

## Evaluation of the estrus synchronization and reproductive performance of Farahani ewes during the breeding season following treatment with letrozole

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### Abstract

This study investigated the efficiency of substituting conventional gonadotropin-based technique with letrozole based regimen in reproductive parameters and hormonal changes in Farahani sheep during the breeding season. For this purpose, 40 Farahani ewes (3-4 years, 44±2.3 kg BW, BCS 3.12±0.5) were treated with intravaginal CIDR for 14 days and then were divided into four groups: one group was considered as the control, the other groups received eCG (400 IU, intramuscularly), hCG (400 IU, intramuscularly) and letrozole (7.5 mg, oral), respectively. Blood samples were taken from three days before CIDR removal. The 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. Estrogen concentrations showed an increasing trend in all treatments. Results of this study showed that letrozole was not a reliable substitute for eCG to increase the ovulation rate in sheep.

**Key words:** Estrus synchronization, Farahani ewes, Letrozole, Reproductive performance

### Introduction

Control of the estrus cycle of small ruminants can be done by numerous treatments and protocols. Some of these protocols manipulate the luteal phase and others the follicular phase of the estrous cycle (Bister et al, 1999; MA, 2020). 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).

The general strategy for estrus manipulation in ewes is the use of intravaginal devices impregnated with progesterone or synthetic progestagen such as Sponges, CIDR (Controlled Intravaginal Drug Release) (Nakafeero et al, 2020). Protocols based on the use of progesterone are associated with an injection of eCG, before (Ali, 2007), after (Moakhar et al, 2012) or at the time of CIDR withdrawal (Abecia et al, 2011;

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Hameed et al, 2020). In these protocols 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.

Based on Lacker's model, circulating estradiol secreted by the follicles provides systemic control over ovulation number (Shilo et al, 2022). In fact, reducing FSH concentrations to below the threshold needed to sustain the other gonadotrophin dependent follicles is one of the mechanism that determines the ovulation number (Scaramuzzi et al, 2011). Letrozole as aromatase inhibitor can reduce the negative feedback of estradiol concentration on gonadotropin production by inhibiting estrogen production (Mitwally & Casper, 2002). Letrozole is a reversible nonsteroidal inhibitor of the aromatase enzyme that regulating the synthesis of estrogen (Bhatnagar, 2007). It is used safely for treatment of estrogen induced diseases such as breast cancer (Cohen et al, 2002), induction of ovulation in women with anovulatory infertility (Gowri et al, 2022) and for ovarian superstimulation in women (Al-Fadhli et al, 2006). It is known Letrozole stimulates FSH surges and the emergence of a new follicular wave in humans (Mitwally & Casper, 2002). But in cattle, Yapura et al. (2012) showed that single and repeated (every 24 h for 3 days) administration of different formulations of letrozole did not affect FSH concentrations.

In Benoit et al, study (1992) administration of a nonsteroidal aromatase inhibitor, every 8 hr maintained decreased secretion of estradiol and altered profiles of gonadotropins. Kivrak et al, (2021) indicated that letrozole following single and repeated (every 24 h for 3 days) intravenous administrations caused an increase in the FSH concentration.

This study was conducted with the aim to evaluate the efficiency of substituting conventional gonadotropin-based protocol with letrozole on reproductive parameters and hormonal changes in Farahani ewes during breeding season.

## Materials and methods

### Animals and management

The experiment was implemented at the Small Ruminant Research Center (SRRC) of Arak University, Arak, Iran from November 2019 to April 2020. A total of 40 non-lactating Iranian Farahani ewes (3-4 years,  $44 \pm 2.3$  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/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, and the ewes were randomly divided into 4 groups (n=10 per group). One group of ewes served as the control; the first group received 400IU PMSG 48 h prior to CIDR removal (eCG protocol); the second group received 400IU hCG 48 h prior to CIDR removal (hCG protocol) and the third group was orally administered with letrozole (Parsian Pharmaceuticals Co, Tehran, Iran) for 3 consecutive days (2.5 mg per day) prior to CIDR removal (Ortiz-Carrera et al, 2019). An ewe was considered in estrus only when she allowed a ram to mount and this was registered as the onset time of estrus. Six

fertile rams with colored bibs were used for daily mating (in the morning and afternoon) for 4 days, starting at 24 h after CIDR removal (Habibzad et al, 2015). The reproductive variables measured were estrus response (%): Percentage of ewes in estrus after sponge withdrawal, pregnancy rate (%): percentage of pregnant ewes to all synchronizaed ewes, lambing rate (%): percentage of ewes lambd from pregnant ewes previously diagnosed, fertility (%): percentage of ewes lambd from the total of ewes mated, multiple lambing (%): percentage of ewes lambd 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 -1 before CIDR removal.

Blood serum was then separated by centrifugation (2500 rpm for 15 min) and stored at -20°C. Estradiol concentration was 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.

#### 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 concentration, a mixed model for repeated measurements was used. Results were expressed as mean ± SEM, and a probability of P≤0.05 was considered as significant.

#### Results

##### Reproductive performance

The data for reproductive performance are presented in Table 1. There were no significant differences in any of the reproductive performance parameters between control and other groups. This indicated that estrous synchronization using the progestin CIDR could be effective in Farahani ewes during the breeding season. The fecundity (40% vs. 0) and multiple lambing (150% vs. 0) were numerically greater for ewes treated with eCG compared to other treatments which showed that the inclusion of eCG into estrous synchronization program can improve reproductive performance.

**Table 1. Reproductive performance of Farahani 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)
Letrozole	10/10(100)	10/10(100)	10/10(100)	10/10(100)	0/10(0)	10/10(100)
hCG	10/10(100)	10/10(100)	10/10(100)	10/10(100)	0/10(0)	10/10(100)
eCG	10/10(100)	10/10(100)	10/10(100)	10/10(100)	4/10(40)	15/10(150)

#### Blood parameters

The results of serum estradiol concentration are presented in Figure 2. All groups had basal serum estradiol concentrations during the days before sponge removal. In all groups, the estradiol concentration showed an increasing trend

over time. No significant difference was observed in estradiol concentrations between groups (P>0.05). However, there were numerical differences between groups.

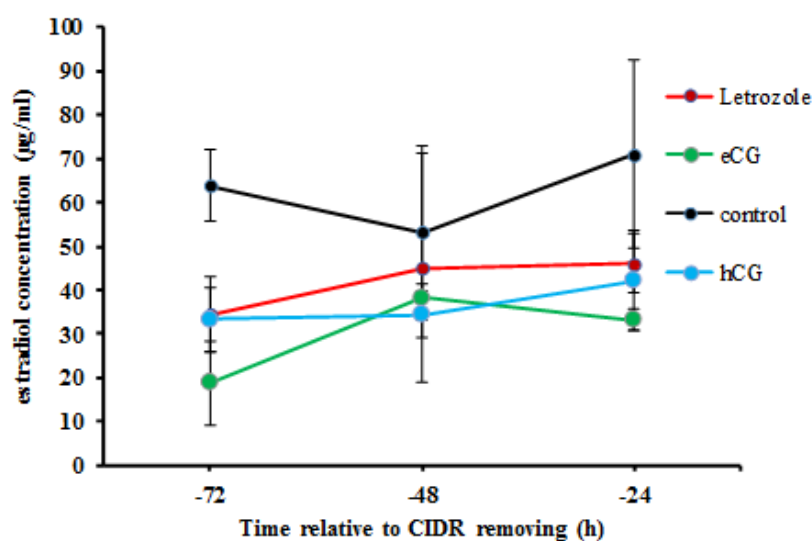


Figure 1. Comparison of serum estradiol concentration between the letrozole, eCG, control and hCG groups ( $P>0.05$ )

## Discussion

Theoretically, there are two practical ways for increasing the number of ovulatory follicles: the supplementation of endogenous gonadotrophins with exogenous products, such as eCG, around the time of mating or reducing the feedback effects of estradiol hormones on gonadotrophins (Land, 1979; Scaramuzzi et al, 2011). This study was designed to test the hypothesis that letrozole treatment, prior to CIDR removal, will reduce the feedback effects of estradiol on gonadotrophins and will improve the reproductive performance in Farahani ewe. Based on the results, orally letrozole treatment did not increase fecundity and multiple lambing compared to eCG treatment. Therefore, using letrozole with our proposed regimen was not a suitable alternative for eCG in order to estrus synchronization in farahani ewe. Abdel Dayem *et al.* (2020) showed that letrozole treatment (using an intravaginal sponge containing 7.5 mg letrozole for five days) did not significantly affect the number of small, medium and large sized follicles in Farafra ewes. Benoit *et al.* (1992) indicated that fadrozole (as a nonsteroidal aromatase inhibitor) treatment (intravenous) had a decreasing and increasing effects on estradiol and FSH respectively in ewes

during the breeding season. In another study, Kivrak *et al.* (2021) showed that letrozole following single and repeated (every 24 h for 3 days) intravenous administrations at 1mg/kg dose caused an increase in the FSH concentration in anestrus Akaraman ewes. The observed differences between the results of this study and others could be attributed to the stage of breeding season, the stage of the follicle wave, letrozole dosage, letrozole administration route and treatment regimen. In most studies, letrozole was administered through intramuscular, intravenous or intravaginal form (Benoit et al, 1992; Kivrak et al, 2021; MA, 2020; Yapura et al, 2018). While in our study, letrozole was used orally. Based on the reports, species differences are also a vital factor that can affect letrozole activity. Akbarinejad et al. (2016) showed that unlike bovine and human, in equine the oral administration of letrozole during the preovulatory period did not affect corpus lutea diameter and progesterone concentration. Yapura et al. (2012), in their study on cattle, indicated that a single treatment or 3-day regimen of letrozole was increased circulating LH while FSH remained unchanged.

There is a positive correlation between serum estradiol levels and the growth of follicles during the estrous cycle (Rahminiwati et al, 2017). In our study, in all treatments, estrogen concentration was significantly increased during the time which reflects the continuous growth of follicles. Based on the results (Figure 1), letrozol did not have a significant effect on reducing estrogen concentration during the treatment time. Insufficient circulating levels of letrozole during the treatment period can be one of the possible reasons for this result. In a study conducted by Abdel Dayem et al. (2020) treatment with letrozole using intravaginal device containing containing 7.5 mg letrozole decreased the serum concentrations of estradiol in ewes. Benoit et al. (1992) showed that fadrozole reduced the E<sub>2</sub>

concentration after 8e10 h following the treatment. However, in studies conducted by yapura et al. (2016) and Garcia-Velasco et al. (2005) on cattle and women respectively, letrozole did not reduce estradiol concentrations. In another study, it was stated that letrozole is less effective in the presence of high estradiol concentrations even if aromatase enzyme is reduced (Cortínez et al, 2005).

Based on the results of this study, using letrozole with our proposed regimen, was not effective in increasing the ovulation rate in Farahani sheep in comparison with the traditional eCG regimens. However, due to the small sample size in this study, further studies with larger sample sizes are needed to evaluate the real potential of letrozole for increasing the ovulation rate in sheep.

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### Conflict of interest

The authors of the article have no conflict of interest.

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## ارزیابی کارائی همزمان سازی فحلی و عملکرد تولیدمثلی میش‌های فراهانی همزمان شده در فصل تولیدمثل با استفاده از لتروزول

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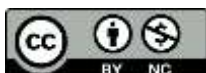
### چکیده

این پژوهش با هدف بررسی تأثیر کارایی جایگزینی گنادوتروپین با لتروزول در برنامه همزمان‌سازی فحلی بر پارامترهای تولیدمثلی و تغییرات هورمونی گوسفند در فصل تولیدمثل انجام شد. برای این منظور، ۴۰ رأس میش فراهانی با دامنه سنی ۳ تا ۴ سال، میانگین وزنی  $44 \pm 2/3$  کیلوگرم و میانگین نمره وضعیت بدنی  $3/12 \pm 0/5$  به مدت ۱۴ روز سیدرگذاری و به سه گروه تقسیم شدند: گروه شاهد (بدون تزریق هورمون)، گروه یکم (تزریق ۴۰۰ واحد ECG، تزریق درون ماهیچه‌ای)، گروه دوم (تزریق ۴۰۰ واحد hCG، تزریق درون ماهیچه‌ای) و گروه سوم ( $7/5$  میلی‌گرم لتروزول به شیوه خوراکی). نمونه خون از سه روز قبل از بیرون آوردن CIDR گرفته شد. فراسنجه‌های عملکرد تولیدمثلی پس از زایش محاسبه شدند. یافته‌ها نشان دادند که اگرچه باروری و بره‌زایی در گروه اول از نظر عددی بیشتر بودند؛ با این وجود تفاوت معنی‌داری بین گروه‌ها برای هیچ یک از فراسنجه‌های عملکرد تولیدمثلی وجود نداشت. غلظت استروژن در تمام تیمارها تا زمان برداشت سیدر روند افزایشی داشتند. به طور کلی، یافته‌های این پژوهش نشان دادند لیتروزول جایگزین قابل اعتمادی برای ECG برای افزایش میزان تخم‌ریزی در گوسفند نیست.

**کلمات کلیدی:** لتروزول، عملکرد تولیدمثلی، همزمان سازی فحلی

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