The effect of 2PGF2α and P4eCG protocol of estrus synchronization on reproductive performance of nulliparous Ghezel ewes

ABOLFAZL HAJIBEMANI[1]*, YOUNES HEYDARI[1], REZA ASADPOUR[1], EZZATOLLAH FATHI[1]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Postal Box 416379828, Tabriz, Iran

SUMMARY

The hormonal protocol is used to increase reproductive efficiency in ewes. The aim of the study was to investigate the effects of 2 prostaglandin F2 alpha (2PGF2α) and compared it with progesterone+ equine chorionic gonadotropin (P4eCG) in nulliparous Ghezel ewes. A total of 132 nulliparous ewes were randomly divided into three groups. The control group (n=42) did not receive any hormonal treatment. The P4eCG group (n=48) used of intravaginal sponge for a 12-day and an injection of 400IU of eCG at the time of sponge removal. The 2PGF2α group (n=42) received the double injection of PGF2α at a 9-day interval. For measurement of the progesterone (P4) concentration, three times (at the start of the experiment, at the time of withdrawal the vaginal sponge and the second injection of PGF_{2a} and on days 20 after mating), blood samples were taken from experimental groups ewes. Pregnancy detection was done on the 30-35 days of pregnancy using B-mode ultrasonography. The estrous rate was obtained 100% in all groups. The highest number of nulliparous ewes were in estrus 48 h after the end of the synchronization program (control: 85.8%, 2PGF2α: 85.7%, P4eCG groups: 58.4%). No significant difference was observed in serum P4 concentration at the time 1 and 3 between the groups. The pregnancy and lambing rates were significantly higher ($P<0.05$) in the P4eCG (85.4% and 85.4%, respectively) and $2PGF_{2\alpha}$ (83.3% and 83.3%, respectively) groups than in the control group (28.6% and 23.8%, respectively). The litter size and twin rates in the P4eCG treatment group (1.19 and 19.5%, respectively) were significantly higher ($P<0.05$) compared to the $2PGF_{2\alpha}$ (1 and 0%, respectively) and control groups (1 and 0%, respectively). The fecundity rate was no statistically significant difference between the P4eCG (1.02±0.08) and $2PGF_{2\alpha}$ (0.83±0.05) groups ($P>0.05$). Based on the results, it was concluded that 2PGF2α injection can be employed to synchronize nulliparous estrus ewes in the late breeding season and is a suitable alternative for the P4eCG protocol.

KEY WORDS

Nulliparous ewes, PGF2α, Progesterone, Estrus synchronization.

INTRODUCTION

Ewes show seasonal reproductive activity. In the Northern hemisphere, the breeding season of ewes starts in early autumn and continues until early winter. The non-breeding season also begins at the end of winter and continues until mid-autumn (1). Today, numerous hormonal protocols are used to increase reproductive efficiency during the breeding season and induce estrus during the non-breeding season in ewes. Using vaginal progestogen compounds with equine chorionic gonadotropin (eCG) is the most common technique for estrus induction and synchronization (2).

The use of intravaginal progestogens causes problems such as vaginitis, environmental impact, affects fertility and animal welfare (2,3). Also, there is a possibility of the sponge falling and foul-smelling discharge following the removal of intravaginal sponges (4). Manes et al. (5) showed that sperm function and viability could be negatively affected by cervical discharge in ewes treated with intravaginal sponges. Moreover, the treatment cost of the intravaginal progestogens (e.g., sponge and controlled internal drug releasing (CIDR)), as well as eCG, are expensive (6,7).

Induction of luteolysis and estrus synchronization using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is one of the alternative ways to manage herd reproduction, especially during the breeding season. It does not interfere negatively with the reproductive response, does not cause reproductive disorders (e.g., vaginitis), and is effective, decreased cost and easy to use (7,8). Two injections of $PGF_{2\alpha}$ in cyclic ewes at 9-12 days interval induces estrus in 72 h after the second $PGF_{2\alpha}$ injection in 95% of the treated ewes (9). Most ewe is in the mid-luteal phase at the time of the second $PGF_{2\alpha}$ injection, therefore, they responded to $PGF_{2\alpha}$ injection (1). The injection of two doses of PGF2α to estrus synchronization is more economical than progesterone sponges in the breeding season. However, this method has shown different fertility rates in various studies (3,6,7).

Therefore, the aim of the present study was to investigate the effects of two injections of $PGF_{2\alpha}$ with intervals of 9 days and progesterone sponge + eCG (P4eCG) in nulliparous Ghezel ewes at the breeding season.

Corresponding Author:
Abolfazl Hajibemani (a.hajibemani@tabrizu.ac.ir)

MATERIALS AND METHODS

Animal

All procedures used in the present study were licensed by the Research Committee of the Department of Veterinary Medicine, University of Tabriz, Tabriz, Iran (99.02.6, 1399/05/01). This study was conducted in Tabriz (East Azarbaijan province, Iran). The Tabriz is located at latitude: 38o4 48 N; 46o4 48 E altitude of 1351 m above sea level. The climate is hot and humid during the summer and temperate during spring and autumn, while the winters are cold with a minimum temperature of - 10 °C. The experiment was performed in nulliparous Ghezel ewes during the breeding season, in the month of November, to achieve the lambing in May. A total of 132 healthy, nulliparous Ghezel lamb-ewe with an average body weight of 51.2 ± 1.5 kg and a body condition score (BCS) of 2.25±0.05 (score 1 for too thin ewes and a score of 5 for too fat ewes), were used in this study. BCS of nulliparous ewe was evaluated by palpation of back vertebral (10). Lamb-ewes were from the same farm.

Feeding experimental ewes

Lamb-ewes were managed as a single group. The ewe was fed from the natural pastures in the spring and summer seasons. Lamb-ewes were 10 hours/day at pasture. The ewes were manually fed with grass hay, barley grain, corn silage, bran, soybean meal and mineral and vitamin supplements in the autumn and winter seasons. The quantity of supplementation was 400gr/ewe/day. The water was access ad libitum.

Experimental design

After evaluation of the age, body weight, and BCS, the ewes were randomly divided into three groups. The first group (control group, n=42), received no any hormonal treatment. The second group (P4+eCG: P4eCG group, n=48) used of intravaginal sponge for a 12-day (Esponjavet, HIPRA, Spain) and intramuscular injection of 400IU of eCG (Gonaser, HIPRA, Spain) at the time of sponge removal. The third group ($PGF_{2\alpha}$+$PGF_{2\alpha}$: $2PGF_{2\alpha}$ group, n=42) received the double intramuscular injection of $PGF_{2\alpha}$ (Vetaglandin, Aburaihan, Iran) with a dose of 75 g at a 9-day intervals.

Oestrus detection

24 h after the sponge removal or the second injection of PGF2α, nulliparous Ghezel ewes were exposed to the twenty-five fertile rams to observe oestrus behaviors. The oestrus was detected every 8 hour for four days. Ewes with standing heat were considered oestrus ewes. In addition to, behaviors including restlessness, teasing the ram and flehmen response was noted (11).

Mating management

The reproductive health of the rams was examined before inserting into the herd. Rams were separated at least one month from the herd before the beginning of the study. After 24 h of the removal of the sponge and the injection of the second $PGF_{2\alpha}$, one healthy and fertile ram was added to the herd for every five nulliparous ewes for mating (ram/ewe ratio of 1:5). This is an average ratio.

Blood sampling

For measurement of the P4 concentration, three times, blood samples were taken from experimental groups ewes. The first time blood sampling was collected at the start of the experiment, the second time blood sampling was collected at the end of the estrous synchronization protocol (at the time of withdrawal the vaginal sponge and the second injection of PGF_{2a}), and the third time blood sampling was taken 20 days after mating. Blood samples were taken by a venoject tube from the jugular vein. Serums of blood samples were separated by centrifugation at 1500 rpm for 15 min. After centrifugation, serums were kept at -20°C until the evaluation of P4 concentration. Serum progesterone concentration was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Progesterone ELISA, Monobind, USA). The sensitivity of this kit is 0.1 ng/mm, and the intra-assay and inter-assay CV were 3.8% and 7.5%, respectively. The ewes with P4 concentrations>1 ng/mL were considered cyclic ewes.

Pregnancy diagnosis

Pregnancy detection was done on the 30-35 days of pregnancy using a B-mode ultrasonography machine (SIUI 800V, China) was equipped with a 5 and 7.5 MHZ linear, rectal transducer. Ewes were restrained in a standing position. For pregnancy diagnosis, the probe was placed at the inguinal region and also inserted into the rectum. The probe was covered with ultrasonography gel before using for pregnancy diagnosis. Pregnancy was determined based on the detection of viable embryonic vesicle within the uterine horn (12).

Evaluation of reproduction indicators

Reproductive performance was statistically analyzed for each experimental group. Variable were included: Estrous rate = (number of ewes showing estrus behaviors/total number of ewes in each group) × 100, End treatment-onset estrus interval (Estrus onset time) = the time between sponges removal or after the second injection of $PGF_{2\alpha}$ and first expression of standing heat), pregnancy rate (number of pregnant ewes/number of ewes which were introduced to a ram in each group), lambing rate (number of lambed ewe/number of ewes which were introduced to a ram in each group), fecundity rate (number of lambs/number of ewes which were exposed to a ram in each group), litter size (number of lambs/number of a lambed ewe), and the twin rate.

Statistical analysis

Statistical analysis of data was performed using SPSS software (Version 22.0, SPSS Inc, Chicago, Illinois). Data were shown as mean ± SEM and percentage. The Chi-square test was used to compare factors of estrous rate, pregnancy and lambing rates, fecundity rate and the gender of the lambs between the studied groups. The Fisher's exact test was used to compare factors of twin rate, and the female rate between the studied groups. The results of the end treatment-onset estrus interval, the concentration of progesterone, the gestation length, and the litter size were statistically analyzed using one-way analysis of variance (ANOVA) and Turkey HSD test as the post hoc test were used. The P-value of < 0.05 was considered significant.

RESULTS

Serum progesterone concentration

The results of serum progesterone concentration of the studied groups are shown in figure 1. No significant difference was observed in serum progesterone concentration between the

Table 1 - Estrous rate (%) and End treatment-onset estrus interval (h) of nulliparous ewes in control and treatment groups.

Groups	Estrous rate (%)	End treatment-onset estrus interval (h)
Control	42/42 (100%)	45.4±2.9[a]
2PGF2α	42/42 (100%)	44.8±2.1[a]
P4eCG	48/48 (100%)	54.00±2.5[B]

[a,b] Different superscript letters within each column indicate significant differences (P < 0.05).

groups when starting the synchronization program (P>0.05). At the end of the synchronization programs, the serum progesterone concentration in the $2PGF_{2\alpha}$ injections treatment group was significantly higher than in the P4eCG treatment group (P<0.05). In the third time of serum progesterone concentration (day 20 after mating), no statistically significant difference was observed between the studied groups (P>0.05).

Reproductive performance

The results regarding the estrous rate in the experimental groups are shown in Table 1. The estrous rate was obtained 100% (132/132) in all groups. All the nulliparous ewes were estrus within four days after the end of the synchronization program in the studied groups. The end treatment-onset estrus interval was significantly lower in the $2PGF_{2\alpha}$ treatment group and the control group than in the P4eCG treatment group (P<0.05) (Table 1). Figure 2 shows the distribution of estrus after the end of synchronization program in the studied nulliparous ewes. The number of ewes in estrus was lower in the P4eCG group compared to the control and 2PGF2α groups after 48 h (P<0.05). (control: 85.8%, $2PGF_{2\alpha}$: 85.7%, P4eCG groups: 58.4%).

There was no statistically significant difference in the gestation length between the treatment groups (Table 2) (P>0.05). The reproductive performance of experimental groups is presented in Table 2. As shown in Table 2, the pregnancy and lambing rates were significantly higher in the P4eCG and $2PGF_{2\alpha}$ treatment groups than in the control group (P<0.05).

The litter size and twin rates in the P4eCG treatment group were significantly higher (P<0.05) compared to the $2PGF_{2\alpha}$ treatment and control groups. The fecundity rate was significantly higher in the P4eCG and $2PGF_{2\alpha}$ treatment groups than in the control group (P<0.05).

DISCUSSION

The results of the present study showed that the use of $2PGF_{2\alpha}$ and P4eCG treatment protocol improved reproductive performance in the nulliparous Ghezel ewes, as has been previously reported in ewes (8,13-16).

In this study, it was shown that the estrus rates were 100% in the studied groups. Estrous response was found in the all groups. Lamb-ewes of the three treatments were managed as one group, therefore could consider that animals of the control group were influenced by the estruses of the animals receiving treatments. In previous studies, the estrus rate was reported after the injection of $2PGF_{2\alpha}$ and P4eCG at 70.91% and 95.97% (17), 85% and 80% (18), 77.8% and 100% (19), 37% and 100% (14), and 83.3% and 75% (20), respectively. The estrus rate in the present study was similar or better to the reference values. The results of a similar this study conducted by Wei et al. (17) revealed that the intervals between the end of treatment and the onset of estrus in the $2PGF_{2\alpha}$ and P4eCG hormonal programs were 45.35±6.16 h and 50.46±7.15 h, respectively, which was significantly higher in the P4eCG group. This is likely because the higher P4 at the time of sponge removal causes that the beginning of preovulatory surge delay and also, the progesterone analogue suppresses secession of luteinizing hormone (LH) and

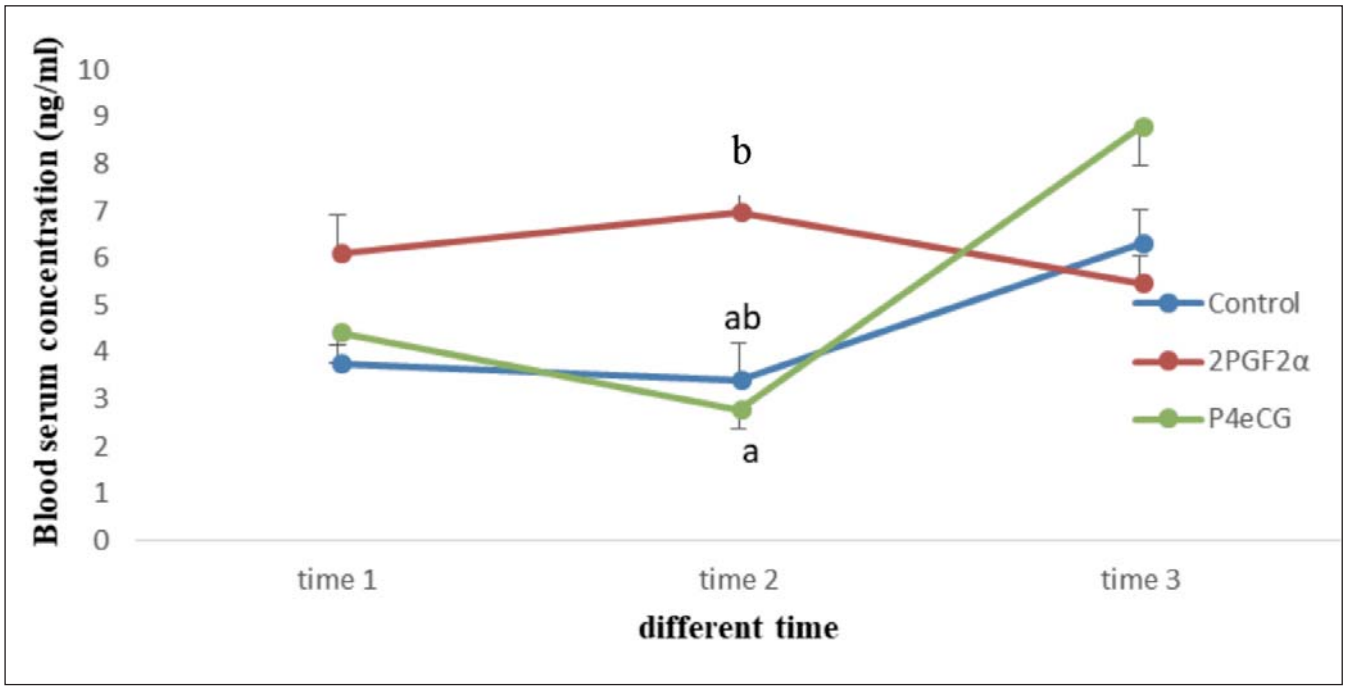

Figure 1 - Serum progesterone (Mean ± SE) concentrations of nulliparous ewes in control and treatment groups in four different times. Time 1: at the start of the experiment, time 2: at the time of withdrawal of the vaginal sponge and the second injection of PGF2a, time 3: 20 days after mating ([a,b] P < 0.05).

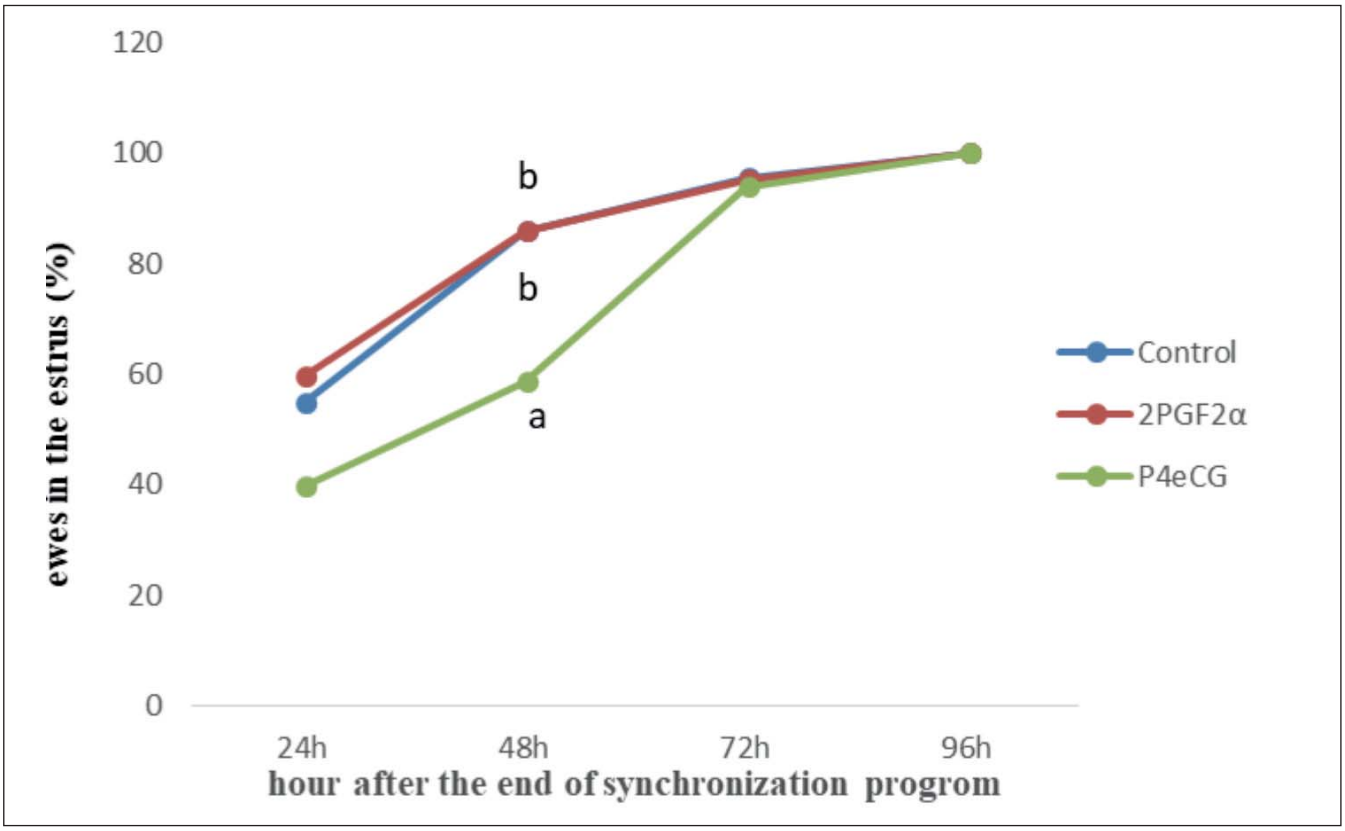

Figure 2 - Distribution of estrus after the end of the synchronization program in control and treatment groups ([a,b] $P < 0.05$).

gonadotropin-releasing hormone (GnRH) more (21,22). Inconsistent with the findings of the present study, some studies indicated that the interval between the end of treatment and the onset of estrus was higher in the $2PGF_{2\alpha}$ treatment group than in the P4eCG treatment group (19,20). However, in these reports, the amount of end treatment-onset estrus interval in the $2PGF_{2\alpha}$ treatment group (38-44 h) was similar to the result of the 2PGF2α group in the current study (42-48 h). In the studies by Ayoub et al. (20) and Danjuma et al. (23) the end treatment-onset estrus interval was insignificant between the two treatment groups. However, they were reported that the duration of the end treatment-onset estrus interval was 44-54 h in the treatment groups, which was similar to data in the current study. In agreement with the present study, the other studies reported a higher percentage of ewes were estrus between 40 and 48 h after the end of treatment (24,25).

These results showed that the use of $2PGF_{2\alpha}$ hormone treatment could cause a pregnancy rate similar to P4eCG hormone treatment and was higher than that in the control group. No negative effects of $2PGF_{2\alpha}$ hormone treatment was presented, because the pregnancy rates were similar in both treatments. In agreement with the results of our study, the findings of some studies showed that the pregnancy rate was similar in the $2PGF_{2\alpha}$ and P4eCG treatment groups in Awassi (55% and 47%, respectively) (18) and tropical hair (53.33% and 60%, respectively) (7) and Lacaune ewes (66.7% % and 76.5%, respectively) (19). These studies showed that $2PGF_{2\alpha}$ hormone treatment could be used to synchronize estrus with an acceptable pregnancy rate. Wei et al. (17) showed that the pregnancy rate in the $2PGF_{2\alpha}$ injection group with a dose of 0.24 mg (92.86%) in some herds

Table 2 - Reproductive performance of nulliparous ewes in control and treatment groups.

Parameters	Control	2PGF2α	P4eCG
Gestation length (days)	149.47±1.57	150.17±0.46	147.04±3.6
Pregnancy rate	28.6% (12/42)[a]	83.3% (35/42)[b]	85.4% (41/48)[b]
Lambing rate	23.8% (10/42)[a]	83.3% (35/42)[b]	85.4% (41/48)[b]
Litter size	1±0[a]	1±0[a]	1.19±0.06[b]
Fecundity rate	0.30±0.05[a]	0.83±0.05[b]	1.02±0.08[b]
Twin rate	0% (0/10)[a]	0% (0/35)[a]	19.5% (8/41)[b]
Single rate	100% (10)	100% (35)	80.5% (33)
Female rate	20.0% (2/10)[a]	57.1% (20/35)[b]	54.2% (26/48)[b]
Male rate	80.0% (8/10)	42.9% (15/35)	45.8% (22/48)

[a,b] Different superscript letters within each column indicate significant differences ($P < 0.05$).

had no statistically significant difference compared to the sponge treatment group with 400 IU eCG at the time of sponge removal (92.31%) which was in agreement with the results of the present study (17). Cueto et al. (26) reported that $2PGF_{2\alpha}$ hormonal protocol with a long interval of 14 days caused a significantly lower pregnancy rate (52.0%) than in the P4-eCG group (76.4%), which was inconsistent with our results. Moreover, in their study, the pregnancy rate was lower in the $2PGF_{2\alpha}$ group than in the present study. The results of the present study showed that the pregnancy rate in the $2PGF_{2\alpha}$ group was better than some previous studies. Differences in pregnancy rates between studies could be due to differences in the season of implementing the hormonal protocol, dose and type of drug and the sheep breed.

In the current study, acceptable lambing rate of ewes was obtained in the 2PGF2α and P4eCG treatment groups. The findings of the present study showed that P4eCG treatment improved the litter size and twin rate. In other similar studies conducted, litter size was significantly higher in the P4eCG treatment group than in the $2PGF_{2\alpha}$ treatment group (14,17) that were similar to the current study; nevertheless, in other studies conducted, this difference was not significant (6,20). The findings of some studies showed that the use of P4 without eCG injection has similar results to $PGF_{2\alpha}$ injections regarding the litter size and twin rate. It has been reported that the injection of eCG with P4 improved multiple births and litter size. Use of eCG at the end of progesterone treatments stimulate follicular growth and improves the rate ovulation response (3,6,9). The results of Fierro et al. [36] study showed that litter size was significantly higher in the P4eCG treatment group than in the treatment group with 2PGF2α injections at a 13-day interval; however, in the treatment group with 2PGF2α injections at 12, 14, 15, and 16 days intervals, it was similar to the P4eCG treatment group. Therefore, based on their study, 2PGF2α injection interval can be effective. Overall, treatments with P4eCG improve the litter size.

The fecundity rate is the most critical reproductive index to assess the herd's reproductive performance, and it was the same rate in the two treatment groups in the present study. Therefore, the results of the fecundity rate showed that using of the $2PGF_{2\alpha}$ hormonal protocol can be a good alternative for the P4eCG protocol with acceptable economic success in the breeding season. But, based on the findings of some studies, the $2PGF_{2\alpha}$ hormonal protocol with an interval of 9, 12, 13, and 14 days significantly could reduce the fecundity rate compared to the P4eCG group; nonetheless, the $2PGF_{2\alpha}$ hormonal protocol with a longer interval had the similar fecundity rate as the P4eCG hormonal program (14,27). In general, the difference in reproductive performance in different studies can be attributed to differences in breed, geographical conditions, nutrition and management, the season conducting the experiment, drug dosage and the duration of P4 treatment and interval of $2PGF_{2\alpha}$ treatment.

In the present study, the serum concentration of progesterone was higher than 1 ng/ml in all treatment groups at the beginning of the program, which indicated that the nulliparous ewes were cyclic (28). According to the results of studies, a new follicular wave developed a few days later the first PGF2α injection and then followed the formation of an active corpus luteum and secreted and increased progesterone hormone (7,29). On the other hand, when the sponge is placed in the vagina the level of progesterone gradually begins to decrease one to two days later. Also, the level of progesterone is low at the time of sponge removal (7,30) which confirmed the results of the present study. The serum level of progesterone increased in the third period, which was 19 days after mating, which is due to the pregnancy and the presence of the mature corpus luteum that produced a large amount of progesterone (16).

CONCLUSION

In conclusion, the use of $2PGF_{2\alpha}$ and P4eCG hormonal protocol to synchronize nulliparous Ghezel ewes improved reproductive performance compared to the nulliparous ewes that no hormonal protocol were used. Furthermore, the $2PGF_{2\alpha}$ injection protocol induced pregnancy, lambing and fecundity rates similar to the P4eCG protocol. The $2PGF_{2\alpha}$ injection protocol can be advised for nulliparous Ghezel ewes due to its short treatment period, lower drug price, high reproductive performance and easy application. Consequently, the $2PGF_{2\alpha}$ injection with a 9-day interval protocol can be employed to synchronize estrus nulliparous Ghezel ewes in the late breeding season and is a suitable alternative for the P4eCG protocol.

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