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Estrus Synchronization Methods during the Non-Breeding Season of Ghezel Ewe Lambs

Research Article

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ABSTRACT

Synchronization can improve the reproductive performance of ewe lambs during the non-breeding season, though seeking a simple method can be more practical for farmers. Accordingly, the study aimed to evaluate the reproductive performance of ewe lambs during the non-breeding season using different clomiphene citrate (CC) protocols compared with progesterone sponge (PS) + equine chorionic gonadotropin (eCG) protocol. In this study, 40 Ghezel ewe lambs were divided into four randomly groups: 1) PS + eCG group: PS insertion (day 0-12) + 500 IU eCG injection on day 12; 2) PS + CC group: PS insertion (day 0-12) + oral administration of 100 mg CC/day (day 7-12); 3) CC + eCG group: oral administration of 100 mg CC/day (day 7-12) + 500 IU eCG injection on day 12; 4) CC group: oral administration of 100 mg CC/day (day 7-12). Ram introduction was done for five days. Blood samples were taken on 1, 11, 14, and 40 days, and serum levels of estradiol and progesterone were measured. Pregnancy and lambing rates were also assessed. Results indicated that pregnancy and lambing rates were the highest in PS + eCG group, while the lowest or no performance was observed in CC groups ($P<0.05$). Based on the overall results of the present study, the best synchronization method during the non-breeding season for ewe lambs was PS + eCG compared with the CC protocols.

KEY WORDS clomiphene citrate, ewe lamb, non-seasonal reproduction, progesterone, reproductive.

INTRODUCTION

Poor feeding management of sheep usually does not allow ewe lambs to gain proper weight for puberty and reproduction in the first breeding season, which delays their pregnancy until the second breeding season (Saadat-Noori and Siah-Mansoor, 1987; Baneh *et al.* 2010; Aktaş *et al.* 2015). However, most ewe lambs of Iranian sheep breeds including Ghezel breed gain enough weight in the first winter of their life (non-breeding season), because of indoor feeding of concentrate and their low body activity (Saadat-Noori and Siah-Mansoor, 1987). Accordingly, breeding of ewe lambs during their first non-breeding season can increase lifetime reproductive performance of ewes and then, increase farmers' income. It has been shown that synchronization during the non-breeding season requires progesterone due to its effect on inducing follicular FSH and LH receptors (Safranski *et al.* 1992; Caraty and Skinner, 1999). Then, using progesterone compounds with or without equine chorionic gonadotropin (eCG) are conventional protocols for estrus synchronization during both non-breeding season and breeding season (Almadaly *et al.* 2016; Ghasemi-Panahi *et al.* 2016; Hasani *et al.* 2018). In previous studies,12 days of insertion of controlled internal drug releasing (CIDR) or progesterone sponge (PS) and 500 IU eCG at the time of CIDR or PS withdrawal in multiparous Ghezel ewes increased pregnancy and lambing rates during the non-breeding and breeding seasons, respectively

(Ghasemi-Panahi *et al.* 2016; Hasani *et al.* 2018). However, no information is available regarding the use of PS + eCG in ewe lambs during their first non-breeding season.

Clomiphene citrate (CC), a treatment for female infertility, has antiestrogenic effects by blocking estradiol receptors, which increases gonadotropin-releasing hormone (GnRH) secretion (by blocking negative feedback of estradiol on the hypothalamus) and in turn, increases FSH and LH secretion (Tiwary, 2006; Heldring *et al.* 2007; EL-Sherry *et al.* 2011; Bukhari *et al.* 2016). Administering CC (100 mg/d for five days) in ewes increased growing follicle numbers, estradiol levels, and estrous (EL-Sherry *et al.* 2011).

Gupta *et al.* (2021) by using a protocol that included oral administration of 150 mg/day of clomiphene citrate for five days in goats during the non-breeding season resulted in higher estrus induction, follicular size, ovulation, conception rate, and lambing rate as compared with those-received clomiphene citrate. Rateb *et al.* (2019) reported that oral administering of 600 mg clomiphene and 20 µg GnRH in goats during the breeding season resulted in a higher conception rate and litter size than that were treated with GC or control ones.

However, Land and Scaramuzzi (1979) reported a small effect of clomiphene citrate on the ovulation of ewes. In another study, nine days of feeding 300 mg CC/day/prepubertal heifer along with 2500 IU hCG/heifer induced ovulation in heifers and their cyclicity (Bukhari *et al.* 2016).

Treating anestrus cows with single injection of 750 IU eCG oral administration of 300 mg CC/d for five days resulted in better results with eCG in comparison with CC for conception rate (Tiwary, 2006). Oral administration of CC is a more simple technique and cheaper method as compared with PS insertion for farmers with low education and low income. However, to our current knowledge, there is no study regarding the use of CC or related protocols as synchronization protocols during the non-breeding season on ewe lambs. Accordingly, the present study was designed to evaluate the reproductive performance of ewe lambs during the non-breeding season using different protocols as compared with PS + eCG protocol.

MATERIALS AND METHODS

Experimental design

This experiment was carried out in Khalat-Pushan Agricultural Research Station, University of Tabriz, Iran from February 2019 to July 2019. Procedures of the present experiment were approved by Animal Care and Ethics Committee synchronized of the University of Tabriz, Iran. To investigate the reproductive performance of ewe lambs during the non-breeding season, 40 Ghezel ewe lambs were divided into four equal experimental groups with an average body weight of 43.89 ± 4.86 kg, body condition score (BCS) of 2.49 ± 0.47, and age of 11.16 ± 0.64. It should be mentioned that winter considered non-breeding season in Ghezel ewes and the breeding season spreads from July to September (Saadat-Noori and Siah-Mansoor, 1987; Rastegarnia, 2007). All animals were housed in group cages (5 m length×2 m width×1.5 m height) and as follows (Figure 1): 1) PS + eCG group treated by intra vaginal insertion of contained 60 mg medroxy progesterone acetate (Esponjavet, Hipra, Spain) from day 0-12 andim injection of 500 IU eCG (Gonaser, Hipra, Spain) on day 12 of the experiment. 2) PS + CC group treated by intra vaginal insertion of from day 0-12 and oral administration of 100 mg CC per day (Iran Hormone Pharmaceutical Co.) from day 7-12 for each animal in the group. 3) CC + eCG group treated by oral administration of 100 mg CC per day from day 7-12 for each animal in the group followed by injection of 500 IU eCG on day 12 of the experiment. 4) CC group only treated by oral administration of 100 mg CC per day from day 7-12 for each animal in the group. The dosage (oral administration of 100 mg CC) and duration of CC treatment (five days) were selected based on the following articles (Dehbashi *et al.* 2006; El-Sherry *et al.* 2011). As animals were in the non-breeding season, days 7-12 were selected to synchronize all groups at the same time. Furthermore, ewe lambs were housed in the barn, in which a couple of rams were already housed in separate cages, because of space limitations.

Rams were introduced to the ewe lambs for five days (from day13-18 of the experiment) (one fertile ram per each 10 ewe lambs), in which rams were switched between groups twice a day to ensure a high fertility rate. Also, all rams' semen was tested already to ensure their fertility. Estrus signs and mating were observed twice a day (from 8-10 a.m. and 2-4 p.m.) for five days (day 13-18). As ewes in the CC groups showed a wider range of estrus signs and mating, rams stayed in groups until observing the last signs of estrus and mating. Accordingly, rams stayed in the groups for five days.

Hormonal evaluations

The blood samples (Jugular vein, non-heparinized tubes) were collected four times from each animal included: at the start of the experiment (day 0), 24 hours before PS removal (day 11), 48 hours after DPS removal (day 14), and 28 days after PS removal (day 40) to evaluate pregnancy of ewe lambs (Akoz *et al.* 2006; Hasani *et al.* 2018). All samples were collected at the same daylight time (11:30 a.m.-12:30 p.m.). Then, blood samples were centrifuged by 3000 × g for 15 minutes at room temperature.

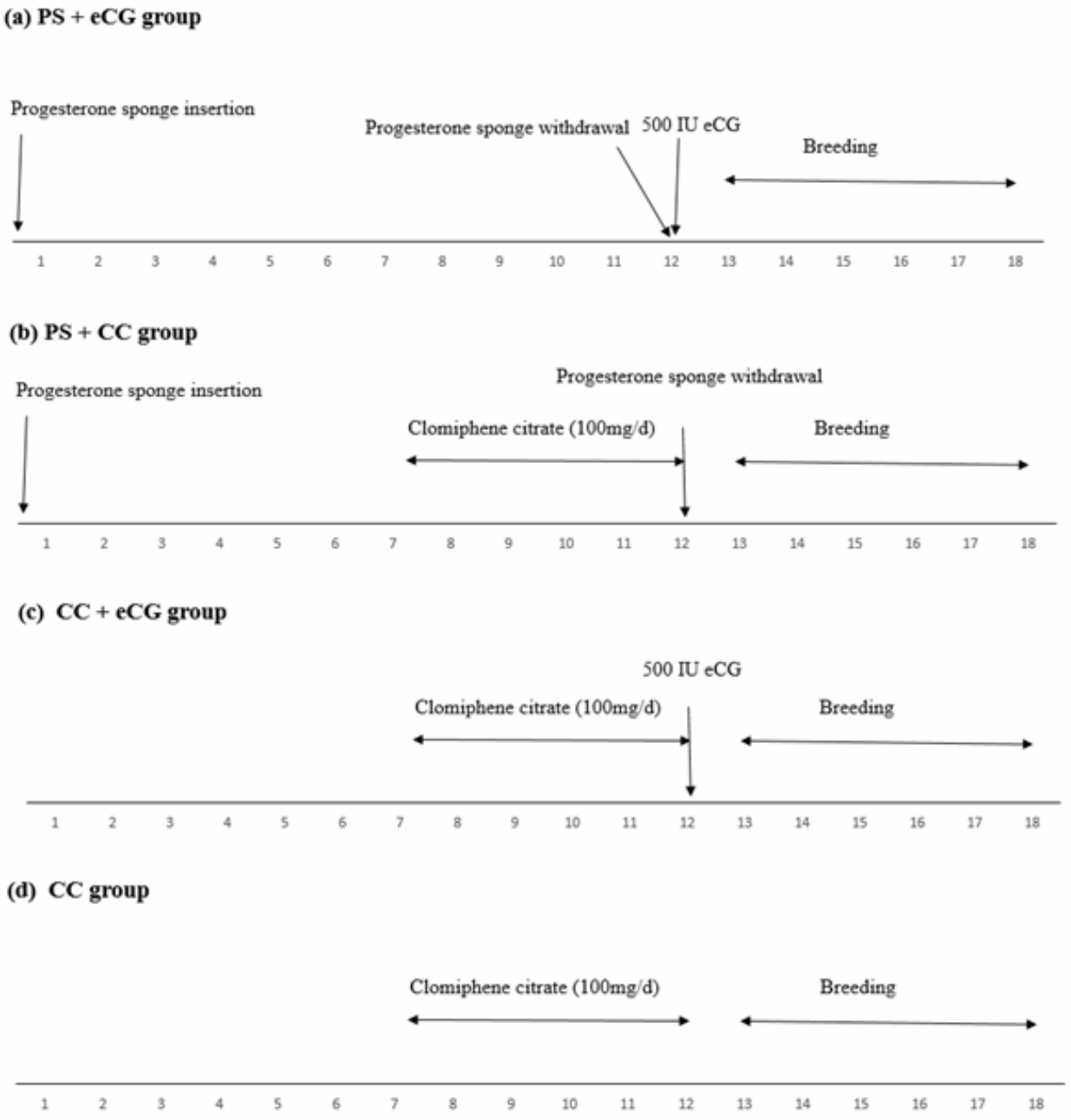

Figure 1 Estrus synchronization protocols included: (a) PS + eCG group: PS insertion (day 0-12) + 500 IU eCG injection on day 12; (b) PS + CC group: PS insertion (day 0-12) + oral administration of 100 mg CC/day (day 7-12); (c) CC + eCG group: oral administration of 100 mg CC/day (day 7-12) + 500 IU eCG injection on day 12 and (d) CC group: oral administration of 100 mg CC/day (day 7-12)

Afterward, serum samples were stored at -20 ˚C till the measurement of estradiol-17β and progesterone concentrations. Progesterone and estradiol-17 β concentrations were measured by using enzyme-linked immunosorbent assay method (ELISA), (Awareness Technology Stat Fax 3200 Microplate Reader, USA). Progesterone kit (Cat. No. 4825-300) was supplied from Mono bind company (USA) with 0.1 ng/mL sensitivity and 23.57% intra-assay coefficients of variation of progesterone. Also, estradiol kit (Cat. No. 4925-300) was supplied from Mono bind company (USA) with 6.5 pg/mL sensitivity and 19.54% intra-assay coefficients of variation of estrogen.

Pregnancy evaluations

Pregnancy was recorded in two stages

A: After evaluating the progesterone concentration of samples of day 40 of the experiment, ewe lambs with progesterone concentrations higher than 3 ng/mL were considered as pregnant. This level of progesterone was considered because the lowest progesterone concentration of ewe lambs

with positive pregnancy during the last trimester of expected pregnancy (using sonography) was 3 ng/mL on day 40 of the experiment.

B: In the last trimester of expected pregnancy (4 months of pregnancy), ewe lambs were evaluated for pregnancy by ultrasound sonography (Ultra Scan 900, AMI Co, Canada)using a 5.0 MHz probe to evaluate the presence of placenta.

The pregnancy rate at day 40 of the experiment and at the last trimester of expected pregnancy was calculated as follows: the number of pregnant ewe lambs in a treatment group/the total number of ewes in that group × 100.

Fertility evaluations

After parturition, the number of lambs, which were born by each ewe lamb was recorded. Lambing rate in each group was calculated based on the total lambs' number/ewe lambs' number ×100. Twin ingrate in each group was calculated as the ratio of twin and triplicate lambs/total number of lambs × 100.

It should be mentioned that one of the ewe lambs in PS + eCG group delivered triplicate and two ewe lambs in this group delivered twines. Also, prolificacy was calculated in each group based on lambs born/ewe lambing (Dzakuma *et al.* 1982).

Data analysis

Proc FREQ and Proc LOGISTICof SAS software (SAS, 2008) were used to analyzereproductive performance data. In addition, the concentrations of progesterone and estradiol were analyzed based on ProcMIXED (SAS 9.2). A significance level of 0.05 was used for comparing treatments.

RESULTS AND DISCUSSION

Estrus signs and mating in PS + eCG group started 12 h after sponge removal and the maximum rate was observed 48 h after sponge removal (no data are available). However, estrus signs and mating in CC, PS + CC, and CC + eCG groups were observed from the beginning of ram introduction and persisted to the last day of ram introduction (from day 13 to 18 of the experiment).

Hormonal-based pregnancy on day 40 of the experiment showed significant effects of different estrus synchronization methods on the pregnancy of ewe lambs ($P<0.05$; Table 1). The highest pregnancy rate at the day 40 of the experiment was observed in PS + eCG group (90%), while the pregnancy rate was lower in the CC groups. Ultrasound-based pregnancy rate at the last trimester of expected pregnancy was significantly affected by synchronization methods and the highest pregnancy rate was observed in PS + eCG ewe lambs ($P<0.05$, Table 1).

The lambing rate was significantly affected by estrus synchronization methods ($P<0.05$) and the highest lambing rate was observed in PS + eCG group (Table 1).

Although the twining rate was not affected by estrus synchronization methods ($P>0.05$), the highest twinning rate was observed in PS + eCG group (Table 1).

Furthermore, estrus synchronization methods did not affect prolificacy ($P>0.05$), though the highest prolificacy was observed in PS + eCG group (Table 1).

Based on the results, blood progesterone concentration was significantly affected by synchronization protocols ($P<0.05$) and progesterone concentration was higher in PS + eCG group than other groups.

Table 1 Impact of different estrus synchronization on reproductive performance of ewe lambs*

Traits	Treatments				P-value
	PS + eCG[1] (n=10)	PS + CC[2] (n=10)	CC + eCG[3] (n=10)	CC[4] (n=10)	
Hormonal based-pregnancy No. (head)[5]	9	3	2	2	-
Hormonal based-pregnancy rate (%)[5]	90.00	30.00	20.00	20.00	0.03
Ultrasound based-pregnancy No. (head)[6]	9	1	0	0	-
Ultrasound based-pregnancy rate (%)[6]	90.00	10.00	0.00	0.00	0.03
Lambs No. (head)	13	1	0	0	-
Lambing rate (%)	130.00	10.00	0.00	0.00	0.03
Twining rate (%)	53.85	0.00	-	-	0.89
Prolificacy	1.44	1	-	-	0.89

[1] PS insertion (day 0-12) + 500 IU eCG injection on day 12.
[2] PS insertion (day 0-12) + oral administration of 100 mg CC/day (day 7-12).
[3] Oral administration of 100 mg CC/day (day 7-12) + 500 IU eCG injection on day 12.
[4] Oral administration of 100 mg CC/day (day 7-12).
[5] Pregnancy of ewe lamb's based on blood progesterone concentration at 40 days of the experiment.
[6] Ewe lamb's pregnancy based on ultrasound at the last expected trimester of pregnancy.
* stated for non-existed data (as ewe lambs in the mentioned groups did not deliver and had no lamb; so, we had no data for the mentioned parts).

Although sampling times did not affect blood progesterone concentration (P>0.05), the interaction of synchronization groups × sampling times was significant (P<0.01; Figure 2). According to the results presented in Figure 2, progesterone concentration was the highest in CC + eCG group as compared with other groups on day 11 of the experiment (P<0.05). Also, PS + eCG group had the highest progesterone concentration on day 40 of the experiment (P<0.05; Figure 2).

The impact of estrus synchronization treatments on blood estradiol concentration was significant (P<0.01).

Serum estradiol concentration was higher in PS + eCG group than CC groups. Also, blood sampling time shad a significant effect on blood estradiol concentration (P≤0.05; Figure 3). Furthermore, the interaction effects of treatments by times on estradiol concentration were significant (P<0.01; Figure 4). Estradiol concentration was significantly different among treatments on sampling times of 0, 11, 14, and 40 days (P<0.05; Figure 4). Estradiol concentration was higher in PS + eCG group than other groups on day 11, 14, and 40 of experiment (P<0.05; Figure 4).

Pregnancy rate of ewe lambs was the highest (90%) in PS + eCG group. Also, results indicated 130% lambing rate, 53.85% twining rate, and 1.44% prolificacy in PS + eCG group. Furthermore, pregnancy (ultrasound based-pregnancy), lambing rate, twining rate, and prolificacy in PS + CC group were 10%, 10%, 0%, and 100%, respectively. However, CC + eCG and CC groups indicated no pregnancy nor lambing rate. Accordingly, all CC protocols were unsuccessful in improving reproductive performance of ewe lambs during then on-breeding season. Results related to PS + eCG ewe lambs were similar to the results of Ghasemi Panahi *et al.* (2016), which reported CIDR and eCG as a useful technique for improving reproductive performance (both pregnancy and lambing rates) of Ghezel ewes during the non-breeding season. Furthermore, in a similar study application of progesterone sponge + eCG in Ghezel ewes during the breeding season resulted in 120% lambing rate (Hasani *et al.* 2018). As 500 IU eCG was administered for ewe lambs with lower body weight as compared with mature ewes of previous studies (Ghasemi Panahi *et al.* 2016; Hasani *et al.* 2018), this may cause higher lambing rate of ewe lambs in the PS + eCG group. In contrary with the present results related to clomiphene citrate, Gupta *et al.* (2021) by oral administration of10 g/day TANUVAS mineral mixture type 2for 20 days followed by five days oral administration of150 mg/day clomiphene citrate and intramuscular injection of 40 µg buserelin acetate in goats during the non-breeding season reported higher estrus induction, follicular size, follicular ovulation, conception rate, and lambing rate as compared with goats with the same condition, though non-receiving clomiphene citrate. Furthermore, Tiwary (2006) with treating anestrus cattle with eCG (750 IU eCG, one injection) and clomiphene citrate (oral administration of 300 mg clomiphene citrate for five days along with 1000 mg copper sulfate) reported 90% estrus exhibition and 50% fertility by the first artificial insemination in eCG group and 80% estrus exhibition and 50% fertility by the first artificial insemination in clomiphene citrate group. El-Sherry *et al.* (2011) by oral administration of 100 mg clomiphene citrate + 2000 IU eCG for five consecutive days in Rahmani sheep reported higher follicle numbers than a group receiving only eCG. Al-Waeli *et al.* (2011) reported reduction in the total follicular number along with failure in maturation of the secondary follicles, which resulted in inhibition of ovulation by administration of clomiphene citrate in mice. Clomiphene citrate is commonly used for stimulating ovulation in humans to cure ovulation disorders, though its side effect include reduction in endometrial artery blood flow, thickness and volume along with lower endometrial receptivity, which causes implantation failure and infertility (Omran *et al.* 2018; Ajdary *et al.* 2020). It was also indicated that clomiphene citrate can suppress follicular growth and induce apoptosis of oocyte in the follicles; so, could not cause a successful pregnancy in human (Chaube *et al.* 2017). Furthermore, it was reported that the improving effect of clomiphene citrate on inducing ovulation of ewes was mainly small and dose-dependent (Land and Scaramuzzi, 1979). On the other hand, exhibiting estrus and mating of ewe lambs in CC groups in the present experiment may be related to the male effect (presence of rams in the same barn with ewe lambs), which was indicated to activate luteinizing hormone secretion and cyclicity of anestrous ewes (Gelez *et al.* 2004). Accordingly, regardless of estrus (no data are available) and mating exhibition of ewe lambs, clomiphene citrate could not cause successful pregnancy and lambing.

Based on the results of the present experiment, blood progesterone concentration was lower in all clomiphene citrate groups as compared with PS + eCG group on day 40 of experiment, which indicated unsuccessful pregnancy in all clomiphene citrate groups. Also, blood estradiol concentration on days14 and 40 of the experiment in the present experiment was the highest in PS + eCG group. After PS withdrawal, a decrease in blood progesterone concentration cause follicular growth and then, an increase in estradiol concentration on day 14 (48 h after PS withdrawal), (Pineda and Dooley, 2003). Because most ewe lambs in PS + eCG group were pregnanton day 40 (28 days after PS removal) and it was indicated that estradiol and progesterone increase during pregnancy (Pineda and Dooley, 2003); then, it was the reason of observing high estradiol concentration on day 40.

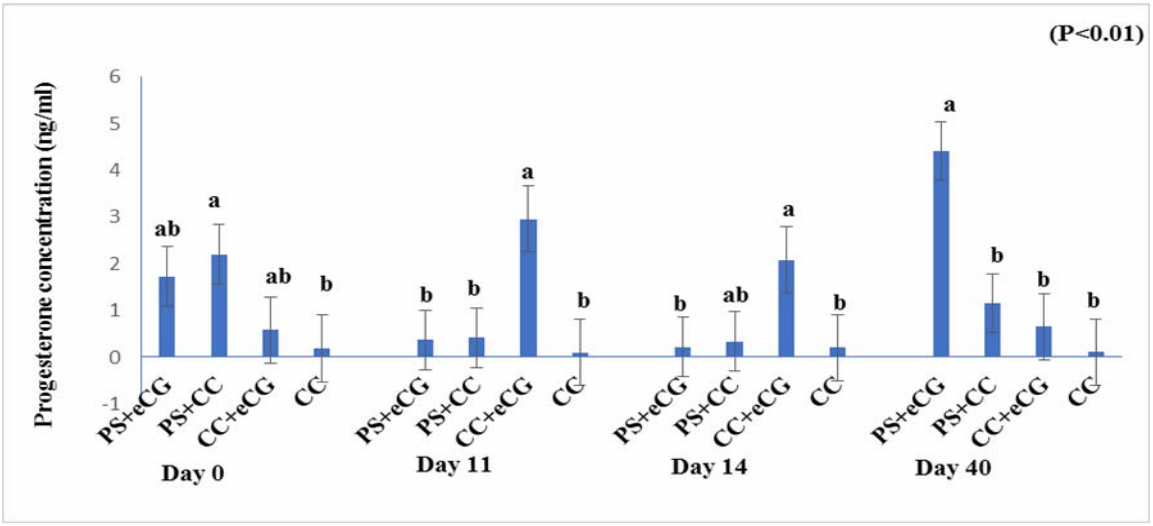

Figure 2 The impact of different estrus synchronization protocols on progesterone concentration, included: PS + eCG group: PS insertion (day 0-12) + 500 IU eCG injection on day 12; PS + CC group: PS insertion (day 0-12) + oral administration of 100 mg CC/day (day 7-12); CC + eCG group: oral administration of 100 mg CC/day (day 7-12) + 500 IU eCG injection on day 12; CC group: oral administration of 100 mg CC/day (day 7-12). Sampling days were also as follows: at the start of the experiment (day 0), 24 hours before PS removal (day 11), 48 hours after PS removal (day 14), and 28 days after PS removal (day 40)

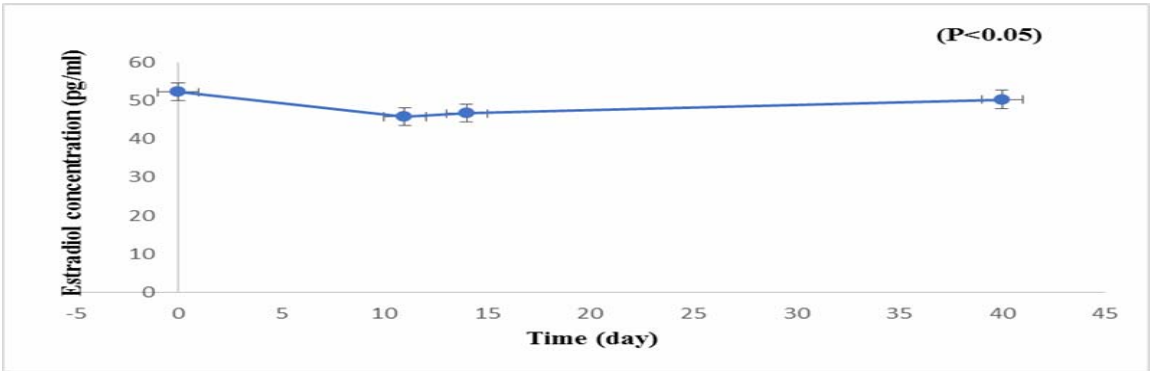

Figure 3 The impact of blood sampling times on serum estradiol-17 β concentration of ewe lambs

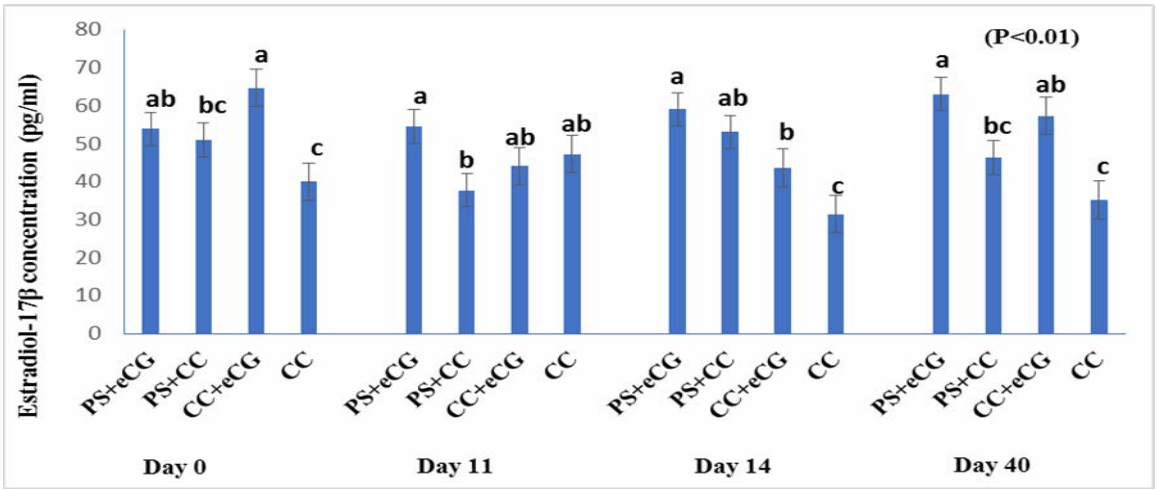

Figure 4 The impact of different estrus synchronization protocols on estradiol-17 β concentration, included: PS + eCG group: PS insertion (day 0-12) + 500 IU eCG injection on day 12; PS + CC group: PS insertion (day 0-12) + oral administration of 100 mg CC/day (day 7-12); CC + eCG group: oral administration of 100 mg CC/day (day 7-12) + 500 IU eCG injection on day 12; CC group: oral administration of 100 mg CC/day (day 7-12). Sampling days were also as follows: at the start of the experiment the start of the experiment (day 0), 24 hours before PS removal (day 11), 48 hours after PS removal (day 14), and 28 days after PS removal (day 40)

In a similar study, El-Sherry *et al.* (2011) by treating Rahmani ewes with oral administration of 100 mg clomiphene citrate and/or 2000 IU of eCG intramuscularly for 5consecutive days indicated no changes in progesterone concentration during these five experimental days by clomiphene citrate and/or eCG treatments, while an increase in estradiol concentration was observed by the treatments. Tiwary (2006) reported higher estradiol concentration on the day of estrous in anestrus cattle treated by eCG (750 IU eCG, one injection) or clomiphene citrate (oral administration of 300 mg clomiphene citrate for five days along with 1000 mg copper sulfate) as compared with control group (intramuscular injection of distilled water or oral administration of copper sulfate). Omran *et al.* (2018) by treating clomiphene citrate in women with unexplained infertility reported no changes in progesterone and estradiol concentrations along with lower peri-implantation endometrial perfusion.

In an experiment, administering clomiphene citrate (300 mg for nine days) and human chorionic gonadotropin (IVF-C 5000 IU on day 10) in pre-pubertal Sahiwal heifers increased progesterone concentration and puberty of heifers (Bukhari *et al.* 2016). It can be concluded that either the level of clomiphene citrate was not sufficient or clomiphene citrate administration is not effective in inducing successful pregnancy and lambing in ewe lambs during the non-breeding season.

CONCLUSION

The overall results of the present experiment showed higher pregnancy and lambing rates in PS + eCG group as compared with all clomiphene citrate groups; therefore, the advisable method for synchronization of ewe lambs during the non-breeding season is progesterone sponge and eCG. Also, administering clomiphene citrate with the present dosage or protocols can not cause successful pregnancy or lambing in ewe lams during the non-breeding season.

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