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Effect of GnRH and hCG injection on the reproductive performance and serum progesterone concentration of ewes during spring season

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Abstract

This study was carried out to investigate the effect of different hormonal programs on the reproductive parameters and serum progesterone concentration of Lake-Ghashghaei ewes in spring season. In this study, 144 ewes were selected from two large nomadic herds. The ewes were synchronized using progesterone sponges. One day before harvesting the sponges, all ewes were injected intramuscularly with 400 IU of eCG. Then ewes were divided into six groups (12 ewes). In the first herd, 1 (GnRH-1 group), 2 (GnRH-2 group), 5 (GnRH-5 group), 7 (GnRH-7 group) and 12 days (GnRH-12 group) after estrus observation, 25.00 µg GnRH was injected intramuscularly to the ewes. In the second herd, the ewes were received 500 IU of hCG on days 1 (hCG-1), 2 (hCG-2), 5 (hCG-5), 7 (hCG-7) and 12 (hCG-12). In each herd, a group of 12 ewes received only eCG and progesterone sponges and was considered as control group. The results showed that twinning rate, the number of born lambs, fecundity and prolificacy rate were higher GnRH and hCG received groups on the second day compared to other groups. The serum progesterone concentration was significantly higher in all groups received GnRH and hCG compared to control group. In general, the results showed that GnRH and hCG two days after mating improved different reproductive activities in addition to increasing serum progesterone concentration. On the other hand, hCG had a greater effect on progesterone concentration, the number of born lambs and the fecundity rate compared to GnRH.

Introduction

Embryonic loss is one of the main reasons for limiting the maximum yield of reproduction in farm animals and it has been reported that the highest number of pregnancy losses occurs in different mammal species at the beginning of pregnancy.[1] In sheep, 30.00 - 40.00% of fertilized eggs are lost during the first three weeks of pregnancy[2] and 70.00 - 80.00% of losses occur between 8 - 16 days after insemination or mating days.[3] Several factors can contribute to this condition, the most important one is the insufficient secretion of progesterone by the corpus luteum,[4] because this hormone is needed in all mammalian species to create and maintain pregnancy[5] and it regulates the vital functions of the endometrium for implantation and placental formation.[6] On the other hand, it has been reported that low concentration of progesterone after mating lead to the production of small and undeveloped embryos which are unable to produce enough interferon-tau during the days of pregnancy detection by mother.[7]

Since the 1980s, various treatment strategies have been used to improve the function of corpus luteum, reduce embryonic mortality during the early days of pregnancy and improve reproductive function.[8] Previous studies have investigated the effect of ovulatory stimuli like gonadotropin releasing hormone (GnRH) and human chorionic gonadotropin (hCG) on stimulating the formation of excess corpus luteum and increasing progesterone concentration, thus, improving the reproductive function of different sheep breeds during different days after mating as an effective solution.[9,10] However, the results are not favorable with the best time to inject these hormones in superovulated ewes with equine chorionic gonadotropin (eCG). It has been reported that the time of GnRH and hCG injection during different days after mating could be very important.[11] On the other hand, the time of hormone therapy, especially up to 12 days after mating or artificial insemination, to increase the

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concentration of progesterone, could be important because it is proportional to the beginning of the lysis of the corpus luteum in the normal estrous cycle.[12] Several studies indicated that applying GnRH on different days after mating in superovulated ewes during reproductive and non-reproductive seasons improved reproductive function, especially ovulation rate, lambing rate and progesterone concentration.[13-16] On the other hand, it has been reported that the use of hCG causes increasing the number of excess corpus luteum on the ovarian surface, increasing luteal tissue volume, increasing progesterone concentration, improving embryonic viability, increasing pregnancy rate and ultimately improving reproductive performance of different breeds of sheep.[5,17,18]

However, some other researchers did not report any significant improvement in pregnancy, fecundity, or multiple birth rates in different breeds of sheep when using GnRH or hCG.[8,19] One study reported that using hCG induced multiple ovulation rates and increased serum progesterone levels compared to the control group, however, various reproductive activities including pregnancy and prolificacy rates were not significantly affected by hormonal treatment.[20] The results of a recent study conducted to investigate the effect of hCG on ovarian response and pregnancy rate showed that the use of this gonadotropin improved ovulation synchronization, however, reduced the pregnancy rate compared to the control group.[21]

Although these hormones have been used in various studies as an effective way to improve reproductive function, however, the results regarding the use of these hormones in different days of estrous cycle and under various experimental conditions have been variable, contradictory and sometimes ineffective.[5] On the other hand, on the basis of current knowledge there is a limited report about the effect of using these hormones on different days after mating during the spring season and comparing the efficiency of using then separately**.** Therefore, the initial object of this study was to determine the best time for using GnRH and hCG after mating on the serum progesterone concentration and various reproductive activities in ewes which was not previously described. The second object was to compare GnRH to hCG used on different days of estrous cycle in ewes super-ovulated with eCG during the spring season.

Materials and Methods

Site of study and breed description. Kohgiluyeh and Boyer-Ahmad province is a mountainous land and Lake-Ghashghaei is the dominant sheep breed in this province. In this province, pasture forage is available for long times in the year. The body color is brown and white. The physical characteristics and productive performances of Lake-Ghashghaei ewes include an adult live weight of 51.00 - 59.00 kg, fat-tail weight of 1.90 - 3.60 kg and milk yield of 45.00 – 68.00 liters per 5 months. Average body weight of rams is 72.00 to 78.00 kg. The ewes are seasonally polyestrous and the breeding season for this breed is from late summer to late autumn and births normally begin in early spring. The ewes usually bear a single offspring. High tolerance to mountainous conditions and highly palatable meat are the other notable characteristics of Lake-Ghashghaei breed.

Animals and experimental procedures. The present study was carried out in Dehdasht city, located in Kohgiluyeh and Boyer-Ahmad province, and laboratory works were 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, 144 Lake-Ghashghaei ewes with an average age of 3 to 4 years and an average weight of 54.00 ± 2.60 kg were selected from two large nomadic herds in a village (72 ewes per herd). At all stages of the research, the environmental conditions were the same for all ewes**.** The estrous cycles of all ewes in both herds were synchronized simultaneously with the use of intravaginal Medroxy-progesterone acetate sponges (60.00 mg; Hipra, Girona, Spain) for a 14-day period. Intramuscular injection of 400 IU eCG (Hipra) was done for all experimental ewes one day before sponge removal. After the sponges were harvested, the superior and intact rams were selected by the nomads, whose reproductive ability were determined by the ranchers and were introduced to the herds with the ratio of one to eight. After observing the signs of estrus (100% of the ewes showed estrus signs), the selected ewes in each herd were randomly divided into six experimental groups (12 ewes in each group) according to the time of GnRH injection after estrus observation. The ewes received 25.00 µg GnRH (Ceva Sante Animale, Libourne, France) intramuscularly on the first (GnRH-1), 2nd (GnRH-2), 5th (GnRH-5), 7th (GnRH-7), and 12th (GnRH-12) days after estrus observation, respectively. In the second herd the ewes were treated by injection 500 IU hCG (Darou Pakhsh Co., Tehran, Iran) intramuscularly on the first (hCG-1), 2nd (hCG -2), 5th (hCG -5), 7th (hCG -7), and 12th (hCG -12) days after estrus observation, respectively. In each herd, a group of 12 ewes received only progesterone sponges and eCG, were considered as control. The reproductive parameters measured for each herd in this study were the rate of return to estrus (number of ewes that did show the signs of estrus after two 17-day periods / number of mated ewes), pregnancy rate (number of ewes that did not show the signs of return to estrus after two 17-day periods / number of mated ewes), parturition rate (number of lambed ewes / number of mated ewes), single birth (n), multi births (n), number of lambs born, death rate of lambs (n), fecundity rate (number of lambs

born / number of mated ewes) and prolificacy rate (number of lambs born / number of ewes lambed).[9,22]

Blood sampling and progesterone analysis. In both herds, blood samples were collected by jugular venipuncture before feeding the ewes in the morning on days 15 and 32 after mating. The samples were immediately placed into cool box and then transported to the lab. Then blood samples were centrifuged at 2,500 *g* for 15 min and serum was collected and stored in 0.50 mL microtubules at – 20.00 ˚C until the evaluation of serum progesterone concentration. Serum progesterone concentration was measured by a commercial ELISA kit (Monobind Inc., Lake Forest, USA) which the sensitivity of the assay was 0.105 ng mL^{-1} and intra- and inter assay coefficient of variation were 4.50% and 9.40%, respectively.

Statistical analysis. In each flock, data for the rate of return to estrus pregnancies and parturitions rate, single, twinning, and multiple births rates were analyzed with the Freq procedure of SAS Software (version 9.10; SAS Institute, Cary, USA), based on the chi-square test. Data related to number of born lambs, 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

The results related to the effect of using GnRH on different days after mating on reproductive performance of ewes are shown in Table 1. There were not any significant difference in the pregnancy rate, parturition rate and the return to estrus among the experimental groups. The results showed that the twinning rate in the GnRH-2 group was significantly higher than the other groups ($p < 0.001$), on the other hand, it was not observed any twining birth on GnRH-12 group. The highest number of born lambs (18 Lambs) and the fecundity and prolificacy rate of GnRH-2 group were significantly higher than the other groups ($p < 0.02$; Table 1).

The results related to the changes of serum progesterone levels are shown in the Tables 2. It shows that serum progesterone concentrations of pregnant, delivered and single birth ewes on 15 and 32 days after mating in all GnRH receiving groups were increased significantly ($p < 0.05$) compared to the control group. On the other hand, serum progesterone concentrations of pregnant, delivered and single birth ewes in all experimental groups were significantly higher on 15 days after mating compared to 32 days of after mating (Table 2).

Table 1 shows the effect of different times of hCG injection on reproductive performance of Lake-Ghashghaei ewes. The results showed that there were not any significant difference in the pregnancy rate, parturition rate and the return to estrus among all experimental groups. On the other hand, the highest number of delivered ewes in the hCG receiver group was on day 2, so that all ewes (12 / 12 ewes) that showed signs of estrus also delivered at the end of the period. Based on the results the multiplicity rate in the hCG receiver group was significantly higher on the second day after mating than in the other groups ($p < 0.009$), however, no significant differences were observed between hCG receiving groups on days one and two after mating. The results showed that hCG-2 group was the only treatment group that had triple births. In this regard, the highest number of born lambs (24 lambs), the fecundity and prolificacy rate in hCG-2 group were significantly higher than the other experimental groups except for the hCG-1 group ($p < 0.0001$). The results showed that none of the 89 born lambs died in all experimental groups after one month (Table 1).

The results of the interaction between treatment and sampling day on the changes of serum progesterone concentrations in pregnant, delivered and single birth-ewes received hCG in different days after mating are shown in Table 2. The results showed that serum progesterone concentration in different sampling days (15 and 32 days after mating) was significantly higher in all hCG receiving group compared to the control group ($p < 0.05$). On the other hand, comparison of the progesterone concentrations treatment groups in different sampling days (15 and 32 days after mating) indicated that serum progesterone concentration on day 32 was significantly lower than day 15 in all experimental groups expect for the control group (Table 2).

The results related to the pairwise comparison of reproductive performance of ewes on the same days (using GnRH and hCG on the same days after mating) showed that the number of born lambs and the fecundity rate in hCG-receiving group on the first day was significantly higher than the use of GnRH on the same day ($p < 0.04$, Table 3). The results of changes in the concentration of progesterone in pregnant, delivered and single birth ewes between the two treatment groups showed that the concentration of this hormone in the hCG group on day one was significantly higher than the GnRH group on the same day ($p < 0.05$; Table 3).

In Table 4, the results of a pairwise comparison of reproductive performance of ewes on the same days (GnRH and hCG use on day two after mating) showed that the number of born lambs and the fecundity rate in hCG-receiving group had tendency to significant ($p = 0.06$) difference compared to GnRH-receiving group. However, the changes of serum progesterone concentrations in pregnant, delivered and single birth ewes were significantly higher in the hCG-receiving group on day two than in the GnRH-receiving group on the same day ($p < 0.05$; Table 4).

Table 1. Effect of different times of GnRH and hCG injection on reproductive performance of Lake-Ghashghaei ewes superovulated with eCG (n =12).

	Parameter	eCG	GnRH-1	GnRH-2	GnRH-5	GnRH-7	GnRH-12	*p*-value
GnRH	Return to estrus (n)	1/12	1/12	1/12	2/12	2/12	1/12	0.95
	Pregnancy rate (n)	11/12	11/12	11/12	10/12	10/12	11/12	0.95
	Parturition rate (n)	10/11	10/11	11/11	10/10	9/10	9/11	0.62
	Single birth (n)	$9/10^{a}$	$8/10^{a}$	$4/11^{b}$	$9/10^{a}$	$8/9^{a}$	$9/9^{a}$	0.01
	Twin births (n)	$1/10^{b}$	$2/10^{b}$	$7/11^{a}$	$1/10^{b}$	$1/9^{b}$	$0/9^{b}$	0.001
	Number of lambs	11^{b}	12^{b}	18^{a}	11^{b}	10^{b}	9^{b}	0.02
	Death of lambs (n)	0	0	0	0	0	0	-
	Fecundity rate (%)	0.92 (91.60)^{b}	1 (100)^{b}	1.50 (150)^{a}	0.92 (91.60)^{b}	0.83 (83.30)^{b}	0.75 (75.00)^{b}	0.02
	Prolificacy rate (%)	1.10 (110)^{b}	1.20 (120)$_{b}$	1.64 (164)$_{a}$	1.10 (110)^{b}	1.11 (111)^{b}	1.00 (100)^{b}	0.002
		eCG	**hCG-1**	**hCG-2**	**hCG-5**	**hCG-7**	**hCG-12**	***p*-value**
hCG	Return to estrus (n)	2/12	0/12	0/12	1/12	1/12	1/12	0.38
	Pregnancy rate (n)	10/12	12/12	12/12	11/12	11/12	11/12	0.38
	Parturition rate (n)	10/10	11/12	12/12	11/11	11/11	10/11	0.54
	Single birth (n)	$8/10^{ab}$	$5/11^{bc}$	$2/12^{c}$	9/11ab	$10/11^{a}$	$9/10^{a}$	0.0005
	Multi births (n)	$2/10^{bc}$	6/11ab	$10/12^{a}$	$2/11^{bc}$	$1/11^{c}$	$1/10^{c}$	0.009
	Number of lambs	12^{c}	17ab	24^{a}	13^{bc}	12^{c}	11^{c}	0.0001
	Death of lambs (n)	0	0	0	0	0	0	-
	Fecundity rate (%)	1.00 (100)^{c}	1.42 (142)^{ab}	2.00 (200)^{a}	1.08 (108)^{bc}	1.00 (100)^{c}	0.92 (91.60)^{c}	0.0001
	Prolificacy rate (%)	1.20 (120)^{b}	1.54 (155)^{ab}	2.00 (200)^{a}	1.18 (118)^{b}	1.09 (109)^{b}	1.10 (110)^{b}	0.0001

eCG: The group received 400 IU of eCG one day before removing the sponge; GnRH-1: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the first day after estrus; GnRH-2: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 2nd day after estrus; GnRH-5: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 5th day after estrus; GnRH-7: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 7th day after estrus; GnRH-12: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 12th day after estrus; hCG-1: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the first day after estrus; hCG-2: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 2nd day after estrus; hCG-5: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 5th day after estrus; hCG-7: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 7th day after estrus; hCG-12: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 12th day after estrus.

abc Different letters superscripts (a,b) within a rows among treatments are statistically significant ($p < 0.05$).

Table 2. Interaction between treatment and day of sampling on serum progesterone concentration (ng mL^{-1}) in pregnant, delivered and single birth Lake-Ghashghaei ewes (LS means ± SE).

	Positions	Sampling days	Treatments					
			eCG	**GnRH-1**	**GnRH-2**	**GnRH-5**	**GnRH-7**	**GnRH-12**
GnRH	Pregnant	15	5.58 ± 0.25^{Ba}	8.11 ± 0.20^{Aa}	8.45 ± 0.24^{Aa}	7.60 ± 0.26^{Aa}	7.67 ± 0.24^{Aa}	7.39 ± 0.25^{Aa}
		32	5.17 ± 0.25^{Ba}	7.05 ± 0.25^{Ab}	7.95 ± 0.22^{Ab}	7.12 ± 0.25^{Aa}	7.13 ± 0.26^{Ab}	6.50 ± 0.22^{Ab}
	Delivered	15	5.68 ± 0.26^{Ba}	8.20 ± 0.24^{Aa}	8.45 ± 0.24^{Aa}	7.60 ± 0.26^{Aa}	7.69 ± 0.27^{Aa}	7.45 ± 0.26^{Aa}
		32	5.27 ± 0.25^{Ba}	7.12 ± 0.26^{Ab}	7.95 ± 0.22^{Ab}	7.12 ± 0.25^{Aa}	7.24 ± 0.27^{Aa}	6.64 ± 0.26^{Ab}
	Single birth	15	5.61 ± 0.21^{Ba}	8.08 ± 0.21^{Aa}	7.64 ± 0.33^{Aa}	7.29 ± 0.23^{Aa}	7.50 ± 0.23^{Aa}	7.45 ± 0.22^{Aa}
		32	5.13 ± 0.21^{Ba}	6.93 ± 0.21^{Ab}	7.05 ± 0.33^{Aa}	6.79 ± 0.23^{Aa}	7.03 ± 0.23^{Aa}	6.64 ± 0.22^{Ab}
			eCG	**hCG-1**	**hCG-2**	**hCG-5**	**hCG-7**	**hCG-12**
hCG	Pregnant	15	5.48 ± 0.19^{Ba}	10.52 ± 0.21^{Aa}	10.45 ± 0.22^{Aa}	10.20 ± 0.21^{Aa}	9.82 ± 0.32^{Aa}	9.91 ± 0.29^{Aa}
		32	5.34 ± 0.23^{Ca}	9.43 ± 0.21^{ABb}	10.28 ± 0.22^{Aa}	9.50 ± 0.23^{ABb}	9.26 ± 0.28^{ABb}	8.96 ± 0.22^{Bb}
	Delivered	15	5.48 ± 0.19^{Ba}	10.65 ± 0.21^{Aa}	10.45 ± 0.22^{Aa}	10.20 ± 0.21^{Aa}	9.82 ± 0.32^{Aa}	10.08 ± 0.21^{Aa}
		32	5.34 ± 0.23^{Ca}	9.49 ± 0.19^{ABb}	10.28 ± 0.22^{Aa}	9.50 ± 0.23^{ABb}	9.26 ± 0.28^{ABb}	9.05 ± 0.21^{Bb}
	Single birth	15	5.35 ± 0.25^{Ba}	10.64 ± 0.23^{Aa}	9.99 ± 0.50^{Aa}	9.80 ± 0.29^{Aa}	9.82 ± 0.22^{Aa}	10.13 ± 0.23^{Aa}
		32	5.15 ± 0.25^{Ba}	9.40 ± 0.23^{Ab}	9.92 ± 0.50^{Aa}	9.14 ± 0.29^{Ab}	9.28 ± 0.22^{Ab}	9.01 ± 0.23^{Ab}

eCG: The group received 400 IU of eCG one day before removing the sponge; GnRH-1: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the first day after estrus; GnRH-2: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 2nd day after estrus; GnRH-5: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 5th day after estrus; GnRH-7: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 7th day after estrus; GnRH-12: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 12th day after estrus. hCG-1: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the first day after estrus; hCG-2: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 2nd day after estrus; hCG-5: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 5th day after estrus; hCG-7: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 7th day after estrus; hCG-12: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 12th day after estrus. Sampling days: Different days of blood sampling after estrus observation.

ABCab Different letters subscripts in each row (uppercase letters) or in each column (lowercase letters in each separate position) indicate difference among treatment ($p < 0.05$).

Table 3. The effect of GnRH and hCG injection on the 1st and 2nd day after estrus observation on the reproductive performance of Lake-Ghashghaei ewes superovulated with eCG (n = 12).

	Parameters	GnRH-1	hCG-1	*p*-value
1st day after estrus	**Return to estrus (n)**	1/12	0/12	0.14
	Pregnancy rate (n)	11/12	12/12	0.13
	Parturition rate (n)	10/11	11/12	-
	Single birth (n)	8/10	5/11	0.14
	Twin births (n)	2/10	6/11	0.15
	Number of lambs	12[b]	17[a]	0.04
	Death of lambs (n)	0	0	-
	Fecundity rate (%)	1.00 (100)[b]	1.42 (142)[a]	0.04
	Prolificacy rate (%)	1.20 (120)	1.54 (155)	0.15
		GnRH-2	**hCG-2**	
2nd day after estrus	**Return to estrus (n)**	1/12	0/12	0.30
	Pregnancy rate (n)	11/12	12/12	0.31
	Parturition rate (n)	11/11	12/12	-
	Single birth (n)	4/11	2/12	0.28
	Multi births (n)	7/11	10/12	0.28
	Number of lambs	18	24	0.06
	Death of lambs (n)	0	0	-
	Fecundity rate (%)	1.50 (150)	2.00 (200)	0.06
	Prolificacy rate (%)	1.64 (164)	2.00 (200)	0.13

GnRH-1: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the first day after estrus; hCG-1: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the first day after estrus. GnRH-2: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 2nd day after estrus; hCG-2: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 2nd day after estrus.
[ab] Different letters subscripts in each row indicate difference among treatments ($p < 0.05$).

Table 4. Overall effect of treatment (GnRH and hCG injection) on 1st and 2nd day after estrus observation on serum progesterone concentration (ng mL^{-1}) in pregnant, delivered and single birth Lake Ghashghaei ewes (LS means ± SE).

Positions	GnRH	hCG
1st day after estrus	GnRH-1	hCG-1
Pregnant	7.58 ± 0.22[b]	9.98 ± 0.21[a]
Delivered	7.66 ± 0.23[b]	10.07 ± 0.22[a]
Single birth	7.50 ± 0.20[b]	10.02 ± 0.24[a]
2nd day after estrus	GnRH-2	hCG-2
Pregnant	8.20 ± 0.25[b]	10.36 ± 0.29[a]
Delivered	8.20 ± 0.25[b]	10.36 ± 0.29[a]
Single birth	7.34 ± 0.34[b]	9.95 ± 0.49[a]

GnRH-1: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the first day after estrus; hCG-1: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the first day after estrus. GnRH-2: The group received 400 IU of eCG one day before removing the sponge and GnRH was injected on the 2nd day after estrus; hCG-2: The group received 400 IU of eCG one day before removing the sponge and hCG was injected on the 2nd day after estrus. [ab] Different letters subscripts in each row indicate difference among treatments ($p < 0.05$).

Discussion

For the first time, this study evaluated the effect two types of hormone therapy on different days after mating on different reproductive activities and the changes of progesterone concentration in Lake-Ghashghaei ewes superovulated during spring season under nomadic breeding conditions. The first reason for doing this study was to identify whether different hormonal programs at different times after mating could improve the concentration of progesterone as a pregnancy-retaining hormone in sheep or not. The results of this study showed that the use of GnRH and hCG during different days (days 1, 2, 5, 7, and 12) after mating in Lake-Ghashghaei ewes significantly increased serum progesterone concentration compared to the control group that was consistent with the results of other studies.[9,17,23] In those studies, despite the time of using these hormones, the concentration of progesterone compared to the control group was increased significantly. hCG has similar activity to Luteinizing Hormone (LH) with a longer half-life than LH and causes luteotropic stimulation in the corpus luteum.[24] These effects can be done either by converting small cells into large corpus luteum or by increasing the size of large luteal cells.[17] Increased progesterone levels can be due to an increase in the number of extra corpus luteum on the surface of the ovary,[6,17] and or increase the number of luteal cells that secrete progesterone.[9,25]

The second reason in this study was to identify whether different hormonal programs could change the concentration of progesterone, and improve various reproductive activities or not and which day or days could be more effective. The results showed that although progesterone concentration in treatment groups was increased, however, reproductive performance in the groups that received GnRH and hCG (5, 7 and 12 days after mating) was not increased at the same times. In accordance with such results, no significant improvement in pregnancy and lambing rate was observed in other studies despite elevating serum progesterone concentrations.[26,27] In some other studies, increasing the pregnancy rate[11,28,29] and the rate of lambing[9] was

reported when using GnRH and hCG in the time of mating or on different days after mating in different breeds of sheep. Differences in results can be attributed to the different protocols used, management systems, nutritional or physiological status of ewes and experimental conditions.[8] In the present study, reproductive performances such as multiple birth rate, the number of lambs and fecundity, in the groups that received GnRH (day 2) and hCG (days 1 and 2) made a significant difference not only compared to the control group but also compared to other groups that received GnRH and hCG during different days after mating. Therefore, obtaining such results can show the importance of using such hormonal programs in different days after mating in connection with obtaining appropriate and successful responses in ewes superovulated with eCG, because it has been reported that the time of using different hormonal programs to improve sheep reproductive performance can also be important.[30]

In the present study, although the characteristics of follicular waves were not investigated, however, the choice of different times for hormonal injections on different days after mating was based on assuming that follicles in different sizes were appeared due to the use of eCG or the emergence of different follicular waves took place during the estrous cycle on the surface of the ovary.[17] On the other hand, the reason for GnRH and hCG injection until 12th day after mating in this study was due to the fact that this day was a very important time in sheep for pregnancy diagnosis by the mother.[12] It was reported that hCG injection before the time of pregnancy diagnosis by the mother (day 12) improved pregnancy rates, lambing rate and prolificacy rate.[18]

It has been reported that the time required for the onset of LH surge is about 8 to 10 hr, while at the time of using GnRH and hCG it takes about 3 to 5 hr.[31] Therefore, it can be assumed that using GnRH and hCG on the first day after the onset of estrus is soon because using these hormones can increase the number of large follicles on the surface of the ovary (follicles grown due to the use of eCG) and ovulation of follicles that have not reached to the stage of sufficient growth for oocyte maturation. Therefore, the results of previous studies showed that using hCG in dairy and beef cows would cause ovulation of small follicles that resulted in the production of a smaller corpus luteum and a reduction in the rate of pregnancy.[32,33] Using these hormones on the first day after estrus has increased serum progesterone concentration and some reproductive performance probably because the released oocytes are not reached to the stage of maturity, hence, it has not significantly improved the various reproductive activities including the rate of twinning. Using GnRH and hCG on the 2nd day after mating induces extra time for follicle growth and oocyte maturation and at the same time viability of the sperm in the ewe reproductive tract is about 30 to 48 hr, [34] therefore, the probability of conception of sperm and oocytes will be increased and the reproductive performance of the ewes will be improved. Using GnRH and hCG on other days increased serum progesterone concentrations compared to the control group and they did not have much effect on various reproductive activities which the reasons of such results are not clear.

The hCG has a longer half-life than GnRH, hence, it can stay in the bloodstream for a longer period of time,[35] because of the more luteotropic nature of hCG compared to GnRH and it can stimulate the development of the main corpus luteum other than the formation of secondary corpus luteum.[28] On the other hand, several studies reported that hCG, compared to GnRH, plays an important role in placental vascularization and increased blood flow to the ovaries especially the corpus luteum in domestic animals.[36,37] A previous study showed that hCG can affect various reproductive activities when the rate of such parameters are low.[38] Since twins and triplet are not common traits in Lake-Ghashghaei ewes, hence, in this study, observing a high level of twins and even observing triplet (two lambs) in hCG-receiving group on the second day after mating was probably related to the administration of this gonadotropin and its injection time. Similar to the results of previous study,[39] the number of born lambs, the rate of fecundity as well as the serum progesterone concentration at the time of hCG injection was significantly increased compared to GnRH injection on the similar day. Therefore, such results indicated the importance of gonadotropin therapy in Lake-Ghashghaei ewes. The results of a recent study showed that the use of GnRH and hCG increased the number of extra corpora lutea (92.00% and 100%, respectively) of the 3-, 4- and 5-mm follicles. However, progesterone concentrations were significantly higher only in the hCG-receiving group compared the control group.[5] Several previous studies reported that hCG increases the size and the length of the embryo, increases the secretion of interferon tau, luteal weight and increases placental weight.[11,29,40] Different results in hCG and GnRH therapy may be depended on the differences between LH concentration after applying these hormones which ultimately causes stimulation the formation of the corpus luteum with the ability of different steroidogenic capacity.[41] The results of a recent study showed that the use of hCG caused the accessory corpus luteum to produce progesterone and subsequently increased the concentration of progesterone in the blood. Although GnRH caused the accessory corpus luteum production but it did not increase serum progesterone.[5] In addition, a previous study did not report increasing in the progesterone concentration after the application of GnRH.[39]

Another important result in the present study was the rate of mortality in lambs up to one month after birth, so that no casualties were observed in any of the

experimental groups. Lamb mortality occurs during the early stages of the pregnancy in mammalian species and can cause economic losses and thus reduce income population in the sheep breeding industry.[42] The results of a previous study reported that the mortality rate of lambs in control groups, GnRH and hCG-receiving groups was 7.10, 4.80 and 2.10%, respectively.[43] On the other hand, the results of another study reported that the mortality rate of the lambs in the control group and the hCG group was 11.80 and 3.60%, respectively.[30] Therefore, one of the important results obtained in the present study was the non-mortality of the lambs in different treatment groups and the reason for this can probably be attributed to the exact time of delivery, good management of delivery time as well as the suitable time for starting and ending time of the study.

The results of this study generally showed that using GnRH and hCG on different days after mating increased serum progesterone concentration compared to the control group. Using these hormones on the 2nd day after estrus caused a significant improvement in various reproductive activities not only compared to the control group but also to the other days of using these hormones during different days after mating. On the other hand, the administration of hCG compared to GnRH on the 2nd day after estrus had a greater effect on progesterone concentration and various reproductive activities including the number of lambs born and faculties in nomadic breeding conditions in Lake-Ghashghaei ewes.

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Conflict of interest

The authors declare no potential competing conflict of interest.

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