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The Effectiveness of Synchronization of Estrus via Prostaglandin $F_{2\alpha}$ Administration and Timed Artificial Inseminaton in Multipar and Nullipar Akkaraman Breed Ewes

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Abstract: In present study it was aimed to investigate the effectiveness of ES via prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) admistration and Timed Artificial Insemination (TAI) in multipar and nullipar Akkaraman breed sheep. The study used a total of 20 animals which were divided into 2 groups: Group 1 = 10 multipar ewes (2-4 years old) and Group 2 = 10 nullipar ewes (1, 5 years old). All animals were injected with 125 μg Cloprostenol (Estrumate® DIF Istanbul Turkey) at nine day intervals. Ewes were inseminated vaginally 42 h after the second $PGF_{2\alpha}$ administration with the semen which were collected from 6 purebreed Akkaraman rams (2-3 years old) and diluted at 150×106 motile spermatozoa / 0.5 mL dose. Progesterone (P_4) levels in blood samples were assessed at the moment of hormone injections and after 3 days. Thirty days after the inseminations, pregnancies were diagnosed via ultrasonography. Pregnancy rates were found to be 40 % for multipar and 20 % for nullipar ewes ($p>0.05$). P_4 levels in multipar and nullipar animals at the first $PGF_{2\alpha}$ administration, three days after the first $PGF_{2\alpha}$ administration, at the second $PGF_{2\alpha}$ administration and three days after the second $PGF_{2\alpha}$ administration were measured as (median) 2.67-3.67 ($p>0,05$), 0.96-0.86 ($p>0,05$), 6.26-5.24 ($p<0,05$) and 0.99-1.25 ng mL^{-1} ($p<0.05$), respectively. It was concluded that multipar animals yielded better responses to ES accomplished through $PGF_{2\alpha}$ administration. The Nullipar animals should be considered as a negative factor for the sucsess of TAI via synchronization of estrus with $PGF_{2\alpha}$ in ewes.

Key words: Ewe, estrus synchronization, $PGF_{2\alpha}$, timed artificial insemination

INTRODUCTION

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 (Colakoglu and Ozbeyaz, 1999; Esen and Bozkurt, 2001). 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 (Leethongdee *et al*., 2006). 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 TAI is an indispensable alternative to estrus detection procedure in order to facilitate flock management. The main problem of TAI (single insemination) with ES or ovulation synchronization applications in ewes is the low fertility (Menchaca *et al*., 2004; Deligiannis *et al*., 2005).

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

Animals and management: In this study 10 multipar (2-4 years old), 10 nullipar (1,5 years old) Akkaraman breed sheep were used. The average body condition scores of the animals, which were measured based on Thompson and Meyer (2007) 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).

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Estrus synchronization, semen collection and artificial insemination: All ewes were given twice, at 9 day interval, intramuscular injections of 125 μg Cloprostenol (Estrumate® DIF Turkey), an analogue of $PGF_{2\alpha}$. Forty two h after the second $PGF_{2\alpha}$ 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 hemositometric method and were diluted in a way to contain at least 150×106 motile spermatozoa in 0.5 mL volume. In the study, an extender was used which was prepared by adding 20 % yolk to a solution of tris (30.28 g), fructose (12.50 g) and citric acid (17.00 g) (Paulenz *et al*., 2002).

Progesteron assays: In order to determine progesteron levels, blood samples were drawn from all animals on the day of the first $PGF_{2\alpha}$ administration, 3 days after the first administration, on the day of the second $PGF_{2\alpha}$ administration and 3 days after the second administration. Samples were allowed to clot at room temperature and were centrifuged within 2 h after collection. Serum was stored at -20°C until hormone determination. Serum P_4 levels were measured on the ADVIA-Centaur Analyzer. The ADVIA Centaur P_4 assay is a competitive immunoassay using direct chemiluminescent technology.

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

Statistical analyses: In order to compare the P_4 levels in multipar and nullipar ewes, a Mann-Whitney test was run. A Wilcoxen-Signed Ranks Test was performed in order to compare the P_4 levels within each group (Nullipars and Multipars), for comparing the pregnancy rates accross the 2 groups, a Chi-square test was conducted.

RESULTS AND DISCUSSION

Pregnancy rates were found to be 40 % (4/10) in multipars and 20 % (2/10) in nullipars. The overall rate of pregnancy in all animals included in the study was 30 % ($p>0.05$).

The P_4 levels prior to the first $PGF_{2\alpha}$ administration were detected as 2.67 and 3.67 ng mL^{-1} for multipar and nullipar animals, respectively ($p>0.05$). The P_4 levels measured three days after the first $PGF_{2\alpha}$ administration were 0.96 and 0.86 ng mL^{-1} on average in multipar and nullipar animals, respectively ($p>0.05$). Nine days after the first $PGF_{2\alpha}$ administration (the day of the second $PGF_{2\alpha}$ administration) the progesteron levels were 6.26 ng mL^{-1} in multipars and 5.24 ng mL^{-1} in nullipars ($p<0.05$). As for the progesteron levels after 3 days of the second $PGF_{2\alpha}$ administration, the measures for multipars and nullipars were 0.99 and 1.25 ng mL^{-1} ($p<0.05$), respectively (Table 1).

Colakoglu and Ozbeyaz (1999) report that they have obtained 89.4-91.6 % 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 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 fluoro-gesteron acetate), different methods are called for. As an alternative to progestagenes, the use of $PGF_{2\alpha}$ as a luteolitic agent has been recommended in ruminants (Menchaca *et al*., 2004; Gonzalez-Bulnes *et al*., 2005).

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 (Deligiannis *et al*., 2005). 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

Table 1: The progesteron leves at different stages of estrus synchronization with $PGF_{2\alpha}$ in Akkaraman ewes

	Multipar ewes Median (Min-Max)	Nullipar ewes Median (Min-Max)	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 admistration (PG1), [2]Progesteron level 3 days after the first PG admistration (PG1-3), [3]Progesteron level before second PG admistration (PG2), [4]Progesteron level 3 days after the second PG admistration (PG2-3)

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 (Greyling *et al*., 1997; Naqvi *et al*., 1998; Anel *et al*., 2006, 2005; Fair *et al*., 2005).

It was observed in this study that on the day of the second $PGF_{2\alpha}$ administration P_4 levels were higher in multipars compared to nullipars animals ($p<0.05$). However, three days after the second $PGF_{2\alpha}$ administration, P_4 levels were lower in multipars compared to nullipars ($p<0.05$).

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

Concerning the decrease rates in P_4 concentrations obtained through ES with 2 $PGF_{2\alpha}$ administrations nine day apart, the current study seems to be in line with Barrett *et al.* (2002) study who reported similar findings with White Face breed sheep. The quantitative discrepancies in P_4 levels can be attributed to the different hormone analysis techniques used and the differences of the two breeds.

It was observed that, while the P_4 levels in multipars 3 days after the first $PGF_{2\alpha}$ administration were below 1ng mL^{-1} in 6 of 10 animals, they were below 1ng mL^{-1} in 6 out of 10 animals after 3 days of the second administration. As for the nullipar animals, the P_4 levels went below 1ng mL⁻1 in 6 animals after 3 days of the first administration. However, although an obvious decrease was observed in the P_4 levels, there were no values below 1 ng mL^{-1} on the 3rd day after the second administration of $PGF_{2\alpha}$. 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) (Anel *et al*., 2006). In the study carried out with Norwegian hybrid breed sheep, Paulenz *et al.* (2003) 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.* (2005) 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 $PGF_{2\alpha}$ (160μg delprostenate) administration seven day apart, Menchaca *et al.* (2004) reported 36.8, 25.8 and 22.6 % pregnancy rates with inseminations applied 42, 48 and 54 h after the second $PGF_{2\alpha}$ administration, respectively. In the present study, the pregnancy rates were 40 % in multipar and 20 % in nullipar animals by inseminations at the 42 h after the second $PGF_{2\alpha}$ administration. The pregnancy rate obtained in Akkaraman breed multipar sheep was found to be higher than those of Menchaca *et al.* (2004). However, it was lower in nullipars. The discrepancies regarding the pregnancy rates between the above mentioned study and the current one could be attributed to the different breeds, hormone preparates used, the synchronization and insemination methods utilized and the concentration of semen used for inseminations.

In the same study Menchaca *et al.* (2004) reported higher levels of onset of estrus following the synchronization 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 $PGF_{2\alpha}$ 11 day apart, Yildiz *et al.* (2003) reported that LH concentrations increased from the basal level at the 54 h and decreased back to the basal level at the 72 h. Furthermore, they reported a significant correlation between LH concentrations 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 acceptable, can be attributed to the wide span of distribution of ovulations in ewes after ES.

CONCLUSION

In conclusion, Akkaraman multipar ewes yielded better responses to ES with $PGF_{2\alpha}$ administration then 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 success of ES with $PGF_{2\alpha}$ administration and TAI.

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