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Evaluation of the estrus synchronization and reproductive performance of Afshari ewes during the breeding season following treatment with clomiphene citrate

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Abstract The present study was conducted to study the efficiency of substituting conventional gonadotropin-based technique with clomiphene citrate (CC) on reproductive parameters and hormonal changes in sheep during the breeding season. For this this purpose, 30 Afshari ewes (3-4 years, 55 ± 1.3 kg BW, BCS 3.04±0.3) were treated with intravaginal CIDR for 14 days and the divided into three groups: one group was considered as the control, the other groups received eCG (300 IU, intramuscularly) and CC (600 mg, oral), respectively. Blood samples were taken from three days before until three days after CIDR removal. Reproductive parameters were calculated after parturition. Fecundity and multiple lambing were numerically higher in the eCG group; however, there was no significant difference in reproductive performance between treatments. Estradiol and progesterone concentrations showed similar trends between treatments. Results of this study showed that CC was not a reliable substitute for eCG to increase the ovulation rate in sheep.

Keywords: clomiphene citrate, estrus synchronization, reproductive performance

Introduction

Estrus synchronization in livestock focuses on the manipulation of either the luteal or the follicular phases of the estrous cycle (Bister et al., 1999). In ewes, the opportunity for estrus manipulation is greater during the luteal phase, due to its higher time period and more manipulative power (Evans, 2003). Two strategies can be used for this purpose: extending the luteal phase by supplying exogenous progesterone or shortening the luteal phase by prematurely regressing the existing corpora lutea (Nakafeero et al., 2020). Extension of the luteal phase by progesterone along with eCG is the most conventional protocol for estrus synchronization in ewes (Hameed et al., 2020). In this protocol eCG improves and increases the estrus response and litter size (Quintero-Elisea et al., 2011). However, the use of eCG can be associated with some constraints; including the occurrence of polycystic ovaries, increased probability of decreased eCG efficiency due to the formation of antibodies against it, and sometimes the high cost of eCG (Rahminiwati et al., 2017). Therefore establishing alternatives compounds for eCG is necessary.

Several pharmacological agents including clomiphene

citrate (CC) (Sudhakar et al., 2022), letrozole (Requena et al., 2008) and gonadotropin releasing hormone (GnRH) (Titi et al., 2010) can induce or augment the ovulation, with varying degrees of success. Clomiphene citrate is a non-steroidal orally-administered substance that was reported to have the capacity to induce FSH discharge from the anterior pituitary (Memon et al., 2011). Being structurally similar to estradiol, CC can bind to estradiol receptors in the brain and reduce the negative feedback of estradiol concentration on gonadotropin production (Kahwanago et al., 1970). Lindsay and Robinson (1970) characterized the action of CC in ewes. Clomiphene citrate suppresses the pituitary activity and induce behavioral estrus. In the study of El-Sherry et al. (2011), administration of CC increased the number of growing follicles and plasma estradiol levels. However, CC administered during superovulation did not increase the number of ovulating follicles and negatively affected the estradiol level. Bukhari et al. (2016) successfully used clomiphene for enhancing ovarian follicular activity in in prepubertal heifers. Rateb et al. (2019) indicated that utilizing CC in combination with $PGF_2\alpha$ and GnRH was efficient in inducing multiple ovulation in goats and can be a reliable substitute to traditional gonadotropin-dependent regimens.

This study was conducted with the aim to evaluate the efficiency of substituting conventional gonadotropin-based protocol with clomiphene citrate (CC) on reproductive parameters and hormonal changes in Afshari ewes during the breeding season.

Materials and methods

Animals and management

The experiment was conducted at the Small Ruminant Research Center of Arak University, Arak, Iran from November 2019 to April 2020. A total of 30 non-lactating Iranian Afshari ewes (3-4 years, 50 ± 1.3 kg BW) with normal estrous cycles were used. The ewes were housed in individual pens with free access to feed (consisting of 10.2% CP, 2.3 Mcal ME/kg DM, 7g/day Ca and 4g/day P) and water.

Experimental design

The estrous cycle was synchronized using CIDR (Controlled Internal Drug Release, EAZI-BREEDTM, CIDR®, New Zealand) for a 14-day period during the breeding season, after which, the ewes were randomly divided into 3 groups (n=10 per group). One group of ewes served as the control; and one group of was orally administered with CC for 3 consecutive days (200 mg per day) prior to CIDR removal and 4 IU GnRH at the time of CIDR withdrawal (CC protocol as per Rateb et al., 2019). The third group received 300IU PMSG 24 h prior to CIDR removal and 4 IU GnRH at the time of CIDR withdrawal (eCG protocol). Six fertile rams were used for daily mating (in the morning and afternoon) for 4 days, starting at 24 h after CIDR removal. The reproductive variables measured were estrus response (%), pregnancy rate (%): percentage of pregnant ewes to all synchronized ewes, lambing rate (%): percentage of ewes lambed from pregnant ewes previously diagnosed, fertility (%): percentage of ewes lambed from the total of ewes mated, multiple lambing (%): percentage of ewes lambed with two or more lambs, fecundity (%): percentage of lamb born per ewe mated.

Blood sampling and hormone determination

To measure changes in estradiol concentration, blood samples were collected from three randomly-selected ewes per group by jugular venipuncture at 9:00 a.m. and the sampling was repeated daily during day −3 to +3 after CIDR removal. To measure progesterone concentration, blood samples were taken from all tested animals on the 60^{th} day after mating.

Blood serum was then separated by centrifugation (2500 rpm for 15 min) and stored at -20°C. Estradiol and progesterone concentrations were determined by ELISA Reader (ELx 808-Ultramicroplate ReaderBio-Tek Instruments INC. U.S.A) using commercial kits (Hangzhou Eastbiopharm CO., LTD. Cat. No: CK-E91162, Hangzhou, China). The intra-assay and inter-assay coefficients of variation were <10% and <12% for estradiol and <2.6% and <4.5% for progesterone, respectively.

Statistical analyses

The experiment was performed in a completely randomized design. The data were analyzed by Proc GLM (SAS, 2003). The statistical model included the fixed effect of treatment and the random effect of ewe. Data on reproductive performance were analyzed using PROC GENMOD. For the analysis of estradiol and progesterone concentrations, a mixed model for repeated measurements was used. Results were expressed as mean ± SEM and a probability of $P \leq 0.05$ was considered as significant.

Results and discussion

Reproductive performance

Based on reproductive parameters in the present study (Table 1), CC was not a suitable alternative for eCG for Afshari ewe estrus synchronization. However, Rateb et al. (2019) showed that utilizing CC or eCG with their proposed protocol (based on $PGF_2\alpha$) did not differ in inducing multiple ovulation in goats. They suggested that CC can be a good alternative for eCG in goat superovulation protocol. In consistent with our results, El-Sherry et al. (2011) showed that in comparison with eCG, administration of oral CC over 5 consecutive days (100 mg per day) did not increase the number of ovulating fol-

licles in Rahmani ewes. Nevertheless, administration of CC increased the number of the growing follicles. Lindsay et al. (1970), in a study on Leicester-Merino ewes, showed that CC had no pituitary-stimulatory or gonadotropin-like activity but induced estrus. Similar findings were reported by Pasqualini et al. (1987).

Table 1. Reproductive performance of Afshari ewes following estrus synchronization by different protocols.

	Estrus response (%)	Pregnancy rate (%)	Lambing rate (%)	Fertility (%)	Multiple lambing (%)	Fecundity (%)
Control	10/10(100)	10/10(100)	10/10(100)	10/10(100)	0/10(0)	**10/10(100)**
CC protocol	10/10(100)	10/10(100)	10/10(100)	10/10(100)	0/10(0)	**10/10(100)**
eCG protocol	10/10(100)	10/10(100)	10/10(100)	10/10(100)	5/10(50)	**15/10(150)**

The observed differences between the results of this study and others could be attributed to the breed of sheep, stage of breeding season, CC dosage, CC administration route, and CC treatment regimen. Land and Scaramuzzi (1979), in a study on Finn-Merino and Blackface ewes, showed that the efficiency of response to CC was affected by breed and breeding season. They also stated that, intermediate doses (between 20-50 µg) compared with high (>50µg) and low-dose (<20µg) CC (in injectable form) may apparently induce the best ovulation rate.

Blood hormones

No difference were observed in serum progesterone concentration between treatment groups (Figure 1). It is known that, the synthetic capacity of corpus luteum to produce progesterone is dependent on the number and functionality of the luteal cells, which in turn, are affected by the size of the preovulatory follicle (Perez-Marin, 2009). Also, Driancourt and Fry (1992) showed that the preovulatory follicles in eCG superovulated ewes had a mean smaller size in comparison with untreated group. Based on this basic knowledges and fecundity results, we suggest the low progesterone concentration in the eCG group can be due to the small size of the preovulatory follicles and the inappropriateness of the CC dose and its effect on the receptors.

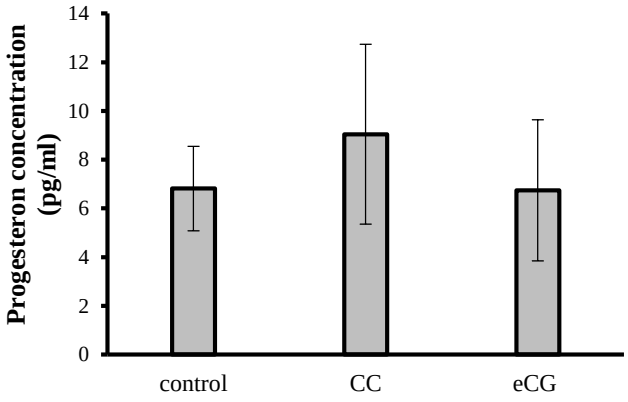

Figure 1. Comparison of serum progesterone concentration between the control, eCG and clomiphene citrate (CC) groups at 60 d after mating (P>0.05)

The results of serum estradiol levels are presented in Figure 2. No significant difference was observed in estradiol concentrations between the treatment (P>0.05). In eCG group, estradiol reached maximum concentration on D_0 and then decreased. There was a significant difference in estradiol level in eCG group with the levels in the control and CC groups on D_0 (P<0.05). Theoretically, there is a positive correlation between serum estradiol levels with the follicular size and presence of more pre-ovulatory follicles during the estrous cycle (Rahminiwati et al., 2017). Therefore, the higher levels of estradiol in eCG group on D_0 can be due to the presence of more pre-ovulatory follicles in comparison with the control and CC groups. Higher fecundity rate in eCG group partially confirms this hypothesis.

Estradiol concentrations showed a decreasing trend over time in control and CC groups. This indicated that CC had no pharmacological effect or had an estradiol effect. However, the second hypothesis is more likely. In this regard, Lindsay et al. (1970) concluded that clomiphene acts in the ewe as a weak estradiol. In another study, Land et al. (1979) showed that by increasing the dose, clomiphene acts as an anti-estradiol compound and then, with further increases, functions as an estradiol so that the ovulation rate is depressed. Accordingly, they indicated that unlike the high and low doses, an intermediate dose of clomiphene raised the ovulation rate in sheep. Pasqualini et al. (1987) stated that the estradiol- or antiestradiol-activities of clomiphene and its isomers are dependent on the species. Rateb et al. (2019), orally administering 200 mg CC in Damascus goats, reported that estradiol concentration gradually increased immediately after CC feeding. Our data did confirm their findings. Species differences and the type of treatment protocol can be considered as reasons for this difference.

Conclusion

Based on the results of this study, using of CC with our proposed regimen, was not effective in increase the ovulation rate in Afshari sheep in comparison with the traditional eCG regimens. However, further studies with other synchronization regimens are needed to evaluate

the real potential of CC for increasing the ovulation rate in sheep.

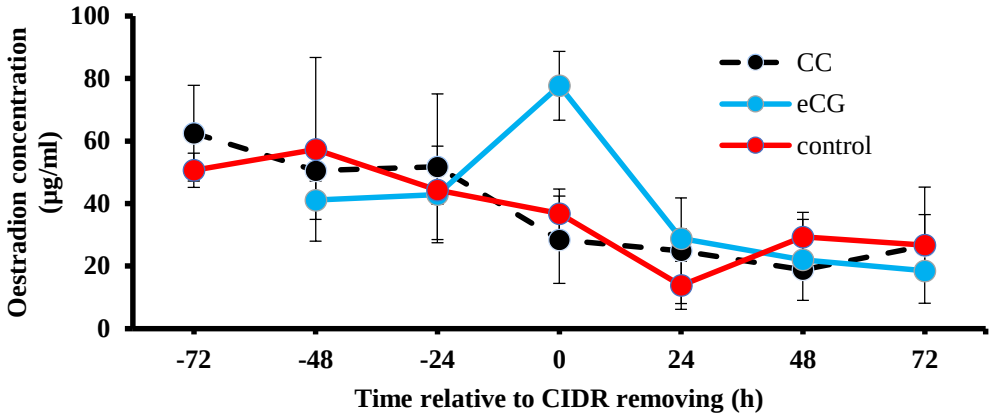

Figure 2. Comparison of serum estradiol concentration between the control, eCG and clomiphene citrate (CC) groups (P>0.05)

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