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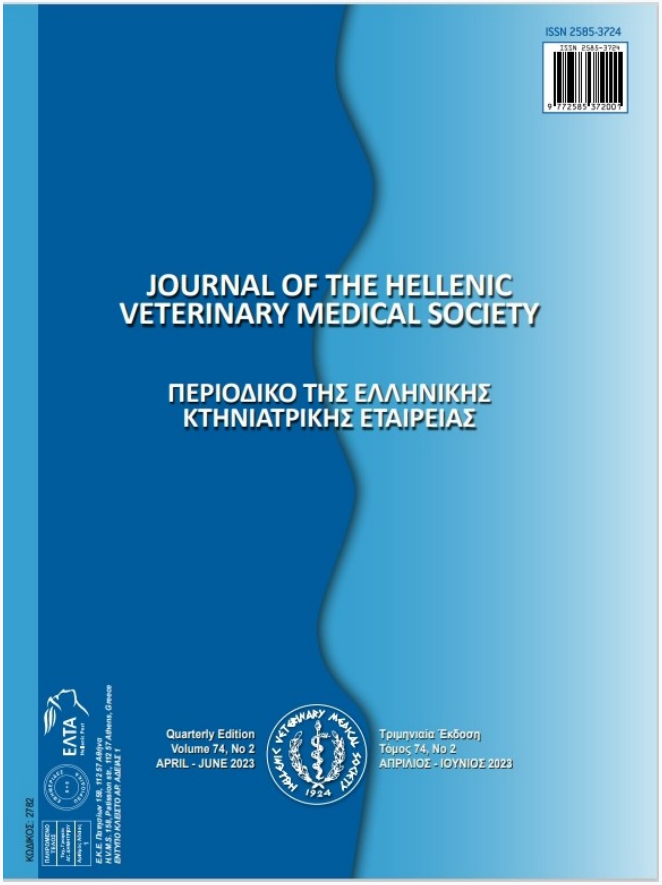

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Effect of hCG administration on ovulation and estrus in Saanen goats subjected to short-term estrus synchronization protocol during the breeding season

I. Dogan[1*], M. Berk Toker[1], A. Aktar[1], M.M. Yilmaz[1], D. Udum[2]

[1]Uludag University, Veterinary Faculty, Department of Reproduction and Artificial Insemination, Bursa, Turkey

[2]Uludag University, Veterinary Faculty, Department of Biochemistry, Bursa, Turkey

ABSTRACT: This study aimed to compare the effect of hCG administered at 24 or 36 h following a short-term estrus synchronization treatment on the ovulation time and estrus parameters in non-lactating Saanen goats during the breeding season. The estrus cycles of does were synchronized with an intravaginal sponge containing 60 mg of medroxyprogesterone acetate (MAP) for six days, and an injection of 125 μg of d-cloprostenol at the time of sponge insertion in addition to an injection of 300 IU of eCG 24h before sponge removal.After removal of the sponges, does were injected intramuscularly either 1 ml physiological saline(0.9% NaCl) solution after 12 h ($Group_1$/Control; *n*=10), 100 IU hCG after 24 h ($Group_2$; *n*=9) or 100 IU hCG after 36 h ($Group_3$; *n*=9). Estrus behavior after sponge removal was observed twice daily for 84 h using teaser bucks and transrectal ovarian ultrasonography was performed twice a day for seven days to determine small, medium and large follicle numbers, luteal development and the time of ovulation.Blood samples were collected on the same days to determine serum progesterone (P4) and estradiol (E2) concentrations. No significant differences were observed in terms of estrus parameters, ovarian structure and serum P4 and E2 concentrations between the hCG-treated groups and the control group.Average values observed for all groups: estrus response (53.57%), the interval from sponge removal to estrus and ovulation (35.2 h and 67.86 h, respectively), duration of estrus (18.4 h), the interval from estrus onset to ovulation (50.37 h), ovulation rate (96.43%), number of ovulations (1.36), ovulatory follicle diameter (6.86 mm), corpus luteum diameter (8.22 mm), follicle and luteal growth rate (1.17 and 0.68 mm/day, respectively). In conclusion, administration of hCG at 24 or 36 h following a short-term estrus synchronization protocol does not affect ovulation time, estrus parameters, and serum P4 and E2 concentrations in goats during the breeding season.

Keywords:Estradiol, Goat, hCG, Progesterone, Ovulation

Corresponding Author:
Ibrahim Dogan
Uludag University, Veterinary Faculty, Department of Reproduction and Artificial Insemination 16059, Gorukle/Bursa Turkey
E-mail address: idogan@uludag.edu.tr

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INTRODUCTION

Goat breeds generally show seasonal breeding behavior depending on latitude, photoperiod and other environmental factors (Fatet et al., 2011; Hameed et al., 2020).This physiological condition limits the production of goat products such as milk and meat throughout the year.For this reason, it is necessary to control the reproductive activities of goats to meet the increasing consumer demand for these products every year.Estrus synchronization using different hormones in goats is an essential tool for controlling reproduction throughout the year (Fatet et al., 2011; Dogan et al., 2016). Intravaginal devices containing progesterone (P4) or synthetic analogs such as medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA) are generally the most widely used exogenous hormones for the synchronization of estrus and ovulation in goats due to their ease of use and availability for farmers (Rubianesand Menchaca, 2003; Dogan et al., 2008a; Abecia et al., 2012; Dogan et al., 2020b;Gonzalez-Bulneset al., 2020).Progestogen-containing intravaginal devices have previously been used for short (5-7 days) or long (12-14 days) periods to induce or synchronize estrus in goats (Dogan et al., 2008a, 2016; Abecia et al., 2012). However,recently short-term administration of progestogens has been preferred as long-term administration of these hormones has been associated with reduced fertility in goats (Rubianes and Menchaca, 2003; Zarazaga et al., 2014).Additionally, synchronization protocols include the administration of a single intramuscular dose of equine chorionic gonadotropin (eCG) at the end of the progestogen treatment to increase both estrus response and ovulation rate in small ruminants (Rubianes and Menchaca, 2003; Abecia et al., 2012). Equine chorionic gonadotropin (eCG) is an essential component of synchronization protocols (Dogan et al., 2008a; Gonzalez-Bulnes et al., 2020), but its repeated administration has been shown to reduce fertility in goats (Sun et al., 2019).Also, since eCG is a hormone derived from pregnant mares, its production may be restricted in the future due to ethical concerns regarding animal welfare (Gonzalez-Bulnes et al., 2020). The conventional P4/progestogen-based protocols combined with eCG have variable fertility rates in small ruminants due to their inability to fully control the time between ovulation and artificial insemination (Wildeus, 2000;Abecia et al., 2012;Gonzalez-Bulneset al., 2020).To overcome this situation, two approaches have recently been proposed by researchers. These approaches rely on the high similarity between human chorionic gonadotropin (hCG) and luteinizing hormone (LH) and their binding to the same LH receptor (Saleh et al., 2012).The first of these approaches has been focused on providing ovulation by inducing the time and concentration of the preovulatory LH surge with hCG administration during the estrus period induced in goats (González-Álvarez et al., 2016; Alvarado-Espino et al., 2016, 2019a, 2019b). The second approach focuses on promoting luteal activity by administering exogenous hCG hormone five days after natural mating,but these treatments did not affect pregnancy rates (Fonseca and Torres, 2005; Fonseca et al., 2006). However, the potential impact of hCG on subsequent ovulation and preovulatory follicle development after the use of a short-term estrus synchronization protocol during the breeding season in goats has not yet been investigated.The objective of the present study was to compare the effect of single-dose hCG administered 24 or 36 h following a short-term estrus synchronization protocol on the ovulatory response, estrus parameters and serum P4 and E_2 concentrationsin non-lactating Saanen goats, during the breeding season.

MATERIALS AND METHODS

Animal management

The study was carried out at the Research and Application Farm of the Veterinary Faculty at Uludag University, located in Bursa (latitude 40° 11`N, longitude 29° 04` E, altitude 155 m), Turkey. A total of 28 clinically healthy, free of reproductive disorders and non-lactating multiparous Saanen does were used during the breeding season in the region. The does were kept indoors in sand/hay-floored pens with access to outdoors in the sheltered paddock under conditions of natural photoperiod and temperature and were fed dry grain wheat hay (1500 g/doe/day) supplemented with commercial pellets(18% crude protein; 800 g/doe/day, 2800 Kcal). No extra food was offered to the goats during the study. Goats were provided *ad libitum* with clean drinking water and mineralized salt,and no changes were made during the experiment. Does and bucks were kept in the same pen, but their physical contact was prevented.All of the methods and management procedures in this study were evaluated and approved by theAnimal Experiments Local Ethics Committee of the Uludag University (approval reference number: B.30.2.ULU.08Z.00.00/41).

Estrus synchronization and hCG treatment

Estrus was synchronized in all goats by the in-

sertion of an intravaginal sponge impregnated with 60 mg medroxyprogesterone acetate (MAP, Esponjavet,Hipra, Spain) for 6 days, coupled with intramuscular injection of 125 μg d-cloprostenol (PGS, Alke, Turkey) at sponge insertion and intramuscular injection of 300 IUeCG (Oviser, Hipra, Spain) 24 h before sponge removal.The removal day of intravaginal sponges was considered as the beginning of the study. Thereafter, all does were divided into three groups according to their age, body weight, and body condition score (scale 0 to 5, according to the model proposed by Morand-Fehr et al, (1989). In groups 1 to 3, the age averaged 38.28±7.95, 31.10±3.76, and 32.42±9.07 months, body weights averaged 45.80±2.12, 43.51±2.27, and 44.69±2.07 kg, and the body condition score averaged2.85±0.15, 2.83±0.17, and 2.67±0.17, respectively; these parameters were not statistically different among the groups. Goats in $Group_1$ (control; n=10) received an intramuscular injection of 1 ml physiological saline solution (0.9% NaCI) 12 h after sponge removal. The rest of the goats received an intramuscular injection of 100 IU human chorionic gonadotrophin (hCG, Chorulon, MSD, Netherlands) 24h ($Group_2$; n=9) or 36h ($Group_3$; n=9) after sponge removal.Estrus behavior was monitored twice a day (8 am-8pm) for 15 min from 12 to 84 h with an aproned buck following sponge withdrawal. Does showing estrus were then taken to a separate section. Estrus onset was defined as the time when the doe first stood to be mounted by the teaser buck. The estrus response rate was calculated by considering the number of does in estrus up to 84h/number of treated does x 100. Estrus duration was defined as the time elapsed between the first and last acceptance of mounting by the teaser buck within the observation period.

Ovarian ultrasonography examination

Examination of the ovaries in all does was conducted with a B-mode transrectal ultrasonographic scanner (RTU, Prosound 2, Hitachi Aloka Medical, Ltd., Tokyo, Japan), equipped with a 7.5 MHz linear array transducer (model UST-660, 7.5-MHz transrectal probe, depth of 4 cm). During the ultrasonic measurement, the goat was placed in a standing position on a raised narrow wooden platform, after which the hydro-soluble contact gel applied transducer was gently guided into the rectum.Briefly, after imaging the urinary bladder and the uterus horns on the monitor, the transducer was rotated 45°-90°clockwise or counterclockwise to observe both ovaries (Ginther and Kot, 1994).The diameters of all follicles ≥2 mm and corpora lutea(CL) in both ovaries were recorded by the same operator once daily (9 am) for 7 days after the sponge removal in all goats. After freezing the image of each ovary on the screen,the measurement was made using the built-in electronic caliper system and each ovary daily schematic map was drawn on a sheet of paper and compared to the previous day. Ovarian data were then combined for both ovaries of each doe. Follicles were classified daily as small (2.0-3.4 mm), medium (3.5-4.9 mm) and large (≥5.0 mm).Ovulation assessments were performed every 12 hours (9am-9 pm) after sponge removal until ovulation was confirmed in all goats.The day of ovulation was defined by the disappearance or collapse of follicles greater than 5 mm in diameter between two consecutive ultrasound examinations (Dogan et al., 2020a) and was considered as the first day (0 day) of the estrus cycle and was followed by the development of a corpus luteum in the ovulation area of the ovary. The last ultrasonic measurement of the preovulatory follicle before its disappearance was considered as the ovulatory follicle diameter.The growth rate (mm/day) of an ovulatory follicle was calculated by dividing the number of days between its appearance as a follicle larger than 3 mm in diameter and its maximum diameter (Dogan et al., 2020a).The number of ovulations per doe was determined by the number of CL on the ovaries according to their oval or round conformation and counted at day 7after sponge removal (Orita et al., 2000).The interval from sponge removal to ovulation, the interval from onset of estrus to ovulation, ovulation rate (the number of ovulated does/total goatsx100) and the number of ovulations per doe were also determined. The growth rate (mm/day) of luteal tissue,considered as the corpus luteum, was calculated by dividing the number of days its diameters measured between the last day of study and after ovulation and measured together with the area of the central cavity (Simões et al., 2007).

Hormonal analysis

Blood samples (8 ml) were collected from all goats by jugular venipuncture into vacuum blood tubes (Ref. Hp. 0013, Hema& Lab. Ankara, Turkey) once daily for 7 days after sponge removal. The tubes were immediately placed on a nice pack, transported to the laboratory, and then centrifuged at 4 °C for 10 min at1500x g. After centrifugation, serum was transferred into 1.5 ml micro-tubes and stored at -20° Cuntilas sayed for progesterone (P4) and estradiol

(E2).Concentrations of P4 (SRB-T-86624) and E2 (SRB-T-87401) in the blood serum of a goat were determined with a commercial ELISA kit and results were read by the ELISA reader (ELX-808IU Ultra Microplate Reader) according to the manufacturer's instructions.Sensitivities of the P4 and E2 assay were 0.048 ng/ml and 0.925 pg/ml, respectively.The mean intra- and inter-assay coefficients of variation were <10% and<12% for P4 and E2, respectively.

Statistical analysis

The data of the study were statistically analyzed by SPSS for Windows, version 20. The distribution of data for every parameter was determined by the Shapiro-Wilk test and an appropriate statistical method was chosen to analyze the parameter considered. The data that were distributed normally were analyzed by ANOVA test followed with the Tukey as post-hoc test. Non-normally distributed data were analyzed by the Kruskal-Wallis test followed by Mann-Whitney U test to spot the differences between the study groups. The results were given as mean ± SEM and differences were considered significant when the P value was below 0,05

RESULTS

Estrus behavior and ovarian structures

A summary of data regarding the estrus behavior and ovarian findings after the synchronized estrus in the goats are shown in Table 1.There was no significant difference between the control and the experimental groups in terms of estrus behaviors and transrectal ovarian ultrasonography findings. Therefore, the data in the study were pooled and analyzed then in relation to time only. The overall estrus response rate within 84 h was 53.57%.

Serum P4 and E2 profile

The blood serum concentrations of P4 and E2 for 7 days after sponge removal for hCG-treated and control goats are shown in Table 2, 3 and Figure 1, 2. Although, serum P4 and E2 concentrations did not differ statistically between all groups for seven days after sponge removal (P>0.05), mean P4 and E2 serum concentrations were found to differ between days (P<0.05). Therefore, the data from P4 and E2 serum concentrations of goats treated with saline and hCG were pooled and combined. After sponge removal, the lowest (0.41±0.30 ng/ml) and the highest (0.97±0.51 ng/ml) mean serum P4 concentration was detected on days 2 and 7, respectively (P<0.05) (Table 2, Fig. 1). The lowest (10.45±3.30pg/ml) and highest (15.13±5.07pg/ml) mean serum E2 concentrations were observed on days 0 and 2 after the sponge removal, respectively P<0.05) (Table 3, Fig. 2).

Table 1. Estrus behaviors and ovarian findings of Saanen goats treated with MAP sponge for 6 days coupled with d-cloprostenol at sponge insertion andeCG 24 h before sponge removal and administration of hCG 36 ($Group_3$) or 24 ($Group_2$) and saline 12 h ($Group_1$) after sponge removal (means±SEM). No differencedetected between groups (p>0.05).

Data	Hormonal groups			Mean
	$Group_1$ (n = 10)	$Group_2$ (n = 9)	$Group_3$ (n = 9)	
Reproductive behavior				
Estrus response (%)	60.0 (6/10)	44.44 (4/9)	55.56 (5/9)	53.57 (15/28)
Duration of estrus (h)	18.00 ± 6.57	18.00 ± 6.93	19.20 ± 6.57	18.40 ± 6.20
Interval from sponge removal to onset of estrus (h)	34.00 ± 11.80	33.00 ±11.49	38.40 ± 13.15	35.20 ± 11.53
Occurrence of ovulation from sponge removal (h)	66.20 ±13.93	64.89 ±10.73	72,67 ± 7,00	67.86 ± 11.18
Estrus onset to ovulation (h)	45.33 ± 23.00	53.11 ± 19.55	52,67 ± 24,76	50.37 ± 21.96
Ultrasonography evaluation				
Ovulation rate (%)	100.0 (10/10)	88.89 (8/9)	88,89 (8/9)	96.43 (27/28)
Mean number of ovulations per doe	1.40 ± 0.52	1.44 ± 0.53	1,22 ± 0,44	1.36 ± 0.49
Ovulatory follicle diameter (mm)	7.04 ± 0.83	6.77 ± 0.85	6,77 ± 0,90	6.86 ± 0.84
Follicular growth (mm/day)	1.19 ± 0.55	1.20 ± 0.48	1,12 ± 0,36	1.17 ±0.46
Large follicle (n)	5.10 ± 4.09	6.00 ± 2.26	4,50 ± 2,25	5.20 ± 3.03
Medium follicle (n)	5.90 ± 2.81	5.90 ± 2.38	7,50 ± 2,37	6.43 ± 2.56
Small follicle (n)	17.70 ± 6.77	18.70 ± 6.62	18,20 ± 5,27	18.20 ± 6.04
Corpus luteum diameter (mm)	8.62 ± 1.73	8.52 ± 1.25	7,41 ± 0,79	8.22 ± 1.39
Luteal growth (mm/day)	0.86 ± 0.57	0.78 ± 0.37	0,37 ± 0,23	0.68 ± 0.45

Table 2. The effect of at 24 and 36 h administration of hCG on the mean (±SEM) of serum P4 concentrations (ng/ml) during after sponge removal in Saanen goats.No difference was detected between groups on the same days (p>0.05).

Days post sponge removal	Hormonal groups			Mean
	$Group_1$ (n = 10)	$Group_2$ (n = 9)	$Group_3$ (n = 9)	
1	0.56 ± 0.39	0.47 ± 0.26	0.45 ± 0.31	0.50 ± 0.32^{ac}
2	0.45 ± 0.38	0.45 ± 0.22	0.34 ± 0.27	0.41 ± 0.30^{a}
3	0.45 ± 0.35	0.41 ± 0.44	0.52 ± 0.26	0.46 ± 0.35^{ad}
4	0.67 ± 0.41	0.74 ± 0.50	0.64 ± 0.34	0.68 ± 0.41^{c}
5	0.64 ± 0.41	0.63 ± 0.35	0.39 ± 0.40	0.55 ± 0.39^{ac}
6	0.48 ± 0.41	0.60 ± 0.41	0.79 ± 0.43	0.62 ± 0.42^{ac}
7	0.79 ± 0 .53	1.07 ± 0.51	1.06 ± 0.47	0.97 ± 0.51^{b}

$^{a, b, c, d}$ Days between row with different superscripts differ significantly (p<0.05)

Table 3. The effect of at 24 and 36 h administration of hCG on the mean (±SEM) of serum E2 concentrations (pg/ml) during after sponge removal in Saanen goats. No difference was detected between groups on the same days (p>0.05).

Days post sponge removal	Hormonal groups			Mean
	$Group_1$ (n = 10)	$Group_2$ (n = 9)	$Group_3$ (n = 9)	
1	13.15 ± 4.65	11.00 ± 3.40	13.54 ± 3.92	10.45 ± 3.30^{a}
2	9.92 ± 3.30	12.31 ± 3.23	12.11 ± 5.06	15.13 ± 5.07^{bce}
3	9.93 ± 3.01	11.52 ± 1.91	11.39 ± 3.63	12.81 ± 3.84^{bc}
4	15.69 ± 6.63	14.37 ± 6.57	15.62 ± 4.01	12.95 ± 4.15^{bce}
5	14.55 ± 4.57	12.67 ± 2.71	13.46 ± 2.42	14.53 ± 3.45^{e}
6	14.57 ± 3.68	14.93 ± 3.39	14.15 ± 2.56	13.41 ± 4.60^{bce}
7	14.36 ± 3.38	16.09 ± 2.79	14.29 ± 3.13	13.88 ± 2.63^{bce}

$^{a, b, c, d, e}$ Days between row with different superscripts differ significantly (p<0.05)

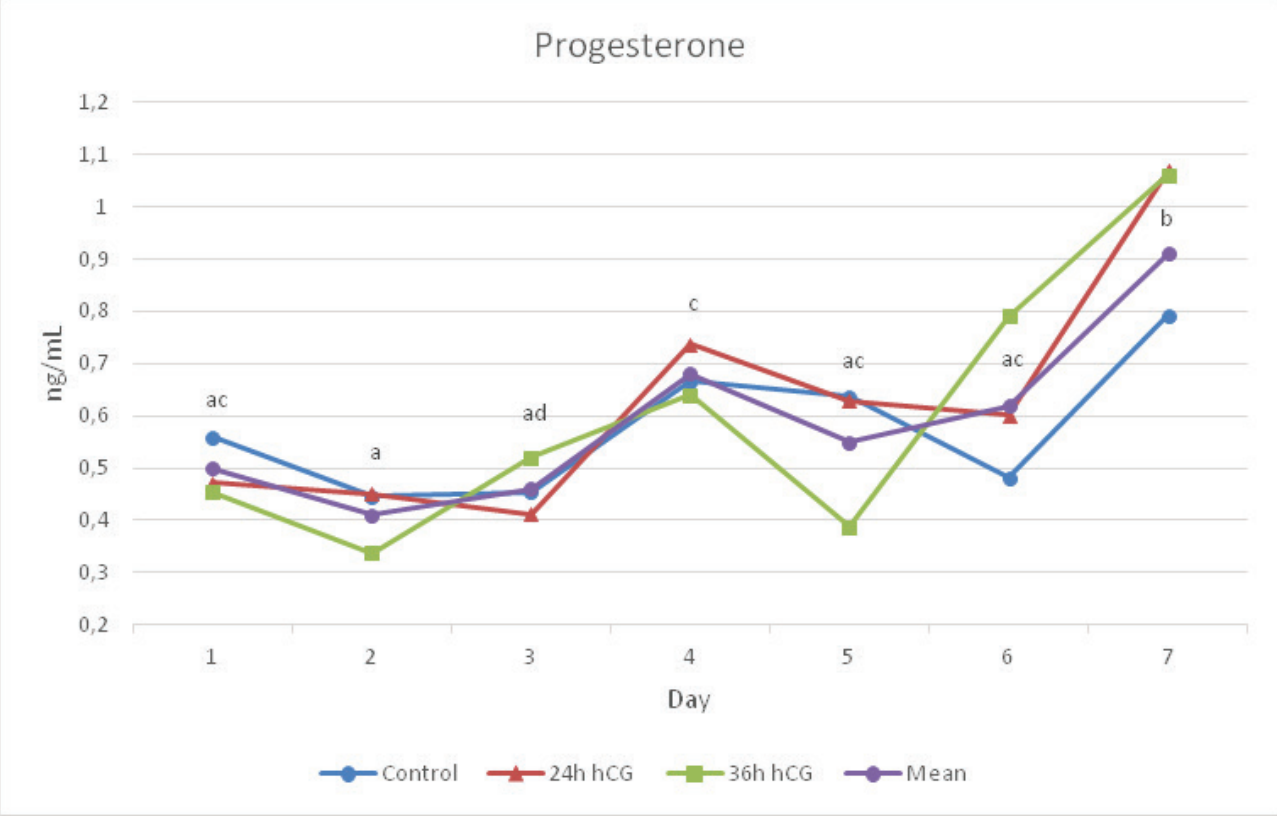

Fig. 1. Mean serum P4 concentrations in Saanen goats receiving 6-day short-term synchronization treatment with saline ($Group_{1\text{-Control}}$) or hCG treatment at 24 ($Group_2$) or 36 h ($Group_3$) (means ± SEM). $^{a, b, c, d}$ Mean values between days with different superscripts differ significantly (p<0.05)

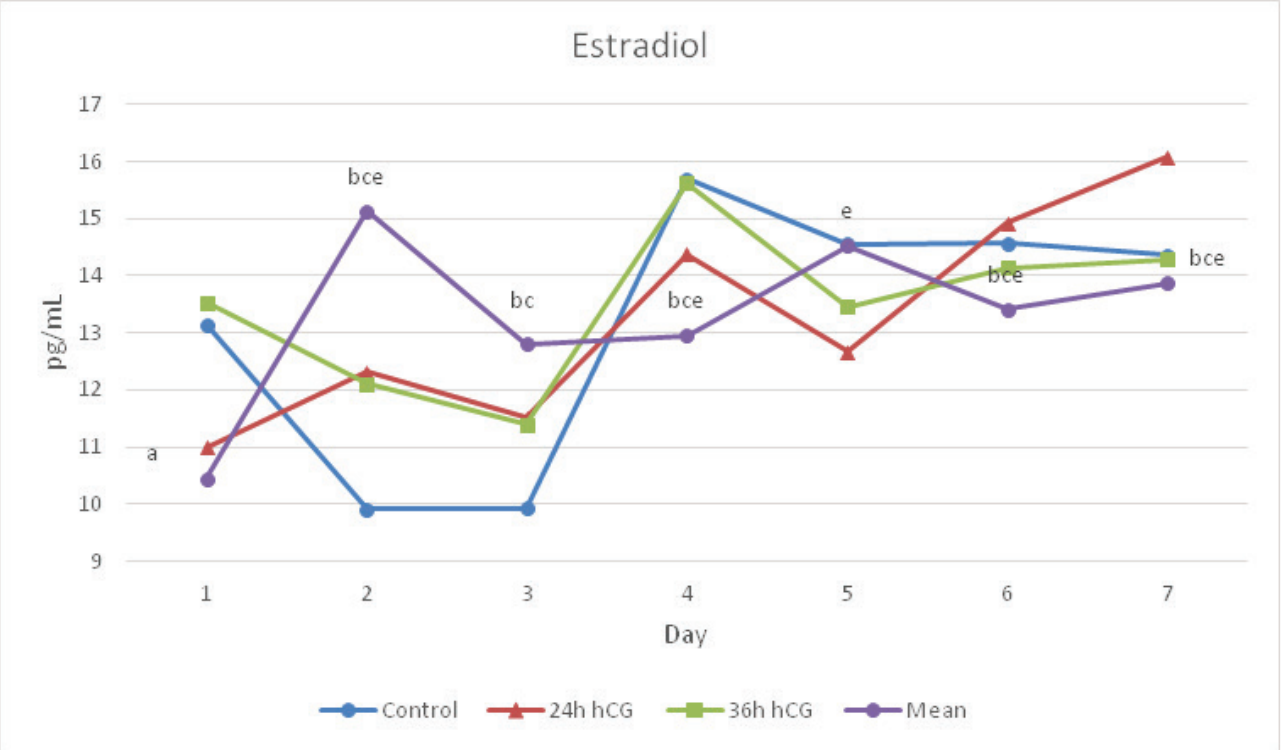

Fig. 2. Mean serum E2 concentrations in Saanen goats receiving 6-day short-term synchronization treatment with saline ($Group_{1\text{-}Control}$) or hCG treatment at 24 ($Group_2$) or 36 h ($Group_3$) (means ± SEM). [a, b, c, d] Mean values between days with different superscripts differ significantly ($p<0.05$)

DISCUSSION

The use of eCG and $PGF_{2\alpha}$along with MAP intravaginal sponge for 6-days, regardless of the hCG treatment, was found to be an efficient protocol for estrus synchronization in the non-lactating Saanen goats during the breeding season in our study. The effectiveness of the protocol was designed such that progestogen inhibits the formation of new *corpora lutea*, while $PGF_{2\alpha}$ treatment on day 0 provides luteolysis and then eCG treatment on day 5 improves follicle development and, the estrus response rate increases as a result (Wildeus, 2000; Abeciaet al., 2012; Fonsecaet al., 2017a).Furthermore, the induction of high P4 concentrations by insertion of progesterone-containing devices into the vagina in goats causes a follicular turnover and the emergence of a new follicular wave within 3 days of insertion of the devices (Rubianes and Menchaca, 2003; Vilariño et al., 2011). In this study, the administration of 100 IU hCG 36 or 24 h after sponge withdrawal compared with the control group had no significant effect on estrus behavior, ovarian findings and serum P4 and E2 concentrations. In small ruminants, estrus response following intravaginal P4 or its analogs during the breeding season is normally defined as acceptable when 90% or more of the treated goats come into estrus within 120 h (Wildeus, 2000;Rubianes and Menchaca, 2003). In the present study, the estrus response was on average 53.57% (15/28) during the 84-h observation period following the cessation of treatment (Table 1). This result was found to be lower than the range of 67.0-100% reported by other researchers using the 6-day short-term progestagen protocol (Dogan et al., 2008b, 2016, 2020b; Pietroskiet al., 2013;Fonseca et al., 2017a, 2017b), orP4-hCG protocol (Alvarado-Espino et al., 2016,2019a, 2019b; González-Álvarezet al., 2016; Rodríguez-Martínez et al., 2018) in different or same breed goats and seasons. Besides, our result is in line with Alvarado-Espino et al. (2016), who reported that administration of 50, 100, and 300 IU hCG 24 h after injection of 20 mg progesterone did not show a statistical difference in the estrus rates in Alpine goats during anestrus to the estrus transition period.The estrus response in small ruminants can vary greatly when intravaginal sponges are administered, depending on factors such as species, breed, co-treatment, season, management, use of gonadotropins and mating system (Wildeus, 2000).Moreover, this difference between current and previous studies may be due to both the difference in feed consumption among goats

and the energy balance of the diet and as a result, this situation may affect ovarian and estrus behaviors.Furthermore,another study has shown that insufficient energy intake in the diet causes delay and suppression of estrus following synchronization in goats (Mani et al., 1996).

In the current study, the interval time from sponge removal to estrus and the duration of estrus were on average 35.2 h and 18.4 h, respectively (Table 1). The time interval from sponge removal to estrus in small ruminants is an important parameter in the application of timed artificial insemination and the development of new protocols (Fonseca et al., 2017b).After using a short-term progestogen-based protocol for 6 days, this interval was shorter than 45.6 h obtained in Saanen goats (Fonseca et al., 2017a) and greater than the 24.3 h (Dogan et al., 2008b), 26.7 h (Pietroski et al., 2013) and 27.4 h (Dogan et al., 2020b) interval observed in the same breed.In a previous study, Alvarado-Espino et al. (2016), using 50, 100, and 300 IU of hCG 24 h after P4 injection in Alpine goats, reported an interval of 60, 54, and 76 h for the onset of estrus, respectively.Similarly, in these previous studies using the hCG-P4 protocol, the time interval to estrus in different goat breeds during the non-breeding season varied between 52.0 h and 65.0 h (González-Álvarez et al., 2016;Rodríguez-Martínezet al., 2018;Alvarado-Espino et al., 2019a,2019b).This variability can be attributed to the use of gonadotropins such as eCG or hCG, the different seasons and the estrus induction protocol. Gonadotropins such as FSH and LH are not required in the early stages of follicular development,but the later stages of follicular development are dependent on gonadotropins (Hunter et al., 2004). The primary follicles or small follicles are responsive to eCG, but not to hCG, whereas middle and large follicles are dependent on both hCG and eCG for their growth (Fatet et al., 2011).Considering the high similarity between eCG and FSH, the medium or large follicles are affected by eCG administration 24h before or at the time of sponge removal, increasing their size while increasing estrogen concentration and LH surge (Abecia et al., 2012), eventually this endocrine response shortens the interval to estrus (Dogan et al., 2008a). Although many factors such as lactation, breed, parity, and season are effective, the dose of eCG affects the time interval to estrus after sponge removal (Fatet et al., 2011).The dose of eCG varies from 300 to 600 IU (Wildeus, 2000), but eCG doses in excess of 400 IU are not recommended for dairy goats as they may result in a lower pregnancy rate or embryo death, yet, the use of exogenous eCG is necessary for induction of estrus in goats during anestrus period (Fonseca et al., 2017b; Gonzalez-Bulnes et al., 2020).

In previous studies using the same protocol as in this study, the duration of estrus varied between 22.3 and34.4 h in the same or different goat breeds (Dogan et al., 2008b, 2016, 2020b; Pietroski et al., 2013). While this duration is approximately 36 h in cyclic dairy goats,it varies between 24 and 58 h depending on many factors such as breed, age, lactating, season, and the presence of a buck (Fatet et al., 2011; Dogan et al., 2016). Similar to the present study, Alvarado-Espino et al. (2016) reported no significant difference in estrus duration between goats treated with 50, 100, and 300-hCG 24 h after P4 administration.On the other hand, other researchers using the hCG-P4 protocol reported that the duration of estrus varied between 29 to 39 h (González-Álvarez et al., 2016;Alvarado-Espino et al.,2016;Rodríguez-Martínezet al., 2018).

In the current study, the intervals from the removal of sponge and the onset of estrus to ovulation were on average 67.86 h and 50.37 h, respectively (Table 1).In previous studies, although the interval from sponge removal to ovulation was similar, the interval from the onset of estrus to ovulation was shorter than in our study (Fonseca et al., 2017a, 2017b).Moreover,our findings were longer than those reported by Dogan et al. (2020b) were found interval from the removal of sponge (48.05 h) and the onset of estrus (21.51 h) to ovulation.

Based on the results of the ultrasonographic examination, the hCG hormone used in this trial had no effect on ovulation time among the treatment groups, but induced ovulation in goats treated with hCG that did not exhibit estrus behavior within 84 h.This may also be due to the fact that the study was carried out during the breeding season.On the other hand, a single injection of hCG has been shown to induce ovulation and CL formation by directly stimulating sexual activity in acyclic goats during the non-breeding season or transition period (Alvarado-Espino et al., 2016, 2019a, 2019b;Rodríguez-Martínezet al., 2018). Besides,it was reported that the frequency of LH pulse release in goats is less in the anestrus period compared to the natural breeding season (Chemineau et al., 1988).The hormone hCG is a glycoprotein hormone that has a similar structure to LH, binds to the same receptor, and has a longer half-life and rapid absorption than LH (39 h vs 11.6 h, respectively) (Saleh

et al., 2012).In addition, hCG is one of the most potent stimulators of final oocyte maturation due to its high LH similarity (González-Álvarez et al., 2016).

In this study, the percentage of goats ovulating and the number of ovulations (number of CL) per doe were on average 96.43% (27/28) and 1.36, respectively (Table 1),almost similar to those reported in previous studies using the 6-day the short-term progestagen protocol (Pietroski et al., 2013; Fonseca et al., 2017a, 2017b; Dogan et al., 2020b) or a P4 injection plus hCG-based protocol (Alvarado-Espino et al., 2016, 2019a, 2019b; González-Álvarezet al., 2016; Rodríguez-Martínez et al., 2018).As a result, hCG administration in the estrus period has triggered ovulation by directly stimulating the ovarian follicles in the advanced stages of development, while indirectly promoting CL formation after ovulation in acyclic goats (Alvarado-Espino et al., 2016, 2019a, 2019b; Rodríguez-Martínez et al., 2018).

In our study,although there was no difference in mean serum P4 and E2 concentrations between all groups for 7 days after sponge removal, a difference was observed between overall means and days (Tab. 2, 3 and Fig. 1, 2). These results are consistent with the results obtained in goats (Orita et al., 2000; Simões et al., 2007; Dogan et al., 2020a).

In addition, the mean diameter of ovulatory follicle(6.86 mm) and follicular growth rate (1.17 mm/day) in our study were similar to those reported in previous studies conducted in different seasons in other (Fonseca et al., 2017b) or in the same goat breeds (Fonseca et al., 2017a; Dogan et al., 2020a, 2020b), using the same protocol. On the contrary, the ovulatory follicle diameter obtained in the present trial was lower than that reported by Rodríguez-Martínez et al.(2018) and Alvarado-Espino et al. (2019a, 2019b) using hCG combined with P4. This difference may be due to different measurement techniques.

Considering the number of follicles of different sizes as observed by transrectal ovarian ultrasonography after sponge withdrawal (Table 1), while there was the highest number of small follicles among the groups, no difference was observed among the experimental groups in terms of the average number of ovarian follicles of different sizes.As seen in the groups treated with hCG in our study, administration of hCG within the estrus period may show that it only induces ovulation due to its similarity with LH, but it has no effect on the number of follicles of different sizes.Our results are in line with Alvarado-Espino et al., (2016; 2019b), González-Álvarez et al.(2016), and Rodríguez-Martínez et al.,(2018). These follicles, which are outside the gonadotropin-dependent follicles, are a dynamic pool of continuously developing antral follicles, one or more of which might grow to a pre-ovulatory size and ovulate, while other follicles regress (Ginther and Kot, 1994;Oritaet al., 2000; Hunter et al., 2004). Subsequently, other follicles under the influence of gonadotropins undergo structural and functional atresia at various stages of development (Rubianes and Menchaca, 2003; Hunter et. al., 2004).

The mean diameter of CL (8.22 mm) and the daily growth rate of luteal tissue (0.68 mm/day) observed per day after ovulation were similar between the control and hCG-treated groups (Table 1). Our findings agree with the finding of another study where no differences were found in CL diameter between multiparous and nulliparous goats treated with hCG (Alvarado-Espino et al., 2019a). In our study, CL was detected on the first day after ovulation by transrectal ultrasonography, similar to the results obtained in Shiba (Orita et al., 2000) and Serrana (Simões et al., 2007) goats. Similarly, although the first detection of the CL by transrectal ultrasonography was approximately 3 days after ovulation, the period was reported to range from 1 to 6 days (Orita et al., 2000; Simões et al., 2007). The mean CL diameter detected in our study was higher than that observed in Shiba goats (6.1 mm on day 3) by Orita et. al. (2000) and in Serrana goats (7.1 mm on day 2) by Simões et al.(2007). This difference may be due to the measuring technique and different goat breeds.On the other hand, the mean growth rate of CL obtained in this study was similar to the results obtained by Simões et al.(2007), who recorded approximate growth rate of 0.7 mm/day.

CONCLUSIONS

This study confirms that short-term progestogen protocol is successful for synchronizing the estrus and ovulation non-lactating goats during the breeding season. A single of injection 100 IU hCG 24 or 36 h after removal of sponge had no effect on estrus behavior, ovarian findings and serum P4 and E2 concentrations. Yet, further studies eliciting the endocrine response should be designed to better define or explain the practical applicability of hCG.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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