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Reproductive Performance and Progesterone Concentration Changes on Mamasani Goats, using Human Chorionic Gonadotropin during 1.5, 2 and 7 Days after Mating on the Spring Season

Research Article

M. Ghaedi[1], J. Habibizad[1*], M. Meamar[1] and M. Muhaghegh-Dolatabady[1]

[1] Department of Animal Science, Faculty of Agriculture, Yasouj University, Yasouj, Iran

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*Correspondence E-mail: j.habibi@yu.ac.ir

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ABSTRACT

Present study was conducted to investigate the effect of different times of using human Chorionic Gonadotropin (hCG) during different days after mating on the reproductive performance and serum progesterone concentration of superovulated Mamasani goats. In this study, 60 goats (3-4 years) with the weight range of 46 ± 2.1 kg were used. The goats were synchronized for a period of 15 days and one day before sponge removal, 400 international units of equine chorionic gonadotropin (eCG) were injected to them. The goats were divided into 4 treatment groups based on not receiving (control) or receiving (350 international units of hCG) on one and a half, two, and 7 days after mating. Blood samples were collected on day 2 before sponging and days 19 and 40 after mating. The results showed that fecundity and prolificacy rate were significantly ($P<0.0001$) higher in the groups receiving hCG during days 1.5 and 2 after mating compared to the other groups. Serum progesterone concentration of pregnant, delivered, single birth and multiple births goats on 19 and 40 days after mating were significantly higher in the groups receiving hCG compared to the control group ($P<0.05$). Serum progesterone concentration of multiple births goats in all groups receiving hCG significantly ($P<0.05$) increased compared to control group in 19 and 40 days of sampling. The results generally showed that reproductive performance and serum progesterone concentration were affected by different times of using hCG and these parameters increased in goats receiving hCG in days 1.5 and 2 after mating.

KEY WORDS eCG, hCG, Mamasani goats, prolificacy rate, reproductive performance, twining rate.

INTRODUCTION

The use of methods that lead to an increase in the ovulation rate and subsequently to an increase in reproductive performance in goats (pregnancy rate, parturition rate and multiple births) can have a significant effect on increasing the amount of incoming generation and subsequently making the goat breeding industry more economical. Among the various methods used to improve goat reproductive performance, equine chorionic gonadotropin (eCG) has a special place. The results of several previous studies showed that the use of progesterone-containing devices to implement the estrus synchronization program, along with eCG in different seasons (reproductive and non-reproductive) led to the improvement of various reproductive activities, including the improvement of the estrous response rate, ovulation rate and finally increase the rate of kidding in different breeds of goats (Contreras-Villarreal *et al.* 2016; Carrillo *et al.* 2019). In a previous study, it has been reported that eCG increases the growth of follicles of differ-

ent sizes by influencing the condition of ovarian follicles, leads to an increase in the rate of ovulation and kidding in goats (Karaca *et al.* 2009).

Although the high half-life of eCG can be considered as an important advantage (one time injection), but this feature causes the presence of a large number of non-ovulatory follicles on the surface of the ovary, that the presence of these follicles can have a negative effect on various parameters of reproduction by increasing the concentration of estradiol (Husein and Ababneh, 2008). In order to eliminate the negative effects of using eCG and stimulating the growth of follicles related to different follicular waves in the reproductive cycle of goats that will not reach the final stage of follicular growth, using human Chorionic Gonadotropin (hCG) which is similar to luteinizing hormone (LH) can have a special effect. It has been reported that hCG inhibits the initial degradation of corpus hemorrhagicum, reducing the number of follicles on the surface of the ovary, and has the property of luteinizing hormone which causes the final growth of follicles and increasing the ovulation rate, concentration of progesterone hormone, pregnancy rate and kidding (Coyan *et al.* 2003; Cortes *et al.* 2021). Despite this, the results of various studies regarding the use of this gonadotropin are contradictory.

Previous studies reported that the use of hCG during 5 days after the onset of estrus did not affect the pregnancy status of goats compared to the control group (Fonseca and Torres, 2005; Fonseca *et al.* 2006). In a study hCG was used at the time of observing signs of estrus and the results showed that it did not affect various parameters of reproduction compared to the control group (Esteves *et al.* 2013). On the other hand, the results of a study about the effect of using hCG on the reproductive performance of different breeds of goats showed that hCG effectively improved the pregnancy rate and the rate of kidding (Rodriguez-Martinez *et al.* 2018). A study investigated the function of the corpus luteum and the pregnancy rate when using hCG on day 7 after the occurrence of estrus in goats superovulated with eCG. The results showed that the use of hCG caused an increase in the number of additional corpus luteum and also significantly increased the pregnancy rate compared to the control group (Cortes *et al.* 2021). The results obtained from another study related to hCG used on 14th day after the artificial insemination program showed that the use of this hormone increases fecundity rate, corpus luteum function and implantation rate (Bustamante-Andrade *et al.* 2021). It can be concluded that the results of different studies regarding the effect of using hCG during different days after mating can be different.

Although various studies have been conducted regarding the effect of hCG on the reproductive performance of different breeds of goats, but so far, there is no report on the use of hCG during different days after mating in a goat breed superovulated with eCG during the spring season. Therefore, determining the use of hCG on different days after mating in goats superovulated with eCG can be valuable and of great importance.

MATERIALS AND METHODS

The present study was carried out in Yasouj city, located in Kohgiluyeh and Boyer-Ahmad province, the laboratory labors were also done in the physiology laboratory of the Department of Animal Sciences, Faculty of Agriculture, Yasouj University, Islamic Republic of Iran (30˚ 41´N latitude and 51˚ 33´E longitude). In order to carry out this study, 60 Mamasani goats with an average age of 3 to 4 years and an average weight of 46 ± 2.1 kg were selected from one large nomadic herd in a village. At all stages of the research, the environmental conditions were the same for all goats. The estrous cycles of all goats were synchronized simultaneously with the use of intravaginal progestin sponges (60 mg Medroxyprogesterone acetate/sponge, Esponjavet, Laboratorios Hipra, S.A., Avda. La Selva, 135, 17170 Amer (Girona) Spain) for a 15-day period. Intramuscular injection of eCG (400 IU, Gonaser, Laboratorios Hipra, S.A., Avda. La Selva, 135, 17170 Amer (Girona) Spain) was done for all experimental goats one day before sponge removal. After the sponges were harvested, the superior and intact bucks were selected by the nomads, whose reproductive ability were determined by the ranchers, and were introduced to the herds with the ratio of 1 to 5. After observing the signs of estrus (100% of the goats showed estrus signs), the selected goats were randomly divided into four experimental groups (15 goats in each group) according to the time of hCG injection after mating. The goats received 300 IU of hCG (Darou Pakhsh Co., Tehran, Iran) intramuscularly, on the 1.5th (hCG-1.5), 2th (hCG-2) and 7th (hCG-7), days after mating (Figure 1). A group of 15 goats received only progesterone sponges and eCG, were considered as control.

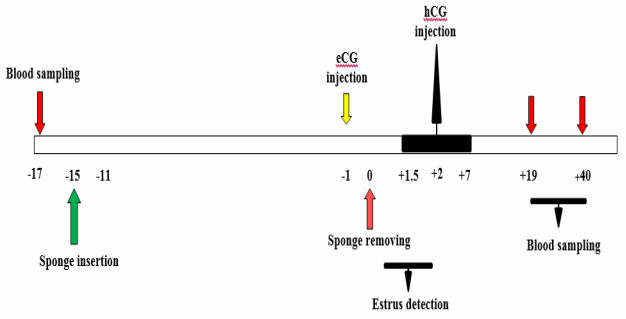

Figure 1 Schematic representation of the activities performed in Mamasani goats

The reproductive parameters measured in this study were: the rate of return to estrus, pregnancy rate (the number of goats that did not show the signs of return to estrus after two 21-day period / the number of mated goats), parturition rate (the number of delivered goats / the number of goats considered pregnant), single birth, multiple births, the number of kids born, the death rate of kids, fecundity rate (the number of kids born / the number of mated goats) and prolificacy rate (the number of kids born / the number of goats delivered) (Sun *et al.* 2024).

Blood samples were collected by jugular venipuncture, before feeding the goats in the morning, 2 days before sponging and days 19 and 40 after mating.

The samples were immediately placed into cool box and then transported to the lab. Then blood samples were centrifuged at 2500 × g for 15 minutes and sera were collected and stored in 0.5 mL microtubules at -20 ˚C until the evaluation of serum progesterone concentration. Serum progesterone concentration was measured by a commercial ELISA kit (ELA-4825-300, Monobind, Inc. Lake Forest, CA (92630) USA) which the sensitivity of the assay was 0.105 ng/mL.

Statistical analysis

Data for the rate of return to estrus, pregnancy and parturition rate; single and multiple births rates were analyzed with the Freq procedure of SAS (2013), based on the chi-square test. Data related to the number of born kids, fecundity and prolificacy rates were analyzed by using the GLM procedure of SAS and the mean values were also compared by the Duncan's multiple range test ($P<0.05$). The data of progesterone concentration were analyzed by Proc Mixed produce of SAS and the means were corrected by least square procedure and compared by Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

The results related to the effect of using hCG during different days after mating on various reproductive parameters are shown in Table 1. In the groups receiving hCG, none of the goats showed signs of return to estrus during two periods of 21 days (equal to the length of the goat's reproductive cycle), but one goat in the control group showed such signs. Proportionally, after two periods of 21 days, all goats receiving hCG and 14 goats from the control group were also considered as pregnant goats. The results related to the number of goats that delivered, showed that all the goats which were considered as pregnant goats in the control group and the groups receiving hCG within 1.5 and 2 days after the occurrence of estrus, delivered at the end of the period. While in the group receiving hCG during the 7th day after the occurrence of estrus, 14 out of 15 heads that were considered as pregnant, delivered at the end of the period (Table 1).

The results showed a statistically significant difference between the different treatment groups regarding the rate of single and multiples births ($P<0.0001$), so that the highest number of single birth was observed in the control group (10 out of 14 goats) and the group receiving hCG on the 7th day (12 out of 14 goats) after the occurrence of estrus. The lowest rate of single birth was observed in the groups receiving hCG during days 1.5 and 2 after the occurrence of estrus. The results related to the rate of multiple births showed that the lowest rate of this parameter was observed in the control group and the group receiving hCG on the 7th day after the occurrence of estrus. Interestingly, the results showed that although the difference between these two groups was not significant, but in the group receiving hCG on the 7th day after the occurrence of estrus, it was less than in the control group (2 compared to 4 vertices). The highest rate of multiple births was observed in the groups receiving hCG during days 1.5 and 2 after the occurrence of estrus, although the difference between these two groups was not significant, but in the group receiving hCG on day 2 was more compared to day 1.5 (13 compared to 12 heads) (Table 1).

The results interestingly showed that in the group receiving hCG on day 1.5, 12 goats delivered multi-births (9 twins, 2 triplets and 1 quadruplets). Regarding the delivery status of the goats in the group receiving hCG on day 2, it showed that 8 (twins); 3 (triplets); 1 (quadruplets) and 1 (quintuplets) delivered at the end of the period. The interesting thing was that, for the first time in the nomadic herds of the region, 5 kids were born, and more interestingly, none of these 5 kids were lost until the kids were sold or kept in the herd as goats.

The results related to the number of kids born, showed that the highest number was observed in the group receiving hCG on day 2 after the occurrence of estrus and the lowest in the group receiving hCG on the 7th day after occurrence of estrus (36 versus 16 heads). From the total of 101 kids born in all different treatment groups, only 2 kids were lost until the end of the period (5-month period), and these two were lost in the early days after birth (Table 1).

The results showed a statistically significant difference between the different treatment groups regarding fecundity and proliferative rate ($P<0.0001$). The results related to these two parameters showed that the highest amount was observed in the group receiving hCG on day 2, which was significantly higher ($P<0.0001$) than the other groups except the group receiving hCG on day 1.5. Although the difference between the two groups receiving hCG during days 1.5 and 2 after the occurrence of estrus was not statistically significant ($P>0.05$) but numerically, it was higher in the

group receiving hCG on the 2nd day after the occurrence of estrus compared to the day 1.5. The results interestingly showed that in the group receiving hCG on the 7th day after the occurrence of estrus, both of these parameters were lower than the control group (Table 1).

The results related to the interaction of treatment and sampling day on changes in serum progesterone concentration in goats pregnant, delivered, single and multiple births are shown in tables 2-5. According to the results, in different reproductive status, the concentration of progesterone on the second day before sponging did not show a significant difference between different treatment groups (P>0.05). The results related to other sampling days (19 and 40) in pregnant and delivered goats showed that serum progesterone concentration was significantly higher in hCG receiving groups than in the control group (P<0.05) (Tables 2 and 3). On the other hand, in goats that received hCG on days 1.5 and 2 after mating, it was significantly higher on both days 19 and 40 than the group that received hCG on day 7 after mating (P<0.05). While the goats that were single and multiple births at the end of the period, the results showed that on the 19th and 40th days after mating, in different treatment groups receiving hCG, serum progesterone concentration was significantly higher than the control group (P<0.05). On the other hand, the concentration of this hormone did not show a significant difference between the treatment groups receiving hCG on different days after mating (1.5, 2 and 7) (P>0.05). But in the group receiving hCG on day 2, it was numerically higher compared to other days receiving hCG.

The comparison of serum progesterone concentration among different days of sampling (days-2, 19 and 40) in each treatment group separately and in different reproductive status showed that the concentration of this hormone was significantly higher on different days of sampling after the occurrence of estrus compared to the period before sponging (day-2) (P<0.05). The results in pregnant goats in all treatment groups and in each treatment separately showed a decreasing trend of serum progesterone concentration changes on day 40 compared to day 19. In all different treatment groups receiving hCG, the difference between the two days was significantly observed (P<0.05). In the control group, although the trend was decreasing, the difference between the two days of sampling was not significant (P>0.05) (Table 2).

The trend of progesterone changes in single birth goats on different days of sampling (19 and 40) in each treatment group showed that although the difference between sampling days was not significant (P>0.05), it was lower on day 40 compared to day 19 of sampling and this process of changes was observed in all different treatment groups separately (Table 4).

The trend of this hormone changes in multiple births goats in the groups receiving hCG (1.5 and 2 days after mating), during different days of sampling (19 and 40), showed a significant difference (P<0.05), so that it was significantly higher on 19th day than on 40th day (P<0.05). However, no significant difference was observed between these two days (19 and 40) in the control group and the group receiving hCG on 7th day after mating (P>0.05) (Table 5).

For the first time, this study has evaluated the effect of using 300 international units of hCG during different days after mating on various parameters of reproduction and changes in progesterone hormone concentration in goats superovulated with eCG during the spring season in nomadic breeding conditions. In this research, in order to stimulate ovulation, 400 international units of gonadotropin of eCG were used one day before progesterone-containing sponge removal. In our previous study, it was suggested to use 500 units of this hormone in order to improve the reproductive performance of goats during the spring season (Habibizad *et al.* 2020). But the reproductive performance of goats receiving this level of hormone was negatively affected compared to the level of 400 units (return to estrus, pregnant and parturition rate). Therefore, it was suggested to use 400 units of this hormone instead of 500 international units of eCG in this breed. It has been reported that the level of eCG used to improve goat reproductive performance can vary from 300 to 600 international units, but it has been suggested that levels of more than 400 eCG units are not recommended for dairy goats because it may lead to reduced pregnancy rates or embryo death (Wildeus, 2000). The use of eCG during the non-breeding season in order to stimulate estrus and ovulation in different breeds of goats has been recommended (Fonseca *et al.* 2017; Gonzalez-Bulnes *et al.* 2020).

Early embryonic loss is an important cause of economic loss and one of the main factors of reducing economic income in goat breeding system (Dogruer *et al.* 2012). In small ruminants, this rate of loss varies from 8.9 to 20.1 percent in sheep (Regassa *et al.* 2007; Saribay and Erdem, 2007) and from 10.8 to 11.0 percent in goats (Engeland *et al.* 1998; Anvar and Ahmad, 1999).

It has been suggested that part of this loss is due to the lack of synchronization between the embryo and the uterine endometrium or insufficient production of interferon tau (IFN-τ) by the embryo (Martinod *et al.* 1991). In animals whose estrus is stimulated, the pregnancy losses due to the formation and insufficient ability to secrete the corpus luteum can reach up to 60% (Gordon, 1997). During the first three weeks of pregnancy, 30-40% of fertilized eggs in sheep and goats are lost (Nancarrow, 1994; Cam and Kuran, 2004).

Table 1 The effect of different times of hCG injection on reproductive performance of Mamasani goats supperovulated with eCG

Parameter	Experimental groups[1]				P-value
	eCG	eCG+hCG$_{1.5}$	eCG+hCG$_2$	eCG+hCG$_7$	
Return to estrus (n)	1/15	0/15	0/15	0/15	0.38
Pregnant rate (n)	14/15	15/15	15/15	15/15	0.38
Parturition rate (n)	14/14	15/15	15/15	14/15	0.39
Single birth (n)	10/14^{a}	3/15^{b}	2/15^{b}	12/14^{a}	0.0001
Multi births (n)	4/14^{b}	12/15^{a}	13/15^{a}	2/14^{b}	0.0001
Number of kids	18	31	36	16	-
Death of kids (n)	1	1	2	0	-
Fecundity (%)	1.2 (120)^{bc}	2.07 (206.6)^{ab}	2.4 (240.0)^{a}	1.06 (106.6)^{c}	0.0001
Prolificacy (%)	1.29 (129)^{bc}	2.07 (206.6)^{ab}	2.4 (240.0)^{a}	1.14 (114.2)^{c}	0.0001

[1] eCG: the group received 400 IU of eCG one day before removing the sponge; eCG + hCG$_{1.5}$: the group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 1.5nd day after mating; eCG + hCG$_2$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 2nd day after mating and eCG + hCG$_7$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 7th day after mating.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 2 The interaction between treatment and day of sampling on serum progesterone concentration (ng/mL) in pregnant Mamasani goats (means±SD)

Sampling days*	Experimental groups[1]			
	eCG	eCG+hCG$_{1.5}$	eCG+hCG$_2$	eCG+hCG$_7$
-2	0.82±0.16^{Ba}	0.81±0.12^{Ca}	0.78±0.16^{Ca}	0.80±0.16^{Ca}
19	7.83±0.96^{Ac}	11.74±1.08^{Aa}	12.31±0.94^{Aa}	10.56±1.18^{Ab}
40	7.39±0.94^{Ac}	10.60±1.14^{Ba}	11.24±0.93^{Ba}	9.42±1.40^{Bb}

[1] eCG: the group received 400 IU of eCG one day before removing the sponge; eCG + hCG$_{1.5}$: the group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 1.5nd day after mating; eCG + hCG$_2$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 2nd day after mating and eCG + hCG$_7$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 7th day after mating.
* Sampling days: different days of blood sampling sponge insertion (-2) and after mating (19 and 40).
$^{a, b, c}$: the means within the same row with different letter, are significantly different (P<0.05).
$^{A, B, C}$: the means within the same column with different letter, are significantly different (P<0.05).

Table 3 The interaction between treatment and day of sampling on serum progesterone concentration (ng/mL) in delivered Mamasani goats (means±SD)

Sampling days*	Experimental groups[1]			
	eCG	eCG+hCG$_{1.5}$	eCG+hCG$_2$	eCG+hCG$_7$
-2	0.82±0.16^{Ba}	0.81±0.12^{Ca}	0.78±0.16^{Ca}	0.79±0.16^{Ca}
19	7.83±0.96^{Ac}	11.74±1.08^{Aab}	12.31±0.94^{Aa}	10.72±1.05^{Ab}
40	7.39±0.94^{Ac}	10.60±1.14^{Bab}	11.24±0.93^{Ba}	9.69±0.95^{Bb}

[1] eCG: the group received 400 IU of eCG one day before removing the sponge; eCG + hCG$_{1.5}$: the group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 1.5nd day after mating; eCG + hCG$_2$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 2nd day after mating and eCG + hCG$_7$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 7th day after mating.
* Sampling days: different days of blood sampling sponge insertion (-2) and after mating (19 and 40).
$^{a, b, c}$: the means within the same row with different letter, are significantly different (P<0.05).
$^{A, B, C}$: the means within the same column with different letter, are significantly different (P<0.05).

Table 4 The interaction between treatment and day of sampling on serum progesterone concentration (ng/mL) in single birth Mamasani goats (means±SD)

Sampling days*	Experimental groups[1]			
	eCG	eCG+hCG$_{1.5}$	eCG+hCG$_2$	eCG+hCG$_7$
-2	0.83±0.14^{Ba}	0.86±0.15^{Ba}	0.85±0.05^{Ba}	0.81±0.16^{Ba}
19	7.43±0.73^{Ab}	11.36±1.10^{Aa}	12.00±0.60^{Aa}	10.59±1.10^{Aa}
40	7.03±0.73^{Ab}	10.20±1.05^{Aa}	10.85±0.35^{Aa}	9.58±0.98^{Aa}

[1] eCG: the group received 400 IU of eCG one day before removing the sponge; eCG + hCG$_{1.5}$: the group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 1.5nd day after mating; eCG + hCG$_2$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 2nd day after mating and eCG + hCG$_7$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 7th day after mating.
* Sampling days: different days of blood sampling sponge insertion (-2) and after mating (19 and 40).
$^{a, b, c}$: the means within the same row with different letter, are significantly different (P<0.05).
$^{A, B, C}$: the means within the same column with different letter, are significantly different (P<0.05).

Table 5 The interaction between treatment and day of sampling on serum progesterone concentration (ng/mL) in multi births Mamasani goats (means±SD)

Sampling days*	Experimental groups[1]			
	eCG	eCG+hCG$_{1.5}$	eCG+hCG$_2$	eCG+hCG$_7$
-2	0.80±0.21^{Ba}	0.80±0.12^{Ca}	0.77±0.17^{Ca}	0.70±0.14^{Ba}
19	8.85±0.71^{Ab}	11.83±1.10^{Aa}	12.36±0.98^{Aa}	11.40±0.28^{Aa}
40	8.30±0.82^{Ab}	10.70±1.18^{Ba}	11.30±0.97^{Ba}	10.35±0.91^{Aa}

[1] eCG: the group received 400 IU of eCG one day before removing the sponge; eCG + hCG$_{1.5}$: the group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 1.5nd day after mating; eCG + hCG$_2$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 2nd day after mating and eCG + hCG$_7$: the group received 400 IU of eCG one day before removing the sponge and hCG injection on the 7th day after mating.
* Sampling days: different days of blood sampling sponge insertion (-2) and after mating (19 and 40).
$^{a, b, c}$: the means within the same row with different letter, are significantly different (P<0.05).
$^{A, B, C}$: the means within the same column with different letter, are significantly different (P<0.05).

In a study, the results showed that about 11% of the goats had fetal losses after the initial pregnancy (Engeland *et al.* 1998). The critical period for fetal detection or pregnancy detection by the mother in goats occurs around the 15th day of pregnancy (Weise *et al.* 1993). The presence of the corpus luteum and the progesterone produced by it are necessary to create and maintain pregnancy until the end of the pregnancy period in goats, and its absence leads to the loss of pregnancy at any stage (Meites *et al.* 1951). Therefore, it has been concluded that the insufficient function of the corpus luteum, followed by the insufficient concentration of progesterone at the beginning of pregnancy, is one of the main and important factors of early fetal loss that leads to the occurrence of fetal death during the critical period of pregnancy diagnosis by the mother or the previous period from implantation (Engeland *et al.* 1998).

Despite several reports suggesting the use of eCG to improve reproductive performance in different breeds of goats synchronized with progesterone-containing devices, the results obtained in different studies are contradictory. Therefore, one of the practical solutions for more effective use (increasing progesterone concentration and improving reproductive performance) during the use of eCG and solving the problems raised in connection with the use of this hormone, as well as an effective solution for reducing early embryonic losses in goats, is the use of hCG after mating in goats. Although hCG is not produced in livestock, its role in various animal species includes increasing the lifespan of the corpus luteum, increasing the synthesis of progesterone by the corpus luteum, stimulating ovulation, and helping to form an additional corpus luteum (Rajamahendran and Sianangama, 1992). However, it is unclear what day hCG should be used after mating, and the results of various studies in this field are contradictory. In the current study, the choice of different days for hCG injection was based on the investigations and results obtained from previous studies that reported that follicular dynamics occur in the form of follicular waves in the reproductive cycle of different breeds of goats (Ginther and Kot, 1994; De Castro *et al.* 1999). In addition, due to the presence of LH receptors in various parts of the reproductive system, hCG can increase or improve the secretory function of the oviduct and uterus during the pre-embryonic and pre-implantation periods (Mishra *et al.* 2003).

The results of a previous study on the use of hCG during the 5th day after mating in Sanen and Alpine goats showed that the use of this hormone, despite the existence of a significant difference between the two breeds in relation to the concentration of the progesterone hormone, but it did not show a statistically significant difference between these two breeds in relation to various parameters of reproduction, including the rate of pregnancy, delivery and prolificacy.

On the other hand, statistically significant differences were not observed in both breeds when using hCG compared to the control group, not only in relation to progesterone hormone concentration but also in relation to various parameters of reproduction (Fonseca and Torres, 2005). In a recent study, there was not observed any significant difference in the number of corpora lutea when using hCG or not using it after mating. The lack of observed differences in this study was attributed to the intravaginal use of hCG instead of intramuscular injection (Rodrigues *et al.* 2023). The results of the study by Saleh *et al.* (2012) showed that the use of hCG intramuscularly has a fast absorption rate, but on the other hand, it is slowly removed from the body system (Saleh *et al.* 2012). In order to investigate the detectable time of the presence of hCG in the blood circulation after using this hormone by injection or intravaginally in the sponge, a study used the β-hCG rapid detection test and they reported that when using hCG by injection or intravaginally, it can be detected in all goats 3 and 72 hours after injection or treatment, respectively. The results of a recent study in Sanen goats during the reproductive season showed that the use of hCG within 24 to 36 hours after the removal of the progesterone-containing sponge did not affect the concentration of the progesterone hormone (Dogan *et al.* 2023). The results of another recent study showed that the use of hCG one day after the occurrence of estrus (artificial insemination day) did not increase the concentration of progesterone hormone and did not improve the pregnancy rate in goats (Rodrigues *et al.* 2022b).

Contrary to the results of some studies, which reported no effect of hCG on the reproductive performance of different breeds of goats, the results of some other studies have shown that the use of hCG during different days after mating can have positive effects on the changes of progesterone concentration and various parameters of reproduction. It has been reported in various studies that the use of hCG can cause the formation of an additional corpus luteum (Fonseca *et al.* 2018; Vergani *et al.* 2020; Cortes *et al.* 2021; Rodrigues *et al.* 2022a), which increase the concentration of progesterone hormone (Fonseca *et al.* 2006; Vergani *et al.* 2020; Rodrigues *et al.* 2022a) and improving reproductive performance in small ruminants, especially increasing the pregnancy rate (Cam and Kuran, 2004; Gomez-Brunet *et al.* 2007; Khan *et al.* 2009; Fonseca *et al.* 2021). The results of a study using 300 international units of hCG during the 7th day after mating in the transition period of goats to the breeding season showed that the pregnancy rate was significantly higher in the group receiving hCG than in the control group. On the other hand, all goats receiving hCG with extra corpus luteum and 83% of goats without extra corpus luteum delivered by the end of the gestation period (Cortes *et al.* 2021). In another previous

study, it has been reported that the use of hCG at the beginning of pregnancy in dairy goats can improve the pregnancy rate by increasing the volume and blood flow of the corpus luteum, stimulating the formation of additional corpus luteum, and thus stimulating the increase in the concentration of progesterone hormone (Rodrigues *et al.* 2022a).

The results of the present study showed that the use of hCG during different days after mating, despite the positive effect on changes in progesterone concentration, can have different effects on various parameters of reproduction, including the rate of multiple births, fecundity and prolificacy. Therefore, it is very important to find the best time of using hCG after mating in goats, so obtaining such results showed the importance of time using hCG in order to improve reproductive performance in goats. The results of present study showed when using this gonadotropin during days 1.5 and 2 after mating compared to day 7, it can have positive effects on various parameters of reproduction. On the other hand, when hCG was used on the 7th day after mating, despite the increase in progesterone concentration, reproductive parameters did not improve but decreased compared to the control group. In the present study, the use of hCG on days 1 and 2 after mating, increased progesterone concentration and improved reproductive performance compared to the control group. In contrast to the results obtained in the present study, several previous studies reported that the use of hCG had no effect on various reproductive parameters including pregnancy rate (Fonseca and Torres, 2005; Fonseca *et al.* 2006). The reason for the observed difference can be attributed to the level of hCG used and the time of injection in the present study compared to previous studies. Reproductive performance improved in the groups receiving hCG during days 1.5 and 2 after mating. It is suggested that in addition to the follicles that were growing due to the high half-life of eCG a follicular wave has also occurred, so it is expected that a large number of follicles will be present on the surface of the ovary during these days. Therefore, with the administration of hCG during these days, which has a structural similarity with LH (about 80%), the number of ovulation and the concentration of progesterone increased. So various parameters of reproduction, including the rate of multiple births, fecundity and prolificacy improved. It has been reported that human placental gonadotropin has a similar structure to LH and binds to its receptors and has a longer half-life and faster absorption than LH (36 hours versus 11.6 hours) (Saleh *et al.* 2012). Therefore, it can be inferred that administered hCG can have a long-term effect on the reproductive organs of LH-responsive goats, potentially affecting both preovulatory ovarian follicles and the developing corpus luteum (Rodrigues *et al.* 2023).

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

Follicular dynamics during the goat reproductive cycle can vary by breed, season, and management (Gonzalez-Bulnes *et al.* 1999). It has been reported in several previous studies that the growth of ovarian follicles throughout the reproductive cycle is carried out in a follicular wave-like pattern (Ginther and Kot, 1994; De Castro *et al.* 1999). The results several studies in different breeds of goats indicated different follicular wave (two to five) on different days of the reproductive cycle (Schwarz and Wierzchos, 1999; Medan *et al.* 2003). On the other hand, in follicular waves with 2 to 5 cases, a follicular wave is observed on day 1 after mating or estrus (Ginther and Kot, 1994). In a previous study, no difference was observed in the onset of the first and second wave for estrous cycles with 3, 4 and 5 follicular waves (Osman *et al.* 2021). Therefore, it is possible that the present results are because of using hCG during days 1.5 and 2 after mating which different types of follicles exist during these days.

In a previous study, it has been reported that the use of hCG can effectively cause ovulation from the dominant follicle of the first follicular wave, which causes the formation of an additional corpus luteum that increases the concentration of progesterone (Fonseca *et al.* 2001). Therefore, if hCG is used during 1.5 or 2 days after mating due to the presence of follicles, it can increase the ovulation rate by affecting them and increase the concentration of progesterone which improves reproductive performance. On the other hand, ovulation occurs on 1.5 or 2 days after mating, adequate sperms with high fertility power are present in the reproductive system of goats. Therefore, the rate of pregnancy and multiple births has increased, in case on the 7th day, with the possibility the appearance of the follicular wave, because during this time period, despite the increase in ovulation, there is no sperm with fertility in the reproductive system during this time period. This could be a suggestion that despite the increase in progesterone concentration when using hCG on day 7 after mating, reproductive performance did not improve.

It has been reported that the mechanism by which hCG improves reproductive performance is through affecting the number of additional corpus luteum created through stimulation of ovulation from dominant follicles due to its structural and functional similarity with LH, effects on the growth and transformation of small luteal cells into large luteal cells and thus increasing the steroidogenic capacity of the corpus luteum, direct effects on the uterine endometrium and embryo viability or through effect on preventing the initial disappear of corpus hemorrhagicum or corpus

luteum (Sanchez *et al.* 2018; Agarwal *et al.* 2021; Cortes *et al.* 2021). The results in a previous study showed that in none of the goats receiving hCG, the primary disappearance of the corpus luteum did not occur, while in the control goats, this parameter was 57.5% (Fonseca *et al.* 2005). It has been assumed that the pregnancy rate obtained in animals whose estrus is artificially stimulated is lower than when estrus occurs naturally due to the insufficient development of the corpus luteum and as a result the synthesis and secretion of progesterone (Fonseca *et al.* 2005). If such an issue is raised as a fundamental problem, then in the present study, a luteotropic agent such as hCG was used, which can create a maternal environment for pregnancy, preservation and development of the embryo by increasing the concentration of progesterone hormone. Therefore, the increase in progesterone concentration during the first days after mating, when the corpus luteum is not yet fully developed, can play an important role in creating and maintaining pregnancy and, as a result, increasing reproductive performance. In this study, the higher concentration of progesterone observed in goats treated with hCG during days 1.5 and 2 due to the possibility of the presence of medium follicles on the surface of the ovary.

CONCLUSION

The results of this study generally showed that:

(i): The rate of multiple births, fecundity, proliferative rate and concentration of serum progesterone (19 and 40 days after mating) were significantly higher in hCG receiving groups during days 1.5 and 2 after mating compared to other groups.

(ii): The concentration of serum progesterone on different days of sampling (19 and 40 after mating) was significantly higher in pregnant, parturition, single and multiple goats in the groups receiving hCG compared to the control group.

(iii): The results of this study generally showed that different parameters of reproduction and progesterone concentration were affected by different times of hCG use, so hCG can be used to improve reproductive performance and progesterone concentration of goats on days 1.5 and 2 after mating.

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