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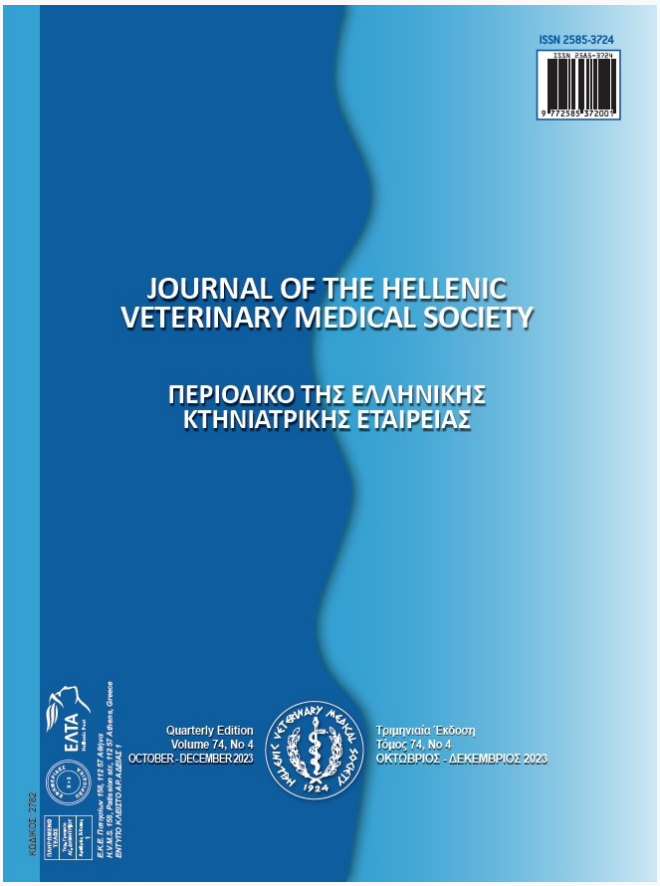

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Research article
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Comparison of the effect of eCG and hCG treatment on the fertility in Saanen goats during the breeding season

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ABSTRACT: This study aimed to compare the effect of hCG or eCG administered at the end of a short-term estrus synchronization treatment on the estrus parameters and pregnancy rates 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 five days and an injection of 125 µg of D-cloprostenol at the time of sponge insertion. Does were injected intramuscularly either 1 ml physiological saline solution (Control-Group; *n*=16), 400 IU hCG (hCG-Group; *n*=14) or 400 IU eCG (eCG-Group; *n*=14) at the time of sponge removal. The estrus behavior was observed using teaser bucks and the ovulation time was monitored using transrectal ultra sonography twice daily for 96 h after the sponge removal. Does in estrus were allowed a single copulation using fertile bucks. Blood samples were collected on the same days to determine serum progesterone (P4) and estradiol (E2) concentrations. No significant differences were observed between the groups in term of estrus parameters, ovarian structure and serum P4 concentrations. Although serum E2 concentration was similar between groups in the first three days. Serum E2 concentrations differed between the eCG and the other two groups on the fourth day, and between the eCG and control groups on the fifth day. As a result, it can be concluded that the effects of hCG or eCG administration on estrus behavior, ovarian findings and pregnancy rate at the time of sponge removal are similar, therefore hCG can be an alternative to eCG.

Keywords: estrus synchronization; eCG; Fertility; Goat; hCG

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INTRODUCTION

Goats are seasonally polyestrus animals that usually exhibit reproductive behavior depending on latitude, photoperiod, and other environmental factors (Fatet et al., 2011; Abecia et al., 2012). Goats are bred for the purpose of meat, milk, fiber and hide in a wide range of production systems that provide important economic inputs for many farmers (Abecia et al., 2012). On the other hand, goats bred in many regions exhibit seasonal reproductive behaviors which is seen as the main limiting factor for year-round production of goat products (Fatet et al., 2011). Therefore, in recent years, various methods have been developed to control the reproductive behavior of goats, such as the administration of exogenous hormones, light control or exposure to a male (Fatet et al., 2011; Abecia et al., 2012). Nowadays, exogenous hormone applications have been the most preferred method for farmers due to its ease of use, applicability and suitability for every shelter system. In this regard, induction or synchronization of estrus using exogenous sex hormones in goats has emerged as an important management tool to control reproductive activity throughout the year (Dogan et al., 2008a,b, 2016;Abecia et al., 2012). The reproductive management of goats all-year round mainly relies on protocols that include natural progesterone or its most common synthetic analogues, such as fluorogestone acetate (FGA) and medroxyprogesterone acetate (MAP). Currently, short-term (5-7 days) administration of progestogens is recommended, as long-term administration of these hormones is associated with low fertility and vaginitis in small ruminant (Rubianes and Menchaca, 2003; Menchaca and Rubianes, 2004; Gonzalez-Bulnes et al., 2020). In addition, administration of a single dose of equine chorionic gonadotropin (eCG), 24 h before or at the end of progestin treatment has combined with these protocols (Rubianes and Menchaca, 2003; Menchaca and Rubianes, 2004; Abecia et al., 2012; Dogan et al., 2020b). eCG is a placental glycoprotein hormone produced by syncytiotrophoblast cells of endometrial cups in pregnant mares between 40 and 120 days of gestation that has more follicle-stimulating hormone (FSH) and less luteinizing hormone (LH)-like activity but in non-equine species, binds to the same receptor within granulosa cells in the ovary, and has a longer half-life than FSH (Abecia et al., 2012; Thach et al., 2022). Thus, these biological properties of eCG have made it a key component of estrus synchronization and induction protocols, particularly because it induces greater estrus rate and ovulation, during the non-breeding season (Dogan et al., 2008a,b; Fonseca et al., 2017b; Gonzalez-Bulnes et al., 2020; Hameed et al., 2020). However, eCG has some disadvantages that limit its use in reproduction. One of the disadvantages of eCG is that it has a long half-life of 21 h in sheep and 45.6 h in cattle (Thach et al., 2022). Therefore, it leads to an increase in the number of unovulated follicles by continuously maintaining antral follicle development as well as premature regression of the corpus luteum, especially when administered at high doses in superovulation protocols (Wildeus, 2000; Menchaca and Rubianes, 2004). Another disadvantage of eCG is that it can lead to low pregnancy rates after repeated or prolonged use in small ruminants (Sun et al., 2019). Also, ethical concerns regarding animal welfare in the European Union may raise the issue of restriction or prohibition of eCG production in the future (Gonzalez-Bulnes et al., 2020). These disadvantages and variable fertility outcomes associated with eCG as well as potential setback for its use in the future have prompted researchers to consider the use of alternative gonadotropin hormones. Human chorionic gonadotropin (hCG), which has a glycoprotein structure like LH, binds to the same receptor, and has a longer half-life than LH, may be an alternative to eCG (Saleh et al., 2012). Researchers used hCG to induce estrus and ovulation at the time removal of intravaginal progesterone-loaded CIDR or 24 h after progesterone injection in ewes (Santos-Jimenez et al., 2021) and goats (Alvarado-Espino et al., 2016, 2019a,b; González-Álvarez et al., 2016; Rodríguez-Martínez et al., 2018) during the non-breeding season or anestrus-to-estrus transition period. On other hand, the potential effect on reproduction parameters of hCG administration with together a short-term estrus synchronization protocol in goats during the breeding season has been limitedly investigated. Therefore, this study aimed to compare the estrus parameters and pregnancy rates of Saanen goats treated with MAP-loaded intravaginal sponges, coupled with either saline, eCG, or hCG during the breeding season.

MATERIALS AND METHODS

Ethics approval

All procedures in this study were approved by the Animal Experiments Local Ethics Committee of the Uludag University (approval reference number: B.30.2.ULU.08Z.00.00).

Animal management

The study was conducted at the Research and Ap-

plication 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 44 clinically healthy, free of reproductive disorders, and non-lactating multiparous Saanen does were used between September and November, considered the breeding season in the region. The does were kept outdoors in a sheltered paddock under natural photoperiod and temperature conditions and were fed dry-grain wheat hay (1500 g/doe/day) supplemented with commercial pellets (18% crude protein; 800 g/doe/day, 2800 Kcal), and were not given any additional food. 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.

Estrus synchronization and hCG or eCG treatment

In all goats, estrus was synchronized by placing an intravaginal sponge impregnated with 60 mg medroxyprogesterone acetate (MAP, Esponjavet, Hipra, Spain) for 5 days, and 125 μg D-cloprostenol (PGS, Alke, Turkey) was administered intramuscularly at the time of sponge insertion. The withdrawal 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 control (saline), hCG and eCG groups, the age averaged 35.79±4.70, 35.64±4.13, and 35.38±3.84 months; body weights averaged 49.51±2.87, 48.22±2.02, and 49.29±2.08 kg; and the body condition score averaged 3.07±0.17, 3.00±0.12, and 3.12±0.10, respectively; these parameters were not statistically different among the groups. Does in the control (n=16), hCG (n=14) and eCG (n=14) groups were received one intramuscular injection of 1 ml of physiological saline solution (0.9% NaCI), 400 IU of hCG (Chorulon, MSD, Netherlands) and 400 IU of eCG (Oviser, Hipra, Spain) respectively, at the time of sponge removal. Estrus behavior was determined twice a day (at 8 a.m. and 8 p.m.) for 20 min from 12 h to 96 h by using an aproned buck following sponge withdrawal. Does showing estrus were immediately taken to a separate section and were then allowed a single mating per doe using one of the four fertile buck. The bucks were about 3 years old, sexually active, and has previously been tested for fertility. 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 96 h/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, as previously described (Dogan et al., 2008a,b).

Ultrasonography of ovaries

In all goats, examination of the ovaries was performed with a B-mode transrectal ultrasonographic scanner (Prosound 2, Hitachi Aloka Medical, Ltd., Tokyo, Japan), equipped with a 7.5 MHz linear array transducer (model UST-660). During the ultrasonographic examination, 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 (Dogan et al., 2020a). The diameters of all follicles ≥4 mm in both ovaries were recorded by the same operator twice a day (at 9 a.m. and 9 p.m.) for 96 h 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. Ovulation assessments were performed every 12 hours (at 9 a.m.-9 p.m.) 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). The last ultrasonic measurement of the preovulatory follicle before its disappearance was considered as the ovulatory follicle diameter (Simões et al., 2006).

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 corpora lutea, which were slightly more anechoic than the echogenic ovarian stroma, were sonographically detected as spherical or elliptical conformations, and the number of ovulations per doe was determined by counting the corpus luteum on the 10th day after sponge removal (Simões et al., 2006; Dogan et al., 2020a). The interval from sponge removal to

ovulation, the interval from onset of estrus to ovulation, and ovulation rate (the number of ovulated does/total goatsx100) were also determined. Number of pregnant does was determined by transrectal ultrasonography (Prosound 2, Hitachi Aloka Medical Ltd., Japan) with a 7.5-MHz probe 35 day after mating and the pregnancy rate (does pregnant/does mated) was calculated for each group.

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 5 days after sponge removal. The tubes were immediately placed on an ice pack, transported to the laboratory, and then centrifuged at 4 °C for 10 min at 1500 x g. After centrifugation, serum was transferred into 1.5 ml micro-tubes and stored at -20°C until assayed 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

SPSS (Version 20 for Windows) was used for analyzing the data of the study. The significance level was chosen as 0,05. Shapiro-Wilk test was used to determine the normality of the data distribution. By the results of Shapiro-Wilk test, either Kruskal-Wallis then Mann-Whitney U tests or ANOVA then Tukey tests were applied to obtain the statistical differences. Proportional values were analyzed by Chi-square test. The results of the experiments were obtained as mean (± SEM) and rate (%) values.

RESULTS

Estrus behavior and ovarian findings

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 combined and analyzed then in relation to time only. The overall estrus response rate within 96 h and the total pregnancy rate were 72.73% (32/44) and 59.38% (19/32), respectively.

Serum P4 and E2 profile

The blood serum concentrations of P4 and E2 for 5 days after sponge removal for hCG or eCG treated and control goats are shown in Table 2, 3 and Figure 1, 2. The serum P4 concentrations and mean P4 and E2 serum concentrations did not differ statistically between the control, hCG-treated, and eCG-treated groups for five days after sponge removal (P>0.05). However, serum E2 concentrations during the first three days after sponge removal did not differ statistically between all groups (P>0.05), serum E2 concentrations differed between eCG and the control and hCG-treated groups on day four, and between eCG and control groups on day five (P<0.05).

Table 1. Reproductive outcomes of Saanen goats treated with MAP sponge for 5 days along with D-cloprostenol at sponge insertion during the breeding season, and receiving either hCG, eCG or saline (control) groups at the time of sponge removal (means ± SEM).

Data	Hormonal groups			Mean
	Control (n=16)	hCG-Group (n=14)	eCG-Group (n=14)	
Reproductive behavior				
Estrus response (%)	81.25 (13/16)	64.29 (9/14)	71.43 (10/14)	72.73 (32/44)
Duration of estrus (h)	21.23±2.41	23.00±5.00	22.73±3.27	21.67±2.07
Interval from sponge removal to onset of estrus (h)	60.00±4.71	43.00±6.35	46.91±6.15	50.33±3.44
Interval from sponge removal to ovulation (h)	70.50±2.87	66.00±2.74	66.86±2.73	67.91±1.61
Estrus onset to ovulation (h)	11.08±2.13	9.00±2.15	15.27±2.34	11.67±1.31
Ultrasonography evaluation				
Ovulation rate (%)	100 (16/16)	100 (14/14)	100 (14/14)	100 (44/44)
Number of CL per doe at day 10 (n)	1.06±0.27	1.07±0.22	1.29±0.22	1.14±0.14
Ovulatory follicle diameter (mm)	5.45±0.16	5.72±0.16	5.63±0.14	5.59±0.09
Follicular growth (mm/day)	0.79±0.13	1.06±0.20	0.66±0.13	0.83±0.09
Pregnancy Rate (%)	61.54 (8/13)	66.67 (6/9)	50.00 (5/10)	59.38 (19/32)

Corpus luteum=CL. No difference detected between groups (P>0.05).

Table 2. The effect of administration of hCG or eCG and saline on the mean (±SEM) of serum P4 concentrations (ng/ml) during after sponge removal in Saanen goats.

Days post sponge removal	Hormonal groups			Mean
	Control	hCG-Group	eCG-Group	
1	2.18 ± 0.19	2.84 ± 0.21	3.93 ± 1.09	2.91 ± 0.36
2	3.00 ± 0.31	2.88 ± 0.30	4.56 ± 0.58	3.44 ± 0.26
3	2.58 ± 0.32	2.89 ± 0.36	5.65 ± 1.42	3.66 ± 0.53
4	2.68 ± 0.27	2.51 ± 0.24	4.02 ± 1.26	3.03 ± 0.41
5	3.02 ± 0.56	2.81 ± 0.36	5.96 ± 1.45	3.85 ± 0.55

No difference was detected between groups or mean values on the same days (P>0.05).

Table 3. The effect of administration of hCG or eCG and saline on the mean (±SEM) of serum E2 concentrations (pg/ml) during after sponge removal in Saanen goats.

Days post sponge removal	Hormonal groups			Mean
	Control	hCG-Group	eCG-Group	
1	44.37 ± 3.91	41.33 ± 4.35	60.49 ± 14.65	48.33 ± 4.92
2	51.33 ± 5.55	50.24 ± 6.99	62.41 ± 11.15	54.36 ± 4.49
3	42.00 ± 4.63	45.19 ± 5.46	64.07 ± 9.47	50.08 ± 4.13
4	45.71 ± 5.67[a]	49.30 ± 7.36[a]	78.34 ± 16.90[b]	56.68 ± 6.34
5	46.07 ± 6.08[a]	48.13 ± 6.29[ab]	61.62 ± 9.34[b]	51.41 ± 4.21

[a,b]Values with different superscripts in the same row are significantly different (P<0.05). No difference was detected between mean values on the same days (P>0.05).

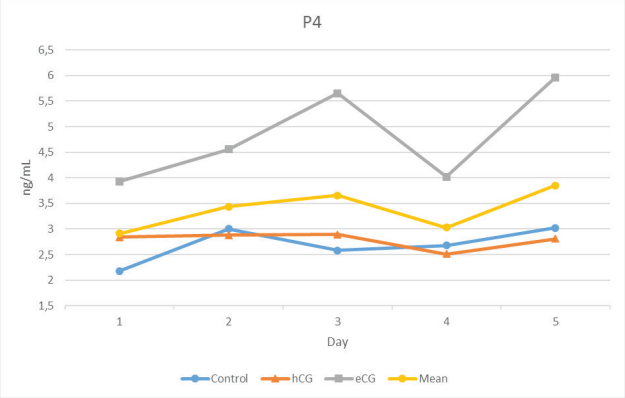

Fig. 1. Mean serum P4 concentrations in Saanen goats receiving 5-day short-term synchronization treatment with saline (Control) or hCG and eCG treatment at the time of sponge removal (means ± SEM). No difference was detected between groups or mean values on the same days (P>0.05).

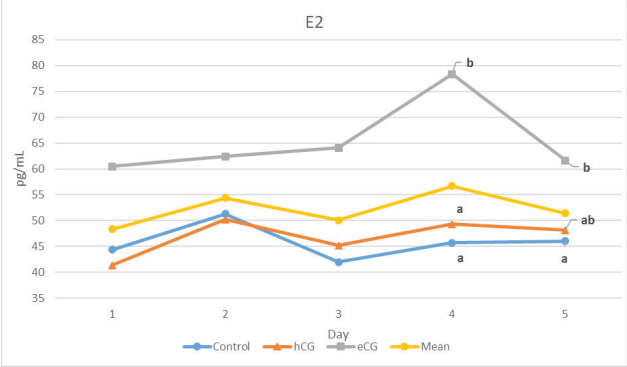

Fig. 2. Mean serum E2 concentrations in Saanen goats receiving 5-day short-term synchronization treatment with saline (Control) or hCG and eCG treatment at the time of sponge removal (means ± SEM). [a, b] Mean values between days with different superscripts differ significantly (P<0.05). No difference was detected between mean values on the same days (P>0.05).

DISCUSSION

The use of $PGF_{2\alpha}$ along with MAP intravaginal sponge for 5-days, regardless of the hCG or eCG 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 short-term estrus synchronization protocol used in this study is designed such that the progestogen inhibits the formation of new corpora lutea, while $PGF_{2\alpha}$ administration on day 0 induces luteolysis of the corpora lutea in the ovaries, and then a single dose administration of hCG or eCG on day 5 accelerates follicle development and the estrus response and fertility increases as a result (Menchaca and Rubianes, 2004; Abecia et al., 2012; Fonseca et al., 2017a; Santos-Jimenez et al., 2021). The deficiencies in ovulatory follicles have also been associated with treatment duration of progestagen-loaded intravaginal devices in small ruminants (Menchaca and Rubianes, 2004; Gonzalez-Bulnes et al., 2020). Exogenous progestagen administration inhibits tonic secretion of LH by negative feedback to the hypothalamus and thus estrus behaviors, preovu-

latory surge of LH and the occurrence of ovulation are prevented until treatment is discontinued (Menchaca and Rubianes, 2004; Menchaca et al., 2007; Gonzalez-Bulnes et al., 2020). On the other hand, the low tonic secretions of LH during progestagen treatment affect directly the survival and development of the large follicles in the ovaries (Gonzalez-Bulnes et al., 2020). The large follicles need LH to survive and grow, and they undergo atresia when the LH concentration is low (Medan et al., 2003). Progestagen secreted from intravaginal devices towards the end of the administration period cannot completely suppress the secretion of LH. This endocrine millennium causing the development of abnormal follicles results in the formation of large persistent follicles containing ageing oocytes in the ovaries, leading to low fertility after ovulation in small ruminants (Viñoles et al., 1999, 2001). To overcome this problem, short-term protocols consisting of 5-7 days of progestogen treatment have been developed (Rubianes and Menchaca, 2003; Menchaca and Rubianes, 2004; Menchaca et al., 2007; Dogan et al., 2020b). The advantage of the short-term treatment is that high blood P4 concentrations that do not fall to the subluteal level produced by the insertion of intravaginal devices follicular turnover supports by suppressing LH release from the anterior pituitary, thus causes the emergence of a new follicular wave that reaches a preovulatory diameter in the ovaries 5-7 days after device insertion (Menchaca and Rubianes, 2004; Menchaca et al., 2007; Abecia et al., 2012; Gonzalez-Bulnes et al., 2020). As a result, while ovulation occurs in a young follicle containing healthy oocytes, fertility outcomes are generally similar or higher compared to long-term protocols (Viñoles et al., 1999; Dogan et al., 2016). Given the possible presence of a corpus luteum in cycling animals, in short-term protocols for 5-7 days, it is also necessary to induce luteolysis with a single dose of $PGF_{2\alpha}$ or its analogues at either insertion or after the removal of intravaginal devices (Menchaca and Rubianes, 2004; Menchaca et al., 2007; Vilariño et al., 2011; Dogan et al., 2016, 2020b; Gonzalez-Bulnes et al., 2020), as applied to goats in this study. In this trial, there was no significant difference in estrus behavior, ovarian findings, and serum P4 and E2 concentrations in the hCG or eCG groups compared to the control group. In small ruminants, estrus rate is defined as acceptable when at least 90% of females treated with intravaginal progestogens (natural or synthetic forms of progesterone) devices in conjunction with eCG during the breeding season exhibit estrus behavior within 120 h (Rubianes and Menchaca, 2003, Menchaca and Rubianes, 2004). Results of the present study indicated that estrus was observed in 32 out of 44 goats (72.73%) within 96 hours of sponge removal. On the other hand, the proportion of goats exhibiting estrus behavior was reported within the range of 65.0 to 100% in other studies using the short-term progesterone/progestogen-based protocol with eCG and $PGF_{2\alpha}$ (Menchaca et al., 2007; Vilariño et al., 2011; Fonseca et al., 2017a,b; Dogan et al., 2008b, 2016, 2020b) or a progesterone injection plus hCG-based protocol (González-Alvarez et al., 2016; Rodríguez-Martinez et al., 2018; Alvarado-Espino et al., 2016, 2019a,b), in different and same breed goats or seasons. It has also been reported that natural or synthetic forms of progesterone do not cause a significant difference in estrus rate in small ruminates (Dogan et al., 2004; Gonzalez-Bulnes et al., 2020). Similar to the present study, Menchaca et al. (2007) reported no significant difference in estrus response between Alpine goats treated with or without eCG at the time withdrawal of intravaginal progesterone device (CIDR) during the breeding season. Besides, our result is in line with Dogan et al. (2008a), who reported that application of cronolone sponge and $PGF_{2\alpha}$ or cronolone sponge plus eCG and $PGF_{2\alpha}$ did not demonstrate a statistical difference in the estrus rate in Saanen goats during transition period. Similarly, Fonseca et al., (2017b), reported no significant differences in estrus response in goats treated with eCG or hCG 24 h before intravaginal sponge removal during the non-breeding season. On the other hand, using the same protocol as in the present study, Santos-Jimenez et al. (2021) reported a significant difference in estrus response in Dorper ewes treated with saline, hCG or eCG (0.0%, 69.3% and 100%, respectively), during the non-breeding season. Although these protocols are used to control estrus in goats throughout the year, differences in estrus response can be explained by the association of breed, season, management, gonadotropins, and nutrition (Wildeus, 2000; Rubianes and Menchaca, 2003; Menchaca and Rubianes, 2004).

The onset interval of estrus following intravaginal progestogen withdrawal is an important data both in the application of timed artificial insemination and in the development of new protocols (Fonseca et al., 2017b). In the present trial, the interval from sponge withdrawal to the onset of estrus and the duration of estrus were similar among the treatments, averaging 50.33 h and 21.67 h, respectively (Table 1). After using short-term (5-6 days) progestogen treatment plus

eCG and $PGF_{2\alpha}$ in same or different breed goats, the interval time from sponge removal to estrus ranged from 24.3 to 48.0 h (Menchaca et al., 2007; Dogan et al., 2008b, 2016, 2020b; Fonseca et al., 2017a). In line with our study, Fonseca et al. (2017b) did not obtain a significant difference between the intervals to onset of estrus in Toggenburg goats after eCG or hCG treatments, (43.0 vs. 30.9 h. respectively), following short-term (6-day) progesterone plus D-cloprostenol treatments during the non-breeding season. On the other hand, Dogan et al. (2008a) reported a significant difference for this parameter between eCG-treated and the untreated goats in their study, (21.8 vs. 39.6 h, respectively) where they applied eCG 24 h before sponge withdrawal in the treated group. In another study, Santos-Jimenez et al. (2021) reported a significant difference in the interval from device removal to the onset of estrus between ewes treated with 300-eCG or 300-hCG at the time CIDR withdrawal (41.45 vs. 52.0 h, respectively), during the non-breeding season. In a previous study, using 50, 100, or 300 IU of hCG 24 h after P4 injection in Alpine breed goats during the transition period Alvarado-Espino et al. (2016), reported an interval of 60, 54, and 76 h for the onset of estrus, respectively, and also a significant difference for this parameter between goats treated with 100 IU and 300 IU hCG. In other similar studies that use the hCG-P4 protocol in goats during the anovulatory season, onset of estrus ranged from 52.0 to 65.0 h (González-Álvarez et al., 2016; Rodríguez-Martínez et al., 2018; Alvarado-Espino et al., 2019a,b).These differences between studies may be due to seasons, goat breeds, different gonadotropins such as eCG or hCG and the doses used. Gonadotropins such as FSH and LH are required in the final stages of follicular development in the ovaries but not in the early stages (Fatet et al., 2011). eCG, whose biological activity is more similar to FSH than LH (Thach et al., 2022), promotes the development of the medium or large follicles when administered 24 h before or at the time of sponge withdrawal, increasing the estrogen concentration and LH surge by supporting their faster growth (Abecia et al., 2012). As a result, the onset interval of estrus becomes even shorter, especially outside the breeding season (Dogan et al., 2008a; Fonseca et al., 2017b; Hameed et al., 2020; Santos-Jimenez et al., 2021). Although this parameter is affected by many factors such as lactation, breed, parity, and season, high doses of eCG further shorten the onset interval of estrus (Dogan et al., 2004; Fatet et al., 2011). The dose of eCG varies from 200 to 600 IU, but doses of eCG greater than 400 IU are not recommended, as they cause decreased fertility in female dairy goats (Wildeus, 2000; Fatet et al., 2011). Besides, for induction of estrus in sheep and goats outside the breeding season, hCG was administered at doses ranging from 50-300 IU 24 h before or during removal of intravaginal devices (Fonseca et al., 2017b; Santos-Jimenez et al., 2021) or 24 h after progesterone injection (González-Álvarez et al., 2016; Alvarado-Espino et al., 2016, 2019a,b; Rodríguez-Martínez et al., 2018), as an alternative to eCG. hCG has more LH-like activity than eCG, on the development of large follicles is probably lower due to less similarity to FSH (Saleh et al., 2012), since hCG shows its effect by binding directly to LH receptors emerging in the granulosa cells as the follicle increases in size (Driancourt, 2001).

In previous studies, after using short-term progestagen-based protocols (Dogan et al., 2008b, 2016, 2020b; Menchaca et al., 2007; Hameed et al., 2020) or P4-hCG protocol (González-Álvarez et al., 2016; Alvarado-Espino et al., 2016; Rodríguez-Martínez et al., 2018), the duration of estrus in goats varied between 22.3 and 40.6 h. Although goats exhibit estrus behavior for an average of 36 h, this duration varies between 24 and 48 h depending on factors such as age, individuals, breeds, season and the presence of a buck (Fatet et al., 2011; Dogan et al., 2016). On the other hand, estrus duration was significantly shortened with eCG treatment in goats during the non-breeding season (Menchaca et al., 2007; Hameed et al., 2020) but not in the transition period (Dogan et al., 2008a).

In the current study, the intervals from the sponge removal to ovulation and from the onset of estrus to ovulation were, on average, 67.91h and 11.67 h, respectively (Table 1). In previous studies, different intervals were reported for these parameters in goats using the short-term (5-7 days) protocol as 64.2 and 17.4 h (Fonseca et al., 2017a), 73.5 and 32.3 h (Fonseca et al., 2017b), 48.25 and 22.75 h (Dogan et al., 2020b), respectively. Similar to the present study, Santoz-Jimenez et al. (2021) reported no significant difference in the interval from device removal to ovulation in Dorper ewes treated with hCG or eCG at the end of CIDR device removal during the non-breeding season. In contrast, Hameed et al. (2021) reported that eCG injection administered at the time removal of the CIDR device had a significant effect on ovulation time in goats during the non-breeding season. In the current trial, ovulations occurring approximately 66 h after hCG or eCG injection compared to saline

injection (70.5 h) were observed following monitoring of the ovaries by transrectal ultrasonography, and no significant difference between the trial and control groups. However, it can be claimed that the long half-life of hCG or eCG may have a permanent effect, directly or indirectly, on the occurrence of ovulation. On the other hand, hCG, in particular, may contribute directly to the development of the corpus luteum after ovulation.

In our study, according to the results of ultrasonographic examination, the percentage of goats ovulating and the rate of corpus luteum (ovulation) per doe were on average 100% (44/44) and 1.14, respectively (Table 1), also ovulations occurred within 96 hours in all goats with or without estrus behavior. Similar results were obtained by Vilariño et al. (2011), González-Álvarez et al. (2016) and Fonseca et al. (2017a) in dairy goats that received the same or different hormonal treatment. However, in the previous reported studies, the proportion of ovulating follicles was almost similar or lower in goats treated with eCG (Menchaca et al., 2007; Dogan et al., 2020b) or hCG (Alvarado-Espino et al., 2016, 2019a,b; Rodríguez-Martínez et al., 2018). In contrast, Santos-Jimenez et al. (2021) reported that the percentage of ewes ovulating within 72 h differed significantly between females treated with hCG (38.5%) and eCG (100%), during the non-breeding season, a response not observed in our study. In regard to number of ovulations per doe, our results are similar or slightly lower than those presented in other studies using the short-term progestagen protocol (Vilariño et al., 2011; Fonseca et al., 2017a,b; Dogan et al., 2020b) or P4-hCG protocol (Alvarado-Espino et al., 2016, 2019a,b; González-Álvarez et al., 2016; Rodríguez-Martínez et al., 2018); many factors such as breed goats, season, dose of gonadotropins could explain differences between studies. Similarly, the researchers reported that the ovulation rate was not significantly different between goats (Fonseca et al., 2017b) or sheep (Santos-Jimenez et al., 2021) treated with hCG and eCG; as observed in our study. In addition, Hameed et al. (2021) reported that the ovulation rate was significantly different between eCG-treated and non-eCG-treated goats, possibly due to differences in breed and season, in contrast to this study.

In our study, there was no difference in mean serum P4 and first three days E2 concentrations between all groups for 5 days after sponge removal, also, no difference was observed between overall means and days (Tab. 2, 3 and Fig. 1, 2). On the other hand, serum E2 concentrations differed between the eCG and the other two groups on the fourth day and between the eCG and control groups on the fifth day (Tab. 3 and Fig. 2). These results are consistent with the results obtained in goats (Medan et al., 2003; Simões et al., 2006; Menchaca et al., 2007; Dogan et al., 2020a).

In the current trial, the mean ovulatory follicle diameter and follicular growth were 5.59 mm and 0.83 (mm/day), respectively, also, the mean growth rate of preovulatory follicles was within the limits of normal distribution (Simões et al., 2006; Dogan et al., 2020a). Using the short-term protocol (Menchaca et al., 2007; Vilariño et al., 2011; Fonseca et al., 2017a,b; Dogan et al., 2020a,b; Hameed et al., 2020) or P4-hCG protocol (González-Álvarez et al., 2016; Alvarado-Espino et al., 2016, 2019a,b; Rodríguez-Martínez et al., 2018), the researchers reported that the diameter of preovulatory follicles ranges from 6.5 to 8.7 mm following progestagen withdrawal in the dairy goats and no significant differences were also observed between goats treated with either eCG and hCG, or eCG and non-eCG (Fonseca et al., 2017b; Hameed et al., 2020). The difference can be explained by the association of the size of the ovulatory follicle and breed, steroid hormone concentrations, season and measurement technique.

Pregnancy rates in this study did not differ among treatments but was similar or lower compared to previous results obtained from the short-term (Dogan et al., 2016; Hameed et al., 2020) or P4-hCG (Alvarado-Espino et al., 2016, 2019b; Rodríguez-Martínez et al., 2018) protocols using natural mating, but resulted in an acceptable the overall pregnancy rate (59.38%). Likewise, using artificial insemination following short-term (Dogan et al., 2008b; Vilariño et al., 2011; Fonseca et al., 2017a,b) or P4-hCG (Alvarado-Espino et al., 2019a) protocols, researchers reported that pregnancy rates ranged from 15.0% to 75.3%, depending on many variables such as breeds, season, insemination method and protocol. In addition, after administration of eCG or hCG during the non-breeding season, Fonseca et al. (2017b) reported no difference in pregnancy rates in goats, but in ewes (Santos-Jimenez et al., 2021). Our finding was similar to the results obtained in goats, but the difference may be due to ewes. On the contrary, the pregnancy rate was significantly higher in eCG-treated goats compared to non-eCG-treated goats, during the non-breeding season (Hameed et al., 2020). In contrast, the pregnancy rate was similar between goats with and without

eCG-treated during the transition period (Dogan et al., 2008a). This situation observed in current studies may be due to the season.

CONCLUSIONS

This study shows that short-term progestogen protocol is successful for synchronizing the estrus and ovulation in non-lactating goats during the breeding season. A single injection of 400 IU of hCG or eCG during sponge removal also appears to have a similar effect on estrus behavior, ovarian findings, and serum P4 and E2 concentrations, with the exception of day four for serum E2 concentrations, so hCG may be an alternative to eCG.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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