

## Estrous Synchronization with Used CIDR-G Devices in Ewes During Non-Breeding Season <sup>[1]</sup>

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### Summary

The objective of this study was to investigate the efficacy of used controlled internal drug release devices (CIDR-G) on estrous synchronization in ewes. This investigation was conducted in 58 fat-tailed ewes during the non-breeding season. Ewes were divided in to four groups. In Group I, CIDR-G devices were inserted to vagina for 12 d (CIDR group). In Group II, previously used CIDR-G devices were inserted into the vagina for 12 d (U-CIDR). After the intravaginal devices had been removed 500 IU equine chorionic gonadotropin (eCG) was injected intramuscularly (i.m.) into ewes of the CIDR and U-CIDR groups. Ewes in Group III (eCG group), 500 IU eCG was injected im. Ewes in Group IV, served as control and received an injection of 3 mL 0.9% NaCl (C Group). None of the control ewes expressed estrus. It was found that, 13/15 (86.67%), 10/15 (66.67%) and 1/13 (7.69%) ewes expressed estrus in CIDR, U-CIDR and eCG groups, respectively. The pregnancy rates in the CIDR, U-CIDR and eCG groups were 8/15 (53.33%), 9/15 (60%) and 1/13 (7.69%) at first breeding, respectively. The interval from eCG application to onset of estrus was 42.6±1.01, 40.13±1.35 and 74 h in the CIDR, U-CIDR and eCG groups, respectively. Average progesterone (P4) levels during P4 treatment were 3.28±0.28 and 2.62±0.14 ng/ml in the CIDR and U-CIDR groups, respectively. It is concluded that, CIDR-G that have been previously used for 12 d could be used for the synchroniation of estrus in ewes during non-breeding season.

**Keywords:** *Estrus synchronization, Ewe, Used CIDR-G*

## Koyunlarda Anöstrus Mevsiminde Kullanılmış CIDR-G Uygulamasıyla Östrusun Uyarılması

### Özet

Bu çalışmanın amacı, koyunlarda daha önce kullanılmış olan CIDR-G'nin östrus senkronizasyonu amacıyla tekrar kullanılabilirliğini araştırmaktır. Çalışma, anöstrus döneminde 58 adet koyunda yapıldı. Koyunlar dört ayrı gruba ayrıldı. Grup I; CIDR-G 12 gün boyunca vaginada bırakıldı (CIDR Grubu). Grup II; daha önce kullanılmış CIDR-G 12 gün boyunca vaginada bırakıldı (U-CIDR Grubu). İnvaginal alet uygulamalarını takiben aletlerin çıkarıldığı gün Grup I ve II'deki koyunlara 500 IU gebe kısrak serum gonadotropini (PMSG), i.m. yolla uygulandı. Grup III'teki koyunlara sadece 500 IU PMSG uygulandı. Kontrol grubu olarak oluşturulan Grup IV'teki koyunlara %0.9 NaCl'den 3 ml uygulandı. Kontrol grubundaki koyunların hiçbirinde östrus gözlenmedi. Östrus görülme oranları CIDR, U-CIDR ve PMSG grupları için sırasıyla %86.67 (13/15), %66.67 (10/15) ve %7.69 (1/13) olarak belirlendi. Gebelik oranları, CIDR grubunda %53.33 (8/15), U-CIDR grubunda %60 (9/15) ve PMSG grubunda %7.69 (1/13) olarak saptandı. PMSG uygulamasından sonra östrus görülüne kadar geçen süreye bakıldığında CIDR, U-CIDR ve PMSG grupları için sırasıyla 42.6±1.01, 40.13±1.35 ve 74 saat olarak kaydedildi. Uygulama süresi boyunca ortalama progesteron seviyesi CIDR grubunda 3.28±0.28 ve U-CIDR grubunda 2.62±0.14 ng/ml düzeyinde oldu. Araştırma sonucunda, daha önce koyunlarda 12 gün süreyle kullanılmış olan CIDR-G'nin koyunlarda anöstrus mevsiminde fertil östruslar uyarılabildiği görüldü.

**Anahtar sözcükler:** *Östrusun senkronizasyonu, Koyun, Kullanılmış CIDR-G*



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## INTRODUCTION

Applications of exogenous hormones for increased reproductive performance in domestic ewes are usually focused on estrous synchronization<sup>1</sup>. Estrous synchronization in goats and sheep is achieved by control of the luteal phase of the estrous cycle, either by providing exogenous P4 or by inducing premature luteolysis<sup>2,3</sup>.

Progestagens, one of the types of hormone used for synchronization in ewes, can be given by oral administration, subcutaneous<sup>4</sup>, or intravaginal insertion<sup>3,5</sup>. Blood progesterone levels increase within 1-4 h according to the route of administration<sup>1</sup>. Traditionally, sponges impregnated with either native P4 or analogues and then inserted into the vagina for a given period of time have been used. Afterwards, eCG may be injected 48 h before or at the time of sponge removal. Controlled internal drug release (CIDR) devices, consisting of a nylon core surrounded by a silicone elastomer that is impregnated with P4, have been developed as an alternative<sup>6,7</sup>. Controlled internal drug release silicone devices can release limited levels of progesterone for a longer time than is recommended for synchronization<sup>4</sup>. Because these devices are discarded after use, this can be a source of economic loss. These devices have been reused recently in cows, and researchers report successful results<sup>8,9</sup>. However, there have been no reports of similar trials in ewes and goats.

Equine chorionic gonadotrophin is used for induction of ovulation and estrus outside the breeding season, and is used more effectively for synchronization during the breeding season. In addition, eCG has been found to increase ovulation rate and twinning in a dose-related manner<sup>10</sup>.

The aim of this study was to investigate the effectiveness of used CIDR-G devices for synchronization in ewes. If successful, the cost of estrous synchronization could be decreased so that farm income can be increased. The implication of this study is to improve CIDR-G based synchronization protocols in sheep.

## MATERIALS and METHODS

### Location

Experiment was performed on Research and Training farm, University of Dicle, Diyarbakir, Turkey

(37°55'01"N latitude and 40°16'46"E longitude). This region, at an altitude of 660 m, is characterized by a temperate climate, with an average annual temperature of 15.7°C.

### Animals, treatments and experimental designs

Fifty-eight non-lactating fat-tailed (Awassi breed), 3-5 years old ewes were used in non-breeding season (postpartum average 70 d). Animals were housed in flock barn accessed to a feeding lot and were fed with grass hay as ad libitum basis. Multiparous ewes were randomly divided in to four groups. Rams were separated one month before the initiation of the enquiry.

*Group I (CIDR group, n=15):* CIDR-G devices (Eazi-Breed CIDR, 300 mg progesterone, Pharmacia Animal Health, Australia) were inserted into the vagina for a 12 d period. Following this, 500 IU eCG (Choronogest PMSG, Intervet, Turkey) was injected intramuscularly (IM) after intravaginal devices had been removed.

*Group II (U-CIDR group, n=15):* Disinfected and previously used CIDR devices inserted into the vagina for 12 d. Immediately after the intravaginal devices were removed, 500 IU eCG was injected IM. Previously used CIDR-G devices were washed with soap and water immediately after being removed and the mucus is cleaned. Then devices established in 0.1% chlorhexidine acetate solution for 30 min. Devices were dried in the room temperature. Devices have been wrapped with aluminium folio, has been stored in the refrigerator. Fifteen days after cleaning, previously used CIDR devices were used.

*Group III (eCG group, n=13):* 500 IU eCG was injected IM alone.

*Group IV (C group, n=15):* ewes served as controls and received 3 ml 0.9% NaCl IM.

### Estrus and pregnancy detection

Sheep were observed for estrus for 1 h twice a day (morning and evening) with a teaser ram (1 ram: 10 ewes), and ewes that expressed estrus were bred. Pregnancy was diagnosed by transrectal ultrasonography (Pie Medical LC 100) 35±2 days after breeding. The day of expression of estrus, the level of blood progesterone (P4), and pregnancy rates in all groups were evaluated.

### Blood sampling

Jugular blood samples were collected every other

day for P4 determination into anticoagulant tubes (4 mL), starting from the day before insertion of the vaginal devices and ending 21 d after devices were removed. Blood samples were centrifuged; the plasma was separated and stored at -20°C until analysis. Plasma samples were analyzed using a double-antibody Enzyme Immuno Assay (EIA) technique for determination of P411. The range of standards for P4 was from 0.25 to 16.0 ng/mL; the intra- and inter-assay coefficients of variation were 8% and 9%, respectively.

### Statistical Analyses

The mix model, GLM model and chi-square analysis sequences of SAS statistical programme version 9.0 were used.

## RESULTS

No CIDR-G was lost throughout the experimental period. None of the ewes in the control group expressed estrus. The estrous response, interval to

estrus, pregnancy rates and hormone levels are presented in *Table 1*. Although more ewes expressed estrus in the CIDR group, more pregnancies were obtained in U-CIDR group; however, this difference was not statistically significant ( $P>0.05$ ).

Ewes expressed estrus within 36-60 h of the removal of the device and eCG injection. Only one ewe expressed estrus in the eCG group (*Figure 1*). Differences in the interval from eCG application to estrus were not statistically significant between the CIDR and U-CIDR groups ( $P>0.05$ ). However, in CIDR-G treated groups the interval from eCG application to estrus was significantly ( $P<0.01$ ) shorter than that in eCG group. When hormone levels were evaluated, there was no significant difference among the groups prior to P4 treatment (day 0;  $P>0.05$ ).

Progesterone levels during treatment were significantly different in the CIDR and U-CIDR groups ( $P<0.01$ ). Average progesterone (P4) levels during P4 treatment were  $3.28\pm 0.28$  and  $2.62\pm 0.14$  ng/ml in the CIDR and U-CIDR groups, respectively (*Figure 2*).

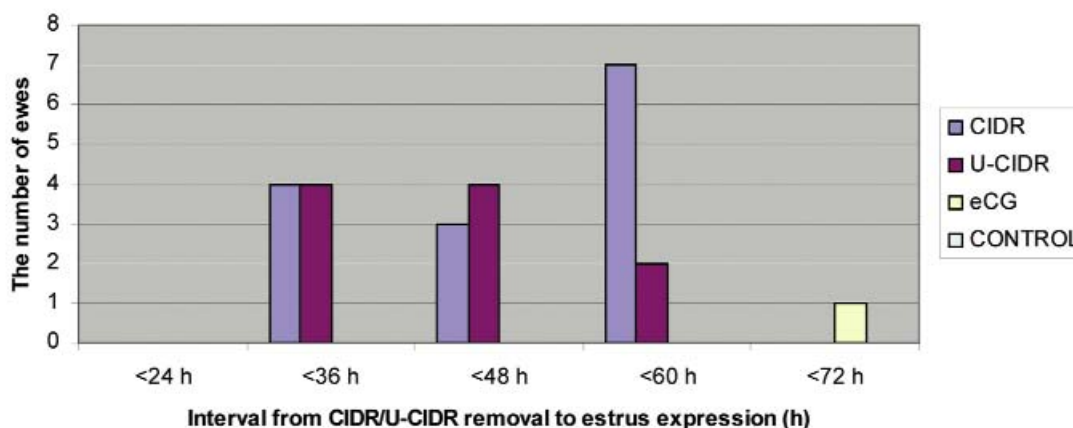
**Table 1.** Results of the study

**Tablo 1.** Araştırmada elde edilen bulgular

Groups	CIDR (n=15)	U-CIDR (n=15)	eCG (n=13)	C (n=15)
Number of ewes express estrus	13/15 <sup>a</sup> (%86.67)	10/15 <sup>a</sup> (%66.67)	1/13 <sup>b</sup> (%7.69)	0
Pregnancy rates	8/15 <sup>a</sup> (%53.33)	9/15 <sup>a</sup> (%60)	1/13 <sup>b</sup> (%7.69)	0
Interval from CIDR-G/U-CIDR-G removal to estrus (h)	42.6±1.01 <sup>a</sup>	40.13±1.35 <sup>a</sup>	74±3.82 <sup>b</sup>	-
P4 levels, prior to devices application (ng/mL)	0.55±0.07 <sup>a</sup>	0.53±0.07 <sup>a</sup>	0.53±0.06 <sup>a</sup>	0.55±0.06 <sup>a</sup>
P4 levels, during devices application (ng/mL)	3.28±0.28 <sup>a</sup>	2.62±0.14 <sup>b</sup>	0.70±0.13 <sup>c</sup>	0.77±0.13 <sup>c</sup>
P4 levels, devices removal to three weeks (ng/mL)	2.98±0.40 <sup>a</sup>	3.59±0.40 <sup>a</sup>	1.90±0.43 <sup>b</sup>	0.98±0.40 <sup>c</sup>

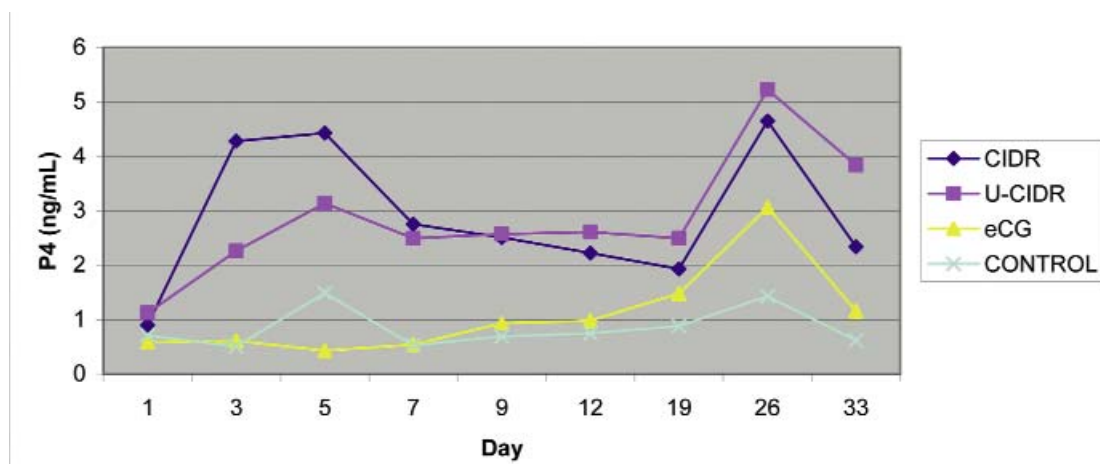
<sup>a, b, c</sup>: Superscripts within a same row indicates significance ( $P<0.01$ )

<sup>a, b, c</sup>: Aynı satırdaki farklı harfler  $P<0.01$  olduğunu gösterir



**Fig 1.** Distribution of ewes that express oestrus to estrus time

**Şekil 1.** Kızgınlık gösteren koyunların, kızgınlıklarının zamana göre kümülatif dağılımı



**Fig 2.** Average P4 levels during research

**Şekil 2.** Araştırma süresince ortalama P4 değerleri

Progesterone levels obtained three weeks after eCG injection were not significantly different in the CIDR and U-CIDR groups; however, the CIDR and U-CIDR groups were significantly different from the eCG and C groups ( $P < 0.01$ ; *Table 1*).

It was determined that the progesterone level was over 2 ng/ml within 24 h of intravaginal device insertion and the level was maintained until device removal. After introduction of the ram, the P4 value in the control group increased slightly, but it subsequently decreased.

## DISCUSSION

The slight increase in the P4 concentration of the ewes in the control group after the introduction of the ram can be attributed to the stimulation of follicular activity by the ram effect<sup>12</sup>. However, the P4 concentration decreased subsequently because of hormonal insufficiency.

Stenbak et al.<sup>13</sup> observed that hormonally induced ewes usually exhibit estrus at 24-48 h after the removal of progestagen implants or pessaries. Greyling and Brink<sup>14</sup> determined that estrus occurred about 31 h after the end of P4 treatment, Van Cleff et al.<sup>15</sup> reported estrus 36 h after the P4 treatment, and Godfrey et al.<sup>16</sup> reported estrus 34-40 h following P4 treatment. In a previous study<sup>17</sup>, we observed that onset of estrus 34.5±2.9 h after devices removal in ewes. In the present study, onset of the estrus following devices removal was similar to those reported by others.

After the treatment of the sheep in the eCG

group, the P4 level increased for the first two weeks but declined in the third week. It has been stated that single or repeated eCG treatment in ewes during the non-breeding season causes abnormal corpus luteum formation because of insufficient follicular maturity<sup>18</sup>. In this study, it is thought that inadequate LH exposure could result in immature follicle development and ovulation failure following eCG treatment during non-breeding season. Other researchers<sup>18,19</sup> have reported that ovulation can be induced after treatment with exogenous eCG in anestrous sheep. This may explain why one ewe in the eCG group expressed estrus and became pregnant.

It was observed that the average level of P4 started to decrease in the third week in ewes that had been given eCG. This decline was more marked in the CIDR group when compared with the U-CIDR group. However, this difference was not statistically significant. It is assumed that the reason for this difference was the rate of pregnancy, which was higher in the U-CIDR group than in the CIDR group.

Vinoles<sup>20</sup> reported that, use of a CIDR device resulted in peak plasma P4 levels of 2.1 ng/ml within 24 h of application, and relatively stable levels between days 1 and 13 (1.9 ng/ml). Güngör et al.<sup>17</sup> previously reported a P4 value of 4.1±0.2 ng/ml in anestrous ewes treated with CIDR-G; however, the P4 values were 3.28 ng/ml and 2.62 ng/ml in the CIDR and U-CIDR groups; respectively, in the current study. The progesterone values obtained in this study were lower than the values obtained in a previous study<sup>17</sup>. Although the previous research was carried out in the same season, the difference might have been because the current

study was performed when the ewes were in deep anestrus, while the ewes studied by Güngör et al.<sup>17</sup> were in mid-anestrus. When the estrus and hormone results were evaluated, it was determined that, the measured concentration was sufficient for synchronization, as demonstrated by the rate of estrus and pregnancy in this group; although the previously used CIDR-G could not achieve blood progesterone levels as high as those induced by the new CIDR-G.

In conclusion, it has been demonstrated that controlled internal drug release silicone (CIDR-G) devices that have been previously used once for 12 days could be reused for the estrous synchronization in ewes. Nevertheless, it would be useful to repeat this study with more ewes in breeding protocols.

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