

*Full Length Research Paper*

# The effects of three methods of synchronization on estrus induction and hormonal profile in Kalkuhi ewes: A comparison study

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Due to the importances of estrus synchronization in sheep reproduction and fertility, the present trial had been carried out to compare the three methods of estrus synchronization in ewes during breeding season. 60 cycling, fat-tailed Iranian Kalkuhi ewes were randomly allocated in equal numbers ( $n = 20$ ) to 3 treatment groups. In two treatment groups, sponges (medroxyprogesterone) and controlled intravaginal drug-releasing devices (CIDR) were inserted for a period of 14 and 12 days respectively; also, another group received two injections of prostaglandin F2alpha (PGF2 $\alpha$ ) (with 11 days interval). At the time of CIDR/sponge removal and second PGF2 $\alpha$  administration, all the animals received 400 IU of pregnant mare serum gonadotropin (PMSG). Blood samples were collected from all of the treated ewes, and progesterone (P4) and estradiol (E2) concentrations were measured by double-antibody enzyme immune-assay (ELA). Estrus synchronization by CIDR was as effective as Sponge and PGF2 $\alpha$  treatments; also CIDR treated ewes exhibited overt signs of estrus earlier than those in other treatment groups. Following hormonal assays, P4 concentrations at first and second sampling and estradiol concentrations at second and third sampling were significantly different ( $P < 0.05$ ).

**Key words:** Ewe, reproduction, synchronization, estradiol, progesterone.

## INTRODUCTION

Successful estrus synchronization programs have a key role in lambing rate efficiency and profitability of sheep holders in semi-intensive production systems (Knights et al., 2001). Using different intravaginal devices impregnated with P4 or synthetic progestogens has been extended all over the world. Controlled internal drug release (CIDR) device impregnated with 300 mg P4 and intravaginal sponges containing with 60 mg medroxyprogesterone acetate (MAP) or 40 mg fluorprogesterone acetate (FGA) are two main

intravaginal devices that are used vastly for ewes estrus synchronization (Ungerfeld and Rubianes, 2002).

PGF2 $\alpha$  and/or its analogue are luteolytic factors and according to its role in regression mechanism of corpus luteum (Turk et al., 2008), double injections (9 to 11 days interval) of PGF2 $\alpha$  is common for ewes' estrus synchronization (Atamani and Akoz, 2006; Wildeus 1999). Also, Dixon et al. (2006) used progesterone and/or its analogue treatment and reported greater estrus observation in progesterone-PGF2 $\alpha$  combination treated ewes than those received PGF2 $\alpha$  alone.

Combination of PMSG and P4 impregnated intravaginal devices causes readily estrus synchronization (Romano 2004). PMSG treatment reduces the interval between the onset of estrus and ovulation (Dogan and Nur, 2006), also in no PMSG received ewes, ovulations occurs later (Barrett et al., 2004). Obtained results by Dogan and Nur (2006) demonstrated that the interval between Sponge or CIDR removal until the estrus onset was shorter in

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**Abbreviations:** CIDR, Controlled intravaginal drug-releasing devices; PGFF2 $\alpha$ , prostaglandin F2alpha; PMSG, pregnant mare serum gonadotropin; ELA, enzyme immune-assay.

progesterone-PGF2 $\alpha$  combination treated ewes than those that received progesterone alone.

One dose of PMSG can stimulate follicular development and higher ovulation rate in ewes (Koyuncu and Ozis Alticekic, 2010). Due to the importance of PMSG in sheep reproduction efficiency and injection of 500 IU PMSG with 40 mg of FGA for estrus synchronization in breeding season, 87% of treated ewes exhibited overt sign of estrus when compared with 48% in the control group (Langford, 1982).

According to the points mentioned above and the importance of estrus synchronization to optimize ewes reproduction efficiency, the aim of the present study was to investigate the effects of three methods of estrus synchronization and their effects on blood plasma concentrations of progesterone and estradiol.

## MATERIALS AND METHODS

The experiment was conducted during the breeding season (October, 2010), at the Islamic Azad University, Saveh Branch farm located in Saveh City. The site is situated at  $50.19 \pm 21^\circ\text{E}$  longitude and  $35.01 \pm 13^\circ\text{E}$  latitude and at an altitude of 1108 m above sea level. The mean annual temperature is  $18.2^\circ\text{C}$  and the annual rainfall in this region ranges from 190 to 210 mm.

### Animals and their management

Sixty cycling, fat-tailed Iranian Kalkuhi ewes, the combination of multiparous and primiparous animals, weighing  $55 \pm 2.5$  kg were used in the trial. All Animals were provided with water and manually fed alfalfa hay, supplemented with grain pellets, *ad libitum*. The ewes were randomly allocated in equal numbers ( $n = 20$ ) to 1 of the 3 treatment groups.

### Treatment protocols

The estrous cycles were synchronized using three different methods: In the first group, progesterone sponges containing 60 mg of medroxyprogesterone (Spongovet/ Hipra) were inserted for a period of 14 days; In second group, CIDR's (EAZI-BREEDTM, CIDR<sup>®</sup>, New Zealand), was inserted for a period of 12 days and the third group received it intramuscularly (i.m). Two injections of PGF2 $\alpha$  (12.5 mg; Vetalyse<sup>®</sup> Iran) with 11 days interval, and also PMSG (400 IU; BIONICHE; Australia) intramuscularly (i.m) was injected into all ewes at CIDR/sponge removal (day 0 = day of CIDR/sponge removal) and after second PGF2 $\alpha$  administration.

For estrus detection, six mature rams with marking-harnesses and abdominal aprons were added to each treatment groups (two rams vs. twenty ewes) at the end of synchronization protocols.

### Blood sampling and hormonal analysis

Blood samples were collected from the jugular vein of ewes three times: (1) Seven days after initiation of synchronization protocols (1st sampling), (2) At the end of synchronization protocols, days 11, 12 and 14 in PGF2 $\alpha$ , CIDR and Sponge groups, respectively (2nd sampling), and (3) Two days after the end of each one of synchronization methods (3rd sampling).

Blood samples were centrifuged (in 3000 rpm for 15 min), and plasma was harvested and stored at  $-20^\circ\text{C}$  until progesterone and

estradiol assays. Plasma P4 and E2 concentrations were measured by double-antibody enzyme immune-assay (ELA) using 96-well ELISA (Monobind<sup>®</sup>; USA) plates according to the method of Kohno et al. (2005).

### Statistical analyses

The P4 and E2 concentrations were analyzed by using the mixed procedure of SAS (9.1). The mean comparison was performed using least square mean method. Effects of weight of ewes were added as a covariate to the model. Significant differences between treatments were determined at the  $P < 0.05$  level. Data were expressed as the mean $\pm$ SEM, unless otherwise stated.

## RESULTS

### Estrus exhibition

From the 60 ewes used in this study, 17 (85.0%), 18 (90.0%) and 16 (80.0%) ewes in sponge, CIDR and PGF2 $\alpha$  treated groups respectively, exhibited overt signs of estrus within the 96 h observation period and no one of the ewes expelled intravaginal devices in the trial. Percentage of ewes exhibiting estrus behavior was not significantly different between treatment groups. Also, CIDR treated ewes exhibited overt signs of estrus around 5 and 10 h earlier than PGF2 $\alpha$  and intravaginal spongers treated ewes, respectively.

### Hormonal assays

The results of hormonal measurement following three synchronization methods are shown in Table 1. The mean progesterone concentrations in first and third time of blood sampling was significantly different between CIDR and sponge or PGF2 $\alpha$  treated ewes ( $P < 0.05$ ) but in the second time of sampling, a significant difference was observed between the progesterone concentrations in Sponge received and PGF2 $\alpha$  /CIDR treated groups ( $P < 0.05$ ).

According to the evaluation of E2 concentrations in collected samples (Table 1), the mean E2 concentrations in the second sampling was significantly higher in PGF2 $\alpha$  treated group than other groups ( $P < 0.05$ ); also, the mean concentration of this hormone in third sampling was significantly different in Sponge treated ewes than the other treatment groups ( $P < 0.05$ ).

## DISCUSSION

In the present study, ewes estrus detection was conducted via teaser rams (with apron) and the results showed that in Sponge treated group ( $n = 20$ ), 17 (85.0%) ewes exhibited estrus behavior and this rate in CIDR ( $n = 20$ ) and PGF2 $\alpha$  ( $n = 20$ ) treated groups was 18

**Table 1.** The mean ( $\pm$ SEM) progesterone and estradiol concentrations at three times of blood sampling in three treatment groups.

Treatment group	1st Sampling		2nd Sampling		3rd sampling	
	P4 (ng/ml)	E2 (pg/ml)	P4 (ng/ml)	E2 (pg/ml)	P4 (ng/ml)	E2 (pg/ml)
Sponge	2.97 $\pm$ 1.16 <sup>a</sup>	4.67 $\pm$ 3.35	0.41 $\pm$ 0.11 <sup>a</sup>	5.80 $\pm$ 2.49 <sup>a</sup>	0.57 $\pm$ 0.18	4.37 $\pm$ 2.91 <sup>a</sup>
CIDR	6.99 $\pm$ 1.05 <sup>b</sup>	6.40 $\pm$ 2.07	3.82 $\pm$ 0.60 <sup>b</sup>	6.62 $\pm$ 3.58 <sup>a</sup>	1.27 $\pm$ 0.50	7.96 $\pm$ 3.70 <sup>b</sup>
PGF2 $\alpha$	2.13 $\pm$ 0.51 <sup>a</sup>	5.33 $\pm$ 1.72	3.76 $\pm$ 0.50 <sup>b</sup>	10.34 $\pm$ 2.01 <sup>b</sup>	0.76 $\pm$ 0.14	7.58 $\pm$ 2.61 <sup>b</sup>

Different superscripts (a,b) in the same column within a time of sampling indicate a significant difference ( $P < 0.05$ ). 1st Sampling: Seven days after initiation of synchronization protocols; 2th sampling: At the end of synchronization protocols, days 11, 12 and 14 in PGF2 $\alpha$ , CIDR and Sponge groups, respectively; 3rd sampling: two days after the end of synchronization protocols.

(90.0%) and 16 (80.0%), respectively. Ozyurtlu et al. (2010) reported that estrus responses following CIDR and Sponge treatment was 90% vs. 87.5% respectively also in Mutiga and Mukasa-Mugerwa (1992) study, the rate of 83% for PGF2 $\alpha$  treated animals was recorded. The percentage of ewes that exhibited estrus behavior was not significantly different between treatment groups.

CIDR treated ewes exhibited overt signs of estrus around 5 and 10 h earlier than PGF2 $\alpha$  and intravaginal sponges treated ewes, respectively. Our findings are in line with Wildeus (1999) result. Also, in another trial, it was demonstrated that in PGF2 $\alpha$  treated ewes (2.5 mg, 12 days interval), animals exhibited estrus behavior six hours earlier than those inserted intravaginal sponges (FGA, 40 mg for 12 days) (Mutiga and Mukasa-Mugerwa, 1992). This can be as a result of the influence of inserted CIDR on elimination of the ovulation rate variations (Wildeus, 1999).

Obtained results from this experiment showed that the mean progesterone concentrations at the first blood sampling were significantly different between CIDR and other groups ( $P < 0.05$ ) and our results are in agreement with previous studies which reported that blood plasma progesterone concentration reached the level of 2.1 ng/ml within twenty-four hours following CIDR insertion (Viñoles et al., 2001) and reached the peak level within seventy two hours after CIDR insertion (Turk et al., 2008). One reason for high progesterone levels in CIDR treated group was the quality of materials and the better P4 absorption than those treated by intravaginal sponges.

Following second blood sampling, the mean progesterone concentrations was significantly different between sponge treated ewes and other treated groups ( $P < 0.05$ ), and our examination values are in line with Godfrey et al. (1999) result; they found that mean progesterone concentration at the time of ram induction in PGF2 $\alpha$  treated ewes was significantly higher than those received sponge or CIDR treatments. Also, progesterone assays following 3rd blood sampling demonstrated that its concentrations in blood plasma declined after intravaginal devices withdrawal, also in PGF2 $\alpha$  treated ewes, following the second administration, the corpus luteum (source of

progesterone secretion) was regressed and P4 concentration declined gradually.

The basal concentration of E2 is generally 7 to 8 pg/ml and increases to 10 to 15 ng/ml at the peak concentrations in breeding season (Iida et al., 2004). Following the E2 measurements of first sampling, E2 concentrations of collected samples were not significantly different between treatments, but a significant difference ( $P < 0.05$ ) was observed in 2d sampling. Our findings are in agreement with previous studies, also, E2 concentrations increases can be related to the creation of first follicular waves. The mean concentration of E2 in PGF2 $\alpha$  treated ewes (2nd sampling) was higher than those received sponge or CIDR. This could be as a result of the development of ovulatory follicles and E2 secretion by them (Carson et al., 1981). The mean E2 concentrations following 3rd sampling were significantly higher ( $P < 0.05$ ) in CIDR and PGF2 $\alpha$  treated groups than that received sponges. Within 96 h after estrus detection, the mean E2 concentrations decreased gradually and same as this principal in our study, mean E2 values at 3rd sampling in comparison with 2nd sampling had been decreased.

In conclusion, estrus synchronization by CIDR method was as effective as Sponge (MAP) and PGF2 $\alpha$  treatment for inducing estrus. Also, CIDR treated ewes exhibited overt signs of estrus earlier than other treatment groups. Using three different types of estrus synchronization method was effective on plasma E2 and P4 profile changes. A further trial is needed to investigate the effects of these synchronization methods {Sponge (MAP), CIDR and PGF2 $\alpha$  on pregnancy and lambing rates by natural mating or AI of treatments.

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