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Efficacy of Pre-Synchronization and CIDR on the Outcome of Short-Term Synchronization Program in Zandi Ewes During the Breeding Season

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Abstract

BACKGROUND: Timed breeding programs are essential to implementing extensive artificial insemination (AI) programs in sheep.

OBJECTIVES: This study aimed to evaluate whether application of pre-synchronization and controlled internal drug releasing (CIDR) before and during fixed time AI protocol, respectively, could enhance estrus synchronization and fertility of ewes.

METHODS: A total of 120 ewes were randomly assigned into four experimental groups (n=30 in each group) considering age, weight, and body condition score (BCS). All ewes received GnRH (25 µg of alarelin acetate), and five days afterwards, $PGF_2\alpha$ (75 µg d-cloprostenol) plus eCG (400 IU). In the control group, ewes received no additional treatment. In Pre-synch group, ewes received two injections of $PGF_2\alpha$ at 9-day interval three days before GnRH administration of main estrus synchronization protocol. In CIDR group, ewes received 5-day CIDR between GnRH and PGF2α of main estrus synchronization protocol. In Pre-synch-CIDR group, ewes received both two injections of $PGF_2\alpha$ at 9-day interval and 5-day CIDR. Blood serum progesterone concentrations were measured in all ewes prior to injection of $PGF_2\alpha$ (day 5). All ewes were subjected to fixed time laparoscopic AI 48 hours after administration of the last $PGF_2\alpha$.

RESULTS: No interaction was found between CIDR and pre-synchronization protocols ($P>0.05$). Progesterone concentration was higher in the CIDR groups than in groups without CIDR ($P<0.0001$). Estrous cycle was not affected by pre-synchronization and CIDR ($P>0.05$). The estrus was earlier in ewes with pre-synchronization compared to ewes without pre-synchronization following the last injection of prostaglandin ($P=0.022$). Pregnancy rate, lambing rate, prolificacy rate, fecundity rate, lamb weight at birth, and lamb gender were not significantly different between the treatment groups ($P>0.05$).

CONCLUSIONS: In our study, estrus rate and reproductive parameters showed no significant differences between different groups, although pre-synchronization advanced onset of estrus expression.

KEYWORDS: Breeding season, CIDR, Ewe, Pre-synchronization, Short-term synchronization

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Introduction

In recent years, there has been an increasing demand for the use of various breeds in domestic sheep herds to implement breeding programs. The high price of imported rams, the sensitivity and mortality of these rams due to the various diseases in domestic herds, as well as the presence of rump in the most Iranian breeds as a cause of inefficient mating prevent the extensive use of such breeds of rams in the Iranian herds (Vodjgani *et al.*, 2017). Artificial insemination (AI) can be applied as a useful method in breeding programs (Sathe, 2018). Due to the special anatomy of ewes' cervix, it is difficult to access inside the uterus and thus, insemination of ewes with frozen semen has been encountered with a big challenge (Sathe, 2018; Noakes *et al.*, 2019).

Laparoscopic AI is currently one of the well-known intrauterine methods of insemination with frozen sperm in sheep (Casali *et al.*, 2017). Successful synchronization of estrus and ovulation is one of the major contributing factors affecting conception rate following laparoscopic AI in sheep (Olivera–Muzante *et al.*, 2019).

In this context, various estrus synchronization protocols have been used in sheep including application of 12- or 14-day progestogen-dependent protocols (Abecia *et al.*, 2012; Ávila-Castillo *et al.*, 2019; Martemucci and D'Alessandro, 2010). Yet, synchronization of estrus by protocols merely including progestogens or prostaglandin F2α (PGF2α) during the breeding season may not lead to the desired outcomes (Martemucci and D'Alessandro, 2010) since prolonged exposure with progestogens (≥ 9 days) reduces the quality of oocytes, and in turn, conception rate, and impairs embryonic development (Fierro *et al.*, 2016). It also affects sperm transport and survival in the female reproductive tract (Martemucci and D'Alessandro, 2010). Moreover, devices containing progestogens could escalate the risk of environmental and tissue contamination (Ávila-Castillo *et al.*, 2019). To overcome these issues, some studies have shown short-term exposure to progesterone; short-term synchronization protocols have shown acceptable results in sheep during breeding as well as non-breeding seasons because they not only enhance estrus synchronization but also culminate in oocytes of higher quality (Titi *et al.*, 2010).

Administration of a single dose of PGF2α during the breeding season does not influence synchrony of estrus and ovulation, because it can induce luteolysis but not synchrony in follicle development. (Martemucci and D'Alessandro, 2011). Nevertheless, two injections of $PGF_2\alpha$ at 7-16-day intervals have been reported to improve synchrony of estrous and ovulation (Fierro *et al.*, 2016; Carvalho *et al.*, 2018), but the interval between two administrations of PGF2α could impact fertility (Ataman and Akoz, 2006). In this sense, administration of GnRH was incorporated at the beginning of the protocol to control follicular growth prior to PGF2α injection (Ataman and Akoz, 2006; Titi *et al.*, 2010; Vallejo *et al.*, 2019). As a result, short-term GnRH-PGF2α-based protocol (5-day interval) has been recommended as a practical alternative to common progestogen-based estrous synchronization protocols during the breeding season (Ataman and Akoz, 2006; Titi *et al.*, 2010; Martemucci and D'Alessandro, 2010; Hashem *et al.*, 2015).

Studies on ewes (Bartlewski *et al.*, 2017; Titi *et al.*, 2010) and cows (Simões et *al.*, 2018) have shown that the use of exogenous progesterone between GnRH and PGF2α injections prevents the spontaneous luteolysis resulted from irresponsiveness of the dominant follicle to GnRH injection. Response to GnRH depends on the stage of the estrous cycle in which the injection is made. Furthermore, exogenous progesterone also increases pregnancy rates and boosts follicular growth (Martinez and Gonzalez, 2019).

In cows, the highest efficiency of Ovsynch program is achieved when there is a dominant responsive follicle at the time of GnRH injection and a suitable corpus luteum at the time of PGF2α injection (Youngquist and Threlfall, 2007). Studies have shown that starting Ovsynch at the beginning and at the end of the estrous cycle has not good results due to the presence of new follicles that are not responsive to GnRH, and premature luteolysis and onset of estrus before the second GnRH administration, respectively (Youngquist and Threlfall, 2007). Therefore, pre-synchronization using two PGF2α injections 14 days apart was used 12 days before the start of the Ovsynch program in cows, until the cows were in ideal phase of estrus cycle at the beginning of the Ovsynch program (days 5-12) (Zarkouny *et al.*, 2004).

The use of pre-synchronization to increase the efficiency of GnRH-PGF2α based programs in sheep is a new practice and only has been carried out sparingly in one study, with two PGF2α injections nine days apart (Ávila-Castillo *et al.*, 2019).

To increase the efficiency of short-term protocols based on GnRH-PGF2α, Ferdowsi *et al.* (2018) evaluated eCG performance at different time intervals to PGF2α. The results of the study showed that the concomitant use of eCG with PGF2α can increase the effectiveness of short-term GnRH-PGF2α-based programs.

This study aimed to increase the efficiency short-term protocols to of GnRH-PGF۲α- eCG achieve the appropriate program for increasing the fertility rate resulting from laparoscopic insemination. Therefore, the effects of pre-,synchronization with two PGF۲α injections nine days apart, three days before the start of GnRH-PGF۲α- eCG-based short-term programs, and the effect of using ٥-day exogenous progesterone were evaluated in this study.

Materials and Methods

Location of Study

The study was carried out in an ewe flock located in Alborz province of Iran in Eshtehard District with a longitude of 50 degrees and 22 minutes and latitude of 35 degrees and 43 minutes during the breeding season.

Animals

In this study, 120 healthy ewes of Zandi breed were used (age range: 2-6 years). Average weight and body condition score (BCS) were 57.3±1.4 kg and 3.1±0.1, respectively. All ewes received the same diet and had ad labium access to fresh water.

Experimental Design

Ewes were randomly assigned into four experimental groups (n=30 in each group) considering age, weight, and BCS. All ewes received GnRH (25 µg of alarelin acetate, Vetaroline, Aboureihan, Iran), and five days afterwards, $PGF_2\alpha$ (75 µg d-cloprostenol, Vetaglandin, Aboureihan, Iran) plus eCG (400 IU; Gonaser, Hipra, Spain) (Ferdowsi *et al.*, 2018). In controls (Group 1), ewes received no additional treatment. In controlled internal drug releasing (CIDR) group (Group 2), ewes received 5-day CIDR between GnRH and PGF2α of main estrus synchronization protocol. In Presynch group (Group 3), ewes received two injections of $PGF_2\alpha$ at 9-day interval three days before GnRH administration of main estrus synchronization protocol. In Pre-synch-CIDR group (Group 4), ewes received both injections of $PGF_2\alpha$ at 9-day interval and 5-day CIDR (Figure 1).

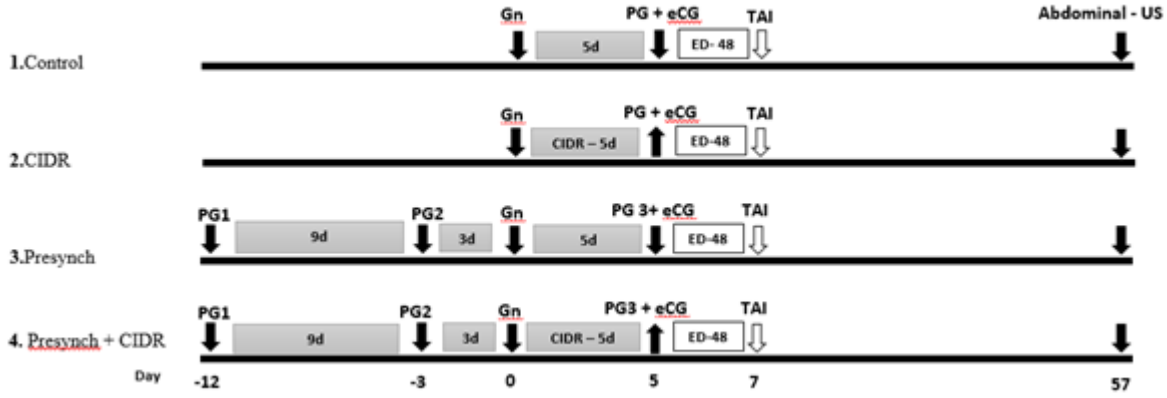

Figure 1. Experimental design including blood sampling (BS), injection and timing in different experimental groups (30 ewes in each group)

Estrus Detection

Estrus detection using teaser rams equipped with aprons and crayons was started six hours after $PGF_2\alpha$ injection and lasted for 48-hour after that (Ferdowsi *et al.*, 2018).

Laparoscopic AI

All the ewes were inseminated based on fixed time laparoscopic AI at 48 hours after administration of the last $PGF_2\alpha$ (Youngquist and Threlfall, 2007). To this end, the ewes were deprived of food and water for 12 h prior to laparoscopy (Sathe, 2018). Animals were sedated with 0.3 ml acepromazine maleate (Neurotanq; 10 mg/mL, Alfasan, Holland) 10–30 minutes before insemination (Sathe, 2018). Ewes were placed in laparoscopic cradles and kept in dorsal recumbency. Local anesthesia was performed by administration of 2 mL lidocaine hydrochloride (Luracaïne; 20 mg/mL; Vétaquinol S.A., France) in the site of abdominal puncture 5 min prior to insemination (Sathe, 2018). Two trocars were inserted about 3 cm on either side of the midline and about 3 cm cranial to udder to allow for introducing telescope and insemination pipette (Sathe, 2018). Following observation of uterine horns, half of the volume of thawed frozen semen (100×10^6 sperm; Animal Breeding Center, Karaj, Iran) was inseminated in each uterine horn. After insemination, all trocar puncture wounds were sprayed with a combined antiseptic and insect repellant (Vetaque, Animal Health Division, Iran). All laparoscopic operations were performed by an experienced operator.

Evaluation of Blood Serum Progesterone Concentration

Blood serum progesterone concentrations were measured in all ewes prior to injection of $PGF_2\alpha$ (day 5). Blood samples (10 mL) were collected from jugular vein, poured into vacuum tubes without anticoagulants (Sunphoria, Mediplus, China; Figure 1), and centrifuged at 3000 rpm for 15 min. Serum was isolated and transferred to 2-mL tubes and maintained at -20°C until measurement of progesterone (Ferdowsi *et al.*, 2018). Progesterone concentration was measured by ELISA kit (DRG Instruments GmbH, Germany) with detection limit of 0.045 ng to 40 ng progesterone per mL. Intra-assay and inter-assay coefficients of variation were calculated as 5.40% and 9.96%, respectively.

Pregnancy Diagnosis

Diagnosis of pregnancy was performed by transabdominal ultrasonographic examination using a B-mode ultrasound machine (V9, Emperor, China) equipped with a 3.5 MHz convex probe, 50 days after laparoscopic AI (Youngquist and Threlfall, 2007).

Evaluation of Reproductive Parameters

The following reproductive parameters were assessed: time of estrus onset, estrus detection rate (number of estrous ewes divided by total

number of ewes × 100), pregnancy rate (number of pregnant ewes divided by number of inseminated ewes × 100), lambing rate (number of lambed ewes divided by number of inseminated ewes × 100), fecundity rate (number of lambs divided by number of inseminated ewes), and prolificacy rate (number of lambs divided by number of lambed ewes × 100). Weight and gender of lambs were also recorded (Vodjgani *et al.*, 2017).

Statistical Analysis

Continuous data (i.e., progesterone concentration, time of estrus onset, and birth weight) were analyzed by General Linear Model (GLM) procedure. Binary data (i.e., estrus detection rate, pregnancy rate, lambing rate, fecundity, prolificacy, and sex ratio) were analyzed using GENMOD procedure including function link logit in the model. Multiple comparisons were performed using LSMEANS statement. All analyses were conducted in SAS version 9.4 (Statistical Analysis Systems, Cary, NC, USA). Data were presented as mean ± SEM or percentage. Differences were considered significant at P-value<0.05.

Results

Pre-synchronization using two administrations of PGF2α nine days apart had no influence on the concentration of progesterone at the time of PGF2α administration in the main synchronization protocol (P>0.05) (Table 1). However, application of CIDR for five days between GnRH and PGF2α injections in the main protocol culminated in higher concentration of circulating progesterone at the time of PGF2α in the main synchronization protocol (P<0.0001) (Table 1), and in turn, progesterone concentration at this time point was greater in CIDR and Pre-synch-CIDR groups as compared with the control and Pre-synch groups (P<0.0001) (Table 1).

Estrus detection rate was not affected by pre-synchronization using two administrations of PGF2α nine days apart, five-day usage of CIDR, and their interaction (P>0.05) (Table 1).

Time to commencement of behavioral estrus expression was advanced by 3.65 hours by the main effect of pre-synchronization using two administrations of PGF2α nine days apart (30.44±1.19 hours in non-pre-synchronized ewes versus 26.79±1.02 hours in pre-synchronized ewes; P=0.022; Table 1). Yet, five-day CIDR and interaction of pre-synchronization using two PGF2α injections nine days apart and five-day CIDR did not impact the estrus onset time (P>0.05) (Table 1).

Pregnancy rate, lambing rate, fecundity, prolificacy, birth weight of lambs, and sex ratio of offspring were not influenced by two administrations of PGF2α nine days apart, five-day usage of CIDR, and their interaction (P>0.05) (Table 1).

Table 1. Reproductive indices of ewes prepared for timed breeding using GnRH- prostaglandin $F_2\alpha_5$ days apart, in association with pre-synchronization (two prostaglandin F_2 ,α_9 days apart) and CIDR during breeding season

	Experimental group				P-value		
Parameter	Control	CIDR	Presynch	Presynch -CIDR	Pre-synch	CIDR	Presynch-CIDR
Progesterone (ng/ml)	2.6 ± 0.33[a]	4.6 ± 0.23[b]	2.4 ± 0.22[a]	4.7 ± 0.15[b]	0.38	< 0.0001	0.62
Estrus detection rate (%)	93.3 (28/30)	93.3 (28/30)	96.7 (29/30)	96.7 (29/30)	0.39	1.00	1.00
Time of estrus onset (hour)	31.1 ± 1.54	29.8 ± 1.82	27.1 ± 1.48	26.5 ± 1.48	0.02	0.53	0.81

	Experimental group				P-value		
Pregnancy rate (%)	50 (15/30)	46.7 (14/30)	56.7 (17/30)	60.0 (18/30)	0.27	0.99	0.71
Lambing rate (%)	50 (15/30)	46.7 (14/30)	56.7 (17/30)	60.0 (18/30)	0.27	0.99	0.71
Fecundity	60 ± 12	47 ± 9	70 ± 12	70 ± 11	0.24	0.55	0.55
Prolificacy	120 ± 11	100 ± 0	120 ± 10	110 ± 8	0.60	0.12	0.42
Birth weight (kg)	4.2 ± 0.19	4.7 ± 0.18	4.4 ± 0.26	4.4 ± 0.22	0.91	0.36	0.21
Sex ratio (%)	50 (9/18)	42.9 (6/14)	55.0 (11/20)	30.0 (6/20)	0.71	0.17	0.44

[a,b]Various letters indicate significant difference ($P<0.05$).

Discussion

The present study aimed to evaluate whether application of pre-synchronization using two $PGF_2\alpha$ injections nine days apart and usage of 5-day CIDR before and during fixed time AI protocol, respectively, could enhance estrus synchronization and fertility of ewes. The efficient estrus and ovulation synchronization is considered as one of the cornerstones of fixed time AI programs in sheep industry (Noakes *et al.*, 2019; Sathe, 2018; Youngquist and Threlfall, 2007).

Pre-synchronization protocol with two injections of $PGF_2\alpha$ with 9-day interval and three days before GnRH administration of main estrus synchronization protocol (Group 3) increased fertility rate by 56.7 %. No significant difference was found between the third group and the controls. While fertility and fecundity rates (60%) improved in the pre-synchronization technique, the progesterone was used in the original protocol for five days; but no statistically significant difference was found between the groups, which might be due to small sample size. These results were consistent with the findings of Ávila *et al.* (2019) who assessed pre-synchronization utilizing two injections of $PGF_2\alpha$ within 9-day intervals. Although no significant difference was found between the pregnancy and normal mating rates in the studied groups, the highest rates belonged to the pre-synchronization protocol with 5-day progesterone administration (90%) (Ávila-Castillo *et al.*, 2019). An explanation for this improvement in fertility could be the fact that pre-synchronization prepares the females to initiate the main protocol at a more appropriate stage of estrous cycle so that the GnRH could act as an agent to eliminate the current follicular wave to pave the way for emergence of the subsequent follicular wave at the favorable time (Navanukraw *et al.*, 2004).

Martemucci and D'Alessandro (2011) assessed the efficacy of short-term 5-day GnRH-FGA-$PGF_2\alpha$-eCG-based synchronization protocol for natural mating and laparoscopic AI. Fertility rates in laparoscopic AI in different treatment groups were reported as 60% and 40%, respectively, within 60 and 52 hours. These results were consistent with the findings of the present study regarding fertility rates (46-60%).

Pre-synchronization with two injections of $PGF_2\alpha$ within 9-day interval prior to five-day GnRH-PGF2α-eCG-based protocol advanced estrus manifestation, although it had no effect on progesterone concentration at the last $PGF_2\alpha$ injection, estrus rate, and other reproductive parameters in ewes. It has been reported that pre-synchronization with two injections of $PGF_2\alpha$

prior to Ovsynch in cattle can enhance the fertility rate in artificial insemination by synchronizing estrus and ovulation (Nowicki *et al.*, 2017). Moreover, El-Zarkouny *et al.* (2004) assessed the effect of pre-synchronization technique with two injections of $PGF_2\alpha$ within 14-day interval prior to Ovsynch protocol; they also assessed the efficacy of CIDR. The results showed that a single use of pre-synchronization and CIDR increased the fertility rate, but combination of CIDR and pre-synchronization had no effect on fertility rate (El-Zarkouny *et al.*, 2004).

Use of progesterone caused a sudden increase in progesterone level followed by decreased secretion of pituitary LH (Noakes *et al.*, 2019), which resulted in follicular atresia and generation of new waves of follicles (Martinez and Gonzalez, 2019). A 5-day progesterone protocol generated regular waves of follicles. Adequate progesterone level during follicular development helps to create healthy oocytes (Martinez and Gonzalez, 2019; Nowicki *et al.*, 2017). However, the 5-day progesterone in the synchronization protocol significantly increased the progesterone level at the time of $PGF_2\alpha$ injection in the original protocol (on day 5); but no significant difference was found in reproductive parameters. This finding was consistent with the findings obtained in the study on cyclic cows (Nowicki *et al.*, 2017). This might be due to the fact that in the main estrus synchronization protocol, GnRH injection induced ovulation and luteinization or luteinization without ovulation in some ewes (Youngquist and Threlfall, 2007), which would lead to increased endogenous progesterone. Therefore, it is possible that elevation of endogenous progesterone by GnRH overrode the potential beneficial effects of exogenous progesterone provided by CIDR. However, Titi *et al.* (2010) used the 5-day progesterone in the GnRH-$PGF_2\alpha$ protocol. The results showed that exogenous progesterone improved estrus and fertility. The difference between the present study and the former study was simultaneous injection of eCG and $PGF_2\alpha$, which did not necessitate progesterone protocol. Furthermore, Martinez *et al.* (2019) showed that 5-day progesterone administration without eCG (the same as 14-day conventional protocol) was more effective than 6- or 7-day progesterone administration in ovulation and fertility. This might be due to ovulation of a dominant follicle induced by progesterone on day 5 without eCG administration. The eCG should be added to the protocol for accurate synchronization of ovulation and estrus. Martinez *et al.* (2019) used the short-term progesterone administration in GnRH-PGF2α-based protocol without eCG in Group 3 and reported the fertility rate in normal mating as 68.4%, which did not significantly differ from the other studied groups.

Conclusion

Given the findings of the present study and the certain length of estrus in ewes (24 to 36 hours, Abecia et al., 2012), estrus might have long passed in some ewes due to early onset of estrus cycle following the last PGF2α injection at the time of AI. Therefore, AI was delayed in pre-synchronized ewes with early estrus cycle. A certain AI time should be determined in pre-synchronized ewes in future studies.

Considering the fertility rate in the different groups in this study (46%-60%), short-term 5-day GnRH-PGF2α-eCG-based protocols can be used during the breeding season to synchronize estrus and ovulation (50% fertility rate in the control group). However, estrus rate and reproductive parameters (e.g., pregnancy rate, lambing rate, fecundity, and prolificacy) did not show significant differences between different groups in this study. Yet, pre-synchronization improved the onset of estrus expression.

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Conflict of Interest

The authors declared no conflict of interest.

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تاثیر پیش همزمان‌سازی و CIDR روی نتایج حاصل از برنامه‌های کوتاه‌مدت همزمان‌سازی در میش‌های نژاد زندی طی فصل تولید مثل

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زمینه مطالعه: برنامه‌های همزمان‌سازی تولید مثل برای اجرای گسترده برنامه‌های تلقیح مصنوعی در گوسفند ضروری است.

هدف فرضیه ما در مطالعه حاضر این بود که، آیا کاربرد پیش‌همزمان‌سازی و CIDR ، به ترتیب قبل و در طول برنامه اصلی تلقیح در زمان ثابت، می‌تواند همزمان‌سازی‌سازی فحلی و باروری را در میش افزایش دهد.

روش کار: میش‌ها به‌طور تصادفی به چهار گروه آزمایشی (۳۰ راس در هر گروه)، با توجه به سن، وزن و نمرهٔ وضعیت بدنی تقسیم شدند. همهٔ میش‌ها GnRH (۲۵ میکروگرم آلارلین استات) و پنج روز پس از آن، PGF2α (۷۵ میکروگرم دی- کلوپرستنول) به همراه eCG (۴۰۰ واحد بین المللی) دریافت کردند. در گروه کنترل، میش‌ها درمان اضافه‌ای دریافت نکردند. در گروه CIDR، میش‌ها ۵ روز CIDR بین GnRH و PGF2α برنامهٔ اصلی دریافت کردند. در گروه Presynch، میش‌ها دو تزریق PGF2α به فاصله ۹ روز، ۳ روز قبل از تجویز GnRH برنامهٔ اصلی دریافت کردند. در گروه Presynch-CIDR، میش‌ها هم دو تزریق PGF2α به فاصله ۹ روز و هم CIDR ۵ روزه دریافت کردند. غلظت پروژسترون سرم خون قبل از تزریق PGF2α (روز ۵) در کلیهٔ میش‌ها اندازه‌گیری شد. ۴۸ ساعت بعد از تجویز آخرین PGF2α تلقیح مصنوعی برای همهٔ میش‌ها به روش لاپاروسکوپی انجام شد.

نتایج: هیچ اثر متقابلی بین CIDR و برنامه‌های پیش‌همزمان‌سازی یافت نشد ($P>0/05$). غلظت پروژسترون در گروه CIDR نسبت به گروه‌های بدون CIDR بیشتر بود ($P<0/0001$) . چرخهٔ فحلی تحت تأثیر پیش‌همزمان‌سازی و CIDR قرار نگرفت ($P>0/05$). بعد از آخرین تزریق پروستاگلاندین، فحلی در میش‌های با پیش‌همزمان‌سازی در مقایسه با میش‌های بدون پیش‌همزمانی سریع‌تر شروع شد ($P=0/022$). سایر شاخص‌های مورد ارزیابی تفاوت معنی‌داری بین گروه‌های درمانی نشان نداد ($P>0/05$).

نتیجه‌گیری نهایی: میزان فحلی و پارامترهای تولید مثل اختلاف معنی‌داری بین گروه‌های مختلف در این مطالعه نشان نداد. با این حال پیش‌همزمان‌سازی زمان شروع فحلی را تسریع کرد.

واژه‌های کلیدی: فصل تولید مثلی، سیدر، میش، پیش‌همزمان‌سازی، روش‌های کوتاه‌مدت همزمان‌سازی.

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