



## Comparison of Pregnancy Rates after Short-Term and Long-Term Synchronization Protocol in Ewes – Pilot Study

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**Abstract:** The objective of the study was to compare the pregnancy rates after short-term and long-term synchronization protocol in Kivircik ewes. Ewes (n=54) were randomly divided into two synchronization groups as ST (short-term; n=27) or LT (long-term, n=27). Intravaginal sponge was inserted on the same day (D0) in both groups. It was removed at 7 days (D7) in the ST group and 13 days (D13) in the LT group. All ewes received 450 IU eCG and 75 µg d-cloprostenol at the time of sponge removal. The characteristics of vaginal discharge were evaluated following sponge withdrawal. For both protocols, 5 fertile rams per 27 ewes were used for natural mating following sponge removal. The overall estrous response and pregnancy rate were 88.9% and 64.8%, respectively. Following the synchronization, the overall estrous response (94.7% vs. 85.7%, respectively) and pregnancy rate (63.1% vs. 65.7%, respectively) were similar in both multiparous and nulliparous ewes. Although the short-term protocol reduced the rate of severe vaginitis from 48.1% to 25.9%, pregnancy rates were not different in both synchronization protocols. Nulliparous ewes had a higher rate (48.6% vs. 15.8%) of severe vaginal discharge than multiparous ewes. However, the alteration of the vaginal discharge severity depending on parity did not affect the overall pregnancy rate after the short-term (66.7%) and long-term (63.0%) protocols. Therefore, the long-term and short-term protocol can be used in both nulliparous and multiparous ewes in small flocks.

**Keywords:** Ewe, fertility, short-term, synchronization, vaginitis

### Koyunlarda Kısa–Sürelili ve Uzun–Sürelili Senkronizasyon Protokolü Sonrası Gebelik Oranlarının Karşılaştırılması – Ön Çalışma

**Öz:** Bu çalışmanın amacı Kivircik koyunlarda kısa süreli ve uzun süreli senkronizasyon protokolü sonrası gebelik oranlarını karşılaştırmaktır. Koyunlar (n=54) KS (kısa süreli; n=27) ve US (uzun süreli; n=27) olmak üzere rastgele iki senkronizasyon grubuna ayrıldı. Her iki gruba da aynı gün intravaginal sünger takıldı. KS grubunda 7. günde ve US grubunda 13. günde intravaginal sünger çıkartıldı. Süngerin çıkarılmasıyla koyunlara 450 IU eCG ve 75 µg d-cloprostenol uygulandı. Süngerin çıkartılmasını takiben vaginal akıntının karakteri değerlendirildi. Her iki protokol için de süngerin çıkarılmasını takiben doğal çiftleşme için 27 baş koyun başına 5 fertil koç kullanıldı. Toplam östrus yanıtı ve gebelik oranı sırasıyla %88.9 ve %64.8 idi. Senkronizasyonu takiben multipar ve nullipar koyunlarda toplam östrus yanıtı (sırasıyla %94.7'ye karşı %85.7), ve gebelik oranı (sırasıyla %63.1'e karşı %65.7) benzerdi. Kısa süreli protokol şiddetli vaginitis oranını %48.1'den %25.9'a azaltmasına rağmen, her iki senkronizasyon protokolündeki tüm vaginal akıntı skorlarına ait gebelik oranları farklı değildi. Nullipar koyunlar, multipar koyunlara göre daha yüksek oranda (%48.6'ya karşı %15.8) şiddetli vaginal akıntıya sahipti. Ancak vajinal akıntı şiddetinin pariteye bağlı olarak değişmesi, kısa süreli (%66.7) ve uzun süreli (%63.0) protokol sonrası toplam gebelik oranını etkilemedi. Dolayısıyla küçük sürülerde uzun süreli ve kısa süreli protokol hem nullipar hem de multipar koyunlarda kullanılabilir.

**Anahtar kelimeler:** Fertilité, kısa-süreli, koyun, senkronizasyon, vaginitis

### Introduction

Reproductive management of ewes is commonly controlled with synchronization protocols rather than natural methods. Synchronization protocols consist of exogenous progestagens is maintained for 12-14 d treatment period by mimicking luteal phase duration of estrous cycle (Abecia et al., 2012). Although long-

term (12-14 d) synchronization protocol promotes high estrous response, fertility outcomes are low compared the estrous response (Özyurtlu et al., 2011). Short-term (5-7 d) synchronization protocol is used as an alternative to reduce the negative effects of long-term protocol (Vinoles et al., 2001). Although previous studies conducting short-term protocol promoted the hypothesis with detrimental effects of long-term protocol (Blaschi et al., 2014; Martinez-Ros et al., 2018; Ungerfeld and Rubianes, 2002), higher pregnancy rate with the use of short-term protocol

was reported in only limited studies (Menchaca et al., 2018).

In addition to the application of shortening the synchronization protocol to improve fertility, the including of ram effect to synchronization protocol is frequently used to increase the reproductive efficacy (Evans et al., 2004). The sudden introduction of rams being isolated at least six weeks is described cheap and natural method for induction of estrous and ovulation (Abecia et al., 2012; Evans et al., 2004). In addition to ram effect, increasing the number of ram per ewe decreases mated ewes (Allison and Davis, 1976). Besides, the isolation of rams from ewes is difficult to indicate the benefit of ram effect even in experimental studies (Evans et al., 2004). Therefore, isolation of rams from flock without the tactile and visual absence, at least 1500 m, may not be possible due to management factors in small herds. In addition, the comparison of the length of synchronization protocol was evaluated as for only comparing the fertility results in previous studies. It was hypothesized that division of the length of synchronization protocol would also increase the pregnancy by decreasing the number of rams for mating. The objective of the present study was to compare the short-term and long-term synchronization protocol by tolerating the insufficient number of rams on fertility outcomes after natural mating in nulliparous and multiparous Kivircik ewes during breeding season.

## Materials and Methods

### Animals and management

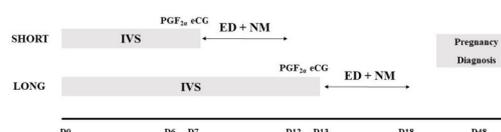
The experimental procedures were approved by the University of Balikesir Animal Care Committee (Reference Number: 2019-7/1). Kivircik ewes display seasonal breeding activity from June to January in Balikesir. The experiment was carried out during breeding season (September-November) on a farm located in a flock of the Balikesir University Experimental Farm (39° 28' N, 28° 02' E) in Turkey.

The herd consisted of 45 nulliparous and 106 multiparous ewes with a total of 151 ewes. Before the experiment, all ewes maintained on natural pasture with an additional 1.5 kg dried grass hay/ewe/day and approximately 1000 g barley/ewe/day as nutritional flushing for one month from August to September. All ewes were allowed to natural mating for about one and a half months with five rams isolated from ewes for five months. At the end of this month, pregnancy examination was performed to determine the non-pregnant ewes with a three-week interval. As a result of the pregnancy examination performed before the experiment, it was found that 64.2% of ewes were pregnant after natural mating for almost one and a half months without synchronization. The present study was initiated after the determination of the non-pregnant ewes. A total of 4 ewes were excluded from

the study - one ewe due to the absence of sponge before the withdrawal of intravaginal sponge, and three pregnant ewes due to the determination that the current pregnancy belonged to previous study at the time of first pregnancy examination.

### Experimental design

This study was carried out in 19 multiparous (2-5 years old), and 35 nulliparous ewes (about 10 months) with a total of 54 non-pregnant ewes in the same flock. The number of rams per ewe was planned to be approximately five (5 rams per 27 ewes) in each synchronization protocol (Martinez-Ros et al., 2018). Five rams were not removed from flock six weeks before the onset of study and all animals (ewes and rams) were housed together. Fifty-four ewes were randomly allocated to two treatment groups as ST (short-term synchronization group; n=27) or LT (long-term synchronization group, n=27). Intravaginal sponge containing 60 mg medroxyprogesterone acetate (MPA; Esponjavet®, HIPRA, Turkey) was inserted on the same day (D0) and it was removed after either 7 days (D7) in ST group or 13 days (D13) in LT group. All ewes received 450 IU equine chorionic gonadotropin (eCG; Gonaser®, HIPRA, Turkey) and 75 µg d-cloprostenol (Gestavet Prost®, HIPRA, Turkey) at the time of sponge removal (Figure 1). The intravaginal sponge was removed in short-term protocol while long-term protocol was ongoing. Five fertile rams with mating marks were used for the daily estrous detection after removal of the vaginal sponge. Five rams was used for natural mating of ewes without any interpretation following the withdrawal of the intravaginal sponge in short-term protocol. Following the mating in the short-term protocol, the vaginal sponge of the ewes was removed and the same mating protocol was applied in the long term protocol (Figure 1).



**Figure 1.** Schematic representation offers a combination of two protocols in the present study. Short-term protocol (ST; n=27) was performed with 7 d short-term protocol between D0 and D7 in while ewes received long-term protocol (LT; n=27) at the same time with short-term protocol between D0 and D13. Estrous detection (ED) was performed with teaser rams after removal of the intravaginal sponge in both groups and ewes exposed to fertile rams for natural mating (NM). Pregnancy diagnosis was performed via transrectal ultrasound at 30 days (D48) following the removal of intravaginal sponge.

The characteristics of vaginal discharge after the withdrawal of intravaginal sponge were evaluated as

**Table 1.** Comparison of synchronization length for estrous response and pregnancy rate in nulliparous and multiparous ewes

Ewes	Parameters	Treatment days			P
		7 days	13 days	Overall	
Nulliparous	Estrous Response % (n/n)	87.5	84.2	85.7	>0.05
		14/16	16/19	30/35	
Multiparous	Estrous Response % (n/n)	100.0	87.5	94.7	
		11/11	7/8	18/19	
Nulliparous	Pregnancy rate % (n/n)	62.5	68.4	65.7	>0.05
		10/16	13/19	23/35	
Multiparous	Pregnancy rate % (n/n)	72.7	50.0	63.1	
		8/11	4/8	12/19	

score according to the amount, odor, and aspect of discharge according to Martinez-Ros et al., 2018. Scores were classified (negligible or no discharge; Score 0, clear and some amount of discharge; Score 1 and abundant purulent or hemorrhagic discharge; Score 2).

Pregnancy examination was performed via transrectal ultrasound (Ibex Pro equipped with a 5.0-7.5 MHz, E.I. Medical Imaging, Loveland, CO) at 30 days following the removal of intravaginal sponge (Figure 1). The presence of a clear anechoic embryonic vesicle with a viable embryo (heartbeat) was evaluated as a positive pregnancy diagnosis. Pregnancies were confirmed at 60 d to determine the pregnancy loss.

**Statistical analysis**

Data were analyzed by using the SPSS® 25.0 package program (SPSS Inc., Chicago, IL, USA) for statistical analyses. Chi-square test was used to compare the estrous response, pregnancy rate, and vaginal discharge score. Pearson Chi-Square or Fisher's Exact Test was used for the significance level of the results. The significance level was considered as P<0.05 for all analyzes.

**Results**

The overall estrous response was 88.9% regardless of the length of synchronization protocol and parity in the present study. Irrespective of the length of synchronization protocol, multiparous ewes had numeri-

cally higher (94.7% vs. 85.7%) estrous response than nulliparous ewes (P>0.05). The estrous responses were not different (100.0% vs. 87.5%) following the withdrawal of intravaginal sponge after short-term and long-term protocol in multiparous ewes (P>0.05). The estrous response was also similar (87.5% vs. 84.2%, respectively) after both protocols in nulliparous ewes (P>0.05) (Table 1). The overall pregnancy rate was 64.8% after synchronization. The difference between estrous response and first pregnancy rate was approximately 24.0% irrespective of the length of synchronization protocol and parity in this study. The length of the synchronization protocol did not alter the pregnancy rate in both multiparous and nulliparous ewes (P>0.05). The pregnancy rates were 66.7% and 63.0% for short-term and long-term synchronization protocol, respectively. Besides, multiparous and nulliparous ewes had similar pregnancy rate (63.1% vs. 65.7%, respectively) irrespective of treatment length (Table 1). The pregnancy rate after the short-term protocol was 72.7% in multiparous ewes and it was 62.5% in nulliparous ewes (P>0.05). The long-term protocol resulted in 50.0% pregnancy rate in multiparous ewes and 68.4% pregnancy rate in nulliparous ewes (Table 1). There was no pregnancy loss between first (D30) and second pregnancy examinations (D60).

Vaginal discharge following the removal of intravaginal sponge was detected in all ewes. The removal of the intravaginal sponges was more difficult in nulliparous ewes than those of multiparous ewes. While

**Table 2.** Percentage of vaginal discharge characteristics and pregnancy rate according to vaginal discharge score in nulliparous and multiparous ewes

Characteristics	Percentage of scores		P	Pregnancy rate		P
	Nulliparous	Multiparous		Nulliparous	Multiparous	
0	0.0 (0/0)	0.0 (0/0)	>0.05	0.0 (0/0)	0.0 (0/0)	>0.05
1	51.4 (18/35)	84.2 (16/19)	<0.05	72.2% (13/18)	62.5% (10/16)	>0.05
2	48.6 (18/35)	15.8 (3/19)	<0.05	58.8% (10/17)	66.7% (2/3)	>0.05
Overall	100.0% (27/27)	100.0% (27/27)	>0.05	65.7% (23/35)	63.1% (12/19)	>0.05

almost half of the nulliparous ewes (48.6%; 17/35) showed purulent or bloody vaginal discharge (Score 2), lower proportion of multiparous ewes (15.8%; 3/19) exhibited severe vaginal discharge ( $P < 0.05$ ). Nulliparous ewes with moderate vaginal discharge (Score 1) had numerically higher pregnancy rate (72.2% vs. 58.8%) compared the nulliparous ewes with severe vaginal discharge ( $P > 0.05$ ). There was similar pregnancy rate (62.5% vs. 66.7%) in multiparous ewes with different scores (Table 2).

Short-term protocol decreased the percentage of severe vaginal discharge (25.9% vs. 48.1%) compared to long-term protocol ( $P = 0.09$ ). This difference numerically improve (71.4% vs. 53.8%) the pregnancy rate at score for short-term protocol. However, overall pregnancy rate did not change (66.7% vs. 63.0%) in favor of the short-term protocol (Table 3).

**Table 3.** Percentage of vaginal discharge characteristics and pregnancy rate according to vaginal discharge score in short-term and long-term protocol

Characteristics	Percentage of scores		P	Pregnancy rate		
	7	13		7	13	P
0	0.0 (0/0)	0.0 (0/0)	>0.05	0.0 (0/0)	0.0 (0/0)	>0.05
1	74.1 (20/27)	51.8 (14/27)	0.09	65.0% (13/20)	71.4% (10/14)	>0.05
2	25.9 (7/27)	48.1 (13/27)	0.09	71.4% (5/7)	53.8% (7/13)	>0.05
Overall	100.0% (27/27)	100.0% (27/27)	>0.05	66.7% (18/27)	63.0% (17/27)	>0.05

### Discussion and Conclusion

Kivircik ewes display seasonal breeding activity from June to January in Marmara and Aegean region. The pregnancy rate was determined in 64.1% of all ewes without synchronization protocol in the middle of the breeding season (August-September) in this herd. However, Kivircik is known as indispensable meat breeds and its fertility based on breeding strategy is an important factor for maintaining the production of Kivircik ewes in Turkey (Koyuncu and Altincekic, 2010). Besides, farmers want to benefit from higher prices by producing lamb in winter (Abecia et al., 2012). Thus, we hypothesized that the use of short-term and long-term synchronization protocol would create uniform lamb production in winter in this study.

Overall estrous response was 88.9% and neither the length of synchronization protocol nor parity affected the estrous response. The estrous response belongs to different breeds following the synchronization ranged from 77.1% to 100% among previous studies (Ataman et al., 2006; Özyurtlu et al., 2011; Ustuner et al., 2007). A high percentage of ewes exhibits estrous after synchronization but fertility results after natural mating is obviously lower than estrous response (Özyurtlu et al., 2011). Consistent with the literature, the difference between overall pregnancy

rate after natural mating and estrous response was 24.1% (88.9% vs. 64.8%) irrespective of the treatment protocol in the present study. This difference firstly revealed depends on the length of the synchronization protocol (Ungerfeld and Rubianes 1999).

The reduction of fertility in long-term synchronization are attributed to some possible reasons such as sub-luteal progesterone levels at the withdrawal time of intravaginal progesterone, extension of the lifespan of the ovulatory follicle, and impaired sperm transport (Vinoles et al., 1999). For this reason, most of the studies have reported the possible reason for preferring short-term treatment by comparing the traditional length of treatment. In our study, pregnancy rates were similar with short-term (66.7%) and long-term synchronization protocol (63.0%) without ram effect in Kivircik ewes. Ungerfeld and Rubianes (1999) re-

ported the similar pregnancy rate (61.5%, 64.3%, and 60.7% for 6, 9, and 13 days, respectively) after the different length of synchronization protocol in Polwarth ewes. Vinales et al. (2001) also obtained the similar pregnancy rate (58% vs. 67%, respectively) after short-term (6 d) and long-term (12 d) treatment protocol in Polwarth ewes. In addition, Ataman et al., (2006) determined the equal pregnancy rate (86.6%) during the breeding season. Authors reported similar pregnancy rate after short-term (85.7%) and long-term (76.9%) synchronization protocol during the non-breeding season in Merino-Akkaraman ewes (Ataman et al., 2006). Besides, long-term and short-term treatment also resulted in similar pregnancy rate (31% vs. 35.7%) after cervical insemination in Awassi ewes during breeding season (Ustuner et al., 2007). In the other previous study the pregnancy rate was not different after short-term (66.7%) and long-term treatment (58.3%) in Awassi ewes (Özyurtlu et al., 2011). However, the short-term treatment resulted in higher pregnancy rate (43.5% vs. 37.8%) compared to the long-term treatment after intrauterine insemination in a comprehensive (1,750 multiparous ewes) study (Menchaca et al., 2018).

Contrary to previous studies revealing similar fertility according to protocol length, the pregnancy rate was statistically higher with 14 d treatment protocol than

those of 9 d and 5 d treatment protocol (83.3% vs. 60.9% and 47.8%, respectively) in crossbred ewes (Blaschi et al., 2014). In a recent study, it was reported only tendency (33% vs. 24%) for higher pregnancy rate after cervical insemination with long-term protocol (11 d) compared to the short-term protocol (7 d) in Santa Inês ewes (Biehl et al., 2019). There has been no consensus for higher fertility with comparison of short-term and long-term protocol in literature (Blaschi et al., 2014; Biehl et al., 2019). In addition to the length of the synchronization protocol, this similarity was not influenced depends on parity in the present study. Synchronization of mature ewes and ewe lambs are practically performed in single groups. However, isolated ewe lambs from mature ewes have higher fertility compared to ewe lambs bred with mature ewes (Corner et al., 2013). However, sexual attractiveness is not main explanation for reproductive differences between mature ewes and ewe lambs. Consistent with the previous study, estrous response and pregnancy rate in mature ewes and ewe lambs was not different in the present study. Rams similarly interest both of them and similar pregnancy rate can be obtained (Ungerfeld and Rubianes, 1999; Ungerfeld, 2016). Our results have corroborated the previous studies revealing similar fertility after comparison of short-term and long-term synchronization protocol in ewes. Thus, the short-term protocol was as effective as the traditional length of synchronization protocol to improve the pregnancy rate. Besides, considering the number of rams per ewe, both protocols with natural mating can be used to achieve optimal pregnancy in non-pregnant Kivircik ewes. In addition to possible benefits of short-term protocol compared to long-term protocol, one of the reasons for preferring short-term protocol is avoiding vaginitis by decreasing the duration of vaginal irritation with sponges (Martinez-Ros et al., 2018). The use of intravaginal sponges for synchronization leads to alteration of vaginal microbiota and provoke vaginitis with the different characteristic of discharge at the sponge withdrawal (Manes et al., 2018). These alterations also decrease the viability of ram semen (Manes et al., 2016). However, the association of length of synchronization protocol and vaginitis has been reported in a limited study (Martinez-Ros et al., 2018). Similar percentage of vaginal mucus discharge was observed after short-term (100%) and long-term treatment (94.4%). However, long-term treatment resulted in higher (83.3% vs. 10.5%) percentage of severe characteristic of vaginal mucus discharge (Score 2) compared to that of short-term treatment in the previous study (Martinez-Ros et al., 2018). In accordance with previous report (Martinez-Ros et al., 2018), all ewes had vaginal discharge after sponge removal and long-term protocol resulted in higher percentage of (48.1% vs. 25.9%) severe discharge compared to that of short-term protocol in the present study. However, the difference between synchronization

protocols was not obvious as in previous report (Martinez-Ros et al., 2018). Although the almost half of nulliparous ewes had severe vaginal discharge (Score 2), low percentage of the multiparous ewes had this type of discharge following synchronization. Vaginal wall of nulliparous ewes was more severely affected by synchronization. However, these differences did not improve the pregnancy rate for the benefit of multiparous ewes in the present study. The expected difference for pregnancy rate may be limited due to the low number of animal in the groups (Martinez-Ros et al., 2018).

In conclusion, the length of synchronization protocol did not affect the percentage of estrous response. Although long-term protocol resulted in higher proportion severe discharge compared the short-term protocol, the pregnancy rate was not statistically different between synchronization groups. Besides, nulliparous ewes more severely responded to irritation of vaginal sponge during synchronization. However, pregnancy rate was not influenced by parity depending on differences in vaginal discharge proportion. Thus, the long-term and short-term protocol can be used by tolerating the insufficient number of rams to obtain optimal fertility in nulliparous and multiparous ewes in small herds.

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