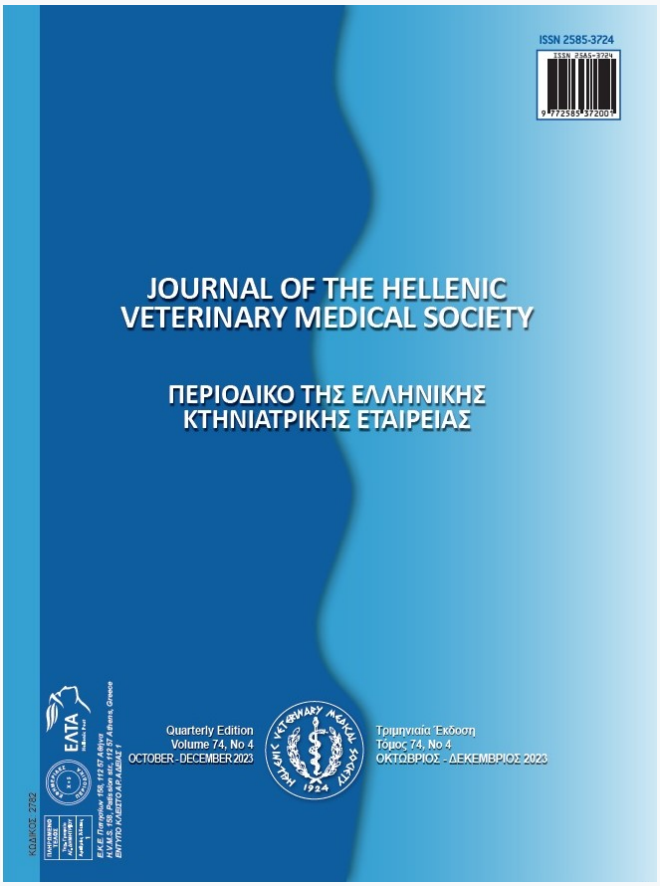
Journal of the Hellenic Veterinary Medical Society

Vol 74, No 4 (2023)



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doi: 10.12681/jhvms.31541

To cite this article:

Dogan, I., Toker, M., Aktar, A., & Yilmaz, M. (2024). Effect of GnRH and hCG Treatment Following a Short-Term Estrus Synchronization Protocol on Ovulation and the Fertility in Merino Ewes, During the Breeding Season. *Journal of the Hellenic Veterinary Medical Society*, *74*(4), 6517–6524. https://doi.org/10.12681/jhvms.31541

J HELLENIC VET MED SOC 2023, 74 (4): 6517-6524
ΠΕΚΕ 2023, 74 (4): 6517-6524

Research article
Ερευνητικό άρθρο

Effect of GnRH and hCG Treatment Following a Short-Term Estrus Synchronization Protocol on Ovulation and the Fertility in Merino Ewes, During the Breeding Season

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ABSTRACT: This study aimed to compare the effect of hCG or GnRH administered 36 h following a short-term estrus synchronization treatment on the ovulation time and pregnancy rates of Merino ewes during the breeding season. The estrus cycles of ewes were synchronized with an intravaginal sponge containing 60 mg of medroxyprogesterone acetate for 6 days, and an injection of 400 IU of eCG and 125 μg of d-cloprostenol 24 h before sponge removal. Thirty-six h after the sponge removal, ewes were injected intramuscularly either 1 ml of physiological saline solution (control-group; *n*=14), 100 IU of hCG (hCG-group; *n*=14) or 0.004 mg of buserelin acetate (GnRH-group; *n*=14). The estrus behavior was observed using teaser rams and the ovulation time was monitored using transrectal ultrasonography twice daily for 96 h after the sponge removal. Ewes in estrus were allowed a single mating using fertile rams. Estrus response was higher (P<0.05) in the control group (92.86%) than in the GnRH group (50.00%). The interval from sponge removal to ovulation and from hCG to ovulation were shorterin the control group than in the hCG group(70.55, 80.83 h; 34.55, 44.83 h, respectively, P<0.05). In conclusion, administration of hCG or GnRH at 36 h following a short-term estrus synchronization protocol did not affect estrus behavior, ovarian findings and pregnancy rate in ewes during the breeding season.

Keywords: Ewe, GnRH, hCG, ovulation, pregnancy rate

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Date of initial submission: 06-10-2022
Date of acceptance: 03-04-2023

INTRODUCTION

Sheep breeds generally exhibit seasonal reproductive cycles, depending on the latitude, photoperiod, and other environmental factors (Bartlewski et al., 2011; Abecia et al., 2012). The seasonal pattern of reproductive activity in sheep is the main factor limiting the production of milk and meat throughout the year. For this reason, it is necessary to control the reproductive activities of sheep to meet the increasing consumer demand for these products every year. In this regard, estrus induction or synchronization using exogenous hormones in sheep is an important tool for controlling the reproductive activity all year round (Dogan and Nur, 2006; Abecia et al., 2012; Dogan et al., 2018). Because of their ease of use and availability for farmers, intravaginal sponges containing synthetic analogues of progesterone such as medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA) are generally the most widely used exogenous hormones for induction or synchronization of estrus and ovulation in sheep (Abecia et al., 2012; Takada et al., 2012; Gonzalez-Bulnes et al., 2020). These hormones have been used for short (5-7 days) or long (12-14 days) periods to induce or synchronize estrus in sheep (Dogan and Nur, 2006; Takada et al., 2012; Balaro et al., 2016; Gonzalez-Bulnes et al., 2020). On the other hand, since long-term administration of progestogens in sheep has been associated with low fertility, short-term administration of progestogens has been currently preferred (Viñoles et al., 2001; Gonzalez-Bulnes et al., 2020). In small ruminants, progesterone (P4)/progestogen-based protocols include injection of one dose of equine chorionic gonadotropin (eCG) 24 h before (Balaro et al., 2016) or at the end of the treatment to increase the estrus response and ovulation rate (Dogan and Nur, 2006; Abecia et al., 2012: Gonzalez-Bulnes et al., 2020).eCG, because of its higher similarity to the follicle-stimulating hormone (FSH), especially during the non-breeding season is an essential component of these protocols (Viñoles et al., 2001; Dogan and Nur, 2006; Gonzalez-Bulnes et al., 2020). On the other hand, the conventional P4/progestogen-based protocols combined with eCG have variable fertility rates in small ruminants because the optimal interval between ovulation and artificial insemination cannot be defined (Evans et al., 2000; Abecia et al., 2012; Gonzalez-Bulnes et al., 2020). Two options have recently been proposed by researchers to overcome low fertility rates in sheep. The first option is to fully induce ovulation by inducing the normal rise of luteinizing hormone (LH) during estrus period by administration of human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH) on the day of mating or artificial insemination (Reyna et al., 2007; Balaro et al., 2016; Dias et al., 2018). The second option is to support the development of luteal function by administering hCG or GnRH 7 to 12 days after natural mating. Neither of these treatments achieved further improvement in pregnancy rates in sheep (Cam and Kuran, 2004; Da Fonseca et al., 2018). However, the potential effect of GnRH or hCG on occurring ovulation following the termination of the short-term estrus synchronization protocol during the breeding season has been limitedly investigated in sheep. The objective of the present study was therefore to compare the effect of a single dose GnRH or hCG administered 36 h after termination of short-term estrus synchronization protocol on the time to ovulation and fertility in Merinos ewes, 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 40º 11`N, longitude 29º 04` E, altitude 155 m), Turkey. A total of 42 clinically healthy, free of reproductive disorders and multiparous Merino ewes were used during the breeding season in the region (from September to October). The ewes were kept indoors in sand/hay-floored pens with access to outdoors in sheltered paddock under conditions of natural photoperiod and temperature and were fed dry grain wheat hay (1500 g/sheep/day) supplemented with commercial pellets (18% crude protein; 800 g/doe/day, 2800 Kcal). No extra food was offered to the ewes during the study. Ewes were provided *ad libitum* with clean drinking water and mineralized salt and no changes were made during the experiment. The ewes and rams 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 the Animal Experiments Local Ethics Committee of the Uludag University (approval reference number: B.30.2.ULU.08Z.00.00/150).

Estrus synchronization, hCG and GnRH treatment

Estrus cycle in ewes was synchronized using 60 mg MAP sponge (Esponjavet, Hipra, Spain) for 6 days. Twenty-four h before the sponge removal, 125

μg d-cloprostenol ($PGF_{2\alpha}$, PGS, Alke, Turkey) and 400 IU eCG (Oviser, Hipra, Spain) were administered intramuscularly (Balaro et al., 2016).The day of sponge removal was considered as the beginning of the experiment. Thereafter, all ewes were divided into three different groups (control group, hCG group and GnRH group) of fourteen animals each 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, hCG, and GnRH groups, the age averaged 34.93±3.79, 34.73±4.32, and 36.29±4.22 months; body weights averaged 62.03±3.09, 63.22±2.09, and 64.83±2.82 kg; and the body condition score averaged 3.08±0.14, 3.23±0.09, and 3.19±0.15, respectively. Ewes in the control group, hCG group and GnRH group received i.m.1 ml of physiological saline solution (0.9% NaCI), 100 IU of hCG (hCG, Chorulon, MSD, Netherlands), and 0.004 mg of buserelin acetate (GnRH, Buserin, Alke, Turkey), respectively, 36 h after the sponge removal.

The estrus was monitored twice a day (at 8 a.m. and 8 p.m.) for 20 min from 12 h to 96 h by using an aproned ram following sponge withdrawal. Ewes showing estrus were immediately taken to a separate section and were then allowed a single mating per ewe using one of the four fertile rams. The rams were about 3 years old, sexually active, and has previously been tested for fertility. Estrus onset was defined as the time when the ewe first stood to be mounted by the teaser ram. The estrus response rate was calculated by considering the number of ewes detected in estrus during the observation period (12-96h)/number of treated ewes x 100. Estrus duration was defined as the time elapsed between the first and last acceptance of mounting within the observation period.

Ovarian ultrasonography examination

Examinations of the ovaries in all ewes was conducted with a B-mode transrectal ultrasonographic scanner (RTU, Prosound 2, Hitachi Aloka Medical, Ltd., Japan), equipped with 7.5 MHz linear array transducer (model UST-660, 7.5-MHz transrectal probe). During the ultrasonic examination, the ewes were 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 (Ravindra et al., 1994).

The diameters of all follicles ≥2 mm and corpora lutea (CL) in both ovaries were recorded once daily (9 a.m.) for seven days beginning at sponge removal in all ewes by the same operator. 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 ewe. 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 (9 a.m.-9 p.m.) until ovulation was confirmed in all ewes. The day of ovulation was defined by the disappearance or collapse of follicles greater than 5 mm in diameter between two consecutive ultrasound examinations (Dias et al., 2018)and followed by the development of a corpus luteum in the ovulation area of the ovary; the day of ovulation was considered as the first day (0 day) of the estrus cycle. 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 its total growth (in mm) by the number of days between its appearance as a follicle larger than 3 mm in diameter and its maximum diameter (Ravindra et al., 1994).

The number of ovulations per ewe was determined by the number of CL on the ovaries according to their oval or round conformation and counted at day sevenafter sponge removal (Evans et al., 2000). Interval from sponge removal to ovulation, interval from onset of estrus to ovulation, ovulation rate (number of ovulated ewes / total ewes x 100) and number of ovulations per ewe were also determined in this study. The luteal tissue considered as the corpus luteum was measured together with the area of central cavity (Dias et al., 2108). Measurements started the day after ovulation and continued until the last day of the study. The growth rate (mm/day) of corpus luteum was calculated by dividing its total growth (in mm) by the number of days ultrasonographic measurements were performed. Number of pregnant ewes 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 (ewes pregnant/ewes mated) was calculated for each group.

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 analyse the parameter considered. The data that distributed normally were analyzed by Analysis of variance (ANOVA) followed by the Tukey as post-hoc test. Non-normally distributed data were analyzed by Kruskal-Wallis test followed by Mann-Whitney U to spot the differences among the study groups. The results were given as mean (± SEM) and differences were considered significant when the P value was below 0.05.

RESULTS

A summary of data regarding the estrus behavior, ovarian findings and pregnancy rate after the synchronized estrus in the ewes are shown in Table 1. Estrus response (%) during the observation period in GnRH group was significantly lower than in the control group (P<0.05). The intervals from sponge removal to ovulation and from hCG application to ovulation were longer in hCG group compared to controls (P<0.05). No other significant differences were recorded between groups regarding the reproductive behavior, ultrasonography findings and pregnancy rate. Therefore, the data in the study were pooled and analyzed then in relation to time only. The overall estrus response rate within 96 h and the total pregnancy rate were 73.81% (31/42) and 51.61% (16/31), respectively.

DISCUSSION

The use of eCG and $PGF_{2\alpha}$ along with MAP intravaginal sponge for six days, regardless of hCG and GnRH treatment, was found to be an efficient protocol for estrus synchronization in the Merino ewes during the breeding season in our study. The effectiveness of the protocol attempting to control the sexual cycle of sheep was designed such that the formation of new corpora lutea is inhibited when given a progestational agent intravaginally, while administration of a dose of $PGF_{2\alpha}$ and eCG at the time of sponge removal provides luteolysis and follicle development and, the estrus response rate increases as a result (Wildeus, 2000; Abecia et al., 2012; Gonzalez-Bulnes et al., 2020). Furthermore, the high P4 concentrations, produced by the insertion of progesterone-containing devices into the vagina in sheep, suppress gonadotropin release from the pituitary, resulting in a follicular turnover and the emergence of a new follicular wave three days after the insertion of the device (Hunter et al., 2004; Abecia et al., 2012; Gonzalez-Bulnes et al., 2020). Short-term progesterone treatments that support the follicular turnover allows the ovulation of a young follicle containing a healthy oocyte, resulting in higher fertility

Table 1. Timing of estrus and ovulation, ovarian findings (ultrasonography exam) and pregnancy rates of Merino ewes treated with MAP sponge for 6 days with eCG plus d-cloprostenol 24 h prior to sponge removal and administration of hCG or GnRH or saline (control) 36 h after sponge removal (means ± SEM).

Data	Treatment groups			Overall
	Control (n=14)	hCG-Group (n=14)	GnRH-Group (n=14)	
Timing of estrus and ovulation				
Estrus response (%)	92.86 (13/14)^{a}	78.57 (11/14)^{ab}	50.00 (7/14)^{b}	73.81 (31/42)
Duration of estrus (h)	18.55±1.89	13.71±1.71	18.00±3.46	16.19±1.29
Interval from sponge removal to onset of estrus (h)	49.09±1.09	53.33±2.91	42.86±3.57	48.89±1.56
Interval from sponge removal to ovulation (h)	70.55±2.35^{a}	80.83±2.60^{b}	73.09±3.78^{ab}	75.00±1.83
Estrus onset to ovulation (h)	23.64±3.04	23.78±3.47	25.71±4.08	24.22±1.93
Interval from hCG and GnRH to ovulation (h)	34.55±2.35^{a}	44.83±2.60^{b}	37.09±3.78^{ab}	39.00±1.83
Ultrasonography evaluation				
Ovulation rate (%)	100 (14/14)	100 (14/14)	100 (14/14)	100 (42/42)
Mean number of ovulations per ewe	1.27±0.14	1.15±0.10	1.15±0.10	1.18±0.66
Ovulatory follicle diameter (mm)	5.74±0.33	5.52±0.24	5.35±0.25	5.52±0.15
Follicular growth (mm/day)	0.84±0.85	0.64±0.10	0.70±0.13	0.72±0.06
Large follicle (n)	1.73±0.62	1.31±0.43	1.36±0.31	1.46±0.26
Medium follicle (n)	4.73±0.66	5.77±0.66	4.85±0.60	5.14±0.37
Small follicle (n)	18.36±1.52	17.77±1.49	15.77±1.80	17.24±0.93
Largest corpus luteum diameter (mm)	8.16±0.44	8.08±0.27	7.94±0.22	8.06±0.17
Luteal growth (mm/day)	0.67±0.11	0.71±0.11	0.66±0.10	0.68±0.06
Pregnancy Rate (%)	46.15 (6/13)	63.64 (7/11)	42.86 (3/7)	51.61 (16/31)

^{a,b}means in the same row, with different subscripts indicate a significant difference (P<0.05)

(Viñoles et al., 1999). In this study, the administration of 100 IU hCG or 0.004 mg buserelin acetate (GnRH) 36 h after sponge removal had no significant effect on ovulation time, ovarian findings and pregnancy rates between the groups. In ewes, estrus rate following intravaginal P4 or its analogs in conjunction with eCG during the breeding season is normally defined as acceptable when 90% ≥ of the treated females come into estrus within 120 h (Wildeus, 2000). Results of the present study indicated that estrus was induced in 73.81% (31/42) of all ewes, within 96 h after sponge removal. In the GnRH group estrus response was significantly lower than in the control group (50.00% vs 92.86%, P<0.05). The estrus response was reported in the range of 65.0 to 100% in other studies using the short-term progestogen-based protocol (Cavalcanti et al., 2012; Balaro et al., 2016; Martinez-Ros and Gonzalez-Bulnes, 2019; Sinimbu et al., 2022) or the long-term progestogen-based protocol (Dogan and Nur, 2006, Türk et al., 2008, Dogan et al., 2018) in different sheep breeds and seasons, without hCG or GnRH. In a previous study, Bruno-Galarrage et al. (2021), using 400 IU of eCG at controlled internal drug releasing (CIDR) device removal or 500 IU of hCG 24 h after CIDR device removal in ewes, reported an estrus response rate of100 and 61.5%, within 84 h, respectively. On the other hand, Balaro et al. (2016) reported no significant differences in estrus rates in Santa Inês ewes treated with (90.0%) or without (78.0%) GnRH (0.025 mg lecirelin) 36 h after sponge removal during the breeding season. Similarly, Martinez-Ros and Gonzalez-Bulnes (2019) indicated that the administration of GnRH (50 μg, gonadorelin acetate) at 56 h after CIDR removal showed no significant effect on the number of ewes exhibiting estrus. A number of factors are known to affect the estrus response in ewes (Wildeus, 2000) might account for these different results.

The time from sponge/device removal to estrus is an important parameter for fertility rates when fixed-time artificial insemination is applied in small ruminants (Fonseca et al., 2017). In the current study, the interval time from sponge removal to estrus and the duration of estrus were on average 48.89 h and 16.91 h, respectively. After using short/long-term progestogen treatments plus eCG and $PGF_{2\alpha}$, the interval time from sponge removal to estrus ranged from 26 to 47.3 h (Dogan and Nur, 2006; Türk et al., 2008; Cavalcanti et al., 2012; Figueira et al., 2015; Balora et al., 2016; Dogan et al., 2018; Dias et al., 2018).Such differences could be related especially to doses of eCG which has a long biological half-life, different seasons, and sheep breeds. 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 (Bartlewski et al., 2011). Considering the high similarity between eCG and FSH, the medium or large follicles are affected by eCG administration 24 h 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 and Nur, 2006; Martinez-Ros and Gonzalez-Bulnes, 2019). Although many factors such as lactation, breed, parity, and season are effective, the dose of eCG is the main factor affecting the interval to estrus after sponge removal (Wildeus, 2000; Gonzalez-Bulnes et al., 2020). The doses of eCG varies from 250 to 500 IU, depending on the age, season and breed, but higher doses of eCG are not recommended for ewes as they may result in lower pregnancy rate or embryo death (Abecia et al., 2012). Yet, the use of exogenous eCG for induction of estrus in ewes is more necessary in the non-breeding season compared to the breeding season (Reyna et al., 2007; Gonzalez-Bulnes et al., 2020).

In contrast, in previous studies using the similar protocol without hCG or GnRH, the duration of estrus in different sheep breeds and seasons varied between 25.0 and 49.3 h (Dogan and Nur, 2006; Türk et al., 2008; Cavalcanti et al., 2012; Figueira et al., 2015; Balaro et al., 2016; Dogan et al., 2018; Dias et al., 2018; Sinimbu et al., 2022). In a previous study, it was reported that natural mating in goats reduces the duration of estrus by 45% (Romano et al., 2016).In our study, the administration of hCG or GnRH at 36 h after the synchronized estrous did not modify the interval to estrus. Cavalcanti et al. (2012) and Balaro et al. (2016) reported that the administration of 25 μg licerelin (a GnRH analogue) 24 or 36 h after progestogen sponge withdrawal had no effect on the interval to estrus in ewes during the breeding season. Similarly, Bruno-Galarraga et al. (2021) reported that administration of 500 IU hCG 24 h after CIDR device withdrawal had no effect on the onset of estrus in ewes during the breeding season.

In the current study, the intervals from the sponge

removal to the onset of estrus and from the onset of estrus to ovulation were on average 75.0 h and 24.22 h, respectively. In contrast, using same protocol (6-day) Figueira et al. (2015) recorded 85.3 h of the interval from sponge removal to ovulation in Santa Inês ewes. In previous studies using GnRH 36 h after sponge removal, Reyna et al. (2007) indicated that the interval from the sponge removal to the ovulation was not significantly different between GnRH-treated (50 h) and non-treated (54 h) ewes. In comparison with the results of Balaro et al. (2016), although the interval from the onset of estrus to ovulation was similar, the interval from sponge removal to ovulation was shorter than in our study. In our study, the interval from sponge removal to ovulation was significantly longer in the hCG group than in the control ewes ($P<0.05$). Moreover, according to our findings these interval were longer than those reported by Dias et al. (2018), who found a 54 h interval from the removal of sponges to ovulation and a 20 h interval from the onset of estrus to ovulation in hCG-treated ewes. This difference between studies may be related to the dose of hCG and the time of administration.

Based on the results of the ultrasonographic examination, no effect on ovulation time was observed between the hCG and GnRH groups, but both induced ovulation within 96 h in ewes that did not exhibit estrus behavior. In the present study, the average interval from administration of exogenous hCG, GnRH or saline solution to ovulation was 39.0 h, and in the control group this interval was significantly shorter than in hCG group ($P<0.05$). This may be due to the fact that the study was carried out during the breeding season. Similarly, Reyna et al. (2007) reported that GnRH synchronized the ovulation time in ewes during the non-breeding season, but not during the breeding season. According to Wildeus(2000), since early administration causes luteinization of the preovulatory follicle and an ovulation, GnRH must be administered at least 24-36 h after progestogen removal or luteolysis.

In this study, the ovulation rate and the number of ovulations (number of CL) per ewe were on average 100% (42/42) and 1.18, respectively (Table 1). A similar percentage (100%) and number of ovulations (1.5/ewe) were reported in the Suffolk ewes during the breeding season using short-term progestogen protocol (Takada et al., 2012). Likewise, Dias et al.(2018) reported no difference in ovulation rates between hCG-treated and the non-treated ewes in their study, where they applied hCG 24 h after device withdrawal. In regard to number of ovulations per ewe, our results are similar to or slightly lower than those presented in other studies (Cavalcanti et al., 2012; Balaro et al., 2016; Dias et al., 2018; Martinez-Ros and Gonzalez-Bulnes, 2019). This variation between studies can be attributed to different breeds of sheep and doses of eCG. The hormone hCG is a glycoprotein hormone that has a similar structure to LH, binds to the same receptor, and has more rapid absorption and a longer half-life than LH (39 h vs 11.6 h, respectively) (Saleh et al., 2012). In this study, hCG was used to synchronize time of ovulation and could only affect this hormonal process but not the number of dominant follicles present at the time of hCG administration, which is one of the most potent stimulators of final oocyte maturation. Furthermore, in their studies with GnRH administered at 36 and 24 h after sponge withdrawal, Cavalcanti et al. (2012) and Balaro et al. (2016)reported no difference in the percentage of ovulation between GnRH-treated and the non-treated ewes. GnRH has been used previously to regulate the time of ovulation in ewes during the non-breeding and breeding season (Reyna et al., 2007). Since GnRH caused an LH surge 1-4 h after intramuscular injection (Cavalcanti et al., 2012), it did not directly regulate the time of ovulation as a result (Bartlewski et al., 2011). Therefore, the ovulatory response to GnRH, due to its short half-life, might depend on the time of treatment and the size of preovulatory follicle (Cavalcanti et al., 2012). Although statistical deference was not reached in our study, the induction of ovulation as a result of the administration of GnRH can be explained by the direct effect of GnRH on follicular development and further increase in estradiol concentrations with onset of estrus then LH surge and ovulation (Hunter et al., 2004; Bartlewski et al., 2011; Martinez-Ros and Gonzalez-Bulnes, 2019).

In previous studies, researchers have reported that the diameter of ovulatory follicles in ewes varies between 5 and 7 mm (Bartlewski et al., 2011), or 4.4 and 8.4 mm (Figueira et al., 2015). In the present trial, the ovulatory follicle diameter and follicular growth averaged 5.52 mm and 0.72 (mm/day), respectively, similar to those previously reported in Suffolk-Cross and Texel ewes (6.2 mm, 0.9 mm/day) (Evans et al., 2000), in Corriedale ewes (5.1 mm, 0.6 mm/day) (Catalano et al., 2012), in Suffolk ewes (5.36 mm, 0.75 mm/day) (Takada et al., 2012) and in Santa Inês ewes (5.96 mm and 0.7 mm/day) (Dias et al., 2018), respectively. No significant difference

was observed in the ovulatory follicle diameter and follicular growth rate between hCG-treated (at 24 or 36 h after removal of the sponges) and non-treated ewes during the non-breeding and breeding season (Catalano et al., 2012; Dias et al., 2018). Likewise, GnRH injection 36 h after sponge withdrawal did not change the ovulatory follicle diameter and follicular growth rate (Reyna et al., 2007; Balaro et al., 2016). Considering the number of follicles of different sizes observed by transrectal ovarian ultrasonography after sponge removal, the small follicles number was the highest in all groups; no difference was observed between the experimental groups in terms of the average number of ovarian follicles of different sizes. Similar results were observed in Suffolk and Texel ewes (Evans et al., 2000; Takada et al., 2012). The ovarian follicular development exhibits wave-like pattern in ewes during the non-breeding and breeding season (Evans et al., 2000; Bartlewski et al., 2011). In addition, Evans et al. (2000) and Takada et al.(2012) have reported that follicles emerge from the follicle pool with a diameter of 2-5 mm in the ovaries and grew before regression process or ovulation. As seen in the groups treated with hCG or GnRH in our study, administration of hCG within the estrus period may only induce ovulation due to its similarity with LH, but it has no effect on follicular development, as the number of follicles of different sizes was similar in hCG and control groups. Similarly, this can be explained by the indirect effect of GnRH on follicular development.

The mean diameter of CL (8.06 mm) and the daily growth rate of luteal tissue (0.68 mm/day) observed per day after ovulation were similar between the control and GnRH or hCG-treated groups. Similarly, the first CL was detected by transrectal ultrasonography on second day of the estrus cycle as an 8-10 mm diameter structure (Ravindra et al., 1994). In addition, a previous study using color Doppler ultrasonography has demonstrated that CL was first detected in the first day of the estrus cycle (Figueira et al., 2015). Ravindra et al. (1994) reported that the mean diameter of the CL increased from 11.5 mm on day 3 of the cycle to 13.3 mm on day 5, but there was no change in diameter between days 5 and 10 of the cycle. The mean CL diameter detected in our study was higher than that observed in Merino ewes (GnRH 8.73 mm and control 8.18 mm on day 10) by Reyna et al. (2007) and in Santa Inês ewes (hCG 9.4 mm and control 9.5 mm on day 12) by Dias et al.(2018). This difference may be due to the measuring technique, the day of measurement, and different sheep breeds. On the other hand, the mean growth rate of CL obtained in this study was slightly lower than the result obtained by Ravindra et al. (1994), who recorded approximate growth rate of 0.9 mm/day.

The overall the pregnancy rate considering all of ewes of the present study was 51.61% (16/31). In contrast, by using the same protocol (6-day) and natural mating, Figueira et al. (2015) obtained 83.3% of the pregnancy rate in Santa Inês ewes. The result observed in our study was higher than the values (29.7 to 45.0%) reported for ewes after fixed-time artificial insemination with fresh diluted semen (Dogan and Nur, 2006; Dogan et al., 2018; Sinimbu et al., 2022), probably due to differences in breeds, season, insemination method and protocol used. Besides, hCG and GnRH treatment in the present study did not improve pregnancy rate. Similarly, other studies have reported no increased pregnancy rate in ewes treated with hCG(Dias et al., 2018) or GnRH (Reyna et al., 2007; Türk et al., 2008; Cavalcanti et al., 2012; Martinez-Ros and Gonzalez-Bulnes, 2019; Mirzaei et al., 2022) after artificial insemination or natural mating.

CONCLUSIONS

As a consequence,the present study indicated that a single injection of 100 IU hCG or 0.004 mg buserelin acetate (GnRH) at 36 h following estrus synchronized by MAP intravaginal sponge plus eCG and $PGF_{2\alpha}$ treatments had no effect on estrus behaviors, ovarian findings and pregnancy rates in Merino ewes during the breeding season.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ACKNOWLEDGMENTS

The authors would like to thank Prof.Dr. Veysi ASLAN and ALKE Co., Ltd for providing d-cloprostenol ($PGF_{2\alpha}$, PGS®).

FINANCIAL SUPPORT

This study was supported by the Uludag University Scientific Research Projects Centre, Bursa, Turkey (Grant No. TGA-2021-432).

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