# Estrous synchronization during the natural breeding season in Anatolian black does

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**ABSTRACT**: The efficiency of medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) sponges with or without $PGF_{2\alpha}$ (cloprostenol) for synchronizing estrous in non-lactating does was investigated during the natural breeding season. Does were treated for 11 days with 60 mg MAP ($n$ = 38) or 40 mg FGA ($n$ = 32) sponges. All does also received intramuscular injections of 500 IU PMSG. In addition, 19 and 14 of the does synchronized with MAP and FGA respectively, were injected with 125 μg cloprostenol and the remaining does from both groups were injected with 1.5 ml of sterile saline solution, 48 h prior the sponge removal. Cervical artificial insemination (AI) with diluted fresh semen was performed at a fixed time (36 and 48 h) following progestagen withdrawal. The different groups estrous response for the first 12 ± 6 h and within 66 h, time to onset and duration of the induced estrous, and pregnancy rate was found to be 52.6%, 92.9%, 20.6 ± 0.8 h, 29.7 ± 1.3 h, and 70.0%, respectively. There were significant differences between groups FGA/PMSG/$PGF_{2\alpha}$ and MAP/PMSG in terms of the duration of induced estrous ($P < 0.05$) and between groups FGA/PMSG/$PGF_{2\alpha}$ and FGA/PMSG in terms of estrous response at the first 12 ± 6 h ($P < 0.05$). These results indicate that, the use of MAP/PMSG and FGA/PMSG intravaginal progestagen treatments with or without cloprostenol are equally efficient in synchronizing estrous in non-lactating hair goats during the natural breeding season.

**Keywords**: goat; cloprostenol; MAP; FGA

In goats, the control of estrous and ovulation is a valuable tool to improve and maintain the production of milk and meat throughout the year. Therefore, estrous synchronization together with AI is extensively applied in the reproductive management of goat (Leboeuf et al., 1998). Progesterone or a progestagen analogue is generally used to synchronize estrous in does during the breeding and non-breeding seasons (Ak et al., 1998). Worldwide, the most common route of progestagen application in goats is via the intravaginal sponge (Bretzlaff, 1997). The most widely used procedures for synchronization and/or the induction of estrous are 12–21 days of FGA or MAP impregnated intra vaginal sponge treatment (Romano, 1996. 1998,2002; Romano and Benech, 1996; Romano and Fernandez Abella, 1997; Leboeuf et al., 1998; Romano et al., 2000) and an intramuscular injection of PMSG at progestagen withdrawal (Ak et al., 1998; Greyling and Van der Nest, 2000; Motlomelo et al., 2002), or 11 days treatment with FGA impregnated intravaginal sponges and an intramuscular injection of PMSG and a synthetic $PGF_{2\alpha}$ analogue 48 h before or at sponge withdrawal (Baril et al., 1993; Freitas et al., 1996a,b, 1997; Ak et al., 1998; Leboeuf et al., 2003). Even though some studies have found these two progestagens to be equally effective in the induction of estrous, ovulation and fertility (Gordon, 1975; Smith et al., 1981), some researchers have found some difference between the effectiveness of the two types progestagen sponges (Evans and Maxwell, 1987).

The objectives of the present study was thus to compare the efficiency of MAP and FGA with or without cloprostenol in synchronizing estrous in non-lactating hair goats during the natural breeding season and compare the fertility rates obtained following AI.

## MATERIAL AND METHODS

The study was carried out at a village located in Balikesir (latitude 39º 06' and 40º 39' N, longitude 26º 39' and 28º 58' E, altitude 139 m) in western Turkey, during September (the natural breeding season) under natural lighting. A total of 70 cyclic hair Anatolian black does ranging in age from 2 to 6 years, weighing 32 to 63 kg and with body condition scores evaluate on a scale of 0 to 5, according to Morand-Fehr et al. (1989) were studied. In addition, 3 Saanen breeding bucks of proven fertility and 5 teaser bucks were used in the present trial. The does were allowed to graze on natural pasture from 07:30 to 11:30 h and from 12:30 to 17:30 h and kept in pens overnight. Water and a mineral salt lick were provided *ad libitum*. The management of the does did not change throughout the entire experimental period.

The experimental does were divided into 4 groups according to the age and body weight. The treatment for estrous synchronization was performed using intravaginal progestagen sponges of 60 mg MAP ($n$ = 38,Vetimex, Bladel, Netherlands) or 40 mg FGA ($n$ = 32, Intervet, Netherlands) for 11 days. On the 9$^{th}$ day, all does were injected im with 500 IU PMSG (Intervet, Netherlands). In addition, on the same day, 19 and 14 of the does synchronized with MAP and FGA respectively were injected with 125 µg cloprostenol (estroPLAN, Parnell Laboratories, Austria). The remaining does from both groups were injected with 1.5 ml of sterile saline solution. Estrous was monitored every 6 h from 12 to 66 h following progestagen sponge withdrawal with the aid of 5 teaser bucks. The does were considered in estrous when they were mounted by the teaser bucks. Estrous duration was defined as the time between the first and last accepted mount, within the same estrous period.

Three ejaculates from each buck were collected by artificial vagina in the presence of a doe in estrous. During collection and examination, the semen was protected from temperature shock. Each ejaculate was immediately evaluated for volume and wave motility (Memon et al., 1997). Only ejaculates with a volume higher than 0.5 ml and good wave motility (≥ 3) were used. The volume was determined from the collection tube, which was graduated in 0.1 ml divisions and the motility was assessed by depositing a drop of semen on a glass slide and examining it on a warm stage (35ºC) under the microscope (×40). The semen sample was scored using a scale ranging from 0 (no wave movement) to 5 (extreme wave movement). Only ejaculates with scores of 3 and higher were used. The semen was diluted to a sperm concentration of $600\times10^6$ motile cells/ml, the density was determined with the aid of a haemocytometer. A one-step dilution was performed at 30ºC with the diluent consisting of sterilized cow skim milk containing 1 000 IU sodium G penicillin and 1 000 µg/ml dihydrostreptomycine sulfate. Thereafter, diluted semen samples were pooled in the same test tube. The diluted semen was then cooled to 16ºC over a 1 h period and kept at this temperature until insemination. Each doe was inseminated intracervically twice at a fixed time 36 and 48 h following sponge withdrawal with a 0.25 ml straw containing $150\times10^6$ spermatozoa. All does were tested for pregnancy 60 days following AI with the aid of a transabdominal ultrasonic scanning apparatus.

The onset of estrous and duration of induced estrous periods were subjected to analyses of variance (one-way ANOVA) and the differences among means were tested for significance by the Fisher's PLSD. Estrous response and pregnancy rates were analyzed using the chi-square test. The 95% significance level was noted. The SPSS 10.0 software was used for all statistical analyses (Instat, 1990–1993).

## RESULTS

The results in terms of estrous response for the first 12 ± 6 h and within 66 h, time to onset and duration of the induced estrous and pregnancy rates are set out in Table 1. Figure 1 shows the intervals between sponge removal and estrous onset. Synchronization parameters and pregnancy rates were not significantly different between the 4 treatment groups, except significant differences between FGA/PMSG/$PGF_{2\alpha}$ and MAP/PMSG groups for the duration of induced estrous ($P < 0.05$) and between FGA/PMSG/$PGF_{2\alpha}$ and FGA/PMSG groups for the estrous response at the first 12 ± 6 h ($P < 0.05$). Thus, the data were pooled. The overall estrous responses for the first 12 ± 6 and within 66 h for 4 groups were 52.6% and 92.9%, respectively. The overall mean time to onset, duration and cessation of estrous following sponge withdrawal in the 4 groups was 20.6 ± 0.8 h and 29.7 ± 1.3 h, respectively. The overall mean pregnancy rate at day 60 following AI for 4 groups was 70.0%, with

Table1. Estrous response and pregnancy rate in hair Anatolian black does after different estrous synchronization treatments and AI at a fixed time

| Treatment group | *n* | Response (%) | | Estrous | | Pregnancy rate (%) |
|---|---|---|---|---|---|---|
| | | 12 ± 6 h | within 66 h | onset (h) mean ± S.E. | duration (h) mean ± S.E. | |
| FGA/PMSG/$PGF_{2\alpha}$ | 14 | (4/10) 71.4[a] | (2/12) 85.7[a] | 18.0 ± 1.9[a] | 34.5 ± 3.0[a] | (4/10) 71.5[a] |
| FGA/PMSG | 18 | (11/7) 38.9[b] | (1/17) 94.4[a] | 22.9 ± 1.6[a] | 29.6 ± 2.5[ab] | (5/13) 72.2[a] |
| MAP/PMSG/$PGF_{2\alpha}$ | 19 | (8/11) 57.9[ab] | (1/18) 94.7[a] | 20.3 ± 1.6[a] | 28.3 ± 2.4[ab] | (5/14) 73.7[a] |
| MAP/PMSG | 19 | (10/9) 47.4[ab] | (1/18) 94.7[a] | 21.0 ± 1.6[a] | 26.3 ± 2.4[b] | (7/12) 63.2[a] |
| Total | 70 | (33/37) 52.6 | (5/65) 92.9 | 20.6 ± 0.8 | 29.7 ± 1.3 | (21/49) 70.0 |

[a,b] means in the same row, with different subscripts indicate a significant difference ($P < 0.05$)

3 of 5 does not showing any estrous response, but diagnosed pregnant two months after AI.

## DISCUSSION

The FGA or MAP treatments and the combination of these with $PGF_{2\alpha}$ were all found to be efficient procedures for estrous synchronization in the does during the breeding season. Estrous response for the first 12 ± 6 h obtained from FGA/PMSG/$PGF_{2\alpha}$ group was the highest (71.4%) and significantly different only from FGA/PMSG group (38.9%). Although there was no significant difference between MAP/PMSG/$PGF_{2\alpha}$ and MAP/PMSG groups, estrous response of MAP/PMSG/$PGF_{2\alpha}$ was superior (57.9% vs 47.4%). From these results it could be concluded that $PGF_{2\alpha}$ application together with FGA or MAP increased estrous response rates. Considering our overall estrous response rate for the first 12 ± 6h (52.6%), our result was higher than the results of Freitas et al. (1996) and Leboeuf et al. (2003). Within 66 h there were no significant differences among 4 groups in terms of estrous response. The high estrous response recorded during the 66 h observation period following the cessation of treatment (overall mean response 92.9%) is within the range of 62.5–100% quoted in treatments with FGA or MAP intravaginal sponges (Romano, 1996, 1998, 2002; Romano and Benech, 1996; Romano and Fernandez Abella, 1997; Greyling and Van der Nest, 2000; Motlomelo et al., 2002) or in combination with $PGF_{2\alpha}$ (Baril et al., 1993; Freitas et al., 1996a,b, 1997; Ak et al., 1998; Leboeuf et al., 2003).

Researchers have reported the onset of estrous to occur within 6–120 h following progestagen withdrawal (Freitas et al., 1996b; Romano, 1998; Greyling and Van der Nest, 2000). In this study, the mean overall interval to the onset of estrous following progestagen removal was 20.6 ± 0.8 h

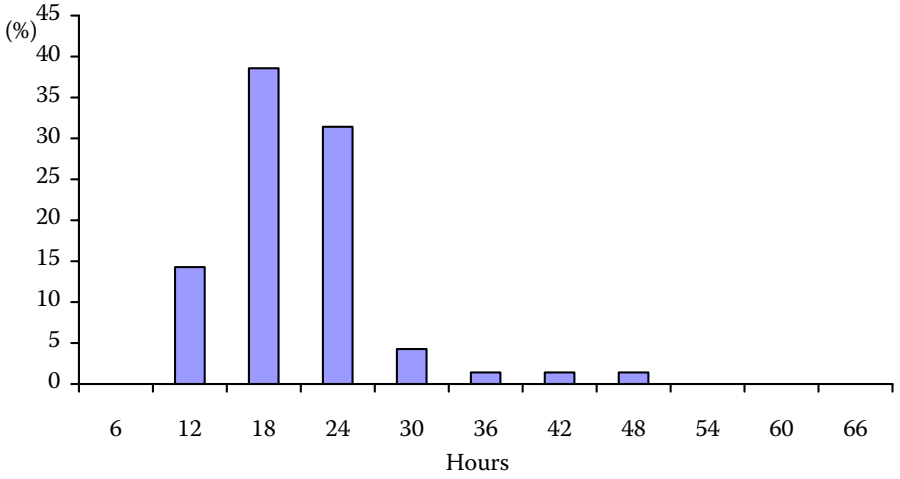


Figure 1. Intervals between sponge removal and estrous onset

with no significant difference between the 4 groups (Table 1). A relatively similar interval was mentioned by Leboeuf et al. (2003), were intravaginal FGA (20 or 45 mg) sponges during the breeding and non-breeding season. With respect to the onset estrous, on the other hand, bigger periods have been reported by Baril et al. (1993), Romano (1996, 1998, 2002), Romano and Benech (1996), Romano and Fernandez Abella (1997), Freitas et al. (1996a,b, 1997), Greyling and Van der Nest (2000) and Motlomelo et al. (2002) in does. The reason for differences is not clear, however it may be attributed to differences in breed, nutrition, season, use of gonadotrophins, presence of females in proestrous/estrous and presence of the male after sponge removal all factors that are known to influence this parameter (Doney et al., 1973; Greyling and Van Niekerk, 1990; Mani et al., 1992; Romano, 1998, 2002; Ahmed et al., 1998).

The mean overall duration of the induced estrous period (29.7 ± 1.3 h) recorded in this study is similar to that reported by Greyling and Van der Nest (2000) and Motlomelo et al. (2002) and lower than that reported by Romano (1996), Romano and Benech (1996), Romano and Fernandez Abella (1997) and Romano et al. (2000). The mean estrous duration was shorter ($P < 0.05$) in the MAP/PMSG treatment (26.3 ± 2.4 h), compared to the FGA/PMSG/$PGF_{2\alpha}$ treatment (34.5 ± 3.0 h). This difference between the two groups may be due to high oestrogen levels in the blood produced following induced luteolysis and stimulation of follicular growth in the ovary by FSH or exogenous PMSG. It appears that high levels of serum oestrogen concentrations are be responsible for a prolonged duration of the estrous period observed in this study which is in agreement with Ahmed et al. (1998).

None of the treatment procedures showed any significant advantage over the other with respect to the conception rate. The overall post-treatment conception rate with fresh diluted semen found in this study was 70.0%. This lies within the range of 51.7 to 87.5% reported for does synchronized with intravaginal progestagen sponges during the breeding and non-breeding season (Freitas et al., 1996b, 1997; Greyling and Van der Nest, 2000; Motlomelo et al., 2002). The logical explanation for this variation may be the detrimental effects of synchronization on sperm transport and survival in the female reproductive tract (Pearce and Robinson, 1985) and differences in the time of occurrence of estrous (Baril et al., 1993).

Three of 5 does did not show any overt signs of estrous, but were diagnosed pregnant two months after AI. Similar findings have been observed by Greyling and Van der Nest (2000). Allison and Robinson (1970) suggested that these silent ovulations may be related to inadequate endogenous progesterone levels.

In conclusion, it can be said that progesterone treatments with MAP or FGA intravaginal progestagen sponges, or combinations with $PGF_{2\alpha}$ are equally efficient in synchronizing estrous in non-lactating hair does during the natural breeding season. The administration of $PGF_{2\alpha}$ may not be necessary as it failed to demonstrate a significant beneficial effect on the response, time interval from the onset and duration of estrous and conception rate, except the induced estrous duration between the FGA/PMSG/$PGF_{2\alpha}$ and MAP/PMSG groups.

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