Different estrous induction methods during the non-breeding season in Kivircik ewes

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ABSTRACT: The efficiency of medroxyprogesterone acetate (MAP) sponges in combination with either pregnant mare serum gonadotrophin (PMSG) or cloprostenol ($PGF_{2\alpha}$) for inducing and synchronizing the estrous cycle in non-lactating Kivircik ewes was investigated during the natural non-breeding season. All ewes (n = 69) were treated with 60 mg MAP sponges for 12 days. In addition, each ewe received an intramuscular injection of either 1.5 ml sterile saline solution (n = 18); 125 μg $PGF_{2\alpha}$ (n = 14); 500 IU PMSG (n = 18) or 500 IU PMSG and 125 μg $PGF_{2\alpha}$ (n = 19), 48 h before the sponge removal. Cervical artificial insemination (AI) with diluted fresh semen was performed at a fixed time (48 and 60 h) following progestagen withdrawal. The different groups estrous response for the first 24 ± 6 h and within 120 h, time to onset and duration of the induced estrous, and pregnancy rate was found to be 36.2%, 81.6%, 41.7 ± 2.3 h, 29.6 ± 1.5 h, and 54.5%, respectively. There were significant differences between groups MAP and MAP/$PGF_{2\alpha}$ and their with the two latter groups (MAP/PMSG, MAP/PMSG/$PGF_{2\alpha}$) in terms of the onset of induced estrous ($P < 0.05$) and between groups MAP and MAP/$PGF_{2\alpha}$ in terms of the duration of induced estrous ($P < 0.05$) and between the first two groups (MAP, MAP/$PGF_{2\alpha}$) and the latter two groups (MAP/PMSG, MAP/PMSG/$PGF_{2\alpha}$) in terms of estrous response at the first 24 ± 6 h ($P < 0.05$). These results indicate that, the use of MAP/PMSG, rather than MAP or MAP/$PGF_{2\alpha}$, was effective in the attainment of early and compact induction and synchronization of estrous in non-lactating Kivircik ewes during the natural non-breeding season.

Keywords: ewes; anestrous; cloprostenol; PMSG; MAP

The majority of sheep breeds perform different reproduction activities depending on season changes, latitude/longitude, the length of the photoperiod and other factors. Therefore, estrous synchronization together with AI in ewes is important in the improvement of reproductive efficiencies and management processes (Gordon, 1999). Controlled breeding of sheep involves artificial control of estrous and ovulation with exogenous hormone treatments (Keisler and Buckrell, 1997). Intravaginal sponges impregnated with progesterone or its synthetic analogues, namely medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) are usually inserted over periods of 6 to 14 day and used in conjunction with PMSG, particularly for out of season, and sometimes prostaglandin $F_{2\alpha}$ injected at time of sponge removal or 48 h prior to sponge removal (Greyling et al., 1997; Rosado et al., 1998; Gordon, 1999; Simonetti et al., 2000; Vinoles et al., 2001; Ungerfeld and Rubianes, 2002). Gonadotrophins such as PMSG administration have been shown to stimulate follicular growth and increases ovulation rate and fertility and induce a tighter synchrony of ovulation in both anestrous and cycling sheep (Gordon, 1999; Cline et al., 2001; Maurel et al., 2003). At the same time, prostaglandin $F_{2\alpha}$ and its synthetic analogs is the luteolytic factor in ewes, as in other ruminants and the use of prostaglandin $F_{2\alpha}$ or one of its analogs causes luteolysis in sheep having a functional *corpus luteum* at the time of treatment (Keisler and Buckrell, 1997; Rosado et al., 1998; Gordon, 1999). Estrous synchronization of sheep has been accomplished using several protocols with

varying degrees of success (Scaramuzzi and Martin, 1984). Therefore, the objective of the present study was to compare the efficiency of MAP sponges in combination with either PMSG or cloprostenol in inducing and synchronizing the estrous cycle in non-lactating Kivircik ewes during the natural non-breeding season and compare the fertility rates obtained following AI.

MATERIAL AND METHODS

The study was carried out at a village located in Inegol, Bursa (latitude 40° 13' E, longitude 29° 00' N, altitude 100 m) in western Turkey, during April (the natural non-breeding season) under natural lighting. A total of 77 non-lactating Kivircik ewes ranging in age from 2 to 4 years, weighing 36 to 61 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, 7 Kivircik breeding rams of proven fertility and 5 teaser rams were used in the present study. The sheep 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*. In addition, the ewes received 0.4 kg concentrate ewe/day during the entire period of study. The management of the ewes did not change throughout the entire experimental period.

The experimental ewes were divided into 4 groups according to the age, body weight and body condition scores. The hormonal treatments comprised of intravaginal sponges impregnated with 60 mg MAP (Esponjavet, Hipra, Spain) inserted for 12 days; intravaginal MAP sponges plus intramuscular injection of 1.5 ml sterile saline solution (MAP, n = 19); intravaginal MAP sponges plus intramuscular injection of 125 µg cloprostenol (Dalmzin, Fatro, Italy) (MAP/$PGF_{2\alpha}$; n = 19); intravaginal MAP sponges plus intramuscular injection of 500 IU PMSG (Intervet, Netherlands) (MAP/PMSG, n = 19); and intravaginal MAP sponges plus intramuscular injection of 500 IU PMSG and 125 µg cloprostenol (MAP/PMSG/$PGF_{2\alpha}$; n = 20) on the 10^{th} day. Estrous was monitored every 6 h from 12 to 120 h following progestagen sponge withdrawal with the aid of 5 teaser rams. The ewes were considered in estrous when they were mounted by the teaser rams. Estrous onset was defined as the time elapsed between sponge removal and the first accepted mount. Estrous duration was defined as the time between the first and last accepted mount, within the same estrous period.

One ejaculate from each ram was collected by electroejaculation. During collection and examination, the semen was protected from temperature shock. Each ejaculate was immediately evaluated for volume and wave motility (Mylne 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 (35°C) 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 800×10^6 motile cells/ml, the density was determined with the aid of a haemocytometer. A one-step dilution was performed at 30°C 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 16°C over a 1h period and kept at this temperature until insemination. Each ewe was inseminated intracervically twice at a fixed time 48 and 60 h following sponge withdrawal with a 0.25 ml straw containing 200×10^6 spermatozoa. All ewes were tested for pregnancy 54 days following AI with the aid of a transrectal 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 24 ± 6 h and within 120 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. One ewe from MAP group, five ewes from

Table 1. Estrous response and pregnancy rate in Kivircik ewes after different estrous synchronization treatments and AI at a fixed time

Treatment group	*n*	Estrous				Pregnancy rate (%)
		response (%)		onset (h) mean ± S.E.	duration (h) mean ± S.E.	
		24 ± 6 h	within 120 h			
MAP	18	(17/1) 5.6[b]	(4/14) 77.8[a]	47.1 ± 2.5[b]	35.1 ± 3.1[a]	(10/8) 44.4[a]
MAP + $PGF_{2\alpha}$	14	(13/1)7.6[b]	(2/12) 85.7[a]	60.5 ± 6.2[a]	22.5 ± 3.1[b]	(6/8) 57.1[a]
MAP + PMSG	18	(5/13)72.2[a]	(2/16) 88.9[a]	31.1 ± 1.8[c]	28.5 ± 2.2[ab]	(4/13) 76.5[a]
MAP + PMSG + $PGF_{2\alpha}$	19	(9/10) 52.6[a]	(5/14) 73.7[a]	32.4 ± 2.5[c]	30.0 ± 3.0[ab]	(10/7) 41.2[a]
Total	69	(44/25) 36.2	(13/56) 81.6	41.7 ± 2.3	29.6 ± 1.5	(30/36) 54.5

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

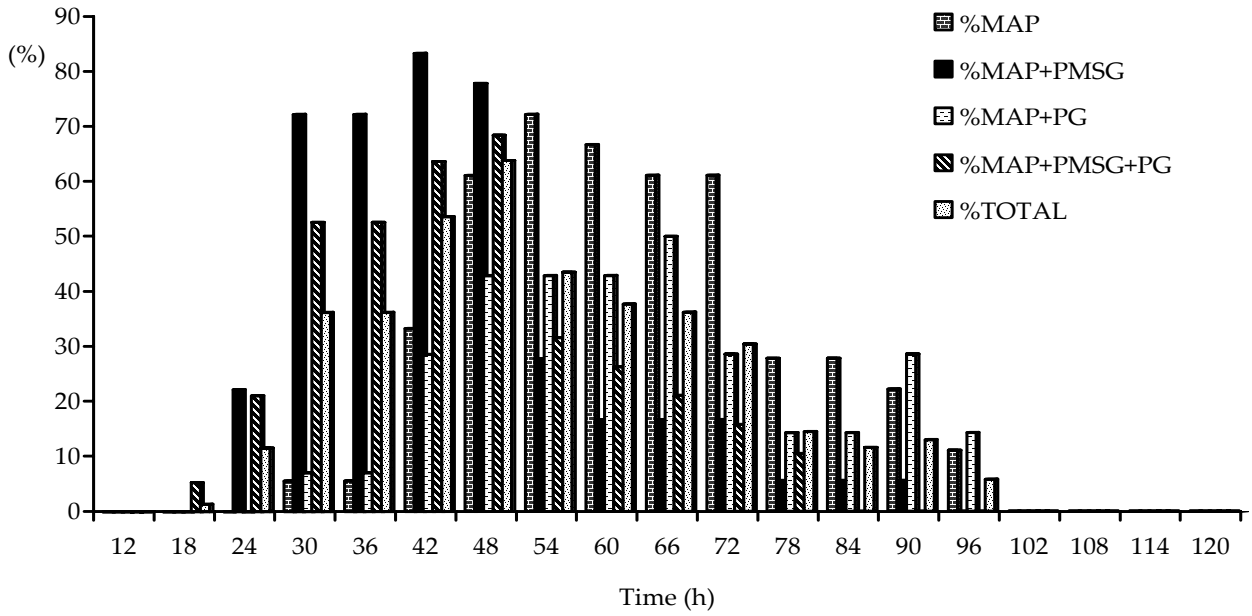

Figure 1. Intervals between sponge removal and estrous

$MAP/PGF_{2\alpha}$ group, one ewe from MAP/PMSG group and one ewe from $MAP/PMSG/PGF_{2\alpha}$, group lost their sponges, so they were excluded from the experiment. In addition, four ewes from MAP group, two ewes from $MAP/PGF_{2\alpha}$ group, two ewes from MAP/PMSG group and five ewes from $MAP/PMSG/PGF_{2\alpha}$ group did not show any overt signs of estrous during the observation period. One ewe from MAP/PMSG group and two ewes from $MAP/PMGS/PGF_{2\alpha}$ group died at day 10–20 following AI, so they were not diagnosed pregnant. Estrous appearance for the other ewes occurred between 18 and 96 h after the end of treatment. Synchronization parameters and pregnancy rates were not significantly different between the 4 treatment groups, except significant differences between groups MAP and $MAP/PGF_{2\alpha}$ and their with the two latter groups in terms of the onset of induced estrous ($P < 0.05$) and between groups MAP and $MAP/PGF_{2\alpha}$ in terms of the duration of induced estrous ($P < 0.05$) and between the first two groups and the latter two groups in terms of estrous response at the first 24 ± 6 h ($P < 0.05$). The overall estrous responses for the first 24 ± 6 and within 120 h for 4 groups were 36.2% and 81.6%, respectively. The overall mean time to onset and

duration of estrous following sponge withdrawal in the 4 groups was 41.7±2.3 h and 29.6±1.5 h, respectively. The overall mean pregnancy rate at day 54 following AI for 4 groups was 54.5%, with 13 of 4 ewes not showing any estrous response, but diagnosed pregnant two months after AI.

DISCUSSION

The use of PMSG in conjunction with intravaginal progestagen treatment, regardless of $PGF_{2\alpha}$ administration, was found to be efficient methods for estrous induction and synchronization in the ewes during the non-breeding season. Estrous response obtained from MAP/PMSG group was the highest (72.2%) for the first 24 ± 6 h and significantly different from MAP (5.6%) and MAP/$PGF_{2\alpha}$ (7.6%) groups ($P < 0.05$), except for MAP/PMSG/$PGF_{2\alpha}$ group. Although there was no significant difference between MAP (5.6%) and MAP/$PGF_{2\alpha}$ (7.6%) groups, and also between MAP/PMSG (72.2%) and MAP/PMSG/$PGF_{2\alpha}$ (52.6%) groups, ewes receiving PMSG showed earlier estrous and closer synchrony. Similarly, Husein et al. (1998) reported the time onset of estrous to be earlier in ewes treated with progesterone sponge and PMSG, compared to ewes treated with alone progesterone sponge, the non-breeding season. In addition, Cline et al. (2001) indicated that PMSG administration had an important effect on the formation of compact estrous and ovulation. From these results it could be concluded that PMSG application together with MAP increased estrous response rates obtained for the first 24 ± 6 h. Considering our overall estrous response rate for the first 24 ± 6 h (36.2%), our result was higher than the results of Ungerfeld and Rubianes (1999), Das et al. (2000), Simonetti et al. (2000) and Vinoles et al. (2001). Within 120 h there were no significant differences among 4 groups in terms of estrous response. The high estrous response recorded during the 120 h observation period following the cessation of treatment (overall mean response 81.6%) is within the range of 33.3–100% quoted in treatment with progesterone (Das et al., 2000) or FGA or MAP intravaginal sponges (Ungerfeld and Rubianes, 1999; Simonetti et al., 2000) or in combination with PMSG (Greyling et al., 1997; Zarkawi et al., 1999; Vinoles et al., 2001; Ungerfeld and Rubianes, 2002; Zeleke et al., 2005) and $PGF_{2\alpha}$ or its analogues (Rosado et al., 1998) in different breeds of ewes under different environmental conditions.

Researchers have reported the onset of estrous to occur within 24–144 h following progestagen or progesterone withdrawal (Ungerfeld and Rubianes, 1999; Das et al., 2000; Simonetti et al., 2000; Vinoles et al., 2001). Ewes came on heat between 18 and 96 h after sponge withdrawal, with the highest incidence of estrous onset occurring between 30 and 60 hours. The distribution of estrous in our groups was similar to that reported by Zarkawi et al. (1999), Ungerfeld and Rubianes (1999) and Simonetti et al. (2000). In present study, the mean overall interval to the onset of estrous following progestagen removal was 41.7 ± 2.3 h and significantly longer in both the MAP and MAP/$PGF_{2\alpha}$ groups, compared to the MAP/PMSG and MAP/PMSG/$PGF_{2\alpha}$ groups ($P < 0.05$) (Table 1). The mean overall interval obtained in this trial is agreement with the findings of Ungerfeld and Rubianes (2002) and Zeleke et al. (2005). The present result is, however, longer when compared with that of Ungerfeld and Rubianes (1999) and Simonetti et al. (2000), but shorter than that of Greyling et al. (1997), Das et al. (2000) and Vinoles et al. (2001). The reason for these discrepancies is indefinite, on the other hand, it may be attributed to differences in breed, nutrition, season, use of gonadotrophins and presence of the male after sponge removal all factors that are known to influence this parameter (Greyling et al., 1997; Rosado et al., 1998; Gordon, 1999; Ungerfeld and Rubianes, 1999; Zeleke et al., 2005).

The mean overall duration of the induced estrous period (29.6 ± 1.5 h) recorded in this study is similar to that reported by Greyling et al. (1997) and higher than that reported by Fuentes et al. (1998), Das et al. (2000) and Zeleke et al. (2005). The mean estrous duration was shorter ($P < 0.05$) in the MAP/$PGF_{2\alpha}$ treatment (22.5±3.1 h), compared to the MAP treatment (35.1 ± 3.1 h). This variation 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 methods 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 54.5%. This lies within the range of 43.75 to 70.5% reported for ewes synchronized with

intravaginal progestagen sponges during the breeding and non-breeding season by using AI (Greyling et al., 1997; Husein et al., 1998; Simonetti et al., 2000). The logical explanation for this difference may be the detrimental effects of synchronization on sperm transport and survival in the female reproductive tract (Pearce and Robinson, 1985), differences in the time of occurrence of estrous (Baril et al., 1993) and extension of the lifespan of the ovulatory follicle (Vinoles et al., 1999).

Four of 13 ewes did not show any overt signs of estrous, but were diagnosed pregnant at day 54 after AI. Such a finding is in agreement with a previous report in Boer and indigenous goats (Greyling and Van der Nest, 2000). Allison and Robinson (1970) suggested that these silent ovulations may be related to inadequate endogenous progesterone levels. Besides, absence of estrus and ovulation may be due to insufficient gonadotrophic hormone released by the pituitary, to a poor response by ovary to the exogenous PMSG or variation in responsiveness of animals to PMSG (Akusu and Egbunike, 1984).

In conclusion, it can be said that PMSG treatment in conjunction with MAP impregnated intravaginal sponges, rather than MAP or MAP/$PGF_{2\alpha}$, can be used to induce estrous and increase the estrus response at the first 24 ± 6 h and occur earlier the onset of induced estrous in non-lactating Kivircik ewes during the non-breeding season, despite similar pregnancy rates among treatments groups.

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