



Different progestagen treatment duration on estrous synchronization during the natural breeding season in non-lactating Anatolian black goats

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Abstract

The objective of this study was to evaluate the duration of four progestagen treatments on estrous synchronization and pregnancy rate in non-lactating Anatolian black goats during the natural breeding season. All does were divided into four groups according to progestagen treatment duration using intravaginal sponges (60 mg MAP): group 1, 13 days (n = 23), group 2, 11 days (n = 25), group 3, 9 days (n = 25) and group 4, 6 days (n = 25). In addition, 24 h before sponges removal, each doe was injected with 0.075 mg of cloprostenol (PGF₂α) and 500 IU eCG. The goats within the same group were naturally mated at fixed time 40 h following progestagen removal, using the same breed fertile bucks (1:5 mating ratio). The total estrous response for the first 12 h, total estrous response within 60 h, time to onset of the induced estrus, duration of the induced estrus and pregnancy rate were 3.0, 96.9%, 26.5 ± 0.7 h, 22.6 ± 0.8 h and 92.0%, respectively. In terms of the onset of induced estrus there were significant differences between group 1 and groups 3 and 4 and between group 2 and group 3 (P < 0.05). The protocols used were equally efficient in synchronizing and inducing estrus in non-lactating Anatolian black does during the natural breeding season. Conclude on pregnancy rate. In addition, pregnancy rates were similar results.

Keywords: cloprostenol, eCG, estrus synchronization, goat, MAP.

Introduction

Most breeds of goats show seasonality in reproduction activities, due to season changes, latitude/longitude, the length of the photoperiod and other factors (Amoah *et al.*, 1996; Simões, 2015). During a year, reproductive activity in goats is composed of non-breeding season, transition period and breeding season (Pierson *et al.*, 2001). Thus, various protocols have been developed to control reproduction activities throughout the year including the buck effect, photoperiod treatments and the use of exogenous hormone treatment (Whitley and Jackson, 2004; Lopez-Sebastián *et al.*, 2014). Exogenous hormones such as progesterone or its synthetic analogues, equine chorionic gonadotrophin (eCG) and prostaglandin F₂α (PGF₂α) are generally used in the reproductive season

of goats (Dogan *et al.*, 2005), outside the natural breeding season (Corteel *et al.*, 1988; Souza *et al.*, 2011; Pietroski *et al.*, 2013) and in the transition period (Dogan *et al.*, 2004, 2008a, b). Estrous synchronization in goats is achieved by control of the luteal phase of the estrous cycle, either by providing exogenous progesterone or by inducing premature luteolysis (Gordon, 1999). Progestagens are the main hormones used for synchronization and/or induction of estrus in goats (Bretzlaff and Romano, 2001). Intravaginal sponges impregnated with different progestagens, mainly medroxyprogesterone acetate (MAP) and fluorogestone acetate (FGA) are the most appropriate hormonal techniques used for controlled breeding in goats (Wildeus, 2000). Traditionally, progestagen treatment in goats is either longer or similar to the lifespan of a cyclic corpus luteum. The most common treatments for estrous synchronization in goats, regardless of stage of the cycle or follicular status of the ovary at the time of treatment or season, are based on long progestagen (progesterone or a synthetic analogue) exposure (11 or 21 days) associated with an intramuscular (i.m.) injection of eCG alone or eCG with PGF₂α given at the end of the treatment (Corteel *et al.*, 1988; Gordon, 1999). Following treatment, a high rate of does show estrus within a relative short time, but fertility rate is generally low or variable (Dogan *et al.*, 2004, 2008a, b; Pietroski *et al.*, 2013). This phenomenon is related to lower concentration of progesterone/progestogen with abnormalities in follicular development, ovulation, oocyte health, luteal function, alteration in sperm transport and fertility (Corteel *et al.*, 1988; Menchaca *et al.*, 2007). Recently, with the objective to avoid prolonged progesterone exposure, the short term protocol using intravaginal devices during 5-7 days has been proposed for goats (Menchaca *et al.*, 2007; Dogan *et al.*, 2008b; Vilariño *et al.*, 2011; Pietroski *et al.*, 2013). The short term protocol uses progesterone/progestogen, PGF₂α and eCG, and is effective for controlling both luteal activity and follicular dynamics (Menchaca *et al.*, 2007) and also resulting in high pregnancy rates following fixed timed artificial insemination (AI; Vilariño *et al.*, 2011) or after natural mating (Souza *et al.*, 2011). The objective of this study was to evaluate different progestagen treatment duration on estrous synchronization and pregnancy rate in non-lactating Anatolian black goats during the natural breeding season.

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Material and Methods

The study was carried out at village located in Inegol, Bursa (latitude 40° 13'E, longitude 29° 00' N, altitude 100 m) in western Turkey, during October (the natural breeding season) under natural lighting. The ambient temperature, relative humidity and the monthly rainfall during the experiment were 15.2°C, 69% and 68.8 kg/m², respectively. A total of 98 cyclic non-lactating hair Anatolian black does ranging in age from 2 to 4 years (3.0 ± 1.1 years), weighing 35 to 50 kg (42.6 ± 7.8 kg) and with 3.0 ± 0.1 body condition (BC) score (evaluated on a scale of 1 to 5; Morand-Fehr *et al.*, 1989) were evaluated. In addition, 20 Anatolian black breeding bucks of proven fertility and 5 teaser bucks were used in the present study. The does were allowed to graze on natural pasture from 07:30 to 11:30 h and from 13:30 to 17:30 h and kept in pen overnight. Water and mineral salt lick were provided *ad libitum*. In addition, goats received 0.5 kg commercial concentrate (15% crude protein) doe/day during the entire period of study. 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, body weight and body condition scores. The does were synchronized by inserting 60 mg MAP (Esponjavet, Hipra, Spain) vaginal sponges for 13, 11, 9 and 6 days in groups 1, 2, 3 and 4, respectively. Goats were allocated in three groups of 25 animals and one group of 23 does. In addition, whatever the group, each goat received 0.075 mg of cloprostenol (PGF2 α , Dalmzin, Fatro, Italy) and 500 IU eCG (Chrono-Gest, Intervet, Netherlands) i.m. 24 h before sponges removal. In group 1, group 2 and group 3 the treatment was initiated 7, 5 and 3 days earlier, respectively, compared to group 4 in order to synchronize sponges removal, PGF2 α and eCG administration. Estrus was monitored every 6 h from 12 to 60 h after sponge removal and with the aid of 5 teaser bucks. A doe was diagnosed in estrus when it stood to be 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 estrus duration as the time between the first and last accepted mount, within the same estrous period. Each doe within the same estrous period was naturally mated in paddock at fixed 40 h following

sponge withdrawal, using 20 Anatolian black fertile bucks (1:5 mating ratio). One mating was performed in each goat. The mating was controlled and observed. All does were tested for pregnancy 60 days following natural mating with the aid of a trans-abdominal ultrasonic scanning apparatus (5.0-MHz linear probe, Honda, HS-1500, Japan). The fetal heartbeat and the fetal image were used in the diagnosis of pregnancy.

The onset of estrus and duration of induced estrous periods were subjected to analyses of variance (one-way ANOVA) and differences between means were tested for significance with the Fisher's PLSD. Estrous response (12 and within 60 h) 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. Grophpad instat copyright (c) Grophpad software).

Results

The results in terms of estrous response for the first 12 h and within 60 h after progestagen treatment, time to onset and duration of the induced estrous and pregnancy rates are set out in Table 1. The Fig. 1 illustrates the intervals between sponge removal and the onset of estrus. One doe from group 1, one doe from group 3 and one doe from group 4 did not show any overt signs of estrus during the observation period but these does were mated by bucks. Estrous appearance for the other does occurred between 12 and 60 h after the end of treatment. Synchronization parameters (estrous response, onset and duration of estrus) and pregnancy rates were not significantly different among the four groups ($P > 0.05$), except for significant differences in the onset of induced estrus period among group 1 and groups 3 and 4 and between group 2 and group 3 ($P < 0.05$). Thus, data were pooled. The overall estrous response for the first 12 h period and within 60 h after progestagen treatment were 3.0 and 96.9%, respectively. The overall mean time to onset and duration of estrus following sponge removal were 26.5 ± 0.7 h and 22.6 ± 0.8 h, respectively. The overall mean pregnancy rate at day 60 following natural mating was 92.0%, with 2 of 3 does not showing any estrous response, but diagnosed pregnant at day 60 after natural mating.

Table 1. Estrous response rate, onset and duration of estrus and pregnancy rate in Anatolian black does following four different progestogen treatments using natural mating at a fixed time during breeding season in non lactating goats

Treatment group	Duration of progestagen treatment (days)	n	Estrous				Pregnancy rate (%)
			Response (%)		Onset (h)	Duration (h)	
			12 h	within 60 h			
Group 1	13	23	(20/3) 13	(22/1) 95.7	23.5 ± 2.2 ^a	23.6 ± 2.	(20/3) 91.
Group 2	11	25	(25/0) 0	(25/25) 100	25.4 ± 0.7 ^{ac}	23.5 ± 1.1	(25/25) 100
Group 3	9	25	(25/0) 0	(24/1) 96	28.8 ± 1 ^b	21.0 ± 1.5	(22/3) 88
Group 4	6	25	(25/0) 0	(24/1) 96	28.3 ± 1. ^{1bc}	22.3 ± 1.6	(22/3) 88
Total		98	(95/3) 3	(95/3) 96.9	26.5 ± 0.7	22.6 ± 0.8	(89/9) 92

Include information about dose and time of administration of eCG and PGF2 α . Each goat received 0.075 mg of cloprostenol (PGF2 α) and 500 IU eCG i.m. 24 h before sponges removal. ^{a,b,c}means in the same column, with different superscripts indicate a significant difference ($P < 0.05$).

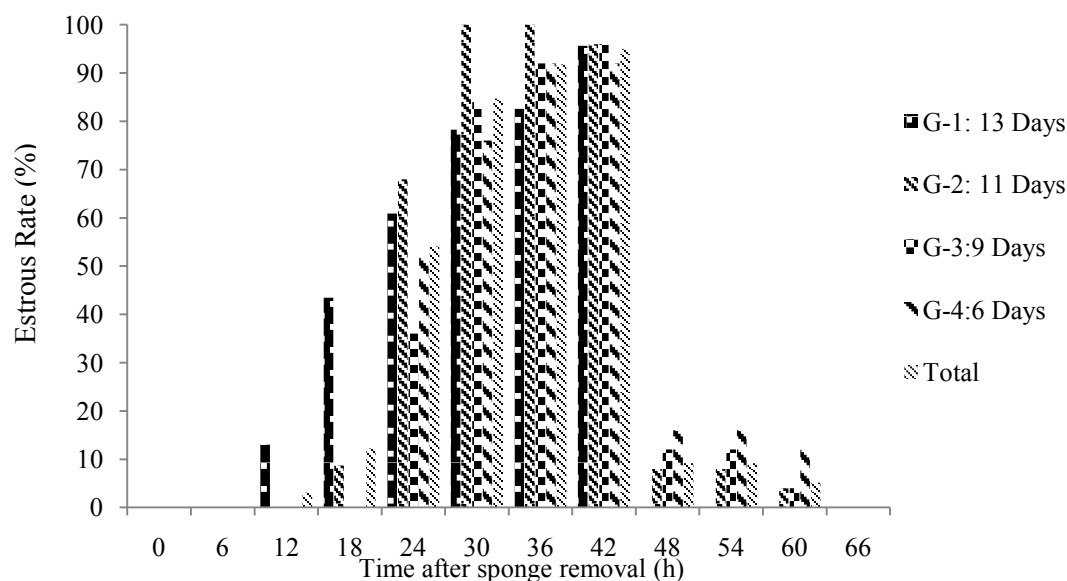


Figure 1. Intervals between sponge removal and onset of estrus in Anatolian black does following four different progestagen treatments using natural mating at fixed time. Complete with the data of treatment used in each group; include title in vertical axis (estrous rate).

Discussion

The four treatments used in this study, regardless of the duration of progestagen treatment, were found to be efficient for estrous induction and synchronization in non-lactating Anatolian black does during the natural breeding season. There were no significant differences in the rate of estrous response in the first 12 h period among the four groups. These results are in agreement with those of Freitas *et al.* (1997) who reported that estrous response for the first 12 h period of lactating Alpine and Saanen goats was 10% when either 45 mg FGA sponge, 1.5 mg or 3 mg norgestomet ear implant for 11 days with 400 or 500 IU eCG and 50 µg cloprostenol were applied to synchronize estrus but in anestrus goats. However, using 20 mg FGA sponge for 6, 9 and 11 days with 500 IU eCG and 0.075 mg cloprostenol, Dogan *et al.* (2008b) reported 20, 15 and 55% estrous response for the first 12 h period in Turkish Saanen does, respectively, during the transitional period. The variation observed in estrous concentration in the first 12 h period after progestagen treatment among authors could be due to differences in the rate of absorption and metabolization of each progestagen and/or differences in reproductive seasonality. Considering overall estrous response for the first 12 h period (3%), our result was lower than those of Dogan *et al.* (2005, 2008b) and Nogueira *et al.* (2011), in which different breeds of goats under different environmental conditions were studied.

Within 60 h after progestagen removal, there were no significant differences among the four groups in terms of estrous response rate. The high estrous response recorded during the 60 h observation period following the cessation of treatment (overall mean

response ~97%) was within the range of 90 to 100% estrous response obtained by Pietroski *et al.* (2013), after treatments with intravaginal sponges containing MAP applied for 12 or 9 or 6 days plus the administration of eCG and PGF2α 24 h before sponges removal. In the present study, the overall mean estrous response was high compared with the result obtained by Fonseca and Torres (2005) and Fonseca *et al.* (2005), who reported 83.1 and 89.5% of animals in estrus after 6-day and 9-day protocols with MAP sponge, respectively, using 200 IU eCG and 22.2 µg d-cloprostenol in goats (include breed). The estrous response in the present study was within the range of 78.6 to 100% quoted in treatments with intravaginal sponges containing FGA or MAP for 11 days or 14 days plus an i.m. administration of eCG and PGF2α 48 h prior to or at sponges removal by Dogan *et al.* (2004, 2005, 2008a, b), Amarantidis *et al.* (2004), Bukar *et al.* (2012) and Omontese *et al.* (2013). In the present study, it was possible to reduce the duration of treatment from 13 days to 11 or 9 or 6 days while maintaining hormonal treatment efficiency on estrous induction and pregnancy rate.

Researchers have reported that onset of estrus in goats occurs within 12-120 h following progestagen withdrawal (Dogan *et al.*, 2004, 2005, 2008 a, b; Fonseca *et al.*, 2005; Lertchunhakiat *et al.*, 2012; Omontese *et al.*, 2013). In the present study, estrous concentration was between 12 and 60 h after sponge withdrawal, with the highest rate of estrus occurring between 36 and 42 h. According to present results, total distribution of estrus was similar to that reported by Fonseca *et al.* (2005) and Dogan *et al.* (2008a, b), who found the highest rate of estrous onset to occur between 36 and 42 h after MAP or FGA sponge withdrawal, but other studies found an earlier occurrence of estrous



onset (Dogan *et al.*, 2005). In the current study, the mean overall interval to the onset of estrus, following progestagen removal, was 26.5 ± 0.7 h. This value was similar to Pietroski *et al.* (2013), who reported a 26.7, 25.2 and 25.2 h interval to estrus in Saanen goats, after using MAP sponge for 6, 9, and 12 days, respectively. Similarly, Dogan *et al.* (2005) using the same protocol as in the present study (11 days), reported 20.6 h interval to onset of estrus in Anatolian black goats during the natural breeding season. Fonseca and Torres (2005), using the same protocol as in the present study (9 days), reported 30 h and 32 h interval to the onset of estrus in Alpine and Saanen goats, respectively. In a previous study, Dogan *et al.* (2004), after using 11-day sponge treatments of MAP or FGA with 750 IU eCG and 125 μ g cloprostenol, reported an interval of 15.4 h to the onset of estrus in non-lactating Saanen goats in the transition period. This greater dose of eCG could have induced greater ovarian activity, which could have decreased the interval to onset of estrus (Amarantidis *et al.*, 2004; Omontese *et al.*, 2013). On the other hand, present results were shorter than those of Omontese *et al.* (2013), who recorded an interval of 44.2 and 29.3 h to onset of estrus with 14 days FGA sponge alone or FGA sponge with eCG, respectively, in Red Sokoto goats. Similarly, using 45 mg FGA sponges for 14 days with 300 IU eCG and 7.5 mg PGF 2α , Bukar *et al.* (2012) reported a 41.1 h interval to estrus in cyclic Boer goats. Fonseca *et al.* (2005), after using eCG plus cloprostenol for 9 or 6 days of MAP sponge treatment, reported an interval of 53.6 and 46.1 h for the onset of estrus for non-lactating Toggenburg does, respectively. The reason for variation in the interval after hormonal treatment to estrous onset might be due to the effect of breed, dose of eCG, nutrition and/or season, which is known to have an influence on plasma progesterone level (Amarantidis *et al.*, 2004). Include reference for time and dose of eCG/PGF 2α (Dogan *et al.* 2008a, b).

The mean overall duration of the induced estrous period was 22.6 ± 0.8 h. According to previous studies in different breeds, it was observed that average estrous duration was in Saanen goats, 32.5 h (Dogan *et al.*, 2004), Greek, 29.7 h (Amarantidis *et al.*, 2004), Toggenburg, 27.2 and 30.0 h (Fonseca *et al.*, 2005), Anatolian black, 29.7 h (Dogan *et al.*, 2005), Turkish Saanen, 29.4 h (Dogan *et al.*, 2008b), Turkish Saanen, 29.3 h (Dogan *et al.*, 2008a), Boer, 36.0 h (Bukar *et al.*, 2012) and in Red Sokoto, 30.8 h (Omontese *et al.*, 2013). The protocols used in the present study did not affect the duration of the induced estrous period, but it was shorter compared with results obtained in other studies. However, Fonseca and Torres (2005) reported a short estrous duration of 14.7 and 17.3 h in Alpine and Saanen goats, respectively. In previous studies, it was reported that mating decreased the duration of estrus (Romano, 1993). One service reduced the duration of estrus by 45%; but estrous duration was not affected whether the goat was served once, twice or three times (Romano, 1994b; Romano *et al.*, 2016). The author suggested that penis intromission stimulates mechanisms involved in estrous shortening (Romano, 1994a). Thus, it appears that the effect of copulation

with fertile buck at 40 h after the onset of estrus could have been responsible for the shortening in the duration of the estrous period.

None of the treatment protocols showed any significant advantage on conception rate. Also, using FGA sponge for 6, 9 and 11 days with eCG and cloprostenol, Dogan *et al.* (2008b) did not report any difference in pregnancy rates in Turkish Saanen goats, using fixed-time AI with frozen-thawed semen. Likewise, using the same protocols as in the present study (6 and 9 days), Pietroski *et al.* (2013) reported that pregnancy rates were not different after AI or natural mating in Saanen goats. The overall post-treatment conception rate obtained with natural mating in this study was 92% and similar to results obtained by Amarantidis *et al.* (2004), who recorded an average pregnancy rate of 86.4% in FGA sponge treated goats, after natural mating with known fertility bucks. Results observed in the present study were higher than values within the range of 51.2 to 75.3% reported for goats synchronized with intravaginal progestagen sponges or progesterone devices during the breeding (Dogan *et al.*, 2005), non-breeding season (Vilariño *et al.*, 2011) and transition period (Dogan *et al.*, 2004) after fixed-time AI with fresh diluted semen. The results in pregnancy rate may differ among authors considering breed, season, natural mating, AI using fresh, diluted or frozen-thawed semen, synchronization protocols, lifespan of spermatozoa and oocyte and overall management conditions.

Two of three does did not show any overt signs of estrus, but were diagnosed pregnant at day 60 after natural mating. Such a finding is in agreement with a previous report in Anatolian black goats (Dogan *et al.*, 2005), synchronized with MAP or FGA sponges. 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 ovary response to exogenous eCG or variation in responsiveness of animals to eCG (Rubianes and Menchaca, 2003).

In conclusion, protocols used in the present study were equally efficient in synchronizing and inducing estrus in non-lactating Anatolian black does during the natural breeding season. Conclude on pregnancy rate. In addition, pregnancy rates did not differ among the groups. According to results of the present study, reduction in the duration of progestagen treatment can be used without affecting reproductive efficiency.

Conflict of interest

There is no conflict of interest to declare.

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