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# Effects of Oestrus Synchronisation by Double FGA-Sponge or Split eCG Administrations upon the Reproductive Traits in Angora Goats During the Breeding Season

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**Abstract:** In this study, the effects of post-mating  $FGA/P_4$  and of split eCG/PMSG injections at different doses were evaluated for the induction of oestrus in Angora goats during the breeding season. In total, thirty-six multiparous does were assigned into two separate synchronisation trials. In Experiment 1 (double FGA), 22 does were administered all firstly an intravaginal sponge containing 20 mg FGA for 11 days. On day 9, the animals then received i.m. injections of 125 mg cloprostenol, and of 400 IU eCG concurrently. On day 6 post-mating, animals were randomly assigned into two groups, as control (n=13), single and double FGA group (n=9), receiving the second FGA for 11 days. In Experiment 2 (split eCG), 14 does were randomly assigned into eCG 750 and eCG 500 groups (n=7 each), with multiple injections (on 5 consecutive days) of 750 (250 and 100 in double and 50 IU at last) or of 500 IU (100 IU daily). The results showed that; i) double/post-mating FGA had no favourable effect upon the reproductive parameters studied, ii) the eCG itself led to a considerably poorer fertility and twinning in Angora does during the breeding season.

Key words: Angora goat, Post-mating progestagen, Split eCG, Synchronisation.

## Ankara Keçilerinde Sezon-içi Östrus Senkronizasyonu Amacıyla Çift Doz FGA Sünger veya Bölünmüş eCG Uygulamalarının Üreme Özellikleri Üzerine Etkileri

**Özet**: Sunulan çalışmada, üreme sezonundaki Ankara keçilerinde çiftleşme sonrası FGA/P<sub>4</sub> ve farklı dozlarda bölünmüş eCG/PMSG enjeksiyonlarının östrus senkronizasyonundaki etkileri araştırıldı. Toplam 36 baş anaç (önceden doğum yapmış) keçi iki ayrı senkronizasyon grubuna ayrıldı. Çalışma 1'de (çift doz FGA), keçilere 11 gün süreyle 20 mg FGA içeren vagina-içi sünger uygulandı. Hayvanlara 9. günde, 125 mg cloprostenol ve 400 IU eCG birlikte enjekte edildi. Çiftleşme sonrası 6. günde, hayvanlar tek (kontrol, n=13) veya çift doz FGA grubu (n=9), ikinci FGA 11 gün süreyle, olmak üzere rastgele iki deneme grubuna ayrıldı. Çalışma 2'de (bölünmüş eCG), 14 baş keçi rasgele tarzda eCG 750 ve eCG 500 gruplarına ayrılarak (n=7, her biri), ilk gruba 750 IU eCG (iki kez 250, iki kez 100 ve son kez 50 IU) verilirken, diğer gruba ise 500 IU eCG (günlük 100 IU) çoklu enjeksiyon tarzında (ardışık 5 gün süreli) olarak verildi. Sonuç olarak; i) çift doz/çiftleşme sonrası FGA uygulamasının çalışılan üreme parametreleri üzerine bir katkısının olmadığı, ii) sadece eCG uygulamasının ise yavru verimi ve ikizlik yönünden belli düzeyde daha düşük oranlara yol açtığı kanısına varıldı.

Anahtar kelimeler: Ankara keçisi, Bölünmüş eCG, Çiftleşme sonrası progestagen, Senkronizasyon.

#### INTRODUCTION

estrus synchronisation has long been one of the main strategies for managing reproduction in farm animals since the early 1960s. In goats, FGA administration combined with the eCG and PGF<sub>2a</sub> injections has been extensively employed (Baril et al., 1998; Baril and Saumande, 2000; Martemucci et al., 2011). However, the fertility rate of FGA- and eCG-treated goats/ewes varied widely, ranging from 11 % to 87 % (Baril et al., 1998; Amarantidis et al., 2004; Martemucci et al., 2011). This variation may be attributable to various factors, including; the administration regime (Ustuner et al., 2007), stage of oestrus period (Inshwar and Pandey, 1990) or season (Martemucci et al., 2011), breed (Greyling and Van Der Nest, 2000), lactation, seasonal changes in buck fertility, time of insemination or a combination of all (Regueiro et al., 1999).

In recent years, therefore, the researchers have been seeking new methods to increase the reproductive outcome in goats. Amongst them, PGF<sub>2</sub> $\alpha$  (Mani et al., 1992), Ovsynch (Holtz et al., 2008) and multiple eCG (Karaca et al., 2009) may be given, as few examples. Using these conventional protocols, acceptable synchronisation results have been achieved so far. Indeed, Karaca et al. (2009), using multiple eCG, reported quite successful rates of oestrus (80 %) and pregnancy (75 %), even outside the breeding season in goats.

During the early pregnancy, the embryonic mortality causes a marked reduction in the reproductive performance in farm animals. Indeed, during the first 3 weeks of pregnancy, 30-40 % of fertilised eggs are lost in sheep and goats (Nancarrow, 1994). The vast majority (70-80 %) of the loss occurs between days 8 and 16 (Sreenan et al., 1996). Undoubtedly, inadequate luteal function (Nancarrow, 1994), specific nutrient deficiency (Sreenan et al., 1996), rations with poor energy and/or protein contents (Silke et al., 2002), excessive milk yield (Sartori et al., 2002), and heat stress (Thatcher et al., 1996) are among the factors that all may affect the embryogenesis more or less. In the literature, therefore, the post-mating  $P_4$  or hCG/GnRH administrations before the time of maternal recognition of pregnancy (Cam et al., 2002) have been employed to compensate for the likelihood of luteal insufficiency or to stimulate the embryonic development during the early pregnancy in ewes (Beck et al., 1994).

Indeed, the post-mating P<sub>4</sub> administration has been reported to increase the embryonic survival and thus pregnancy rates, with conceptus development and size improvement when given between day 5 and 9 in cows (Mann and Lamming, 1999). Likewise, in ewes, the early post-ovulatory P<sub>4</sub> increased the administration conceptus development and the synthesis of  $IFN_t$  (interferon tau), a protein needed for the maternal recognition of pregnancy (Thatcher et al., 1996). However, in an exceptional study in goats (Trujillo et al., 2008), a similar but non-beneficial effect of post-breeding P<sub>4</sub> has been reported based on the pregnancy rate achieved therein.

The objective of the present study was therefore to investigate the effectiveness of double (post-mating) FGA administration and split (multiple) injections (on consecutive days) of eCG at different doses (without  $P_4$ ) for the induction of oestrus in Angora goats during the breeding season.

### MATERIALS and METHODS

#### Location and Animals Used

This study was conducted in Angora goats at the Experimental Research and Practice Farm, Faculty of Veterinary Science, University of Ankara (longitude 32° 53' N; latitude 39° 57' E; altitude 850 m), during the breeding season. A total of 36 goats, 2-3 years old and 30-35 kg live weight were used in this study. The animals were kept in an open pen, fed with alfalfa hay and a commercial concentrate supplement, together with drinking water *ad libitum*.

## Experiment 1 (Double FGA)

In twenty-two does, Fluorogestone acetate (FGA, 20 mg) sponges (Chronogest<sup>®</sup> CR/Sponge, INTERVET, Istanbul, Turkey) were inserted intravaginally for 11 days. On day 9, goats were injected i.m. with 125 mg cloprostenol, a  $PGF_{2\alpha}$  analogue (Estrumate<sup>®</sup>, DIF, Istanbul) and 400 IU eCG (Chronogest<sup>®</sup>/PMSG, INTERVET) together.

Six days post-mating, goats were randomly assigned into two groups as; control (single FGA) group (n=13), no further administration, and post-mating (double) FGA group (n=9), receiving a second sponge for 11 days (between days 6 and 17).

## Experiment 2 (Split eCG)

Fourteen goats were randomly assigned into two groups with numerous eCG injections (without  $P_4$  pre-treatment). To does in the first group (eCG 750; n=7), a total dose of 750 IU eCG in five consecutive days (250 IU twice, 100 IU twice, and 50 IU once, resp.) was injected i.m. For the second group (eCG 500; n=7), 500 IU eCG was given in five consecutive days (100 IU, daily).

#### **Oestrus Detection, Mating and Fertility**

Categorisations of behaviours observed commonly to distinguish between the oestrous (i.e. attractivity and receptivity, mainly standing for a mounting buck) and non-oestrous does were made by using the method of Uçar et al. (2005). The signs of oestrus (mainly mounting) were monitored every 6 h (between 18 and 72 h) upon the first sponge withdrawal or following the last eCG injection for a minimum period of 15 min each, by using teaser bucks. Once detected, the oestrous goats were then separated from the rest of flock and they were hand-mated at least once each with 3 fertilityproven bucks used rotationally.

To detect does that returned to oestrus, all the animals were monitored again daily from day 12 to 25 post-mating. Non-returns were presumed 'pregnancy'. The rates of pregnancy, kidding and twinning were recorded following the 150 ± 5 days of mating.

### **Statistical Analyses**

The present data (mean  $\pm$  SEM) from the reproductive parameters (i.e. the oestrus rate, interval and duration of oestrus as well as pregnancy/kidding and twinning rates) of does receiving FGA- or eCG-based synchronisation programmes were analysed by Regression analysis using MINITAB (Version 11.2, MINITAB Inc., Pennsylvania, USA). Differences between the groups were considered significant (when P≤0.05).

### RESULTS

Briefly, no significant effect of post-mating FGA supplementation was found upon any of the reproductive parameters studied (Experiment 1; Table 1). However, the interval of oestrus was significantly (P≤0.05) shorter in high dose eCG group (Experiment 2; Table 2).

In Experiment 1, there were no significant effects of the number of FGA sponge administrations (single or double) upon any of the reproductive parameters studied. The oestrus rate, interval and duration of oestrus, the rates of pregnancy/kidding and twinning for single and double FGA groups were 84.60 vs. 88.90 %, 29.45 vs. 26.25 h, 25.64 vs. 25.50 h, 81.80 vs. 75.00 %, and 27.30 vs. 25.00 %, respectively.

In Experiment 2, however, the interval of oestrus was relatively significantly (P $\leq$ 0.05) shorter (34.00 ± 2.53 h) in the eCG 750 group as compared to those (42.00 ± 2.68 h) in the low dose group. Nevertheless, as with the former trial, no further significant difference was observed for any other

**Table 1.** Comparative effects of single or double (post-mating) FGA-sponge administration for oestrus synchronisation upon the reproductive parameters in Angora does during the breeding season (Experiment 1)

**Tablo 1.** Östrus senkronizasyonunda tek veya çift doz (çiftleşme sonrası) FGA-sünger uygulamasının üreme sezonundaki Ankara keçilerinde üreme parametreleri üzerine karşılaştırmalı etkileri (Çalışma 1)

	Synchronisation Groups		Statistics		
	FGA-Single (n=13)	FGA-Double (n=9)	F-ratio	P value	Significance
Parameters					
Oestrus, %	84.60 ± 10.40	88.90 ± 11.10	0.08	0.787	N.S.
*Interval (onset) of oestrus, h	29.45 ± 2.35	26.25 ± 1.58	1.08	0.313	N.S.
*Duration of oestrus, h	25.64 ± 0.85	25.50 ± 0.98	0.01	0.918	N.S.
*Pregnancy / Kidding rate, %	81.80 ± 12.20	75.00 ± 16.40	0.12	0.737	N.S.
*Twinning rate, %	27.30 ± 14.10	25.00 ± 16.40	0.01	0.918	N.S.

N.S.: not significant (P>0.05).

\* The number of animals considered for each parameter was 19 only; since a total of 3 does (of 2 does from FGA-Single and of 1 doe from FGA-Double group) showed no signs of oestrus, they were then excluded from these analyses.

**Table 2.** Comparative effects of multiple/split injections (on five consecutive days) of eCG at two different doses (750 vs. 500 IU, in total) for oestrus synchronisation upon the reproductive parameters in Angora does during the breeding season (Experiment 2)

**Tablo 2.** Östrus senkronizasyonunda farklı iki dozdaki (750 ve 500 IU, toplam) eCG'nin çoklu/bölünmüş enjeksiyonunun (ardışık 5 gün süreli) üreme sezonundaki Ankara keçilerinde üreme parametreleri üzerine karşılaştırmalı etkileri (Çalışma 2)

	Synchronisation Groups		Statistics					
	eCG 750 (n=7)	eCG 500 (n=7)	F-ratio	P value	Significance			
Parameters								
Oestrus, %	85.70 ± 14.30	85.70 ± 14.30	**	**	**			
*Interval (onset) of oestrus, h	$34.00^{a} \pm 2.53$	$42.00^{b} \pm 2.68$	4.71	0.055	P≤0.05			
*Duration of oestrus, h	28.00 ± 1.26	29.00 ± 1.84	0.20	0.664	N.S.			
*Pregnancy / Kidding rate, %	66.70 ± 21.10	50.00 ± 22.40	0.29	0.599	N.S.			
*Twinning rate, %	$0.00 \pm 0.00$	$0.00 \pm 0.00$	**	**	**			

N.S.: not significant (P>0.05).

<sup>a,b</sup> Means (± SEM) having different superscripts within the same row are significantly different from each other (P<0.05).

\* The number of animals considered for each parameter was 12 only; since a total of 2 does (of 1 doe from each group) showed no signs of oestrus, they were then excluded from these analyses.

\*\* No statistical analyses could be made since the values for each group were identical.

parameters studied, such that the oestrus rates, duration of oestrus, the pregnancy/kidding and twinning rates for the groups were 85.70 % both, 28.00 vs. 29.00 h, 66.70 vs. 50.00 %, and zero % both, respectively.

#### DISCUSSION

In the present trials, the effects of post-mating FGA and of split eCG given at different doses were studied for oestrus synchronisation in Angora goats during the breeding season. Briefly, we found in does that; i) the post-mating FGA had no favourable effect upon the reproduction, ii) the higher dose of split eCG itself shortened the interval of oestrus, but iii) it led to a considerably inferior fertility when used alone.

Regarding single/double FGA administration at first, the single sponge, together with  $PGF_{2\alpha}$ -eCG, administration was quite effective in season, yielding 85-89 % oestrus rate, as in parallel well with the 81 % reported in Saanen goats (Dogan et al.,

2004). Likewise, in another study (Greyling and Van Der Nest, 2000), with the MAP-eCG, 85 and 75 % oestrus rates were observed in Boer and indigenous goats, respectively. Nevertheless, a higher (up to 100 %) oestrus rate was also reported in other breeds, e.g. Nadooshani (Bitaraf et al., 2007) and indigenous Greek goats (Amarantidis et al., 2004). The variations might be related to differences in the eCG dose, the source of  $P_4$ , schedule/type of treatment, breed and housing-feeding used.

Additionally, the interval of oestrus (around 30 h) in does receiving the conventional programme (FGA-PGF<sub>2 $\alpha$ </sub>-eCG) used herein was less than those (36 to 41 h) in previous studies (Greyling and Van Der Nest, 2000; Kilboz and Karaca, 2010). Furthermore, the duration of oestrus (around 26 h) with this protocol used was similar to the 25 h in Alpine and Saanen breeds (Freitas et al., 1997). However, the present durations were numerically shorter than the 31 and 32 h in Boer and indigenous goats, respectively (Greyling and Van Niekerk, 1991), 34 h in indigenous Greek (Amarantidis et al., 2004) and 36 h in Damascus breeds (Zarkawi et al., 1999). Nevertheless, the present durations observed were considerably higher than the 15 h in Saanen goats reported during the transition period (Dogan et al., 2004). These variations might mainly be due to; the physiological statues/breeding season (Fonseca, 2002), breed (Baril et al., 1993), age (Leboeuf et al., 2003) and nutrition (Romano, 2004), as all would influence the plasma  $P_4$  levels (Pierson et al., 2001). Moreover, interestingly, the 'late oestrus' related to the anti-immunogenic potency may also lower the routine success rates of synchronisation on the farm because of its recent popularity (Baril et al., 1998).

Furthermore, the fertility rate with the conventional protocol (FGA-PGF<sub>2α</sub>-eCG) varies greatly, but its administration (for 9-11 days) has generally been very effective, as leading to acceptable conception rates herein. The overall pregnancy/kidding rates with the conventional

methods were acceptable, as being 82 % (single FGA) and 75 % (double FGA), and these satisfactory values agree well with those (over 60 %) of earlier reports (Baril et al., 1998; Michels et al., 1998). A relatively higher (86 %) pregnancy rate was also noted elsewhere (Amarantidis et al., 2004). In an exceptional study of Trujillo et al. (2008), an unfavourable effect of post-mating  $P_4$ supplementation on the pregnancy rates was reported, as was the case herein. However, the present fertility rates with single or double sponge administration were considerably higher (82 vs. 75 %, resp.) as compared to those (55 vs. 44 %, resp.) reported in the latter study. Obviously, the pregnancy rates were numerically (7 %) lower with further P₄ supplementation post-mating, representing similarly a numerical reduction of fertility in both studies. In this respect, the longterm progestagen treatments (10 to 21 days) have been shown to be associated with low fertility in goats (Martemucci et al., 2011). Thereby, in one hand, it was attributed eventually to the lower  $P_4$ concentration, persistency (senescence) of dominant follicle and impaired sperm transport in vivo following the insemination. On the other hand, we may further presume that other co-factors (e.g. season and breed) might also modify the sustainability of embryogenesis.

Considering the split eCG administration, we found that the higher dose (750 vs. 500 IU) of eCG was relatively markedly superior for an earlier onset of oestrus (34 h vs. 42 h, resp.) and for the fertility rate (67 vs. 50 %, resp.). In goats, it has been reported that the later the onset of oestrus initiates (following the exogenous hormone administration in synchronisation protocol) the lower the fertility rate is achieved (Baril et al., 1998). However, no difference was observed for the rate (86 % both) and duration of oestrus (around 28.5 h both) herein. These findings may indicate, for the first time, that the protocol of multiple/split eCG injections on 5 consecutive days could be used as an alternative

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synchronisation method in goats during the breeding season. Furthermore, Karaca et al. (2009), conducting the only relevant study in the literature, reported that the eCG injections could also successfully stimulate the oestrus (80 %) leading to sufficient pregnancy (75 %) in Angora goats outside the breeding season. It was noteworthy that, unlike the considerable twinning rates (around 26 %) observed with conventional FGA protocols both, there was no twinning at all using the eCG only (without any  $P_4$  pre-treatment). In theory, we may presume that the P<sub>4</sub> pre-treatment may well prepare the reproductive tract, as eventually leading to superior fertility outcomes (Fonseca et al., 2005). Hence, we may presume that, although using the eCG only may result in acceptable rates of oestrus synchrony of cycling Angora does, the protocol should be supported, at least with the P<sub>4</sub> (Baril and Saumande, 2000; Amarantidis et al., 2004; Martemucci et al., 2011) for achieving a superior reproductive outcome. This combination would allow not only for a higher fertility with some twinning but also be used outside the breeding season (Amarantidis et al., 2004).

Overall, the present findings suggest that; i) a single conventional FGA-sponge administration would be enough for the oestrus synchronisation, ii) without any FGA (or P<sub>4</sub>) pre-treatment, multiple/split eCG injections (on five consecutive days) at a higher dose (750 IU vs. 500 IU) considerably shortened the interval of oestrus upon the sponge withdrawal, and hence iii) the protocol of multiple eCG injection itself may be an alternative method for synchronisation especially in mating season, but iv) it yields inferior outcome (lower pregnancy rate and no twinning) as compared to those with the conventional methods (single/double FGA-based) in Angora goats during the breeding season. However, our results should be confirmed by using a higher number of goats from different breeds both in- and outside the breeding season before more reliable conclusions could be drawn.

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