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The Effects of Short-Medium and Long-Term Applications of Fluorogestone Acetate (FGA) on Reproductive Performance of Kıvırcık Ewes at The Onset of The Breeding Season

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Abstract: The aim of the present study was to investigate the efficiency of different treatments of progestogen with Pregnant Mare Serum Gonadotropin (PMSG) in Kıvırcık ewes at the onset of the breeding season. A total of 111 ewes, 3 to 5 years old, weighing on average 47.7 kg and with a body condition score of 3.5 to 4.0 were used in this study. The ewes were randomly divided into 3 equal groups (n= 37 per group). Intravaginal progestogen sponges containing 30 mg FGA were inserted intravaginally in the ewes and left for a different number of days; 7 days for the short-term treatment, 10 days for the medium-term treatment and 14 days for the long-term treatment. Additionally, 500 IU of PMSG was injected administered intramuscularly when the sponges were removed. The estrous response, lambing rate, rate of multiple births, fecundity and litter size were 81.8 %, 70.3 % , 19.2 %, 0.86 and 1.23 in the short-term group; 89.2 %, 83.8 %, 12.0 %, 1.24 and 1.48 in the medium-term group; and 94.6 % , 91.2 % , 35.3 %, 1.30 and 1.41 in the long-term group, respectively. The duration of sponge treatment had a significant effect on estrous response, lambing rate, fecundity, litter size ($P<0.05$), and the rate of multiple births ($P<0.01$). Occurrences of oestrous response, lambing rate, fecundity, litter size were significantly higher in the medium-term and long-term groups than in the short-term group; on the other hand, the rate of multiple births was significantly ($p<0.01$) higher in the long-term group than in the medium-term and short-term groups. No difference in the fertility parameters in the medium (10 days) or long (14 days) term application were detected. Short term (7 days) application were not found as successful other two practice.

Keywords: Breeding season, Estrous synchronization, Fertility, Kıvırcık ewes, Progesterone

Üreme Mevsimindeki Kıvırcık Koyunlarının Döl Verimi Üzerine Kısa, Orta ve Uzun Süreli FGA (Fluorogestone Acetate) Uygulamasının Etkileri

Özet: Bu çalışmanın amacı, üreme mevsimi başlangıcında Kıvırcık koyunlarında gebe kısrak serumu gonadotropin (PMSG) ile progesteronun farklı uygulamalarının etkinliğinin araştırılmasıdır. Araştırmada yaşları 3 - 5, vücut kondisyon puanı 3.5-4.0 arasında değişen ve canlı ağırlıkları ortalama 47.7 kg olan toplam 111 baş koyun kullanılmıştır. Koyunlar rastgele 3 eşit gruba (n=37) ayrılmıştır. Gruplarda yer alan koyunlara FGA (30mg) içeren vaginal süngerler kısa (7 gün), orta (10 gün) ve uzun (14 gün) süreli olarak uygulanmıştır. Tüm gruplarda yer alan koyunlara süngerlerin çıkarıldığı gün 500 IU PMSG kas içi olarak enjekte edilmiştir. Östrus oranı, kuzulama oranı, çoğuz doğum oranı, döl tutma oranı ve yavru verimi kısa süreli grupta sırasıyla % 81.8, %70.3, %19.2, 0.86 ve 1.23; orta süreli grupta % 89.2, %83.8, %12.0, 1.24, 1.48; uzun süreli grupta % 94.6, %91.2, %35.3, 1.30, 1.41 olarak saptanmıştır. Gruplar arasında östrus görülme oranı, kuzulama oranı, döl tutma oranı ve yavru verimi ($P <0.05$) ve çoğuz doğum oranı ($P <0.01$) istatistiki olarak önemli bulunmuştur. Diğer yandan kısa ve orta süreli gruplardaki çoğuz doğum oranı, uzun süreli gruptan önemli düzeyde düşük bulunmuştur ($P<0.01$). Uygulamalardan orta (10 gün) veya uzun (14 gün) süreli olmasının döl verimi parametreleri üzerinde önemli bir fark meydana getirmediği, kısa süreli (7 gün) uygulamanın döl verimi açısından diğer iki uygulama kadar etkili olmadığı görülmüştür.

Anahtar kelimeler: Döl verimi, Kıvırcık koyunu, Kızgınlık senkronizasyonu, Progesteron, Üreme mevsimi

Introduction

The number of lambs obtained from breeding ewes directly affects the yields obtained in livestock production (Akçapınar 2000). Thus, breeders demand more lamb births per ewe (Koyuncu et al. 2001). The majority of sheep breeds perform different reproduction activities depending on season changes, latitude/longitude, the length of the photoperiod and other factors. In local sheep breeds in Turkey, the ewes generally exhibit oestrus during the autumn season, when the days start to become shorter. Synchronisation of the oestrous cycle allows producers to plan the process of oestrus and ovulation according to a desired time slot. Synchronisation and control of oestrus are valuable instruments for increasing the reproductive effectiveness of ewes, when applied successfully (Kusina et al. 2000). With oestrous synchronisation, control of the luteal or follicular phase is achieved (Jainudeen et al. 2000; Kusina et al. 2000). The progestogens that are most commonly used for the purpose of inducing synchronisation of oestrus during the breeding season are synthetic progesterone impregnated vaginal sponges such as fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) (Fukui et al. 1999). Progesterone blocks FSH and LH secretion by suppressing the hypothalamus and also indirectly the pituitary anterior lobe and temporarily stops follicular development. This suppression dissappears with the removal of the sponges, and oestrous behaviours are observed along with follicular development. To obtain a high rate of oestrus and ovulation, PMSG has to be applied together with progestogen impregnated sponges. Ovulation takes place in the 48-72 hours after the injection of PMSG, which is administered when the sponge is removed (Zarkawi 2001). Although the exhibition of oestrus is reportedly higher in ewes that have been treated with an oestrous synchronisation protocol the fertility level is lower than in ewes that exhibit spontaneous oestrus. Some researchers have stated that this decrease in the fertility rate is caused by changes that take place during the transportation of sperm and by hormonal changes that lead to desynchronisation between oestrus and ovulation (Pearce and Robinson 1985; Scaramuzzi et al. 1988). Long time progesterone applications have had suppressive effects on fertility and thus short period progesterone applications have been suggested as an alternative (Vinoles et al. 2001; Ali 2007; Husein et al. 2007). Short-term sponge applications, such as for 5-7 days, have produced good results in establishing oestrus for ewes both in and out of the mating season (Beck et al. 1993; Vinoles et al. 2001; Ataman et al. 2006).

The Kıvırcık ewe is a breed that is commonly raised in the Marmara Region and Thrace section of Turkey (Yalcin 1986). The purpose of this study was to evaluate the effects of the application of vaginal sponges on some reproductive parameters of ewes of the Kıvırcık breed during the mating season for 7 (short), 10 (medium) and 14 (long) days.

Materials and Methods

Location

The study was conducted at the Application and Research Farm of the Agricultural Faculty, Uludag University in Bursa Province (southern Marmara Region). This farm is located in the humid lowland tropics at an altitude of 100 m above sea level and at longitude 29 °E and latitude 40 °N (average minimum temperature 9.0 °C, average maximum temperature 20.2 °C, and annual rainfall 713.1 mm).

Treatment Schedule

A total of 111 ewes were used in the experiment. Body weights and condition scores of these animals were recorded prior to the experiment. The 3 to 5 year old Kıvırcık ewes, which weighed on average 47.7 kg and had a body condition score (BCS) of 3.5 to 4.0 were used in the study, which was conducted in the months of June and July. The experimental flock was maintained under standard farm management practices. The ewes were grazed daily for 8-10 h on a pasture consisting of a mixture of common vetch (*Vicia sativa L*), Hungarian vetch (*V. pannonica L*), alfalfa (*Medicago sativa*) and sainfoin (*Onobrychis sativa*) mixtures. In addition, the ewes received 200 g concentrate (wheat 75 %, sunflower oilcake 23 %, limestone 1.4 %, salt 0.5 % and premix 0.1%) /head/day during the entire study. Animals were routinely drenched for fluke and roundworm and vaccinated for pasteurellosis and clostridial infections. Clean water was available throughout the study period.

The lengths of the progesterone administration (long term: 14 days; medium term: 10 days; and short term: 7 days) and the PMSG treatment in relation to the progesterone removal were studied. The Kıvırcık ewes were randomly divided into 3 equal groups. Groups were designated as long term, medium term and short term. Groups were synchronised using 30 mg FGA (fluorogestone acetate) progestogen sponges inserted intravaginally and left for a different number of days (7, 10 and 14). Additionally 500 IU of PMSG injected administered intramuscularly when the sponges were removed.

Kıvırcık rams, 3 to 4 years old and of proven libido and fertility, were added to the experimental breeding groups at the beginning of the experiment. The ratio of one ram to 15 ewes. Ewes and rams were kept together for a 45-day period in the experimental groups. Ewes were kept indoors at night and outside for most of the day. While indoors the animals were offered diets outside they had free access to natural grazing. All ewes were naturally mated during the synchronised oestrus starting 7 June, and mating was allowed to continue until 21 July for those ewes not conceiving at the induced oestrus.

The following parameters were recorded

The parameters calculated following the FGA sponge removal were oestrous response (percentage of ewes showing oestrus/total ewes treated), lambing rate (percentage of ewes lambing/ewes mated), rate of multiple births (percentage of multiple lambing/total lambing), fecundity (percentage of lambs born/total ewes mated), and litter size (number of lambs born/ewes lambing).

Differences in reproduction performance among the groups were analysed using the General Linear Models (GLM) procedure of SAS (SAS 1991). A chi-square test was used to analyse the oestrous response, fecundity, lambing and rate of multiple births among the groups at the 5 % and 1 % level of significance.

Results and Discussion

All the FGA sponges remained in place until the time of removal. Of the 111 ewes used in the trial, 98 (88.3 %) exhibited overt signs of oestrus during the 48-72 h observation period. The incidence of oestrous response (81.1, 89.2 and 94.6 %, $P<0.05$) in the 7-day, 10-day and 14-day groups respectively, showed that the response was significantly higher in the 14-day group than in the 10-day, and 7-day groups (Table 1).

Table 1. Reproductive response following sponge removal in Kıvırcık ewes treated with 7-d, 10-d and 14-d FGA.

Parameters	Experimental groups			Overall
	7-days	10-days	14-days	
Ewes exposed (n)	37	37	37	
Estrus response (%)	81.1^{b}	89.2^{a}	94.6^{a}	88.3
Lambing rate (%)	70.3^{b}	83.8^{a}	91.2^{a}	81.8
Multiple birth rate (%)	19.2^{d}	12.0^{d}	35.3^{c}	22.2
Fecundity	0.86^{b}	1.24^{a}	1.30^{a}	1.13
Litter size	1.23^{b}	1.48^{a}	1.41^{a}	1.37

[a,b:] Means in the same row, with different superscripts indicate significant difference ($P<0.05$)
[c,d:] Means in the same row, with different superscripts indicate significant difference ($P<0.01$)

Applications that aim to increase the rate of reproduction appear to offer the best opportunity for increasing the number of lambs produced by breeding ewes, thereby increasing the effectiveness of lamb production. Methods that promote reproduction intend to increase the rate of multiple births by generally increasing the rate of twin ovulations (Wilkins 1997). The percentage of ewes exhibiting oestrus in this trial was comparable to values reported in the literature (Amer and Hazzaa 2009; Ustuner et al. 2007; Karaca et al. 2009; Santos et al. 2010; Ozyurtlu et al. 2011). Ustuner et al. (2007) stated that as result of the intravaginal application of sponges for 6 and 12 days in Awassi ewes, they observed an oestrous response of 77.1% in the short-term group and 100% in the long-term group. Ataman et al. (2006) reported that with 12 and 7 day intravaginal FGA sponge applications in ewes in their breeding season, a 100% oestrous rate was obtained in both groups. Husein et al. (2007) identified an oestrus-exhibiting rate

of 100% for ewes in a group treated with an FGA sponge + PMSG for 12 days and as 83% for the ewes in a group treated with an FGA sponge + PMSG for 4 days. Ungerfeld and Rubianes (2002) stated that in terms of fertility in this study short-term applications of MAP or FGA sponges are as effective as long-term applications; this results suggest some flexibility, which can be advantageous in applications performed in field conditions. Although the short-term application of intra-vaginal sponges offers some advantages, it has a disadvantage in that the possibility of becoming pregnant decreases because the progestogen used for oestrous synchronisation is at a low level. The reason for this decrease is explained by the prolonged life of the ovulatory follicle when progestogen is applied at a low level (Vinoles et al. 1999). Nevertheless, Ungerfeld and Rubianes (2002) stated that no difference was available in terms of oestrous response in ewes in which intravaginal sponges were applied for 7 or 14 days and that short-term progestogen application was adequate in terms of inducing fertile oestrus.

The overall lambing rate, rate of multiple births, fecundity rate and litter size recorded following different lengths of progestogen treatments were 81.8, 22.2, 113.3 % and 1.37, respectively. The PMSG administration relative to the different days until sponge removal, had a significant effect on lambing, fecundity rates and litter size ($P<0.05$), and on the rate of multiple births ($P<0.01$). The highest lambing, multiple birth and fecundity rates were recorded following sponge removal in ewes treated with FGA for 14-day. Furthermore, higher litter sizes occurred in ewes in which the sponge was removed at 10-day than in those in which it was removed at 7-day and 14-day. The results obtained in the present study are shown in Table 1.

Ustuner et al. (2007) reported that after short and long-term sponge applications in ewes of the Awassi breed, the induction of oestrus took place at a rate of 77% and 88%, respectively. Ozyurtlu et al. (2011) noted that 7-day long progesterone applications in Awassi ewes were as effective as 14-day long progesterone applications. They also reported pregnancy rates of 66.7% and 58.3% and lambing rates of 83.3% and 66.7% for applications of 7 and 14 days, respectively. Ataman et al. (2006) stated that the effects of progesterone applications for lengths of 6 or 12 days during the mating season and during anoestrous were the same in terms of pregnancy and lambing rates. However, in this study, close results were obtained with the medium and long-term applications in terms of oestrous response and lambing rates, whereas the results of short-term applications were found to be a bit lower. In general, the values obtained are similar to the 75.6% reported by Koyuncu and Altincekic (2010), higher than the 64.6% reported by Amer and Hazzaa (2009), and lower than the 86.0% reported by Santos et al. (2010). Differences among reports in the literature may be due to differences in synchronisation procedures, the amount of PMSG applied, and breed differences in the ewes studied.

The rate of multiple births in Kıvırcık ewes is 10-20% on average, which is low (Kaymakci 2006). With hormonal applications, the rate of multiple births increased to 51.6%. As a result of the increased lambing rate, oestrous synchronisation protocols are preferred by sheep breeders in semi-intensive production systems and provide economic profit. In this study, although the values obtained for the rate of multiple births were similar for short and medium-term applications, values in the long-term group were higher ($p<0.01$). However, fewer data were obtained than in other reports in the literature concerning this subject. In various studies, differences that appear in terms of multiple births may be associated with GnRH injections, which affect the number of synchronised follicles by promoting a new follicular growth prior to short-term progestogen applications (Karaca et al. 2009).

A low sperm retention rate in ewes is affected by the health of the reproductive organs of the ewes, as well as by factors such as breed, genetics, environment, and care and feeding (Husein and Kridli 2003). Ovulation, sperm retention and early embryonic mortality rates are among the factors that affect the number of lambs produced (Beck et al. 1993). Hüseyin and Kridli (2003) reported that with the application of progesterone impregnated sponges, GnRH or $PGF_{2\alpha}$ the oestrus and inception rates in ewes during seasonal anoestrous have clearly increased. Therefore, the inclusion of GnRH with the application of short term progestogen is believed to contribute to the increase in the rate of multiple births and litter size. Fitzgerald et al. (1985) reported that 20 mg $PGF_{2\alpha}$ application with a 60 mg MAP sponge for 7 days was as successful as when the sponge was applied for 14 days. Beck et al. (1993) stated that in terms of reproductive parameters, the application of $PGF_{2\alpha}$ after the 5-day application of a progesterone impregnated sponge produced similar results to an application of a progesterone impregnated sponge for 8 day. Ataman et al. (2006) reported that the application of eCG together with the application of an FGA sponge for 7 days was effective in inducing successful oestrous synchronisation and a high pregnancy and

lambing rate. However, the values identified for the medium and long-term groups in terms of fecundity and litter size are close to each other whereas the values obtained for the short-term group were lower than for the medium and long-term groups. Ataman et al. (2006) reported lamb effectiveness of 1.7 in the ewe group in which they used a sponge for 12 days during mating season and 1.8 for the group in which they used a sponge for 7 days. Sönmez et al. (2009) noted that in their studies, they obtained lamb effectiveness at a rate of 1.63. The rates of lamb effectiveness obtained in the current study were lower than the ones reported in the literature, and progestogen applications for various lengths of time did not have an effect on lamb effectiveness. This result likely arises from breed, care and feeding differences.

Conclusions

In this study, oestrus was succesfully synchronised when vaginal sponges containing FGA were used for long, medium and short periods in combination with PMSG. Medium or long-term progesterone applications did not have significant differences in fertility parameters; however, short-term applications were not as effective as medium or long-term applications in terms of fertility. Relevant reports from the literature show that the effectiveness of reproductive results, which are obtained by the synchronisation of oestrus in ewes, may change based on the breed and the method used. Nonetheless, we are of the opinion that it is appropriate to support the results obtained here with future studies that explore different synchronisation methods with more ewes and more breeds.

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