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Technical Note

Effects of increased exposure time to eCG on reproductive performance in estrus-synchronized Farahani ewes

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Abstract It seems that; long exposure to equine chorionic gonadotropin (eCG) in ewe synchronization programs would stimulate the growth of ovarian follicles resulting in better reproductive performance. Therefore, the aim of present study was to expose Farahani ewes to longer duration of eCG by using an eCG-alhydrogel mixture as a slow-release eCG. Fifty Farahani ewes (3-4 years, 44 ± 1.3 kg BW, BCS 3.04±0.3 on scale 1 to 5) were treated with controlled internal drug release (CIDR) for 14 days. The experimental groups consisted of: control group receiving no eCG, and four groups of ewes receiving either 400 IU eCG or eCG-alhydrogel preparation (i.m.) at 24 h (-24S, and -24SR groups, respectively), or 48 h (-48S and -48SR groups, respectively) prior to CIDR removal. Blood samples were taken from two days before until one day after CIDR removal. Reproductive performance was recorded at lambing. There was no difference (P>0.05) between groups in terms of the pregnancy rate, lambing rate, fertility, multiple birth and fecundity. However orthogonal contrasts showed that the fecundity and multiple birth were higher (P<0.05) in eCG-alhydrogel ewes. In all groups, estradiol concentration showed an increasing trend with time (P<0.05). Estradiol concentration was significantly higher in the -48SR compared with the -48S group; no difference was observed between 24S and -24SR ewes (P>0.05). The findings indicated that in an estrous synchronization protocol, administration of slow- release eCG preparations might improve the fecundity and multiple births in sheep.

Keywords: alhydrogel, estrous synchronization, Farahani ewe, reproductive performance

Paper type: Technical Note

Introduction

To date, exogenous progesterone in combination with gonadotropin is the most accepted protocol for inducing and synchronizing the estrous cycle in ewes during the breeding and anestrous seasons (Hameed et al., 2020). Exogenous progesterone regimen is applied using intravaginal devices, such as sponges and controlled internal drug release (CIDR), which remain for 12 to14 days (Dias et al., 2015). Also, eCG is the most widely used hormone in this protocol (Ali, 2007); eCG can improve fer-

tility, as well as increase the twinning rate. Many parameters can influence the efficiency of eCG in synchronization protocols, including the dose (Quintero-Elisea et al., 2011), route of administration (Boshoff and Burger, 1973), season (Langford et al., 1983), repeated treatments (Driancourt et al., 1991) and the timing of administration relative to the CIDR removal (Ali, 2007).

Generally, there is an interaction of timing of eCG treatment and the stage of the estrous cycle on the efficiency of estrous synchronization (Robinson, 1951). It has been suggested that reproductive efficiency in ewes may be improved when eCG is treated several days before sponge removal (Gordon, 1997). Previous studies reported that administration of eCG 24 h prior to the sponge removal, shortened the time of ovulation (Eppleston et al., 1991), increased pregnancy rate (Zhang and Yuan, 1988) and improved fertility (Zeleke et al., 2005). Also, eCG administration before sponge removal resulted in more predictable and tight estrus (Quintero-Elisea et al., 2011). Increased estrous response by earlier eCG treatment is probably related to changes in the pattern of follicular development (Ali, 2007). In this regard, as a suggested mechanism for eCG action, Driancourt and Fry (1992) showed that, eCG, at low concentration, can recruit follicles less than 3 mm only up to 24 h after injection whereas increasing dosages of eCG extend recruitment of these follicles even 24 h after injection. This mechanism indicated that the duration of access to the effective concentration of eCG is an important factor for increasing the efficiency of estrous synchronization. In addition to the dose of eCG, the pattern of eCG release is also very important (Bó and Mapletoft, 2020).

Sustained release formulations of proteins such as, liposomes, cross linked hydrogels and implants have been evaluated as a suitable way to improve the pattern of proteins release (Yahyaei et al., 2017, 2018). Simple mixing of proteins with various types of adsorbents is a simple and inexpensive method that results in sustained release of proteins (Bó and Mapletoft, 2020). One of these adsorbents is aluminum hydroxide gel that can adsorb proteins and release them gradually (Kimura, 2016; Bó and Mapletoft, 2014).

Although a large number of studies have been carried out to evaluate different estrous synchronization protocols based on eCG application, the numbers of studies associating eCG exposure time and efficiency of estrous synchronization protocols are limited. Therefore, the aim of this study was to test the hypothesis that in an estrous synchronization protocol, the simple mixing of eCG with alhydrogel may improve the reproductive performance in low-fecund Farahani sheep breed.

Materials and methods

Experimental design and treatments

The experiment was conducted from November 2018 to April 2019 at the Small Ruminant Research Center (SRRC) of Arak University, Arak, Iran. A total of 50 non-lactating Farahani ewes (3-4 years, 44 ± 1.3 kg BW, BCS 3.04±0.3 on scale 1 to 5) were housed in individual pens with free access to water and feed (consisting of 10.2% crude protein (CP), 2.3 Mcal/kg dry matter (DM), 7g Ca/day Ca and 4g P/day).

Estrous cycle in experimental ewes was synchronized using CIDR (EAZI-BREED™, CIDR®, New Zealand) for a 14-day period. The experimental groups (n=10 ewes per group) consisted of: a control group receiving no eCG, and four groups of ewes receiving either 400 IU eCG or eCG-alhydrogel preparation at 24 h (-24S, and -24SR groups, respectively), or 48 h (-48S and -48SR groups, respectively) prior to CIDR removal. Equine chorionic gonadotropin (eCG; Gonaser®, Hipra, Spain) and eCG- alhydrogel (alhydrogel® adjuvant, Merck) were injected intramuscularly in 2mL volumes.

Twelve fertile rams were introduced 24 h after CIDR removal and were mated with ewes twice daily (morning and evening). The reproductive parameters measured were: estrous response (%; the percentage of ewes exhibiting estrus after sponge withdrawal), pregnancy rate (%; proportion of synchronized ewes diagnosed as pregnant), lambing rate (%; number of ewes lambed per pregnant ewes), fertility rate (%; number of ewes lambing per number of ewes mated, multiple lambing (%; percentage of ewes lambing with two or more lambs), fecundity (%; percentage of lambs born per ewe mated (Quintero-Elisea et al., 2011).

Blood sampling and hormonal assay

Daily blood samples were collected from three ewes randomly selected from each group via jugular vein at 9:00 a.m. between days −2 to +1 days after CIDR removal and inserted into the test tubes containing anticoagulant (EDTA). Blood samples were centrifuged at 700×g for 15 min and plasma was stored at -20°C until assayed for estradiol concentration. Estradiol concentration were determined by an ELISA Reader (ELx 808-Ultramicroplate ReaderBio-Tek Instruments INC. U.S.A) using a commercial kit (Hangzhou Eastbiopharm CO., LTD.Cat.No:CK-E91162,Hangzhou,China). The intra-assay and inter-assay coefficients of variation were <10% and <12%, respectively. The sensitivity of the assay was 0.92 ng/L.

Statistical methods

The experiment was performed using a completely randomized design. Data for reproductive performance were analyzed using PROC GENMOD. For the analysis of blood estradiol, a mixed model for repeated measurements was used (SAS, 2002). Results were expressed as mean ± SEM, and a probability of P≤0.05 was considered to be significant. The Tukey's test was used for mean comparison (P≤0.05).

Results

Reproductive performance

There was no significant difference in estrous response, pregnancy rate, lambing rate, and fertility when estrus-synchronized ewes were administered with eCG or eCG-alhydrogel preparation for 24 and 48 h prior to CIDR removal (P>0.05); the reproductive parameters was equal to 100% in all treatments. The fecundity and multiple lambing were numerically higher in -24SR (130%) and -48SR (150%) groups (P>0.05). Orthogonal contrasts showed that the fecundity and multiple lambing was significantly increased (P<0.05) by longer eCG exposure time (Table 1).

Plasma estradiol

Plasma estradiol concentrations are presented in Figures 1-3. Plasma estradiol concentration was higher in -48SR group than in -48S ewes but there was no significant difference between the control and -48S and -48SR groups (Figure 2). Based on the orthogonal contrasts, estradiol concentration was significantly higher in the slow-release group compared with the eCG groups. No difference was observed between the control, eCG and

Table 1. The effect of timing of eCG injection and duration of eCG exposure in an estrus-synchronization program on reproductive parameters of Farahani ewes (orthogonal contrasts)

	Estrous response	Pregnancy rate	Lambing rate	Fertility	Multiple lambing	Fecundity
Contrasts	P-value					
-24S vs -24SR	0.54	0.54	0.54	0.54	0.45	0.43
-48S vs -48SR	0.82	0.82	0.82	0.82	0.53	0.49
S vs SR	0.79	0.79	0.79	0.79	0.04	0.02

-24S: injection of eCG (400IU) 24 h prior to CIDR removal, -24SR: injection of slow *release* eCG (400IU) 24 h prior to CIDR removal, -48S: injection of eCG (400IU) 48 h prior to CIDR removal (-48S), -48SR: injection of slow release eCG (400IU) 48 h prior to CIDR removal.

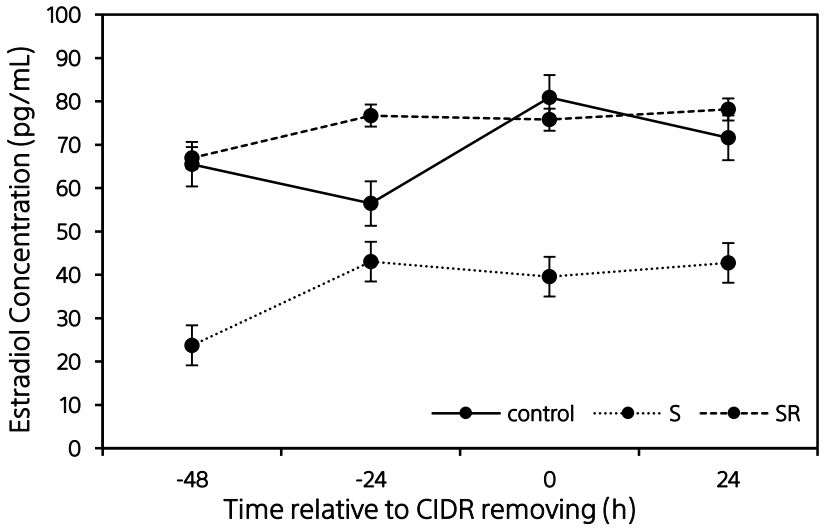

Figure 1. Comparison of serum estradiol concentrations (mean±SEM) between the single (S, 400 IU eCG), slow release (SR, 400 IU eCG-alhydrogel preparation) and control groups. The difference between the S and SR groups was significant (P<0.05). The regression of estradiol concentrations on time was significant.

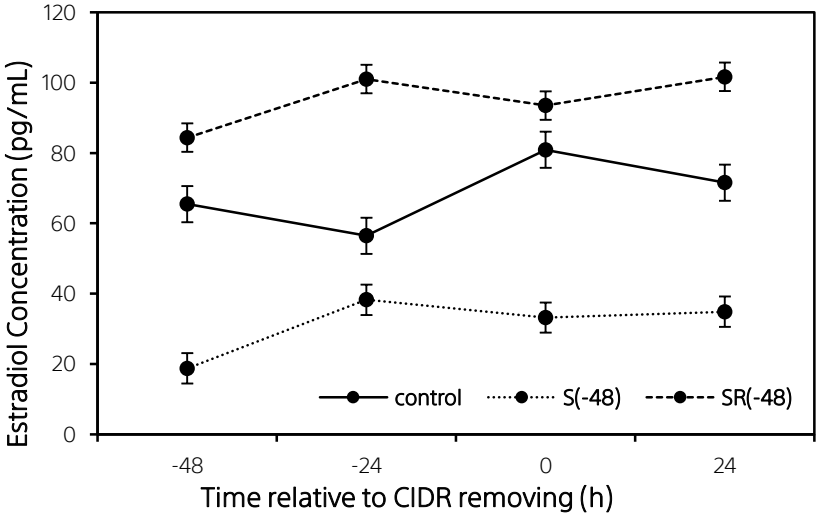

Figure 2. Comparison of serum estradiol concentrations (mean±SEM) between the single (S, 400 IU eCG), slow release (SR, 400 IU eCG-alhydrogel preparation) and control groups when eCG was injected 48 h prior to CIDR removal. The difference between the S (-48) and SR (-48) groups was significant (P<0.05). The regression of estradiol concentrations on time is significant.

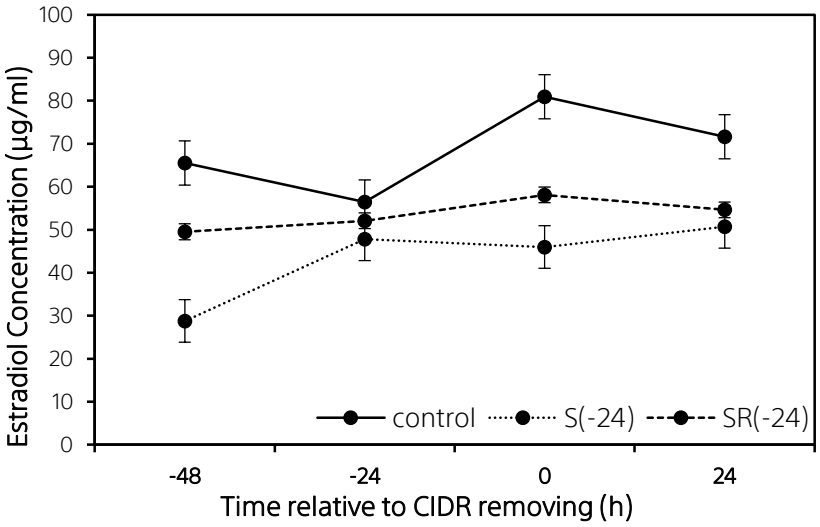

Figure 3. Comparison of serum estradiol concentrations (mean±SEM) between the single (S, 400 IU eCG), slow release (SR, 400 IU eCG-alhydrogel preparation) and control groups when eCG was injected 24 h prior to CIDR removal. The difference between the S (-24) and SR (-24) groups was not significant (P>0.05). The regression of estradiol concentrations on time is significant.

slow-release groups (Figure 1). No significant differences were observed between -24S, -24SR and control groups (Figure 3). Estradiol concentration was significantly affected by time in all groups.

Discussion

The objective of this study was to test whether long exposure to eCG improves the effect of synchronization programs on reproductive performance in low-fecund Farahni ewes. Duration of exposure to eCG did not affect the reproductive performance. This is consistent with previous reports. It has been shown that eCG treatment 48 h prior to sponge removal did not improve the estrous response and pregnancy rate in Ossimi ewes (Ali, 2007).

Quintero-Elisea et al. (2011) reported similar pregnancy rates, lambing rate and fertility between Pelibuey and Blackbelly ewes receiving eCG for 48 or 24 h prior to sponge removal. Also Koyuncu and Altıcekic (2010) indicated that the estrous response and lambing rate were not affected by timing of eCG (24 prior to sponge removal versus at sponge removal) in Kivircik ewes.

Previous research also reported similar multiple lambing and fecundity rate in ewes treated with eCG either prior to sponge removal (-24 h or -48 h) or at time of sponge removal (Ali, 2007; Koyuncu and Altıcekic, 2010; Quintero-Elisea et al., 2011). Based on the orthogonal contrasts, a simple mixing of eCG with alhydrogel was highly efficacious in improving the reproductive performance in Farahani ewes. Possibly, a combination of eCG with alhydrogel can affect the pattern of eCG release from the injection site. Circulating concentrations of eCG following injection depend on absorption from the site of administration, and metabolic clearance rate from the circulation. (Tríbulo et al., 2011). According to metabolic clearance rates (Demoustier et al., 1988), it would be expected that the clearance rate of eCG from blood is independent of the method of administration and eCG concentration. As a result, rapid absorption at the injection site should result in rapid eCG clearance. Therefore, the length of time during which the effective concentration of eCG is available to affect the antral follicles will be so short to sustain the follicular growth until ovulation.

It has been shown that a mixture of FSH and aluminum hydroxide gel led to prolonged FSH peak through slowing the rate of absorption (Demoustier et al., 1988). Scaramuzzi et al. (2011) suggested maintaining the effective FSH concentration for longer duration as a strategy for increasing the number of ovulatory follicles in ewes. The finding of this study indicates that the exposure time to gonadotropins would have greater impacts on the number of ovulatory follicle rather than the dose of application. In this regard, it was shown that at the same dose, continuous exposure to FSH, in comparison with single administration, increased the number of ovulatory follicles (Schipper et al., 1998). Driancourt and Fry (1992) showed that, an increased dose of eCG (which is associated with increased circulating eCG concentration) significantly extended the recruitment of follicles less than 3mm even 24 hours after injection. Kimura et al. (2007) found that follicular growth and ovarian weight in rats were significantly increased when single treatment of pFSH in saline was replaced with single treatment of pFSH in Al-gel.

There is a positive correlation between the number of antral/ tertiary/ /preovulatory follicles and plasma estradiol concentration (Andriyanto et al., 2017). In our study, estradiol concentration increased over the time which reflects the continuous growth of follicles. Higher concentration of estradiol in -48SR and SR groups could be related to the increased number of pre-ovulatory follicles. Nevertheless, there was no significant difference between the control group and SR, -48SR groups. It seems, there is a relationship between the timing and exposure time of eCG with estradiol synthesis. In this regard, Andriyanto et al. (2017) indicated that with management of the time and dose of eCG, it is possible to increase the secretion of estrogen without inducing a superovulatory response.

Conclusions

In summary, administration of 400IU eCG in alhydrogel resulted in comparable fecundity and multiple lambing to 400IU eCG in Farahani breed which is characterized by a low twinnig rate. The reason for this improvement is unclear but it may be assumed that mixing of eCG with alhydrogel improved the pattern of eCG release from the injection site. However, further studies are needed to investigate this issue.

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