Synchronization of Ewe Lambs During Non-Breeding Season: Comparing Clomiphene Citrate-Based Protocols With Progesterone+eCG Protocol

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Abstract

Synchronization can improve reproductive performance of ewe lambs during non-breeding season. Accordingly, the aim of this study was to evaluate reproductive performance of ewe lambs during 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 experimental groups based on the completely randomized design. Experimental groups were as follows: 1- PS (12 days)+eCG (500 I.U. eCG); 2- PS (12 days)+CC (100 mg CC, 5 days); 3- CC (100 mg CC, 5 days)+eCG group (500 I.U. eCG); 4- CC (100 mg CC, 5 days). Ram introduction was done between 13 and 18 days. Blood samples were taken on 1, 11, 14, and 40 days, and serum levels of estrogen and progesterone were measured. Pregnancy was evaluated at the last trimester of expected pregnancy (by sonography). Based on the results, the effects of treatments and the interaction of treatments $\times$ time on estrogen and progesterone concentrations were significant ($P<0.05$). Pregnancy and lambing rates were significantly affected by estrus synchronization treatments and the highest pregnancy was observed in PS+eCG group ($P<0.05$). Based on the overall results of the present study, the best synchronization method during non-breeding season for ewe lambs was PS+eCG.

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 gain enough weight in the first winter of their life, because of indoor feeding of concentrate and their low body activity (Saadat-Noori and Siah-Mansoor, 1987). Accordingly, breeding of ewe lambs during the first winter (non-breeding season) can increase their lifetime reproductive performance.

It has been shown that synchronization during non-breeding season requires progesterone due to its effect on inducing follicular FSH and LH receptors (Burke and Kissler, 1988; Caraty and Skinner, 1999). Then, using progesterone compounds with or without equine chorionic gonadotropin (eCG) are conventional protocols for estrus synchronization during non-breeding season and breeding season (Almadaly et al. 2016; Ghasemi-Panahi et al. 2016; Hasani et al. 2018). In previous studies, controlled internal drug releasing (CIDR) or progesterone sponge (PS) was used for 12 days, and 500 I.U. eCG were intramuscularly injected at the time of CIDR or PS withdrawal in ewes (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 estrogen receptors, which increases gonadotropin-releasing hormone (GnRH) secretion (by blocking negative feedback of estrogen on hypothalamus) and in turn, increases FSH and LH secretion (Tiway, 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, increased estrogen levels, and induced estrous; however, CC

had no effect on ovulating follicles and estrogen levels when administered during superovulation with 2000 I.U. of eCG (EL-sherry et al. 2011). In another study, nine days of feeding 300 mg CC/day/prepubertal heifer along with intravenous injection of 2500 I.U. hCG/heifer induced ovulation in heifers (Bukhari et al. 2016). Treating anestrous cows with single injection of 750 I.U. eCG or oral administration of 300 mg CC/d for five days reported better results with eCG in comparison with CC for conception rate (Tiwary, 2006). To our information, there is no study regarding the use of CC or related protocols during non-breeding season on ewe lambs. So, the present study was designed to evaluate reproductive performance of ewe lambs during non-breeding season using different CC protocols as compared with PS+eCG protocol.

Material And Methods

Experimental design

This experiment was performed using ewe lambs and rams housed 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 of University of Tabriz, Iran. To investigate the reproductive performance of ewe lambs during non-breeding season, 40 Ghezel ewe lambs were divided into four experimental groups with parallel body weight (43.89 ± 4.86 kg). It should be mentioned that ewe lambs were housed based on a group cages in a barn which already had a couple of rams in that barn with separate cages. Groups of ewe lambs were randomly assigned to synchronization protocols as follows: 1- Control group (Hasani et al. 2018) or PS (progesterone sponge: Esponjavet, Hipra, Spain, which contained 60 mg medroxyprogesterone acetate)+eCG (equine chorionic gonadotropin: Gonaser, Hipra, Spain) group (inserting PS for 12 days and intramuscular injection of 500 I.U. eCG on day 12); 2- PS+CC (clomiphene citrate: Iran Hormone Pharmaceutical Co.) group (inserting sponge for 12 days and orally receiving of 100 mg CC during 7-12 days); 3- CC+eCG group (orally receiving of 100 mg CC during 7-12 days and intramuscular injection of 500 I.U. eCG on day 12 of trial); 4- CC group (orally receiving of 100 mg CC during 7-12 days). Rams were introduced to the ewe lambs between 13 and 18 days of the experiment (one ram per each 10 ewe lambs), in which rams were replaced twice a day to ensure high fertility rate. Estrus signs in PS+eCG group were started 12h after sponge removal and the maximum rate was observed 48h after sponge removal. However, estrus signs 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 13 to 18 days of experiment).

Hormonal evaluations

Blood samples of all four groups were collected on days 0 (start of the experiment), 11 (24 hours before progesterone sponge removal), 14 (48 hours after progesterone sponge removal), and 40 of the experiment (28 days after progesterone sponge removal to evaluate pregnancy of ewe lambs). All samples were collected at the same daylight time (11:30 a.m.). Then, blood samples were centrifuged by $3000 \times g$ for 15 minutes at room temperature. Afterward, serum samples were stored at -20°C till the

measurement of estrogen and progesterone concentrations. Serum progesterone and estrogen concentrations were measured in one stage and based on enzyme-linked immunosorbent assay method (ELISA) using ELISA reader (Awareness Technology Stat Fax 3200 Microplate Reader, USA). Progesterone kit (Cat. No. 4825-300) was supplied from Monobind company (USA) with 0.1 ng/ml sensitivity and 23.57% intra-assay coefficients of variation of progesterone. Also, estrogen kit (Cat. No. 4925-300) was supplied from Monobind company (USA) with 6.5 pg/ml sensitivity and 19.54% intra-assay coefficients of variation of estrogen.

Pregnancy evaluations

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

Pregnancy rate 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 ewe lambs in that group $\times$ 100.

Lambing 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 $\times$ 100. Twinning rate in each group was calculated as the ratio of twine and triplicate lambs/total number of lambs $\times$ 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 the total number of lambs/the numbers of delivered ewe lambs $\times$ 100.

Data analysis

Proc FREQ and Proc LOGISTIC of SAS software (SAS 9.2) were used to analyze reproductive performance data. In addition, the concentrations of progesterone and estrogen were analyzed based on Proc MIXED (SAS 9.2). A significance level of 0.05 was used for comparing treatments.

Results

All ewe lambs showed signs of estrous 24 h after sponge withdrawal, though mating in clomiphene citrate groups lasted more till day 18 of experiment (individual data was not recorded). Ultrasound based pregnancy rate at the last trimester of expected pregnancy was significantly affected by synchronization methods ($P<0.05$). The highest pregnancy rate was observed in PS+eCG ewe lambs (90%), while PS+CC group indicated 10% pregnancy rate and CC+eCG and CC groups showed no pregnancy (0%), (Table 1).

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

Traits	Treatments				P value
	PS+eCG ¹	PS+CC ²	CC+eCG ³	CC ⁴	
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	-	-	0.97
Prolificacy (%)	144.44	100.00	-	-	-
¹ Embedding progestogen sponge for 12 days+eCG injection (500 IU) at 12 days of experiment (n=10).					
² Embedding progestogen sponge for 12 days+oral receiving of 100 mg CC during 7-12 days (n=10).					
³ Oral receiving of 100 mg CC during 7-12 days+eCG injection (500 IU) at 12 days of experiment (n=10).					
⁴ Oral receiving of 100 mg CC during 7-12 days (n=10).					
⁵ Pregnancy of ewe lamb's based on blood progesterone concentration at 40 days of experiment.					
⁶ Ewe lamb's pregnancy based on ultrasound at the last expected trimester of pregnancy.					

Lambing rate was significantly affected by estrus synchronization methods ($P<0.05$) and the highest lambing rate was observed in PS+eCG group with 130% lambing rate (Table 1). On the other hand, 10% lambing rate was observed in PS+CC group and 0% lambing rate was observed in CC+eCG and CC groups (Table 1).

The impact of estrus synchronization methods on twining rate was not significant ($P>0.05$); however, twining rate was higher in PS+eCG group (53.85%) compared with PS+CC group (0%), (Table 1).

Furthermore, prolificacy in PS+eCG and PS+CC groups was 144.44% and 100%, respectively.

Based on the results, estrus synchronization treatments had a significant effect on blood progesterone concentration ($P<0.05$), in which progesterone concentration was 1.68 ± 0.32 ng/ml in PS+eCG group, 1.56 ± 0.36 ng/ml in CC+eCG group, 1.03 ± 0.32 ng/ml in PS+CC group, and 0.153 ± 0.36 ng/ml in CC group. Although blood progesterone concentration was not affected by blood sampling times ($P>0.05$), the interaction effects of estrus synchronization treatments by sampling times on serum progesterone

concentration was significant ($P<0.01$), (Fig. 1). Different treatments had a significantly different progesterone concentration on 0, 11, 14, and 40 days of experiment ($P<0.05$), (Fig. 1). On day 11 of the experiment, progesterone concentration was the highest in CC+eCG group as compared with other groups ($P<0.05$), (Fig. 1). Also, PS+eCG group had the highest progesterone concentration on day 40 of the experiment ($P<0.05$), (Fig. 1).

The impact of estrus synchronization treatments on blood estrogen concentration was significant ($P<0.01$). Serum estrogen concentration was 57.55 ± 3.23 pg/ml in PS+eCG group, 52.38 ± 3.61 pg/ml in CC+eCG group, 46.96 ± 3.23 pg/ml in PS+CC group, and 38.41 ± 3.61 pg/ml in CC group. Also, blood sampling times had a significant effect on blood estrogen concentration ($P\leq0.05$), (Fig. 2). Furthermore, the interaction effects of treatments by times on estrogen concentration were significant ($P<0.01$), (Fig. 3). Estrogen concentration was significantly different among treatments on sampling times of 0, 11, 14, and 40 days ($P<0.05$), (Fig. 3). Estrogen concentration was higher in PS+eCG group than other groups on days 11, 14, and 40 of experiment ($P<0.05$), (Fig. 3).

Discussion

Pregnancy rate of ewe lambs was 90% in PS+eCG group and 10% in PS+CC group. Also, results indicated 130% lambing rate, 53.85% twinning rate, and 144.44% prolificacy in PS+eCG group. Furthermore, lambing rate, twinning rate, and prolificacy in PS+CC group were 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 non-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 accompanied with flushing diet as a useful technique for improving reproductive performance (both pregnancy and lambing rates) of Ghezel ewes during non-breeding season. Furthermore, in a similar study application of progesterone sponge+eCG in Ghezel ewes during breeding season resulted in 120% lambing rate (Hassani et al. 2018). In contrary with the present results related to clomiphene citrate, Tiwary (2006) with treating anestrus cattle with eCG (750 I.U. 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 I.U. 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 (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). Accordingly, it can be concluded that clomiphene citrate negatively affected follicular maturation and development along with endometrial implantation in ewe lambs and then, 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 estrogen concentration on days 14 and 40 of experiment in the present experiment was the highest in PS+eCG group, while the lowest amount was observed in CC group. In a similar study, El-sherry et al. (2011) by treating Rahmani ewes with oral administration of 100 mg clomiphene citrate and/or 2000 I.U. of eCG intramuscularly for 5 consecutive days indicated no changes in progesterone concentration during these five experimental days by clomiphene citrate and/or eCG treatments, while an increase in estrogen concentration was observed by the treatments. Tiwary (2006) reported higher estradiol concentration on the day of estrous in anestrus cattle treated by eCG (750 I.U. 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 estrogen 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 I.U. on day 10) in prepubertal Sahiwal heifers increased progesterone concentration and puberty of heifers (Bukhari et al. 2016). Regardless of observing estrous signs in clomiphene citrate groups, because of low estradiol concentration on day 14 of experiment in clomiphene citrate groups, it can be concluded that either the level of clomiphene citrate was not sufficient or it requires simultaneous administration with copper sulfate to be effective and cause a successful pregnancy in ewe lambs during 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 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 lambs during non-breeding season.

Declarations

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Conflicts of interest/Competing interests Authors declare no conflict of interest

Availability of data and material Data will be available based on journal's request.

Code availability Codes will be available based on journal's request.

Authors' contributions Authors contributed in the article as follows: Marziyeh Ebrahimi (designing the experiment and writing the article), Roghayeh Aghamohammadzadeh (performing the experiment), Gholamali Moghaddam (designing the experiment), Hossein Daghigh Kia (designing the experiment), Babak Ghasemi-Panahi (performing sonography and sponge+eCG synchronization protocol), Davoud Kianifard (writing the article)

Consent to participate Not applicable

Consent for publication All authors consent to publish the article in the journal.

Ethics approval All procedures of the present experiment were based on Animal Care and Use Committee (University of Tabriz, Iran).

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Figures

Figure 1

The impact of different estrus synchronizations included: PS+eCG (embedding progestogen sponge for 12 days+eCG injection (500 IU) at 12 days of experiment), PS+CC (embedding progestogen sponge for 12 days+oral receiving of 100 mg CC during 7-12 days), CC+eCG (oral receiving of 100 mg CC during 7-12 days+eCG injection (500 IU) at 12 days of experiment), and CC (oral receiving of 100 mg CC during 7-12 days) on blood progesterone concentrations of ewe lambs at day 0 (start of the experiment), 11 (24 hours before progesterone sponge removal), 14 (48 hours after progesterone sponge removal), and 40 of the experiment (28 days after progesterone sponge removal to evaluate pregnancy of ewe lambs).

Figure 2

The impact of time of blood sampling on serum estrogen concentrations of ewe lambs.

Figure 3

The impact of different estrus synchronizations included: PS+eCG (embedding progestogen sponge for 12 days+eCG injection (500 IU) at 12 days of experiment), PS+CC (embedding progestogen sponge for 12 days+oral receiving of 100 mg CC during 7-12 days), CC+eCG (oral receiving of 100 mg CC during 7-12 days+eCG injection (500 IU) at 12 days of experiment), and CC (oral receiving of 100 mg CC during 7-12 days) on blood estrogen concentrations of ewe lambs at day 0 (start of the experiment), 11 (24 hours before progesterone sponge removal), 14 (48 hours after progesterone sponge removal), and 40 of the experiment (28 days after progesterone sponge removal to evaluate pregnancy of ewe lambs).