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Comparing CIDR with progesterone injections and eCG with human recombinant FSH for synchronizing estrous cycle in ewes

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Abstract

Background: It is desirable in estrus synchronization in sheep to avoid intravaginal devices and to shorten the program from 14 to 6 days. Moreover, replacement of eCG with safe, cheap, and efficient gonadotropin is in worldwide demand. **Aims:** This study investigates the possibility of replacing eCG with human recombinant FSH (hrFSH) and CIDR with progesterone injections for estrous synchronization in ewes. **Methods:** Assaf and Lacaune ewes (n=170) were divided into two groups and synchronized with either progesterone injections for 6 days or CIDR for 14 days. Ewes assigned in the injection group, received progesterone (37.5 mg; SC) and GnRH analogue (7.5 µg Alarelin acetate; IM) on day 0 of the experiment. On days 3 and 6, ewes received 25 and 12.5 mg progesterone (SC), respectively. On day 6, ewes in both groups received prostaglandin $F_2\alpha$ (250 µg Cloprostenol; IM), and were divided into two subgroups to receive either hrFSH (75 IU Follitropin alfa; SC) or eCG (400 IU; IM). On day 7, fertile rams were introduced to ewes for 21 days. Data were analyzed using GLM and Glimmix. **Results:** There was no difference in the respective lambing rates, prolificacy, and fecundity between CIDR (71.1, 1.63, and 1.16%) and injections (66.7, 1.55, and 1.03%); between eCG (71.4, 1.60, and 1.14%), and hrFSH (66.3, 1.58, and 1.05%, P>0.05). **Conclusion:** In conclusion, 6-day progesterone injection-based protocol produced similar results to 14-day CIDR program and hrFSH could be an effective alternative for eCG during estrus synchronization in ewes.

Key words: CIDR, eCG, Human recombinant FSH, Progesterone injections, Sheep

Introduction

Synchronization of estrus during the non-breeding season in ewes plays an essential role in optimizing breeding programs, ensuring reproductive efficiency, and maximizing genetic gains (Hogue, 1987; Raoul and Elsen, 2020). Currently, the estrus synchronization programs mostly rely on progesterone-releasing intravaginal devices (controlled intravaginal drug release also known as CIDR and sponge) for 12 to 14 days (Robinson and Smith, 1967; Gomez et al., 2006; Swelum et al., 2015). Apart from the costs, there are several health issues and ethical concerns about using intravaginal devices, including vaginitis, purulent secretion, foul-smelling fluids, and subsequent impacts on ewe health and welfare (Godfrey et al., 1997; Martinez-Ros et al., 2018). Additionally, there is a risk

of environmental contamination due to residual progesterone and the necessity of antibiotic use to prevent vaginitis (Viñoles et al., 2011). To overcome these limitations, we have developed a progesterone injection-based protocol consisting of three injections of progesterone on days 0, 3, and 6 in association with GnRH and prostaglandin $F_2\alpha$ on days 0 and 6, respectively (Payan et al., 2022; Seidi Samani et al., 2023a, b).

To enhance prolificacy and fecundity, equine chorionic gonadotropin (eCG), Robinson and Smith, 1967; Langford et al., 1983; Pearce and Robinson, 1985; Ferdowsi et al., 2020), hMG (Seidi Samani et al., 2023b), and follicle stimulating hormone (FSH; Knights et al., 2001b) were used toward the end of estrus synchronization programs. Several concerns regarding eCG could restrict the widespread use of this

gonadotropin including high cost, ethical controversies surrounding pregnant mare blood, and potential immune responses, which could diminish its efficacy with repeated use, resulting in a decline in fertility (Bodin *et al*., 1997; Roy *et al*., 1999; Animal Welfare Foundation, 2022). Alternatively, hMG provided promising outcomes to substitute eCG in combination with progesterone injection-based estrus synchronization programs (Seidi Samani *et al*., 2023b). However, hMG high cost causes a limitation to use in large sheep flocks.

FSH produces by the pituitary gland. Bovine FSH (Graff *et al*., 2000; Boscos *et al*., 2002) and porcine FSH (Knights *et al*., 2001a, b, 2003) have been used to enhance the ovulation rate in ewes. Tissue-derived gonadotropins, due to the variations in purity and potency, could be associated with variable ovarian responses (Murphy *et al*., 1984; Kanitz *et al*., 2002). Besides, gonadotropins derived from animal tissues may also be vectors for infectious diseases (Murphy *et al*., 1984; Manning *et al*., 1987; Phillips *et al*., 1993; Ludwig *et al*., 2002; Galli *et al*., 2003; Wrathall *et al*., 2008).

Recombinant FSH could remove many concerns regarding tissue-derived FSH (Daya, 2004; Lunenfeld, 2004). Recombinant FSH has been used for superovulation in ewes (Kendall *et al*., 2004; Rutigliano *et al*., 2014); however, there are limited and inconsistent studies about using recombinant FSH to enhance reproductive performance of ewes (Yavuzer *et al*., 2010; Zeitoun *et al*., 2020a, b). To the best of our knowledge, there is no study using human recombinant FSH in combination with progesterone injection-based estrus synchronization program in ewes.

Cinnal-f® is a new recombinant human FSH (Follitropin alfa), synthetized in genetically modified Chinese hamster ovary cells, which received an approval from Iranian Food and Drug Administration in June 2013 (IRC: 3125387962296341). It is significantly more cost-effective compared to Folltropin-V and has been used successfully in cattle for superovulation (Khodadadi *et al*., 2022).

The objective of this study was to investigate the effectiveness of human recombinant FSH (Cinnal-f) compared to eCG for enhancing reproductive performance of Shal ewes using progesterone injection-based or CIDR protocols for estrus synchronization in ewes.

Materials and Methods

Ethics statement

The present study was approved by the Animal Ethics Committee of the Faculty of the Veterinary Medicine, University of Tehran, Tehran, Iran (SRT45/08.02.2022).

Experimental location and animals

This study was performed at the sheep flock of Avingen Company, Firuzkuh, Iran (latitude: 35°76'93" N; longitude: 52°93'17" E; altitude: 2180 m; Max temp: 38°C; Min temp: -21°C; breeding season: between August and November) between January and February,

2023. Healthy, non-pregnant and non-lactating Assaf and Lacaune ewes (n=170) were selected for this experiment. Thirty-two adult rams, with a previous history of sound fertility, were used for this study.

Experimental design

Healthy, non-pregnant and non-lactating Assaf and Lacune ewes (n=170) were assigned randomly into four experimental groups, considering their breed, weight, and parity, using 2 (injection; n=87, or CIDR; n=83) by 2 (FSH; n=86, or eCG; n=84) factorial design (Fig. 1).
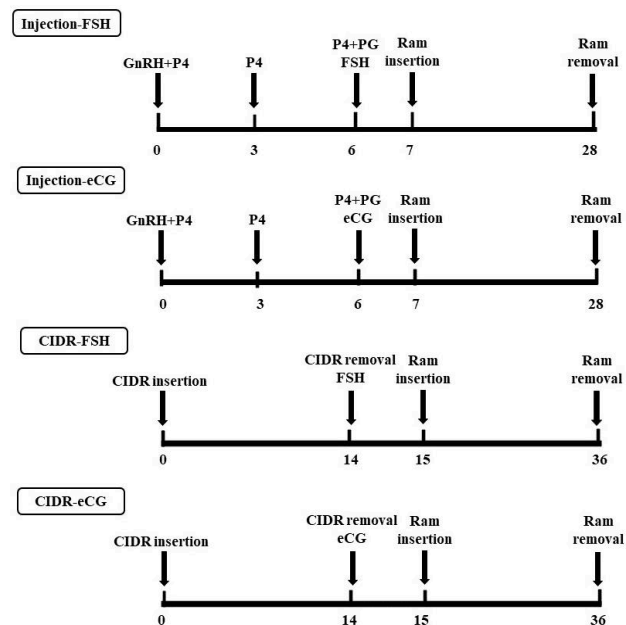

Fig. 1: Comparing CIDR *vs* progesterone injections and hrFSH *vs* eCG for estrus synchronization in ewes. Ewes received CIDR for 14 days (CIDR group; n=83) or three injections of progesterone on days 0, 3, and 6 in association with GnRH on day 0 and prostaglandin F$_2\alpha$ on day 6 of the experiment (injection group; n=87). Ewes in each group were then divided and received either eCG (n=84) or FSH (n=86) at the end of progestogen treatment

Ewes were divided into two groups and were synchronized by inserting CIDR for 14 days or using the progesterone injection-based protocol, according to our previous study (Payan *et al*., 2022). In brief, on day 0 of the experiment, ewes received progesterone (37.5 mg; SC; Aburaihan Pharmaceutical Company, Iran) and GnRH analogue (7.5 µg Alarelin acetate; IM; Aburaihan Pharmaceutical Company, Iran). On days 3 and 6 of the experiment, ewes received subcutaneous injections of 25 and 12.5 mg progesterone, respectively. All ewes received prostaglandin F$_2\alpha$ analogue (250 µg Cloprostenol; im; Nasr Pharmaceutical Company, Iran) on day 6 (Fig. 1). Concurrent with the final days of progestogen treatment, each group of ewes were divided into two subgroups and received either 75 IU hrFSH (sc; Cinnal-f®; Cinnagen, Iran) or 400 IU eCG (im; Gonaser®, Hipra, Spain). The day after progestogen treatments, fertile rams (1:5) were introduced to ewes and remained for 21 days (Fig. 1).

Table 1: Reproductive performance in two groups of synchronized ewes that received CIDR for 14 days (n=83) or three injections of progesterone on day 0, 3, and 6 of the experiment, in association with GnRH on day 0 and prostaglandin F$_2\alpha$ on day 6 of the experiment (injection group; n=87)

Group	Lambing rates (%)	No. of lambs			Total lamb	Prolificacy (%)	Fecundity (%)
		Single	Twin	Triple			
CIDR	59/83 (71.1)	25	31	3	96	1.63	1.16
Injection	58/87 (66.7)	27	30	1	90	1.55	1.03

Table 2: Reproductive performance in two groups of synchronized ewes that received eCG (n=84) or FSH (n=86) at the end of progestogen treatment

Group	Lambing rates (%)	No. of lambs			Total lamb	Prolificacy (%)	Fecundity (%)
		Single	Twin	Triple			
eCG	60/84 (71.4)	26	32	2	96	1.60	1.14
FSH	57/86 (66.3)	26	29	2	90	1.58	1.05

Semen analysis

Prior to introducing the rams, semen was collected by electro-ejaculator and the quality of semen was evaluated according to the methods described by Evans and Maxwell (1987). Rams with the mass motility of ≥3.5 and the individual motility of ≥70% were used in this study.

Reproductive parameters and statistical analysis

Data with discrete nature including lambing rate (ewes lambed per total number of ewes ×100), prolificacy (lambs born per ewes lambing) and fecundity (lambs born per total number of ewes) were analyzed using the Glimmix procedure including logistic regression (log) as Link Function and Binomial (for lambing rate) or Poisson (for prolificacy and fecundity) statements as type of distribution in the model. Treatments were considered as fixed effects and breed as random effects. The percentage of events was calculated using the Frequency procedure. Data with continuous nature including the time intervals to particular events were analyzed using GLM procedure after testing for normality (Shapiro-Wilk) using univariate normal plot procedure. Tukey Studentized Range (HSD) test was used for pair-wise comparisons. Data were presented as mean±SEM and percentage. Data were analyzed using SAS, 2016.

Results

There was no interaction between the main factors: progestogen treatments and gonadotropin treatments (P>0.05). Therefore, the results of the main effects were evaluated and presented (Tables 1 and 2). There was no difference in lambing rates, prolificacy, and fecundity between CIDR and progesterone injections (Table 1; P>0.05) and eCG and hrFSH (Table 2; P>0.05).

Discussion

The main objective of this study was to investigate the effectiveness of hrFSH to enhance the reproductive performance of ewes. In the current study, hrFSH treated Lacaune and Assaf ewes had similar results in the respective prolificacy and fecundity (1.58 and 1.05) compared to eCG treated ewes (1.60 and 1.14). Yavuzer et al. (2010) reported that the administration of hrFSH (10 IU, Puregon, Organon, Turkey), 24 h prior to prostaglandin F$_2\alpha$ injection, was associated with greater twin pregnant Awassi ewes (FSH treated: 57.1% vs control: 4.5%; Yavuzer et al., 2010). There was no information on the prolificacy and fecundity of experimental groups in the latter study. Recently, the effect of hrFSH (Gonal-F) on twinning rates of ewes was investigated in two similar studies (Zeitoun et al., 2020a, b). Due to the high doses of FSH used in the latter studies (133.33 Folltropin-V and 180 IU rh-FSH), close to the dose recommended for superovulation in ewes (176 to 256 mg Folltropin-V; Bartlewski et al., 2016), the rate of mortality in lambs was high. Accordingly, the recommended dose of FSH is not suitable to induce twin ovulation in ewes. Varying doses of Folltropin-V (48, 55, 64 mg) prescribed at different times (12, 24, and 36 h) before intravaginal progestogen removal could affect follicle growth and ovulation; however, it was unable to enhance the prolificacy of ewes during the non-breeding season (Knights et al., 2001a, b and 2003). The respective optimal prolificacy and fecundity were reported to be 1.3 to 1.6 and 1.2 to 1.5 during the breeding season (Youngquist and Threlfall, 2007). During the non-breeding season, fertility and fecundity might be 20% to 30% lower than during the breeding season (Davies, 2019). Therefore, the reproductive indices of the current study could be within the normal range.

In the present study, hrFSH was administered at the time of the last progesterone injection. Our previous results showed that hMG and eCG have to be administered at 24 or 48 h after the last progesterone injection (Payan et al., 2022; Seidi Samani et al., 2023b). This is because, increasing FSH concentrations during the preovulatory period, when the progesterone concentration is approaching its basal concentration, can be associated with an increase in ovulation rates (Henderson et al., 1988; Knights et al., 2003). Due to our previous study, it took a maximum of 72 h for plasma progesterone to reach to the basal concentration after the progesterone injection (Seidi Samani et al., 2023b). However, after removal of CIDR, it takes only 4 h for the

plasma progesterone to reach to the basal concentration (Ainsworth and Downey, 1986). Therefore, the time of eCG administration is recommended before or concurrent with the intravaginal progesterone device removal (Evans and Maxwell, 1987; Ali, 2007) or prostaglandin $F_2\alpha$ administration (Ferdowsi et al., 2020); whereas, the time of gonadotropin administration for the progesterone injection protocol should be concurrent, 24 and 48 h after the last progesterone injection (Payan et al., 2022; Seidi Samani et al., 2023b). In our recent study, the time of estrous expression, lambing rates, and prolificacy were similar when eCG was administered concurrently with and 24 h after the last progesterone injection (Payan et al., 2022). Therefore, in order to reduce the labor intensity, hrFSH was injected concurrently with the last progesterone injection.

In the current study, the lambing rates were similar between CIDR (71.1%) and progesterone injection (66.7%) groups, FSH (66.3%), and eCG (71.4%). Similar result following 26 to 30 days mating period was achieved in synchronized ewes using 42 (76%), and 68 (79%) mg Folltropin-V during the non-breeding season (Knights et al., 2003). The recommended lambing rate goal during the non-breeding season after one and two cycles was around 50% and 70%, respectively (Youngquist and Threlfall, 2007), which is close to the results obtained in the current experiment.

In conclusion, hrFSH (Cinnal-f) could replace eCG, and progesterone injection-based protocol could replace CIDR for estrus synchronization program in ewe. This in turn, not only eliminates the negative impacts and concerns following eCG administration but also reduces the overall cost of the estrus synchronization protocol in ewes.

Acknowledgements

Conflict of interest

The authors declare that they have no conflicts of interest.

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