

DIFFERENT ESTROUS INDUCTION PROTOCOLS DURING THE TRANSITION PERIOD IN LACTATING TURKISH SAANEN DOES FOLLOWING AI

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The objective of this study was to evaluate the duration of three progestagen treatments for estrous synchronization in lactating Turkish Saanen goats during the transitional period from anestrus. All does ($n=60$) were divided into three equal groups and the estrous period of the does was synchronized using intravaginal sponges (20 mg FGA) for either 11 days (Group 1), 9 days (Group 2) or 6 days (Group 3). In addition, 24h before sponge removal (on the 10th day, 8th day and 5th day, respectively) each doe was injected with 0.075 mg cloprostenol (PGF_{2 α}) and 500 IU eCG. Cervical artificial inseminations (AI) with frozen-thawed semen were performed at fixed intervals (36 and 48 h) following progestagen withdrawal. The total estrous response following the first withdrawal was 12 ± 6 h within 66 h. Time to onset and duration of the induced estrus, and pregnancy rates were recorded to be 30.0%, 100%, 23.9 ± 0.7 h, 29.4 ± 1.3 h, and 28.3%, respectively. There were significant differences between Group 1 and the other groups, in terms of the onset of induced estrus ($P < 0.05$) and estrous response for the first 12 ± 6 h ($P < 0.05$) and between Group 2 and Groups 1 and 3 in terms of the duration of induced estrus ($P < 0.05$). Each of the three protocols was effective in inducing and synchronizing estrus in lactating Turkish Saanen goats.

Key words: Turkish Saanen goat, transition period, cronolone, cloprostenol, eCG

INTRODUCTION

Most breeds of goats show seasonality in reproduction activities, due to photoperiod (Chemineau *et. al.*, 1992). Therefore, various treatments have been developed to control reproduction activities throughout the year including the buck effect, photoperiod treatments and the use of exogenous hormone treatments (Whitley and Jackson, 2004). Exogenous hormones such as

progesterone or its synthetic analogues, eCG and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are generally used in the reproductive management of goats in and out of the natural breeding season (Leboeuf *et al.*, 1998). Estrous synchronization in goats is achieved by control of the luteal phase of the estrous cycle, either by being provided by exogenous progesterone or by inducing premature luteolysis (Gordon, 1999). Progestagens are the main hormones used for estrous synchronization and/or the induction of estrus in goats (Bretzlaff and Romano, 2001). Intravaginal sponges impregnated with progestagens, namely medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) are the most appropriate hormonal techniques used for in goats controlled breeding (Bretzlaff, 1997). Traditionally, progestagen treatment in goats is either longer or similar to the lifespan of a cyclic corpus luteum. Long-term progestagen treatments (11 to 21 days) are generally used in goats to induce and synchronize estrus regardless of the stage of the cycle or the follicular status of the ovary at the time of treatment or season (Gordon, 1999; Greyling and Van der Nest, 2000; Dogan *et al.*, 2005; Lehloenya *et al.*, 2005). Following treatment, a high percentage of does show estrus within a relative short time, but fertility rate is generally low or variable (Dogan *et al.*, 2004). This phenomenon has been attributed to changes in the hormonal milieu that results in an asynchrony between estrus and ovulation, with a subsequent alteration in sperm transport (Corteel *et al.*, 1988). Recently, short-term progestagen treatments (6 to 9 days) in does has been shown to be as effective as long-term treatment to induce and synchronize estrus (Fonseca *et al.*, 2005), the subsequent recorded fertility rate to was also high (Fonseca and Torres, 2005).

The aim of the present study was to compare three different durations of treatment with intravaginal cronolone sponges, in combination with eCG and cloprostenol in inducing and synchronizing estrous in lactating Turkish Saanen does during the transition period from non-breeding to natural breeding season, as well as the fertility rates obtained following AI with frozen-thawed semen.

MATERIALS AND METHODS

The study was carried out in a village located in Canakkale, (latitude $39^{\circ} 27'$ and $40^{\circ} 45'$ N, longitude $25^{\circ} 40'$ and $27^{\circ} 30'$ E, altitude 2 m) in western Turkey, during July (the transition from non-breeding to natural breeding season in the region) under natural lighting. A total of 60 lactating Turkish Saanen does (body weight = 48.0 ± 9.7 kg; age = 2.5 ± 1.1 years; BCS = 2.9 ± 0.2) were used for the experiment, together with 6 teaser bucks. At the beginning of the study, the does BCS was evaluated by palpation of the lumbar and sternal region (Morand-Fehr *et al.*, 1989). The does were allowed to graze on natural pasture and were kept in pens overnight. Water and a mineral salt lick were provided *ad libitum*. In addition, the does received 1 kg concentrate daily for the entire duration of the study. All lactating does were milked twice a day at 06:00 and 18:00 and the management of the does did not change throughout the entire experimental period.

The experimental does were divided into three equal groups according to age, body weight and BCS. The does were synchronized by inserting 20 mg FGA

(Chronogest CR, Intervet, Netherlands) vaginal sponges for 11, 9 and 6 days respectively for Groups 1, 2 and 3. In addition, whatever the group, each goat received 0.075 mg of cloprostenol (PGF_{2 α} - Dalmazin, Fatro, Italy) and 500 IU eCG (Chrono-Gest, Intervet, Netherlands) intramuscularly 24 h before sponge removal. In Group 1 and Group 2 the treatment was initiated 5 and 3 days earlier, respectively, compared to Group 3 in order to synchronize sponge removal, PGF_{2 α} and eCG administration at the same time as Group 3. Estrus was monitored every 6 h from 12 to 66 h following sponge removal with the aid of 6 teaser bucks. Does were considered in estrus when mounted by a teaser buck. The onset of estrus was defined as the time between sponge removal and the first accepted mount of the doe and estrous duration as the time between the first and last accepted mount, within the same estrous period.

The frozen buck semen used in this study was imported from a commercial AI centre in Canada, supplied in 0.5 mL straws, and stored at -196°C until AI. Semen was collected by artificial vagina from a Saanen buck, assessed for sperm concentration and initial motility and subsequently diluted with an extender, cooled to 4°C and packed in straws (0.5 mL) containing 100 x 10⁶ motile sperm. The straws were placed in liquid nitrogen vapor from 4°C and the temperature slowly decreased to -150 to -160°C, plunged into liquid nitrogen (-196°C) and stored. Each straw contained a minimum of 35 to 40 million motile spermatozoa post-thawing per straw. A straw was evaluated for motility and sperm concentration before AI (Memon *et. al.*, 1997) and the same rates were confirmed with the results reported by the company. 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 phase contrast microscope (x40). The concentration in spermatozoa was determined with the aid of a haemocytometer. Straws were thawed in water in a thermos at 37°C for 30 s., then dried with a paper towel, and loaded into an insemination gun, and covered with a plastic sheath before AI. Each doe was inseminated intracervically twice at fixed time intervals i.e. 36 and 48 h following sponge withdrawal. All does were restrained in a standing position and with the aid of a speculum and a head lamp, the external opening of the cervix was located and the AI gun was carefully inserted as far as possible into the cervical canal without force, where the semen was slowly deposited. All does were inseminated by a single inseminator. All does were tested for pregnancy 104 days following AI with the aid of a trans-abdominal 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 between means were tested for significance with the Fisher's PLSD. Estrous response and pregnancy rates were analyzed using the *chi-square test* and *Fisher's exact test*, respectively. The 95% significance level was noted. SPSS 10.0 software was used for statistical analyses (Instat, 1990-1993).

RESULTS

The results in terms of estrous response for the first 12 \pm 6 h and with 66 h, time to onset and duration of the induced estrous and pregnancy rates are set out

in Table 1. Figure 1 illustrates the intervals between sponge removal and the onset of estrous. Estrous appearance for the does occurred between 18 and 66 h after the end of treatment. Synchronization parameters and pregnancy rates were not significantly different between the three groups, except for the significant differences between Group 1 and Groups 2 and 3 in terms of the onset of the induced estrus period ($P < 0.05$) and estrous response at the first 12 ± 6 h ($P < 0.05$) and between Group 2 and the other two groups (Group 1 and Group 3) in terms of the duration of induced estrus ($P < 0.05$). The overall estrous response for the first 12 ± 6 h period and within 66 h for three groups was 30.0% and 100%, respectively. The overall mean time to onset and duration of estrous following sponge withdrawal in the three groups was 23.9 ± 0.7 h and 29.4 ± 1.3 h, respectively. The overall mean pregnancy rate recorded at day 104 following AI in the three groups was 28.3%, with no difference between treatments.

Table 1. The mean (\pm S.E.) estrous response and pregnancy rate in Turkish Saanen does following different estrous synchronization treatments and AI

Treatment group	n	Estrous				Pregnancy rate (%)
		response (%)		onset (h)	duration (h)	
		12 \pm 6 h	within 66 h			
Group 1	20	(9/11) 55.0 ^a	(0/20) 100	21.0 \pm 0.8 ^b	31.8 \pm 2.0 ^a	(12/8) 40.0 ^a
Group 2	20	(17/3) 15.0 ^b	(0/20) 100	26.4 \pm 1.4 ^a	24.0 \pm 1.6 ^b	(14/6) 30.0 ^a
Group 3	20	(16/4) 20.0 ^b	(0/20) 100	24.3 \pm 1.1 ^a	34.4 \pm 2.4 ^a	(17/3) 15.0 ^a
Total	60	(42/18) 30.0	(0/20) 100	23.9 \pm 0.7	29.4 \pm 1.3	(43/17) 28.3

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

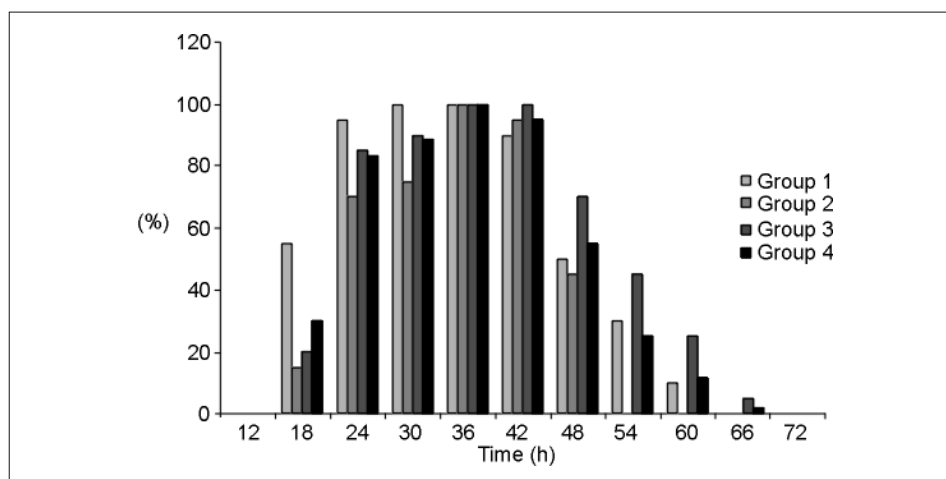


Figure 1. Intervals between sponge removal and onset of estrous

DISCUSSION

The protocols, regardless of the duration of progestagen treatment, were effective in inducing and synchronizing estrus in lactating Turkish Saanen does during the transition period. The estrous response obtained in Group 1 (55.0%) was the highest for the first 12.0 ± 6.0 h period and significantly different for Group 2 (15.0%) and Group 3 (20.0%) ($P < 0.05$). From these results it can be concluded that an 11-day progestagen treatment, rather than the 9-day or 6-day treatment induced earlier estrus in does. Considering the overall estrous response for the first 12.0 ± 6.0 h period (30.0%), the results were higher than those of Freitas *et al.* (1996) and lower than Dogan *et al.* (2005), in which different breeds of goats under different environmental conditions were studied. The higher estrous response recorded during the 66 h observation period following the cessation of treatment (overall mean response 100%) was within the range of 83.1-89.5%, following treatment the intravaginal sponges containing MAP applied for 6 days or 9 days plus the intramuscular administration of eCG and PGF_{2 α} 24 h before sponge removal (Fonseca *et al.*, 2005; Fonseca and Torres, 2005). Similarly, the estrous response was within the range of 85.7 to 100% quoted in the treatment with intravaginal sponges containing FGA or MAP for 11 days plus an intramuscular administration of eCG and PGF_{2 α} 48 h prior to sponge removal by Baril *et al.* (1993), Freitas *et al.* (1996), Freitas *et al.* (1997), Dogan *et al.* (2004), Dogan *et al.* (2005), Salvador *et al.* (2005). In the present study, it was possible to reduce the duration of treatment from 11 days to 9 or 6 days while maintaining the efficiency of estrus induction of the treatment.

Researchers have reported the onset of estrus in goats to occur within 12-120 h following progestagen withdrawal (Baril *et al.*, 1993; Freitas *et al.*, 1996; Freitas *et al.*, 1997; Greyling and Van der Nest, 2000; Dogan *et al.*, 2005). The peak in estrus was between 18 and 66 h after sponge withdrawal, with the highest total incidence of estrus occurring between 36 and 42 h. According to the results of the present study, the total distribution of estrus was similar to that reported by Greyling and Van der Nest (2000), who found the highest incidence of the onset of estrus to occur between 25 and 48 h after whole or halved MAP sponge was withdrawn, during the breeding season, but other studies found an earlier occurrence of estrus (Baril *et al.*, 1993; Freitas *et al.*, 1996; Freitas *et al.*, 1997; Dogan *et al.*, 2005). In the present study, the mean overall interval to the onset of estrus, following progestagen removal was 23.9 ± 0.7 h. This period was significantly longer in both Group 2 and Group 3, compared to Group 1 ($P < 0.05$) (Table 1). In a previous study, Dogan *et al.* (2004) reported an interval of 15.4 h to the onset of estrus in Saanen goats, using 750 IU of eCG per doe. This greater dose of eCG could have induced greater ovarian activity, which could have decreased the interval to onset of estrus. Similarly, the use of 500 IU eCG decreased the interval to the onset of estrus in Anatolian black goats (Dogan *et al.*, 2005). A linear relationship was recorded between the eCG dose and ovulation rate and 200 to 700 IU eCG was considered to be sufficient to stimulate ovulation without inducing a high incidence of multiple ovulations (Bretzlaff, 1997). On the other hand, Fonseca and Torres (2005) using the same protocol used in the

present study (9 days), reported a 30.0 h and 32.0 h interval to the onset of estrus in Alpine and Saanen goats, respectively. Fonseca *et al.* (2005), after using 200 IU eCG plus 22.5 µg cloprostenol for 9 or 6 days of MAP treatment, reported an interval of 53.6 h and 46.1 h for the onset of estrus for non-lactating Toggenburg does, respectively. Similarly, using FGA for 11 days along with 400 IU eCG and 50 µg cloprostenol, Freitas *et al.* (1997) reported a 33.0 h interval to estrus in Alpine and Saanen goats. In a similar study, using 400 or 500 IU eCG plus 50 µg cloprostenol and 11 days of FGA treatment, Freitas *et al.* (1996) reported 27.7 h and 29.2 h to estrus in Alpine and Saanen goats, respectively. Thus, differences in breed response following hormonal treatments do exist and the differences of season and nutrition are factors to be considered.

The mean overall duration of the induced estrous period for the does was 29.4 ± 1.3 h. In previous studies, a duration of estrus of 27.2 h and 30.0 h was reported in non-lactating Toggenburg (Fonseca *et al.*, 2005), 29.7 h in non-lactating Anatolian black (Dogan *et al.*, 2005), 37.0 h and 29.9 h in Boer and Nguni, respectively (Lehloenyne *et al.*, 2005), 32.5 h in Saanen (Dogan *et al.*, 2004), 31.1 h and 31.5 h in Boer and indigenous goats, respectively (Greyling and Van der Nest, 2000). Protocols used in the present study did not affect the duration of the induced estrous period, when compared to obtained results from previous studies. However, Fonseca and Torres (2005) reported an interval of 14.7 h and 17.3 h for the duration of estrus in Alpine and Saanen goats, respectively. The mean duration of estrus was shorter ($P < 0.05$) in Group 2 (24.0 ± 1.6 h), compared to the other two groups. This variation may be due to high oestrogen levels in the blood following induced luteolysis and stimulation of follicular growth in the ovary due to FSH or exogenous eCG. It appears that the high levels of serum oestrogen concentrations are responsible for the prolonged duration of the estrous period observed in this study which is in agreement with Ahmed *et al.* (1998).

None of the treatment protocols showed any significant advantage over the other with respect to conception rate. The overall post-treatment conception rate obtained with frozen-thawed semen in this study was 28.3%. This lies within the very wide range of 33.0 to 85.5% reported for goats synchronized with intravaginal progestagen sponges during the breeding (Gacitua and Arav, 2005) and non-breeding season (Baril *et al.*, 1993; Freitas *et al.*, 1996; Freitas *et al.*, 1997; Salvador *et al.*, 2005) by using AI with frozen-thawed semen. Several factors in the synchronization and induction estrus can influence fertility including the detrimental effects of synchronization on sperm transport and sperm survival in the female reproductive tract, differences in the time of occurrence of estrus and extension of the lifespan of the ovulatory follicle (Baril *et al.*, 1993; Leboeuf *et al.*, 1998; Whitley and Jackson, 2004).

In conclusion, it can be said that the 11-day estrous-synchrony protocol, rather than the 6-day protocol or the 9-day protocol should be used to induce estrus and increase the estrus response at 12.0 ± 6.0 h period. Earlier onset of induced estrus in lactating Turkish Saanen does during the transition period was obtained, despite similar pregnancy rates among treatments groups. According to the results of the present study, the reduction in the duration of treatment with

progesterone can be employed without a reduction in estrous parameters and pregnancy rates.

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PRIMENA RAZLIČITIH PROTOKOLA ZA INDUKCIJU ESTRUSA KOD SANSKIH KOZA IZ TURSKE U LAKTACIJI

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SADRŽAJ

Cilj ovih istraživanja je bio da se proceni efekat tri različita tretmana progestagenima korišćenim za sinhronizaciju estrusa kod Sanskih koza iz Turske tokom prelaznog perioda iz anestrusa. Sve ženke (n=60) su bile podeljene u tri jednake grupe i estrus je sinhronizovan intravaginalnim sunderima sa 20 mg FGA tokom 11 dana (Grupa 1), 9 dana (Grupa 2) ili 6 dana (Grupa 3). Dodatno, 24h pre uklanjanja sundera (desetog, osmog i petog dana) svim ženkama je aplikovano po 0.075 mg kloprostenola (PGF_{2α}) i 500 IU eCG. Veštačko osemenjavanje je izvedeno aplikacijom otopljenog semena u cerviks u fiksnim vremenskim intervalima (36 i 48h posle uklanjanja progestagena). Uočene su značajne razlike između Grupe 1 i ostalih grupa u pogledu momenta ispoljavanja estrusa (P<0.05) i između grupe 2 i ostalih grupa u pogledu trajanja indukovano estrusa (P<0.05). Sva tri primenjena tretmana su bila efikasna u indukciji estrusa koza.