

## SHORT COMMUNICATION

# Oestrus synchronization with short-term and long-term progestagen treatments in goats: the use of GnRH prior to short-term progestagen treatment

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## Abstract

The aim of the present study was to determine the efficacy of the synchronization of oestrus using short- and long-term progestagen treatments in Hair goats at the onset of the breeding season, and to evaluate the effect of the exogenous GnRH administration immediately prior to short-term progestagen treatment on the reproductive performance. A total of 75 Hair goats, aged 2.5-5 years-old were used in this experiment. Goats were divided equally into three groups (n=25 per group). Animals in LT-FGA (long-term progestagen treatment), ST-FGA (short-term progestagen treatment) and Gn-ST-FGA (GnRH-short-term progestagen treatment) groups received an intravaginal sponge (day 0) containing 30 mg fluorogestone acetate (FGA) for 14, 8 and 8 days, respectively, plus 75 µg cloprostenol i.m. 24 h before sponge removal and 400 I.U. equine chorionic gonadotrophin (eCG) i.m. at the time of sponge removal. In addition, the goats in the Gn-ST-FGA group received 10.5 µg busereline acetate i.m. at the time of sponge insertion (day 0). Oestrus response for all treatment groups was 100%. The mean interval from sponge removal and the onset of oestrus for the LT-FGA, ST-FGA and Gn-ST-FGA groups was 28.0±1.0 h, 28.8±1.1 h and 33.1±2.0 h, respectively. No significant difference in onset of oestrus among groups was recorded. The pregnancy rate, kidding rate, multiple kidding rates and litter size were 72.0, 61.1, 45.5% and 1.6 in the LT-FGA, 70.8, 76.5, 69.2% and 1.8 in

the ST-FGA and 58.3, 78.6, 63.6% and 1.6 in the Gn-ST-FGA groups, respectively. The pregnancy rates were similar in the LT-FGA (72.0%) and ST-FGA (70.8%). However, the kidding rate, multiple kidding rates and litter size were numerically higher in the ST-FGA (76.5%, 69.2% and 1.8, respectively) group than in the LT-FGA (61.1%, 45.5% and 1.6, respectively) group. Although not statistically different, pregnancy rate and litter size was lower in the Gn-ST-FGA group (58.3% and 1.6, respectively) compared with the ST-FGA (70.8% and 1.8, respectively).

In conclusion, it can be said that oestrus synchronization with long-term progestagen treatment (14 d-FGA- PGF<sub>2α</sub>- eCG) could be replaced with short-term progestagen treatment (8 d-FGA-PGF<sub>2α</sub>- eCG) without a reduction in oestrus rate and fertility parameters in lactating goats at the beginning of breeding season. However, the use of GnRH prior to short-term progestagen treatment (8 d-FGA-PGF<sub>2α</sub>- eCG) do not have a positive effect on fertility parameters in goats.

## Introduction

Intravaginal sponges impregnated fluorogestone acetate (FGA) or medroxyprogesterone acetate (MAP) are used for oestrus synchronization in goats (Whitley and Jackson, 2004). A routinely synchronization method is used for a period of 11-19 days of FGA vaginal sponges, combined with injections of equine chorionic gonadotrophin (eCG) and an analogue of PGF<sub>2α</sub> 48 h before or at sponge removal (Freitas *et al.*, 1997; Amarantidis *et al.*, 2004). However, short periods of progestagen sponge treatment for as short as 6-9 days have been reported to be successful in inducing/synchronizing estrous in goats (Fonseca *et al.* 2005; Dogan *et al.* 2008). Release of progesterone from the sponges declines over time. Therefore, a short-term treatment provides higher average concentrations of progesterone during the treatment period. Such treatments (5-8 days) have shown to be effective during breeding season (Öztürkler *et al.*, 2003).

Recently, single injection of gonadotropin releasing hormone (GnRH) has been widely used to manipulate patterns of ovarian follicle development in cattle (Macmillan *et al.*, 2003). GnRH treatments at the onset of progestogen treatment increase follicle turnover with recruitment of a new dominant follicle (Thatcher *et al.*, 1989). Ryan *et al.* (1995) reported that estrous synchronization resulting in both a

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high oestrus response and high pregnancy rate is achievable following a 8-day progestagen treatment in cows, if GnRH is given at the start of treatment and prostaglandin on the day before progestagen withdrawal. Also, Karaca *et al.* (2009) informed that the use of GnRH at the beginning of a short-term progestagen (7 days) treatment increased the multiple birth rate and litter size in the ewes. However, similar studies in goats have not been reported yet.

The aim of the present study was to compare effectiveness of the short-term and long-term progestagen treatments, and also to evaluate the effect of GnRH treatment combined with short-term progestagen treatments on reproductive performance of lactating goats at the onset of the breeding season.

## Materials and methods

The present study was conducted during the first 2 weeks of September (autumn) at a private farm in Antakya, in the eastern Mediterranean region of Turkey. This period is accepted as it represents the onset of the natural breeding season of goats in region. The research unit is situated at 85 m altitude and between 36° 53' N latitude and 35° 40' E longitude. The average annual rainfall in the area

ranges between 570 and 1160 mm and the mean annual minimum and maximum temperatures in September vary between 21.0°C and 31.1°C, respectively.

A total of 75 lactating Hair goats, aged 2.5-5 years-old were used in this study. Six healthy bucks that did not have a history of fertility problems and two teaser bucks were also used. One month before beginning of the study, each goat was subjected to general physical examination, and the goats were dewormed orally with anthelmintic containing oxfendazole and oksiklozanid (Oksan bolus, DİF, Turkey). The goats were maintained graze on natural pasture from 06:30 to 18:00 h and kept in pens during nights. Water and a mineral supplement were available *ad libitum*. The management of the goats did not change throughout the experimental period. The goats were lactating and were milked by hand once a day in the mornings. Mean milk production of the goats was about 400 mL during trial period. The bucks were separated from goats 15 days before the study beginning and kept indoor until the end of the treatments. They were fed with dry grass plus and the barley break at an average rate of 500 g/animal daily.

The goats were randomly divided into three equal groups, and were registered by attaching numbers on their ears. Animals in LT-FGA (long-term progestagen treatment), ST-FGA (short-term progestagen treatment) and Gn-ST-FGA (GnRH-short-term progestagen treatment) groups received an intravaginal sponge (Chronogest, Intervet, Turkey) containing 30 mg fluorogestone acetate (FGA) for 14, 8 and 8 days, respectively. Goats in the LT-FGA, ST-FGA and Gn-ST-FGA groups received 75 µg cloprostenol (Dalmazin, Vetaş, Turkey) i.m. 24 h prior to sponge removal and 400 I.U. equine chorionic gonadotrophin (Folligon, Intervet, Turkey) i.m. at the time of sponge removal. In addition, the goats in the Gn-ST-FGA group received 10.5 µg busereline acetate (GnRH, Receptal, Intervet, Turkey) i.m. at the time of sponge insertion. Applications of hormones used for the oestrus synchronization were made between 18:00 and 19:00 h in all groups.

Starting from 12 h after removal of the intravaginal sponges in all groups, the occurrence of behavioral oestrus signs was monitored twice (06:00-07:00 and 18:00-19:00 h) daily, using two bucks equipped with a canvas apron. The goats were considered to be in oestrus only if they stood while being mounted by the bucks. Each goat showing oestrus was hand-mated with the bucks at the time of oestrus detection. The mated goats were recorded and separated from the main flock.

All the goats were examined by trans-

abdominal ultrasonography with a 5-7.5 MHz convex probe at 50 days after mating for pregnancy detection. The goats were kept under close observation to detect the number of abortions until parturition. The reproductive parameters calculated following the treatment, were as follows:

- onset of oestrus (interval from sponges removal to time of first estrus identification);
- oestrus response (number of goats showing oestrus/total number of goats treated in each group X 100);
- pregnancy rate (number of pregnant goats/number of mated goats in each group X 100);
- abortion rate (number of aborted goats/number of pregnant goats in each group X 100);
- kidding rate (number of kidding goats/number of pregnant goats in each group X 100);
- multiple kidding rate (number of goats kidding twin or triplet/number of kidding goats in each group X 100) and
- litter size (number of total kids/number of kidding goats kidded in each group).

The time to oestrus onset and litter size between treatment groups were compared by one-way ANOVA. The significance of mean differences among groups was tested by Duncan. Oestrus response, pregnancy rate, abortion rate, kidding rate and multiple kidding rate were analyzed by  $\chi^2$ -test. The confidence interval used was at the 95% level of confidence. The SPSS/PC program (version 10.0; SPSS, Chicago, IL, USA) was used for all the statistical analyses.

## Results and discussion

All the intra-vaginal sponges remained in place until the time of withdrawal (no losses). No oestrus symptom was detected while the vaginal sponges were in place. However, three goats in the Gn-ST-FGA group showed continuously oestrus behaviours until 5 day after the intra-vaginal sponge removal, and none of these goats became pregnant. Two goats, one from the ST-FGA and one from Gn-ST-FGA groups, were excluded from the experiment due to dying and genital organ infection. The mean time to onset of oestrus, oestrus response, pregnancy rate, abortion rate, kidding rate, multiple kidding rate and litter sizes in

each group is given in Table 1.

In all groups 100% of goats showed oestrus as observed in other studies (Freitas *et al.*, 1997; Motlomelo *et al.*, 2002; Al-Merestani *et al.*, 2003; Romano, 2004; Dogan *et al.*, 2008), where FGA sponges, in period changed between 6-16 days, with combination of eCG and PGF<sub>2α</sub> or both, were used in goats during the transition period or breeding season.

In the present study, the mean interval from sponge removal and the onset of oestrus for the LT-FGA and ST-FGA and Gn-ST-FGA groups was 28.0±1.0 h, 28.83±1.1 h and 33.1±2.0 h, respectively. No significant difference in onset of oestrus between groups was recorded. The mean interval from sponge removal and the onset of oestrus in the LT-FGA, ST-FGA and Gn-ST-FGA groups is consistent with time to oestrus onset obtained in goats by Freitas *et al.* (1997) (27.8±5.0 h), Motlomelo *et al.* (2002) (30.9±0.4 h) and Romano (2004) (32.9±9.7 h). However, the average intervals to oestrus were longer than those (18.0±1.9 h) of Dogan *et al.* (2005), who used 500 IU eCG 2 days before the sponges removal (FGA), but were shorter than those (49.7±15.5 h) of Fonseca *et al.* (2005), who used 200 IU eCG 24 h before sponge removal (MAP). These differences may be associated with the dose of eCG used in the present study. The eCG is known to reduce the interval between sponge removal and onset of oestrus (Greyling *et al.*, 1985). Regueiro *et al.* (1999) informed that the use of 500 IU eCG decreased the interval to oestrus onset in Saanen, Nubian and cross-breed goats. However, it was reported that there was not a significant effect of the type of progestagen sponges or time of PMSG administration on the time to oestrus onset in ewes (Zelege *et al.*, 2005; Ustuner *et al.*, 2007).

In the present study, no difference was observed between the short-term and long-term progestagen treatments combined with eCG and PGF<sub>2α</sub> in terms of reproductive parameters studied (Table 1) in the goats at the onset of breeding season. The pregnancy rates were similar ( $P>0.05$ ), being 72.0% and 70.8% for LT-FGA and ST-FGA, respectively. However, the kidding rate, multiple kidding rates and litter size were numerically higher (but not statistical) in the ST-FGA (76.5%, 69.2% and 1.8, respectively) group than the LT-FGA (61.1%, 45.5% and 1.6, respectively) group. It has been reported that the fertility rate was significantly higher in the short-term (7 d) progestagen treated ewes (87.3%) than in long-term (12 d) treated ewes (71.6%), at the onset of the breeding season (Karaca *et al.*, 2009). Conversely, Ataman *et al.* (2006) reported that no difference was observed between short-

**Table 1. Mean time to onset of oestrus, oestrous response, pregnancy rate, abortion rate, kidding rate, multiple kidding rate and litter size for LT-FGA, ST-FGA and Gn-ST-FGA treatment groups.**

| Parameters                | LT-FGA <sup>a</sup> | ST-FGA <sup>a</sup> | Gn-ST-FGA <sup>a</sup> |
|---------------------------|---------------------|---------------------|------------------------|
| Onset of oestrus*, h      | 28.0±1.0            | 28.8±1.1            | 33.1±2.0               |
| Oestrous response, %      | 100 (25/25)         | 100 (24/24)         | 100 (24/24)            |
| Pregnancy rate, %         | 72.0 (18/25)        | 70.8 (17/24)        | 58.3 (14/24)           |
| Abortion rate, %          | 38.9 (7/18)         | 23.5 (4/17)         | 21.4 (3/14)            |
| Kidding rate, %           | 61.1 (11/18)        | 76.5 (13/17)        | 78.6 (11/14)           |
| Multiple kidding rates, % | 45.5 (5/11)         | 69.2 (9/13)         | 63.6 (7/11)            |
| Number of kids            |                     |                     |                        |
| Single                    | 6 (54.5%)           | 4 (30.8%)           | 4 (36.4%)              |
| Twins                     | 3 (27.3%)           | 8 (61.5%)           | 7 (63.6%)              |
| Triplets                  | 2 (18.2%)           | 1 (7.7%)            | 0 (0%)                 |
| Litter size               | 1.6 (18/11)         | 1.8 (23/13)         | 1.6 (18/11)            |

\*Mean ± SE, <sup>a</sup>No significant difference between treatment groups ( $P>0.05$ ).

term (7 d) and long-term (12 d) progestagen treatments for fertility parameters (conception rate, lambing rate and litter size) in Akkaraman crossbred ewes in breeding season. Likewise, Ustuner *et al.* (2007) informed that short-term (6 d) and long-term (12 d) progestagen treatments resulted in similar pregnancy results in Awassi ewes during the breeding season. The results of the present study are compatible with the findings of Ataman *et al.* (2006) and Ustuner *et al.* (2007), but short-term progestagen treatment does not improve fertility of goats, as it has been reported in ewes (Viñoles *et al.*, 2001; Karaca *et al.*, 2009). The reason for this variation is not clear, it may be associated with the eCG used at the time of sponge removal in goats in the onset of breeding season. Noël *et al.* (1994) reported that the administration of eCG when progestagen treatment was terminated could compensate the deleterious effect of long-term progestagen treatment on follicular dynamics in cyclic ewes. Also, Barrett *et al.* (2004) revealed that 500 IU of eCG given at the end of a 12-day treatment with progestogen-impregnated intravaginal sponges limited effects on the dynamics of ovarian follicular waves in anoestrus ewes.

It has been reported that exogenous progestin treatment during the interval between GnRH and PGF<sub>2α</sub> injections prevents premature oestrus and increases oestrus response and conception rates of anoestrus cows (Thompson *et al.*, 1999; Stevenson *et al.*, 2000). Lamb *et al.* (2001) informed that adding a progesterone insert at the time of the GnRH injection improves fertility, especially in cows not cycling at the onset of the breeding season. Therefore, in the present study it was used a GnRH injection on the same day as sponge insertion in the goats treated with short-term

progestagen (Gn-ST-FGA group), to control the follicular dynamics in goats at onset of breeding season. In the present study, the combination of GnRH with short-term progestagen treatment did not have a positive effect on the fertility parameters in goats. On the contrary, the pregnancy rate and litter size were numerically decreased (but not statistical) in the Gn-ST-FGA group (58.3% and 1.6, respectively) compared with the ST-FGA (70.8% and 1.8, respectively) group. Three goats in the Gn-ST-FGA group exhibited continuously oestrus behaviours until the fifth day after insert removal, but this situation was not observed in other groups. Follicular cystic structures may be a possible reason for the continuously oestrus behaviours observed in these goats. Follicular cysts are one cause of infertility in small ruminants, including goats, and results in a shortened oestrus cycle of between 3 and 7 days or continuous heat (Matthews, 1999). DeJarnette *et al.* (2001) reported that a limited number of persistent follicles were still being developed in the progestin-based protocols despite of the GnRH injections at treatment beginning. However, we are not clearly sure that the cause of follicular cysts is related with the GnRH injection during the sponge insertion. Viñoles *et al.* (2001) informed that eCG provoked the development of follicular cysts in some cases in short-term treated ewes, probably because endogenous progesterone levels were still high at the time of eCG injection. Therefore, the reduction in pregnancy rate and litter size observed in the Gn-ST-FGA group may be associated with the development of follicular cysts. Clearly, further studies are necessary to clarify the effect of the GnRH injection on the same day as the intra-vaginal sponge insertion (FGA) on the fertility in goats.

The mean litter size obtained in the ST-FGA

(1.8), LT-FGA (1.6) and Gn-ST-FGA (1.6) groups was comparable with that (1.3-1.9) reported in earlier studies (Freitas *et al.*, 1997; Kusina *et al.*, 2000; Amarantidis *et al.*, 2004).

The mean abortion rate observed in the present study was 27.9%. The abortions occurred after ca. day 100 of gestation. In the aborted goats, no symptoms of infectious disease were observed. This rate was comparable with the incidence of foetal loss (3-38%) that was reported in the different goat herds by Engeland (1998). It was thought that the abortions may arise from the general problems with the management and nourishment of animals, as no significantly differences between treatments groups were found. In the farm where the present study was carried out, goats were usually fed depending on pasture and continued to be milked until the beginning of the winter. Osuagwu (1992) reported that the West African Dwarf goat has been shown to be sensitive to nutritional stress between days 61-120 of gestation when the foetal growth rate is maximal.

## Conclusions

In conclusion, it can be said that oestrus synchronization with long-term progestagen treatment (14 d-FGA-PGF<sub>2α</sub>-eCG) could be replaced with short-term progestagen treatment (8 d-FGA-PGF<sub>2α</sub>-eCG) without a reduction in oestrus rate and fertility parameters in lactating goats at the beginning of breeding season. However, the use of GnRH prior to short-term progestagen treatment (8 d-FGA-PGF<sub>2α</sub>-eCG) do not have a positive effect on fertility parameters in goats. Confirmation of this claim may, however, require a large scale field study.

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