

THE EFFECTIVENESS OF SYNCHRONIZATION OF ESTRUS VIA
PROSTAGLANDIN F_{2α} ADMINISTRATION AND TIMED ARTIFICIAL
INSEMINATION IN MULTIPAR AND NULLIPAR AKKARAMAN BREED EWES

Multipar ve Nullipar Akkaraman Irkı Koyunlarda Prostaglandin F_{2α} ile Östrus
Senkronizasyonu ve Sabit Zamanlı Tohumlamanın Etkinliği

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Summary : In present study it was aimed to investigate the effectiveness of synchronization of estrus via prostaglandin F_{2α}(PGF_{2α}) administration and timed artificial insemination (TAI) in multipar and nullipar Akkaraman breed sheep. The study used a total of 20 animals which were divided into two groups: Group 1=10 multipar ewes (2-4 years old) and Group 2=10 nullipar ewes (1.5-2 years old). All animals were injected with 125µg Cloprostenol (Estrumate® DİF İstanbul, TURKEY) at nine day intervals. Ewes were inseminated vaginally 42 hours after the second PGF_{2α} administration with the semen which were collected from six purebred Akkaraman rams (2-3 years old) and diluted at 150x 10⁶ motile spermatozoa/0.5 ml dose. Progesterone levels in blood samples were assessed at the moment of hormone injections and after three days. Thirty five days after the inseminations, pregnancies were diagnosed via ultrasonography. Pregnancy rates were found to be 40 % for multipar and 20 % for nullipar ewes (p>0.05). Progesterone levels in multipar and nullipar animals at the first PGF_{2α} administration, three days after the first PGF_{2α} administration, at the second PGF_{2α} administration, and three days after the second PGF_{2α} administration were measured as (median) 2.67-3.67 ng/ml (p>0.05), 0.96-0.86 ng/ml(p>0.05), 6.26-5.24 ng/ml (p<0.05) and 0.99-1.25 ng/ml (p<0.05) respectively. It was concluded that multipar animals yielded better responses to ES accomplished through PGF_{2α} administration. The nullipar animals should be considered as a factor for effectiveness of timed artificial insemination via synchronization of estrus with PGF_{2α} in ewes.

Key words: Akkaraman ewes, multiparous, nulliparous, estrus synchronization, PGF_{2α}, timed artificial insemination

Özet : Sunulan çalışmada; prostaglandin F_{2α} (PGF_{2α}) ile östrus senkronizasyonu ve sabit zamanlı tohumlamanın multipar ve nullipar Akkaraman ırkı koyunlarda, etkinliğinin araştırılması amaçlandı.

Araştırmada 10 adet multipar (2-4 yaşında, Grup 1) ve 10 adet nullipar koyun (1,5-2 yaşında, Grup 2) olmak üzere iki grupta toplam 20 adet hayvan kullanıldı. Tüm hayvanlara 9 gün arayla 125 µg Cloprostenol (Estrumate® DİF İstanbul Türkiye) enjekte edildi. Sperma altı adet koçtan (2-3 yaşında) suni vagen yardımı ile alındı ve 150 milyon/0,5 ml dozda sulandırıldı. Koyunlar ikinci PGF_{2α} uygulamasından 42 saat sonra intravaginal yolla tohumlandı. Kandaki progesteron düzeyleri hormon enjeksiyonları anında ve 3'er gün sonrasında değerlendirildi. Gebelikler, tohumlamadan 35 gün sonra, ultrasonografi ile teşhis edildi. Gebelik oranları multipar koyunlarda % 40, nulliparlarda % 20 olarak belirlendi (p>0.05).

Birinci PGF_{2α} uygulaması öncesinde, birinci PGF_{2α} uygulamasından üç gün sonra, ikinci PGF_{2α} uygulamasından önce ve ikinci PGF_{2α} uygulamasından üç gün sonra multipar-nullipar koyunlarda ortalama progesteron düzeyleri sırasıyla, 2.67-3.67 ng/ml (p>0.05), 0.96-0.86 ng/ml (p>0.05), 6.26-5.24 ng/ml (p<0.05) ve 0.99-1.25 ng/ml (p<0.05) ölçüldü. Sonuç olarak, multipar hayvanların PGF_{2α} uygulaması ile yapılan östrus senkronizasyonuna daha iyi cevap verdiği kanaatine varıldı. Nullipar hayvanlar, koyunlarda PGF_{2α} ile östrus senkronizasyonu ve sabit zamanlı tohumlamaların etkisiz kalmasında, bir faktör olarak değerlendirilmelidir.

Anahtar kelimeler: Akkaraman koyunu, multipar, nullipar, östrus senkronizasyonu, PGF_{2α}, sabit zamanlı tohumlama

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Under normal circumstances Akkaraman breed ewes yield considerably high fertility rates through the conventional method of introducing fertile rams in to the flock during the breeding season in Turkey (1, 2). However, this method presents such problems as elongation of the parturition season, increases in labor costs and lack of uniformity in lambs put up for sale. The estrus synchronization (ES) and artificial insemination (AI) techniques seem to be effective in resolving such economical problems. Furthermore, AI programs provides several advantages such as widespread use of rams, making use of younger rams, and protection of flock from genital diseases transmitted through mating (3). Synchronization of estrus appears to be the most important factor for reducing the cost and labor of AI application. However, the estrus detection procedure requires high spent-time labour in the AI programmes with ES in large flocks. Therefore, the timed artificial insemination (TAI) is an indispensable alternative to estrus detection procedure in facilitating flock management (4).

The main problem of TAI (single insemination) with ES or ovulation synchronization applications in ewes is the low fertility (4, 5).

The object of the present study is to compare the effectiveness of ES with TAI in multipar and nullipar Akkaraman breed ewes.

MATERIALS AND METHODS

In this study 10 multipar (2-4 years old), 10 nullipar (1.5-2 years old) Akkaraman breed sheep were used. The average body condition scores of the animals, measured based on Thompson and Meyer (6) were 3.25 and 2.65 respectively. The animals were fed with dry hay and concentrate. The study was carried out between October 10 and November 30 (within the breeding season) in Sivas (37:02 E, 39:45 N).

All ewes were given twice, at 9 day interval, intra-

muscular injections of 125 µg Cloprostenol (Estrumate® DIF Turkiye), an analogue of PGF_{2α} Forty-two hours after the second PGF_{2α} administration, the animals were inseminated vaginally.

The semen used for inseminations were collected from 6 rams of identical-breed by using an artificial vagina. Each ejaculate was assessed with respect to motility and concentration. Ejaculates with motilities of 70 % and over were mixed. The concentrations of the mixed ejaculates were identified via hemocytometric method and were diluted in a way to contain at least 150x10⁶ motile spermatozoa in 0.5 ml volume. In the study, an extender was used which was prepared by adding 20 % egg yolk to a solution of tris (30.28 g), fructose (12.50 g), and citric acid (17.00g) (7).

In order to determine progesteron (P₄) levels, blood samples were drawn from all animals on the day of the first PGF_{2α} administration, three days after the first administration, on the day of the second PGF_{2α} administration, and three days after the second administration. Samples were allowed to clot at room temperature and were centrifuged within two hours after collection. Serum was stored at -20 °C until hormone determination. Serum P₄ levels were measured on the ADVIA-Centaur Analyzer. The ADVIA Centaur P₄ assay is a competitive immunoassay using direct chemiluminescent technology.

Pregnancies were diagnosed 35 days after the insemination via transrectal ultrasonography using the 5 mHz probe on the Mindray 3300-DP-VET ultrasound equipment.

In order to compare the P₄ levels in multipar and nullipar ewes, a Mann-Whitney test was run. A Wilcoxon-Signed Ranks Test was performed in order to compare the P₄ levels within each group with Benferroni correction (Nullipars and Multipars) For comparing the pregnancy rates between the two groups, a Chi-square test (Fisher's exact test) was conducted. p<0.05 is considered as statistically significant.

RESULTS

Pregnancy rates were found to be 40 % (4/10) in

multipars, and 20 % (2/10) in nullipars ($p>0.05$). The overall rate of pregnancy in all animals included in the study was 30 %. The P₄ levels prior to the first PGF_{2 α} administration were detected as 2.67 ng/ml and 3.67 ng/ml for multipar and nullipar animals, respectively ($p>0.05$). The P₄ levels measured three days after the first PGF_{2 α} administration were 0.96 ng/ml and 0.86 ng/ml on average in multipar and nullipar animals respectively ($p>0.05$). Nine days after the first PGF_{2 α} administration (the

day of the second PGF_{2 α} administration) the progesteron levels were 6.26 ng/ml in multipars and 5.24 ng/ml in nullipars ($p<0.05$). As for the progesteron levels after three days of the second PGF_{2 α} administration, the measures for multipars and nullipars were 0.99 ng/ml and 1.25 ng/ml ($p<0.05$), respectively (Table I).

DISCUSSION

Colakoglu and Ozbeyaz (1) report that they have

Table I. The progesteron leves at different stages of estrus synchronization with PGF_{2 α} in nulliparous and multiparous Akkaraman ewes

	Multipar ewes Median (Min-Max) ($\mu\text{g/dL}$)	Nullipar ewes Median (Min-Max) ($\mu\text{g/dL}$)	p*
PG1 ¹	2.67 (1.11-8.32)	3.67 (0.51-7.08)	0.971
PG1-3 ²	0.96 (0.49-4.51)	0.86 (0.47-2.15)	0.796
PG2 ³	6.26 (4.73-7.63)	5.24 (4.17-6.62)	0.043
PG2-3 ⁴	0.99 (0.79-1.12)	1.25 (0.01-2.61)	0.003
p ⁺ (PG1-PG2)	0.014	0.131	
p ⁺⁺ (PG1-3-PG2-3)	0.547	0.232	

p* Mann-Whitney-U Test.

p⁺, p⁺⁺ Wilcoxon Signed Ranks Test.

¹ Progesteron level before first PG administration (PG1)

² Progesteron level 3 days after the first PG administration (PG1-3).

³ Progesteron level before second PG administration (PG2).

⁴ Progesteron level 3 days after the second PG administration (PG2-3).

obtained 89.4 % to 91.6 % pregnancy rates in Akkaraman breed ewes through mating during the breeding season. In light of the findings of their study, the fertility rates in ewes through mating with rams under normal conditions in breeding season appear to be considerably high. However, considering such disadvantages as the elongation of the birth season, increases in labor costs, and lack of uniformity in lambs put up for sale, the AI of ewes seems to be an indispensable method.

Estrus synchronization with hormones is a commonly used method for AI application in ewes.

Recently, progestagen for ES has been extensively used in ruminants. However, due to several fertility inconveniencies and residue outputs (mostly fluorogesteron acetate), different methods are called for. As an alternative to progestagens, the use of PGF_{2 α} as a luteolytic agent has been recommended in ruminants (4, 8, 9).

The ES in AI applications is also essential in enabling concentration of labor within a few days and simultaneous insemination of the whole herd. To this end, considerable research has been underway in order to achieve as good fertility rates, through

estrus or ovulation synchronization and AI, as natural service (5, 10).

The semen can be deposited via vaginal, cervical and transcervical insemination by laparoscopy methods in ewes. The quality of the sperm and the location of deposition have direct influences on fertility. It has been reported that the best results of fertility are obtained via intrauterine insemination by laparoscopy, rather than vaginal and cervical insemination techniques. However, due to reasons of practicality and high costs of materials required for laparoscopic application, this method causes potential problems for field applications. Currently, considering the field conditions, the use of chilled or diluted ejaculate by vaginal insemination proves to be the most practical and cost-effective method in AI of large flocks (9, 11-14).

The serum P₄ concentrations prior to the first PGF_{2α} administration were detected as 2.67 ng/ml and 3.67 ng/ml on average (Median) in multipar and nullipar ewes, respectively. Three days after the first administration, the P₄ concentrations were 0.96 ng/mL for multipars and 0.86 ng/ml for nullipars. The P₄ levels on the day of the second PGF_{2α} administration and three days later were 6.26 ng/mL, 5.24 ng/mL, and 0.99 ng/mL and 1.25 ng/mL on average for multipar and nullipar animals, respectively.

It was observed that on the day of the second PGF_{2α} administration P₄ levels were higher in multipars compared to nullipars animals. However, three days after the second PGF_{2α} administration, P₄ levels were lower in multipars compared to nullipars.

In light of these findings it was concluded that in ES carried out with PGF_{2α} administration in Akkaraman breed ewes, multipars yielded better responses than nullipars.

Concerning the decrease rates in P₄ concentrations obtained through ES with two PGF_{2α} administrations nine day apart, the current study seems to be in line with Barrett et al's (15) study who reported similar findings with White Face breed sheep. The quantitative discrepancies in P₄ levels can be attributed to the different hormone analysis tech-

niques used and the differences of the two breeds.

It was observed that, while the P₄ levels in multipars three days after the first PGF_{2α} administration were below 1ng/ml in six of ten animals, they were below 1ng/ml in seven out of ten animals after three days of the second administration. As for the nullipar animals, the P₄ levels went below 1ng/ml in six animals after three days of the first administration. However, although an obvious decrease was observed in the P₄ levels, there were no values below 1ng/ml on the third day after the second administration of PGF_{2α}. It is thought that the differences of pregnancy rates and responses to ES in multipar and nullipar animals may account for this situation.

The efficiency of AI depends on the preservation of the sperm and the method of application (vaginal, cervical and intrauterin) (12). In their study carried out with Norwegian hybrid breed sheep, Paulenz et al. (16) report that, they obtained a 52.3 % pregnancy rate within the season through vaginal insemination using sperm diluted in a tris based extender, by determining the estrus alone with no synchronization of any kind. In another study, with the same breed of animals, Paulenz et al. (17) reported a 71.3 % pregnancy rate through vaginal insemination with frozen-thawed semen (200 million spermatozoa). The pregnancy rates in the present study are lower compared to the above mentioned studies, which could be due to the fact that the present study utilized the ES method and animals were inseminated without estrus detection.

In their ES using Corriedale hybrid breed sheep with two-dose PGF_{2α} (160µg delprostenate) administration seven day apart, Menchaca et al. (4) reported 36.8 %, 25.8 % and 22.6 % pregnancy rates with inseminations applied 42, 48 and 54 hours after the second PGF_{2α} administration, respectively. In the present study, the pregnancy rates were 40 % in multipar and 20 % in nullipar animals by inseminations at the 42nd hour after the second PGF_{2α} administration. The pregnancy rate obtained in Akkaraman breed multipar sheep was found to be higher than those of Menchaca et al. (4). However, it was lower in nullipars. The dis-

crepancies regarding the pregnancy rates between the above mentioned study and the current one could be attributed to the different breeds, hormone prepa- rates used, the synchronization and insemination methods utilized, and the concentration of semen used for inseminations.

In the same study Menchaca et al. (4) reported higher levels of onset of estrus following the syn- chronization in multipars than in nullipars (94%- 82%). This may be considered a significant finding in accounting for the differences of pregnancy rates in multipars and nullipars.

In a study with Morkaraman breed sheep using 2 dose PGF_{2α} eleven day apart, Yildiz et al. (18) re- ported that LH concentrations increased from the basal level at the 54th hour and decreased back to the basal level at the 72nd hour. Furthermore, they reported a significant correlation between LH con- centrations and body condition scores. The finding of the current study that the body condition scores of multipars are higher than nullipars is thought to be significant in accounting for differences between pregnancy rates. In addition, the finding that the pregnancy rate is somewhat low, although it is ac- ceptable, can be attributed to the wide span of distri- bution of ovulations in ewes after ES.

CONCLUSIONS

In conclusion, Akkaraman multipar ewes yielded better responses to ES with PGF_{2α} administration than nullipars. By and large, this study suggests that the number of nullipar ewes in the flock could be though to be one of the factors on lowering the suc- cess of ES with

PGF_{2α} administration and TAI.

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