations in hCG-treated does after D7 can most likely be explained by the existence of forming (ie, not fully functional) luteal structures that did not contribute to the total P_4 production as much as the post-ovulatory corpora lutea (CL). Moreover, there were no apparent correlations among luteal tissue vascularity and peripheral P_4 concentrations in either control or hCG-treated does. These results must be interpreted with caution as they were obtained only from a random fraction of gestating animals in the present study. Most importantly, however, the measurement of serum P_4 concentrations revealed that a rise in its synthesis by the luteal structures occurred after the period of maternal recognition of pregnancy in all animals under study (D21). This is intriguing and may suggest that i. the attainment of only minimal (ie, threshold) concentrations of luteal P_4 but not necessarily an increase in P_4 production is necessary to effectively maintain pregnancy in goats or ii. there exists a luteal product other than P_4, which enhances viability or stimulates secretory function of preimplantation embryos and interrupts the onset of a luteolytic cascade. Results of recent studies in polyovulatory species may be supportive of this notion because there is indirect evidence to suggest that, within physiological ranges, the production of luteal P_4 in pigs has limited effect on embryo development although the numbers of CL remain correlated with the numbers of viable embryos in pigs [49]. In cows, impaired luteal function is associated with low levels of insulin-like growth factors, which can create a suboptimal microenvironment in the uterus and culminate in embryo losses [50]. This theory remains to be tested in small ruminants.

One of the causes of premature luteal regression in small ruminants is the untimely induction of the luteolytic signal [44,51–53]. Premature luteolysis is a leading cause of low pregnancy rates in estrous-induced dairy goats raised under similar management systems and climatic conditions as those in our study [10,11,41]. Failure of proper CL formation can be detected with laparoscopy or transrectal ultrasonography in cycling and superovulated goats by D7 after the onset of estrus [9,54]. However, ultrasonographic

Table 3
Correlation coefficients (r) and *P* values for correlations (Pearson product moment) between total luteal area (TLA) or mean vascular area (MVA; color Doppler signal) and serum P_4 concentrations in pregnant does with or without prior hCG injection on D7 after estrus (control vs hCG-treated).

Days	Correlated variables	Control (n = 9)	hCG-treated (n = 12)
D7	TLA (cm^2) vs P_4 concentrations (ng/mL)	r = 0.81, *P* = 0.008	r = 0.62, *P* = 0.03
	MVA (%) vs P_4 concentrations (ng/mL)	r = −0.61, *P* = 0.08	r = −0.22, *P* = 0.49
D13	TLA (cm^2) vs P_4 concentrations (ng/mL)	r = 0.67, *P* = 0.05	r = 0.18, *P* = 0.56
	MVA (%) vs P_4 concentrations (ng/mL)	r = −0.06, *P* = 0.85	r = 0.13, *P* = 0.68
D17	TLA (cm^2) vs P_4 concentrations (ng/mL)	r = 0.78, *P* = 0.01	r = 0.47, *P* = 0.12
	MVA (%) vs P_4 concentrations (ng/mL)	r = −0.22, *P* = 0.56	r = 0.14, *P* = 0.66
D21	TLA (cm^2) vs P_4 concentrations (ng/mL)	r = 0.70, *P* = 0.05	r = 0.37, *P* = 0.24
	MVA (%) vs P_4 concentrations (ng/mL)	r = −0.03, *P* = 0.94	r = 0.42, *P* = 0.17

Transrectal ovarian ultrasonography and blood sampling were performed on D7, D13, D17, and D21.

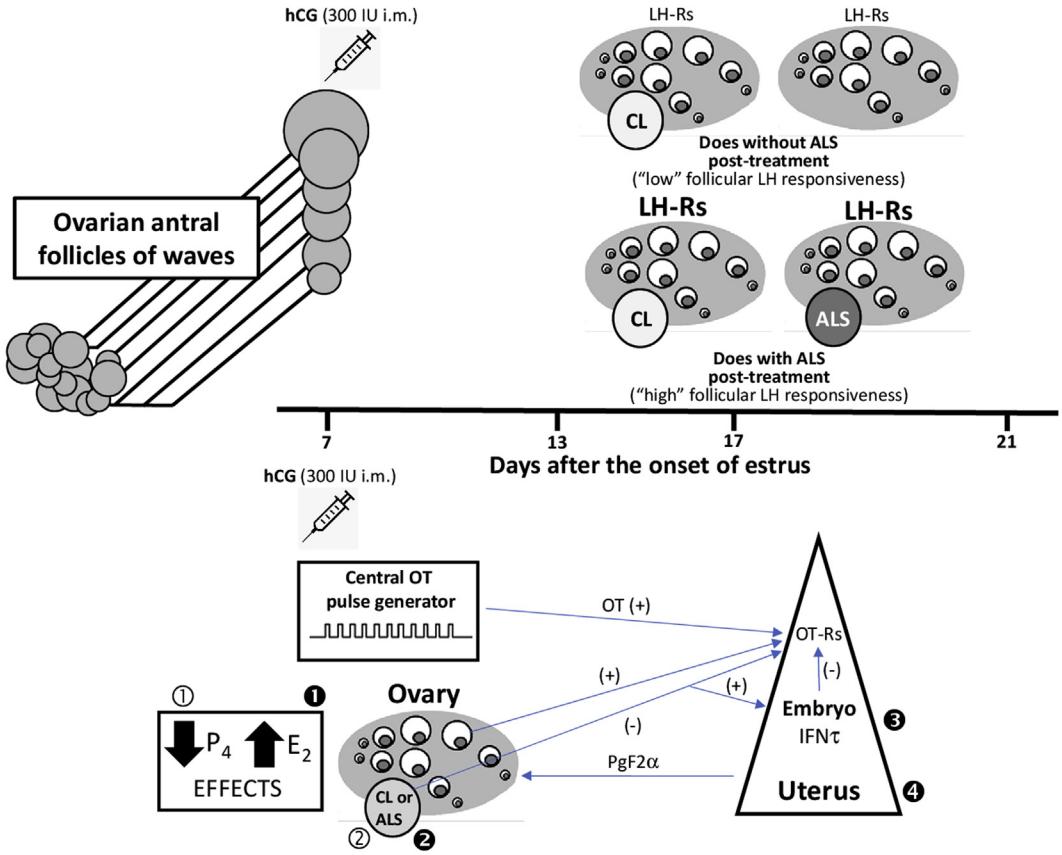

Fig. 6. A diagrammatic figure summarizing the reproductive effects and putative mechanisms of hCG action in early pregnant goats. An upper portion denotes diminished ovarian LH sensitivity (due to photoperiodic influences or an absence of a responsive ovarian antral follicle in its growing phase) as a major plausible cause of a lack of accessory luteal structures (ALSs) after hCG injections (300 IU i.m.). A lower portion summarizes the putative and unlikely modes of hCG effects based on the results of the present study: ❶ diminished follicular estrogen (E_2) biosynthesis due to LH-like effects of hCG could have prevented the premature onset of the oxytocin (OT)-driven luteolytic cascade, ❷ hCG-induced production of luteal factors other than progesterone (P4) that inhibit endometrial oxytocin receptor (OT-R) synthesis, possess embryotropic effects, ❸ possible direct embryotropic effects of hCG, ❹ stimulation of histotrophic function of the uterus. The following 2 mechanisms are disputed considering the findings of this experiment: ① an increase in circulating P4 concentrations during the period of maternal recognition of pregnancy in does and ② potentially increased P_4 release due to enhanced luteal vascularity. IFNτ, embryonic interferon tau; PgF2α, prostaglandin F2-alpha (luteolysin).

examination conducted on D7 in the goats of the present study confirmed that all does that had been mated also had functional CL. Interferon-tau produced by caprine embryos between gestational days 16 and 18 prevents the pulsating release of prostaglandin $F_{2\alpha}$ and CL regression [20]. An increase in peripheral estradiol (E_2) concentrations stimulates the synthesis of endometrial oxytocin receptors resulting in release [52]. A suppression of estradiol 17-β production by nonovulatory follicular waves may lead to a delay in the onset of functional luteolysis, increasing the window of time for embryonic development and maternal recognition of pregnancy [55]. Growing antral follicles are a main source of E_2, and so reducing their numbers or ability to synthesize estrogens could help sustain normal luteal function and pregnancy. The present ultrasonographic observations on the changes in antral follicle numbers are intriguing and may shed a light on the mechanism of the maternal recognition of pregnancy in goats. The numbers of small antral follicles from which growing antral follicles of waves are recruited declined in pregnant animals of both groups (controls and hCG-treated) from D7 to D13 and then remained low until D21. Moreover, even though there were no significant differences in mean daily numbers of ovulatory-sized (large) antral follicles, the number of medium-sized follicles declined by D17 and D13 in all control and pregnant hCG-treated goats, respectively. This decline occurred earlier in pregnant hCG-treated ewes due mainly to accelerated growth of medium and large ovarian follicles manifesting in a rapid increase in maximum follicle size 4 d after the hCG injection. It is attractive to speculate that hCG-induced changes in ovarian antral follicles (eg, a reduction in numbers of estrogenic follicles by accelerating their terminal growth and promoting luteinization or ovulation) are a major mechanism whereby exogenous hCG treatment enhances pregnancy rates in goats. This hypothesis would also explain why hCG given on D5 after breeding was not as effective in maintaining pregnancy in goats [28] as hCG administered on D7; the largest follicles of waves on D5 may have not been large or mature enough (LH responsiveness) to luteinize or rupture, but they

continued to grow and produced large amounts of estrogens (Fig. 6).

The effects of exogenous hCG on caprine embryonic development and secretory function that are independent of P_4 synthesis cannot, therefore, be ruled out. The hCG consists of 2 subunits: hCG-α and hCG-β, and the latter exhibits 85% amino acids homology to the LH-β subunit, allowing the 2 hormones to bind to the same receptor [56,57]. Transcripts for the LH receptor were detected in human oocytes, zygotes, morulas, and blastocysts [58], indicating a potential physiological role of LH in the oocyte maturation and preimplantation embryo development. Moreover, hCG stimulates the production of membrane P_4 receptor type 7 and C-X-C chemokine receptor type 4 in the maternal endometrium and promotes the expression of proangiogenic factors in fetal extra-embryonic membranes [59]. Nephew et al [60] demonstrated that hCG given on D11.5 stimulated/improved uterine secretion, conceptus growth (longer blastocysts), and pregnancy rate in sheep. All these data suggest that in the present study, the mechanism whereby hCG positively affected the pregnancy rate in dairy goats was at least partly due to a direct effect on embryos, maternal endometrium or both. More studies are needed in that area in gestating goats.

In conclusion, to recapitulate, a single dose of 300 IU of hCG on D7 after estrus in dairy goats resulted in an increase in pregnancy rates by approximately 16% compared with untreated, estrous-induced animals in anestrus. Although the formation of ALSs occurred between D13 and 21 in 46.5% of hCG-treated goats and the TLA increased by D13, serum P_4 concentrations did not rise before the period of the maternal recognition of pregnancy (D15-D17) as circulating P_4 concentrations were not correlated with the TLA after the hCG treatment. Luteal vascularity did not change over time in pregnant animals of both groups, and it declined on D21 in all nonpregnant animals, probably reflecting the occurrence of luteolysis. An hCG injection resulted in a transient increase in maximum follicle diameter (accelerated follicle growth) and earlier (on D13) decline in the number of presumptive estrogenic (medium-sized) antral follicles. Collectively, our present observations may be interpreted to suggest hCG given on D7 significantly enhanced the pregnancy rate in dairy goats by reducing the numbers of “proluteolytic” estrogenic follicles, increasing the production of luteal factors other than P_4 with embryotropic and placentation-facilitating properties (eg, insulin-like growth factors) or via a direct effect of hCG on uterine endometrium or embryo viability.

CRediT authorship contribution statement

L.R. Côrtes: Methodology, Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization. **J.M.G. Souza-Fabjan:** Methodology, Writing - review & editing, Project administration. **D.S. Dias:** Methodology, Investigation. **B.B. Martins:** Methodology, Investigation. **A.L.R.S. Maia:** Methodology, Investigation. **M.O. Veiga:** Writing - review & editing. **E.K.N. Arashiro:** Formal analysis. **F.Z. Brandão:** Resources. **M.E.F. Oliveira:** Methodology, 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.

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References

[1] Haenlein GFW. Past, present, and future perspectives of small ruminant dairy research. J Dairy Sci 2001;84:2097–115.
[2] FAOTAST, Food and agriculture organization corporate statistical database. http://www.fao.org/faostat/en/#data/QA. [Accessed 5 August 2018].
[3] Costa-Júnior L. Increasing goat production in Brazil: overview and challenges. https://www.iga-goatworld.com/blog/increasing-goat-production-in-brazil-overview-and-challenges/. [Accessed 5 August 2018].
[4] IBGE. Pesquisa pecuária municipal. https://sidra.ibge.gov.br/pesquisa/ppm/. [Accessed 5 August 2018].
[5] Balaro MFA, de Mello SGV, Santos AS, Cavalcanti LM, Almosny NRP, Fonseca JF, Brandão FZ. Reproductive seasonality in Saanen goats kept under tropical conditions. Trop Anim Health Prod 2019;51:345–53.
[6] Gordon I. Controlled reproduction in sheep and goats. Cambridge, UK: University Press; 1997.
[7] Meites J, Webster HD, Young FW, Thorp Jr F, Hatch RN. Effects of corpora lutea removal and replacement with progesterone on pregnancy in goats. J Anim Sci 1951;10:411–6.
[8] Lonergan P, Forde N, Spencer T. Role of progesterone in embryo development in cattle. Reprod Fertil Dev 2016;28:66–74.
[9] Camp JC, Wildt DE, Howard PK, Stuart LD, Chakraborty PK. Ovarian activity during normal and abnormal length estrous cycles in the goat. Biol Reprod 1983;28:673–81.
[10] Saharrea A, Valencia J, Balcázar A, et al. Premature luteal regression in goats superovulated with PMSG: effect of hCG or GnRH administration during the early luteal phase. Theriogenology 1998;50:1039–52.
[11] Netto MM, Balaro MFA, Cosentino IO, et al. Use of two cloprostenol administrations 11.5 days apart efficiently synchronizes oestrus in photostimulated multiparous dairy goats in the non-breeding season. Reprod Domest Anim 2020;55:965–73.
[12] Lassoued N, Khaldi G, Cognié Y, Chemineau P, Thimonier J. Effet de la progestérone sur le taux d'ovulation et la durée du cycle ovarien induits par effet mâle chez la brebis Barbarine et la chèvre locale tunisienne. Reprod Nutr Dev 1995;35:415–26.
[13] Theodosiadou E, Goulas P, Kouskoura T, Smokovitis A. Oestrogen and progesterone concentrations in plasma and oviductal tissue of ewes exhibiting a natural or induced oestrus. Anim Reprod Sci 2004;80:59–67.
[14] Rhind SM, Chesworth JM, Robinson JJ. A seasonal difference in ovine peripheral plasma prolactin and progesterone concentrations in early pregnancy and in the relationship between the two hormones. J Reprod Fertil 1978;52:79–81.
[15] Bartlewski PM, Beard AP, Cook SJ, Chandolia RK, Honaramooz A, Rawlings NC. Ovarian antral follicular dynamics and their relationships with endocrine variables throughout the oestrous cycle in breeds of sheep differing in prolificacy. J Reprod Fertil 1999a;115:111–24.
[16] Bartlewski PM, Beard AP, Rawlings NC. Ovarian function in ewes at the onset of the breeding season. Anim Reprod Sci 1999b;57:67–88.
[17] Bartlewski PM, Beard AP, Rawlings NC. Ovarian function in ewes during the transition from the breeding season to anoestrus. Anim Reprod Sci 1999c;57:51–66.
[18] Newton GR, Ott TL, Woldesenbet S, Shelton AH, Bazer FW. Biochemical and immunological properties of related small ruminant trophoblast interferons. Theriogenology 1996;46:703–16.

[19] Bazer FW, Wu G, Spencer TE, Johnson GA, Burghardt RC, Bayless K. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Mol Hum Reprod 2010;16:135–52.
[20] Bazer FW, Burghardt RC, Johnson GA, Spencer TE, Wu G. Mechanisms for the establishment and maintenance of pregnancy: synergies from scientific collaborations. Biol Reprod 2018;99:225–41.
[21] Lonergan P, Forde N. Maternal-embryo interaction leading up to the initiation of implantation of pregnancy in cattle. Animal 2014;8:64–9.
[22] Fonseca JF, Silva Filho JM, Palhares MS, Ruas JRM, Pinto Neto A. Plasma progesterone concentration in recipient heifers submitted to administration of rbST, GnRH or hCG on day five of the estrous cycle. Arq Bras Med Vet Zootec 2001;53:1–8.
[23] Nascimento AB, Bender RW, Souza AH, Ayres H, Araujo RR, Guenther JN, Sartori R, Wiltbank MC. Effect of treatment with human chorionic gonadotropin on day 5 after timed artificial insemination on fertility of lactating dairy cows. J Dairy Sci 2013;96:2873–82.
[24] Fonseca JF, Silva Filho JM, Palhares MS, Ruas JRM, Pinto Neto A. Pregnancy rates of recipient heifers submitted to administration of rbST, GnRH or hCG on day five of the estrous cycle. Arq Bras Med Vet Zootec 2001;53:1–6.
[25] Fernandez J, Bruno-Galarraga MM, Soto AT, de la Sota RL, Cueto MI, Lacau IM. Hormonal therapeutic strategy on the induction of accessory corpora lutea in relation to follicle size and on the increase of progesterone in sheep. Theriogenology 2018;105:184–8.
[26] Fernandez J, Galarraga MMB, Soto AT, Sota RL, Cueto MI, Lacau-Mengido IM. Effect of GnRH or hCG administration on day 4 post insemination on reproductive performance in Merino sheep of North Patagonia. Theriogenology 2019;126:63–7.
[27] Fonseca JF, Castro ACR, Arashiro EKN, Oliveira MEF, Zambrini FN, Esteves LV, Brandão FZ, Souza-Fabjan JMG. Effects of hCG administration on accessory corpus luteum formation and progesterone production in estrous-induced nulliparous Santa Inês ewes. Anim Reprod 2018;15:135–9.
[28] Fonseca JF, Maffili VV, Rodrigues MT, Santos ADF, Rovay H, Pinto NA, Brandão FZ, Torres CAA. Effects of hCG on progesterone concentrations and fertility in cyclic, lactating Alpine goats. Anim Reprod 2006;3:410–4.
[29] Amorim EAM, Torres CAA, Fonseca JF, Amorim LS, Maffili VV, Bruschi JH, Guimarães JD, Cecon PR, Alves NG. Estrous synchronization with recycled CIDR in lactating Toggenburg does treated with recombinant bovine somatotropin (r-bST). Arq Bras Med Vet Zootec 2008;60:51–7.
[30] Ginther OJ, Kot K. Follicular dynamics during the ovulatory season in goats. Theriogenology 1994;42:987–1001.
[31] De Castro T, Rubianes E, Menchaca A, Rivero A. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. Theriogenology 1999;52:399–411.
[32] Peel MC, Finlayson BL, McMahon TA. Update world map of the Koppen–Geiger climate classification. Hydrol Earth Syst Sci 2007;11:1633–44.
[33] Detweiler G, Gipson T, Merkel R, Goetsch A, Sahlu T. Body condition scores in goats. Annual Goat Field Day, 23. Langston: Langston University; 2008. p. 127–33.
[34] Souza JMG, Torres CAA, Maia ALRS, Brandão FZ, Bruschi JH, Viana JHM, Oba E, Fonseca JF. Autoclaved, previously used intravaginal progesterone devices induces estrus and ovulation in anestrous Toggenburg goats. Anim Reprod Sci 2011;129:50–5.
[35] Oliveira MEF, Bartlewski PM, Jankowski N, Padilha-Nakaghia LC, Oliveira LG, Bicudo SD, Fonseca JF, Vicente WRR. Relationship of antral follicular blood flow velocity to superovulatory responses in ewes. Anim Reprod Sci 2017;182:48–55.
[36] Figueira LM, Fonseca JF, Arashiro EKN, Souza-Fabjan JMG, Ribeiro ACS, Oba E, Viana JHM, Brandão FZ. Colour Doppler ultrasonography as a tool to assess luteal function in Santa Inês ewes. Reprod Domest Anim 2015;50:643–50.
[37] Cochran WG, Cox GM. Experimental designs. New York: Wiley; 1950.
[38] Fonseca JF, Torres CAA, Maffili VV, Borges AM, Espeschit CJB, Balbinot PZ, Oliveira RFM, Leite PAG. Desempenho reprodutivo de cabras alpinas tratadas com hCG cinco dias após o acasalamento. Rev Brasil Zootec 2005;34:508–13.
[39] Fonseca JF, Torres CAA. Administration of hCG 5 days after breeding and reproductive performance in nulliparous dairy goats. Reprod Domest Anim 2005;40:495–9.
[40] Menchaca A, Rubianes E. New treatments associated with timed artificial insemination in small ruminants. Reprod Fertil Dev 2004;16:403–13.
[41] Carvalho-de-Paula CJ, Souza-Fabjan JMG, Gonçalves JD, Dias JH, De Souza GN, Oliveira MEF, Fonseca JF. Effect of a 12-h increment in the short-term treatment regimen on ovarian status, estrus synchrony, and pregnancy rate in artificially inseminated dairy goats. Anim Reprod Sci 2020;221:106571.
[42] Amorim EAM, Torres CAA, Amorim LS, Fonseca JF, Bruschi JH, Guimarães JD, Cecon PR, Alves NG. Follicular dynamics of lactating Toggenburg does treated with recombinant bovine somatotropin. Arq Bras Med Vet Zootec 2007;59:1500–8.
[43] Sharma A, Sood P. Follicular dynamics in goats. Haryana Vet 2019;58:1–7.
[44] Bartlewski PM, Beard AP, Chapman CL, Nelson ML, Palmer B, Aravindakshan J, Cook SJ, Rawlings NC. Ovarian responses in GnRH-treated anoestrous ewes: follicular and endocrine correlates with luteal outcome. Reprod Fertil Dev 2001;13:133–42.
[45] Prashad RK, Guraya SS. Effect of human chorionic gonadotropin and follicular fluid agglutinability on goat ovarian granulosa cells. Indian J Anim Sci 1987;57:1187–90.
[46] Farin CE, Moeller CL, Mayan H, Gamboni F, Sawyer HR, Niswender GD. Effect of luteinizing hormone and human chorionic gonadotropin on cell populations in the ovine corpus luteum. Biol Reprod 1988;38:413–21.
[47] Gnatek GG, Smith LD, Duby RT, Godkin JD. Maternal recognition of pregnancy in the goat: effects of conceptus removal on interestrus intervals and characterization of conceptus protein production during early pregnancy. Biol Reprod 1989;41:655–63.
[48] Arashiro EKN, Viana JHM, Fonseca JF, Camargo LSA, Fernandes CAC, Brandão FZ. Luteal dynamics in goats: morphological and endocrine features. R Bras Zootec 2010;39:1937–42.
[49] Liu X, Schwarz T, Murawski M, Tayade C, Kridli R, Granados AMP, Sharma C, Bartlewski PM. Measurements of circulating progesterone and estrone sulfate concentrations as a diagnostic and prognostic tool in porcine pregnancy revisited. Domest Anim Endocrinol 2020;71:106402.
[50] Leroy JLMR, Opsomer G, Van Soom A, Goovaerts IGF, Bols PEJ. Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? Part I. The importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. Reprod Domest Anim 2008;43:612–22.
[51] Beard AP, Hunter MG. Effects of bovine follicular fluid and exogenous oestradiol on the GnRH-induced short luteal phase in anoestrous ewes. J Reprod Fertil 1994a;100:211–7.
[52] Beard AP, Hunter MG. Effects of progesterone pretreatment on the oxytocin receptor concentration and the response to oxytocin during the stimulated early luteal phase in ovariectomized Ewe. J Reprod Fertil 1994b;102:57–63.
[53] Beard AP, Lamming GE. Oestradiol concentration and the development of the uterine oxytocin receptor and oxytocin induced PgF2α release in ewes. J Reprod Fertil 1994;100:469–75.
[54] Souza-Fabjan JMG, Rosa RM, Balaro MFA, Pinto PHN, Santos GB, Arashiro EKJ, Fonseca JF, Ungerfeld R, Brandão FZ. Effect of different hormonal combinations on follicular wave emergence and superovulatory response in sheep. Theriogenology 2017;103:24–9.
[55] Newcombe JR, Peters AR. The buserelin enigma; how does treatment with this GnRH analogue decrease embryo mortality? J Vet Sci Technol 2014;5:151.
[56] Keay SD, Vatish M, Karteris E, Hillhouse EW, Randeva HS. The role of hCG in reproductive medicine. BJOG 2004;111:1218–28.
[57] Makrigiannakis A, Vrekoussis T, Zoumakis E, Kalantaridou S, Jeschke U. The Role of hCG in Implantation: a mini-review of molecular and clinical evidence. Int J Mol Sci 2017;18:1305.
[58] Patsoula E, Loutradis D, Drakakis P, Michalas L, Bletsa R, Michalas S. Messenger RNA expression for the follicle-stimulating hormone receptor and luteinizing hormone receptor in human oocytes and preimplantation-stage embryos. Fertil Steril 2003;79:1187–93.
[59] Coleson MPT, Sanchez NS, Ashley AK, Ross TT, Ashley RL. Human chorionic gonadotropin increases serum progesterone, number of corpora lutea and angiogenic factors in pregnant sheep. Reproduction 2015;150:43–52.
[60] Nephew KP, Cárdenas H, McClure KE, Ott TL, Bazer FW, Pope WF. Effects of administration oh human chorionic gonadotropin or progesterone before maternal recognition of pregnancy on blastocyst development and pregnancy in sheep. J Anim Sci 1994;72:453–8.