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Research article
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Effect of GnRH treatment following a short-term estrous induction protocol on estrus and ovulation in Saanen goats, during the transitional period

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ABSTRACT: The objective of this study was to compare the effect on the ovulation time and estrus parameters of a GnRH agonist (buserelin acetate) administered 24 or 36 h following a short-term estrus induction and synchronization treatment in non-lactating Saanen goats during the transitional period. Goats received 20 mg FGA sponges for 6 days plus 300 IU eCG and 125 μg d-cloprostenol 24 h prior to sponge removal. After removal of the sponges, goats were given either 1 ml physiological saline (0.9% NaCI) solution ($Group_1$; n = 9) after 12 h, 0.004 mg GnRH ($Group_2$; n = 10) after 24 h or 0.004 mg GnRH ($Group_3$; n = 10) after 36 h. The follicle development and ovulation in the ovaries were monitored by transrectal ultrasonography starting from the sponge application until the fifth day of the estrus cycle. Blood samples were collected on the same days to determine the plasma concentrations of progesterone (P_4) and estradiol (E_2). No statistical differences among groups were detected in any synchronization parameters, ultrasonic evaluations and plasma P_4 and E_2 concentrations. The evaluation of pooled data showed that the response of Saanen goats to treatments was comparable to the results of other published trials. Results of this study indicate that administration of GnRH at 24 h or 36 h after sponge removal, at the end of a short estrus induction and synchronization protocol, does not affect plasma P_4 and E_2 concentrations, estrus parameters and ovulation time in goats, during the transition period.

Keywords: buserelin acetate, estradiol, goat, progesterone, ovulation

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INTRODUCTION

Reproductive activity in goats is usually seasonal (Fonseca and Torres, 2005) regulated by the photoperiod and other environmental factors (Fatet et al., 2011). Thus, various protocols have been developed to control reproductive activity all-year-round, including exogenous hormone treatment, the male effect and photoperiod treatments (Whitley and Jackson, 2004; Lopez-Sebastián et al., 2014). Exogenous hormones such as progesterone or its synthetic analogues in combination with equine chorionic gonadotrophin (eCG) in goats are generally used more often in control of reproductive activity during the non-breeding season and transition period (Dogan et al., 2008a; Souza et al., 2011; Abecia et al., 2011; Pietroski et al., 2013). However, repeated use of eCG may lead to antibody formation (Baril et al., 1996), resulting in delays in estrus, LH surge and ovulation time and consequently to reduction in pregnancy rates (Baldassarre and Karatzas, 2004; Fatet et al., 2011). Nevertheless, conception rate in the non-breeding season is lower compared to the breeding season (Wildeus, 2000). The main factors which influence variation in the pregnancy rates in goats are genetics, type of estrus induction protocol, follicular status at the beginning of treatment, ovulation time, season and nutritional status (Gonzalez-Bulnes et al., 2003). These factors may affect the quality of oocytes or embryos and are directly or indirectly related to low pregnancy rates. As a result, the cost of sperm and hormones spent per goat per fertilization/pregnancy increases (Souza et al., 2011).

After estrus induction oocytes that have completed development are present in large follicles. On the other hand, significant variation is observed in the time of ovulations. Accurate control of peri-ovulatory events would provide more efficient estrus induction or synchronization programs in goats. Knowledge of the timing of ovulation would permit more precise timing of mating or artificial insemination (Freitas et al., 1997; Simões et al., 2008). More close synchronization of ovulation(s) could be achieved by supporting the preovulatory LH peak with exogenous hormones such as the gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG), as in sheep (Balaro et al., 2016) and cows (Fonseca et al., 2001). Similarly, a single injection of GnRH 24 h after norgestomet implant removal has been used to induce ovulation of dominant follicle in Baladi goats during the non-breeding season (Medan et al., 2002). On the contrary Riaz et al. (2012) reported that the administration of GnRH at the time of breeding did not promote ovulation induction in the goats. However, the potential of GnRH in synchronizing ovulation and follicular emergence after the use of a short-term estrous synchronization protocol has not been assessed during the transitional period in goats. The current study was therefore designed to compare the effect on the ovulatory response, estrus parameters and plasma P_4 and E_2 concentrations of a GnRH agonist (buserelin acetate) administered 24 or 36 hours following a short-term estrous induction and synchronization protocol in non-lactating Saanen goats, during the transition period.

MATERIALS AND METHODS

Location and Animals

The study was carried out at the Research and Application Farm of the Veterinary Faculty at Uludag University, located in Bursa (latitude 40° 11`N, longitude 29° 04` E, altitude 155 m), Turkey. A total of 29 sexually mature Saanen does (20 nulliparous and 9 non-lactating multiparous) were used during the transition period from the non-breeding to breeding season in the region. The does were kept indoors in sand/hay-floored pens with access to outdoors in sheltered paddock under conditions of natural daylight and temperature and were fed dry oats hay supplemented with commercial pellets (18% crude protein; 1000 g/goat/day). No extra food was offered to the goats during the study. Clean water and mineral salt were provided *ad libitum* and no changes were made during the experiment. The does were separated physically and visually from the bucks. The experiment was approved by the Animal Ethics Committee of Uludag University (References No. B.30.2.ULU.0.8Z.00.00/09).

Synchronization of estrus and GnRH treatment

Estrus was synchronized in all goats by means of intravaginal sponges (Chronogest, Intervet Productions SA., France) impregnated with 20 mg fluorogestone acetate (FGA) inserted for 6 days, coupled with 125 μg of d-cloprostenol (PSG, Alke, Turkey) and 300 IU of equine chorionic gonadotropin (eCG, Chrono-Gest; MDS Animal Health, Netherlands) i.m. 24 h before sponge removal. The application day of intravaginal sponges was defined as day 0 of the experiment. After sponge removal, all does were divided into 3 different groups according to their age, body weight and body condition score (scale 0 to 5, according to model proposed by Morand-Fehr et al. (1989). Age, body weights and body condition score for the

1, 2 and 3 Groups averaged 21.67 ± 4.52, 22.30 ± 3.72 and 24.40 ± 4.33 months, 45.54 ± 4.22, 46.40 ± 3.41 and 46.70 ± 2.50 kg and 3.00 ± 0.14, 2.95 ± 0.12 and 3.00 ± 0.07, respectively, so there were not differences in these parameters between groups.

Goats in $Group_1$ (control; n=9) received 1 ml physiological saline solution (0.9% NaCI) i.m. 12 h after sponges removal. The rest of the goats received 0.004 mg buserelin acetate (GnRH, Buserin, Alke, Turkey), i.m., either 24 h ($Group_2$; n=10) or 36 h ($Group_3$; n=10) after sponge removal.

Estrous was monitored with the aid of 2 teaser bucks, every 12 h (8 am – 8 pm) from 12 to 72 h following sponge withdrawal. Estrous onset was defined as the time of the first accepted mount. Estrous duration was defined as the time between the first and last accepted mount.

Ultrasonographic examination and follicular assessment

The ultrasound examination of the ovaries in all does was performed using a real-time B-mode ultrasound scanner (RTU, Prosound 2, Hitachi Aloka Medical, Ltd., Tokyo, Japan), connected with a transrectal 7.5 MHz linear array probe (model UST-660-7.5) and designed for human prostatic examination. Transrectal ovarian ultrasonography was performed every 24 hours from sponge insertion until sponge removal and then every 12 h, from sponge removal until ovulation was confirmed. After ovulation, ultrasonography was performed again every 24 h until Day 5 of the estrus cycle. The day of ovulation was defined by the disappearance of follicles ≥5mm between two successive observations and was considered as the 0 day of the estrus cycle. The preovulatory follicle diameter was the last measurement obtained. The mean number of ovulations per goat was determined by the number of corpora lutea (CL) on the ovaries. After ovulation, the CL number was counted on the 5th day of the estrus cycle. During the examination, the goats were restrained in the standing position and the probe was gently inserted to the rectum, after appling hydrosoluble contact gel. In brief, after the probe was passed the urinary bladder and the uterine horns, it was rotated laterally 90° clockwise and then 180° counter-clockwise to observe both ovaries and their structures, as previously described by Simões et al. (2006). The ultrasonography evaluations were performed always by the same person.

All follicles ≥ 2 mm in diameter with spherical or oval conformation and, smooth walls as well as and the CL were measured. The measurement was carried out once daily throughout the test period and the ovary map was drawn on paper and compared with the previous day. The follicles were classified as small (2.0–3.4 mm), medium (3.5–4.9 mm), and large (≥5.0 mm). The follicles measured for the first time with a diameter of 4 mm were thought to have a diameter of 3 mm in previous day (Ginther and Kot, 1994) and counted as small follicle for the previous day.

Blood collection and hormonal analysis

Blood samples (10 ml) were collected from the jugular vein into heparinized tubes (BD Vacutainer®, Becton Dickinson, Plymouth, UK) once a day for 13 days, from sponge insertion to approximately 4 d after ovulation. Centrifugation was performed at 1500 x g for 15 min at 4 °C, and plasma was separated and stored frozen (-20°C) until assayed for P_4 and E_2. Concentrations of P_4 and E_2 in plasma were assessed with a goat P_4 (SRB-T-86624) and E_2 (SRB-T-87401) enzyme-linked immunosorbent assay (ELISA) kit and results were read by ELISA reader (ELISA, ELX-808IU Ultra Microplate Reader). The standard range of P_4 and E_2, kits used were 0.05-8.0 ng/ml and 1-300 pg/ml, respectively. The inter- and the intra-assay coefficients of variation (CV_s) for P_4 and E_2, were 2.7, 2.7, 4.6 and 4.6, respectively. The sensitivities of P_4 and E_2 assays were 0.048 ng/ml and 0.925 pg/ml, respectively. Plasma P_4 concentration (≥1 ng/ml) was considered as an indication of a functional corpus luteum (Thimonier, 2000).

Statistical analysis

SPSS for Windows, Version 20 was used to conduct the statistical analyses. ANOVA followed by Tukey was used to compare the onset of estrous and duration of induced estrous periods, the onset of ovulation from sponge removal and the onset of ovulation from the onset of induced estrus periods, ovulations and also the largest and second largest follicle diameter and number of small, medium and large follicles. Estrous response and the percentage of goats ovulating were analyzed using the chi-square test. Results are given as mean (± SEM) and the differences were considered significant when $P<0.05$.

Table 1. Clinical observations and ovarian findings (ultrasonography evaluation) of Saanen goats treated with FGA sponge for 6 days with eCG plus d-cloprostenol 24 h prior to sponge removal and administration of GnRH 36 (Group 3) or 24 (Group 2) h and saline 12 (Group 1) h after sponge removal (means ±SEM). No differences detected between groups (p>0.05, Tukey test, *chi square test).

Data	Treatment groups			Mean
	Group 1 (n = 9)	Group 2 (n = 10)	Group 3 (n =10)	
Clinical observations				
Estrus response (%)*	100.0 (9/9)	80.0 (8/10)	100.0 (10/10)	93.10 (27/29)
Duration of estrus (h)	34.67 ± 1.33	31.50 ± 3.89	34.80 ± 3.32	33.66 ± 2.85
Interval from sponge removal to onset of estrus (h)	29.33± 4.06	25.50 ± 1.50	27.60 ± 2.56	27.48 ± 2.71
Occurrence of ovulation from sponge removal (h)	48.25 ± 1.33	47.00 ± 1.39	48.89 ± 1.00	48.05 ± 1.24
Estrus onset to ovulation (h)	22.75 ± 1.92	20.88 ± 1.88	20.89 ± 3.30	21.51 ± 2.37
Ovarian findings (Ultrasonography evaluation)				
The percentage of goats ovulating (%)*	88.89	100.0	90.00	93.10 (27/29)
Mean number of ovulations per doe	2.33 ± 0.17	1.90 ± 0.23	1.80 ± 0.25	2.01 ± 0.22
Largest follicle diameter (mm)	6.64 ± 0.21	6.89 ± 0.30	7.02 ± 0.26	6.85 ± 0.26
Second largest follicle diameter (mm)	5.97 ± 0.16	6.25 ± 0.25	6.14 ± 0.30	6.12 ± 0.24

RESULTS

Estrous and ovarian findings

A summary of data regarding the estrous behavior and ultrasonography evaluation after the induced estrus in the does are set out in Table 1. All estrous behaviors and transrectal ovarian ultrasonography parameters were not significantly different among the 3 treatment groups. Thus, the data were pooled and analysed in relation to time only. The overall estrus response rate within 72 h was 93.10%.

Plasma P_4 and E_2 concentrations

Since the plasma concentrations of both hormones were not different between groups during the experiment period, data on hormone concentrations were combined. During the study period, both P_4 and E_2 plasma concentrations were affected by the day (P<0.05). According to plasma P_4 measurements, 37.93% (11/29) of goats had a functional corpus luteum at the beginning of the study (1> ng/ml). Although there was no statistical difference between plasma P_4 concentrations among the six days of sponges application in all goats, the highest mean plasma progesterone concentration was detected on the fifth day (2.46 ± 0.33 ng/ml). The lowest and highest mean P_4 concentrations were observed on days 2 and 5 after the withdrawal of the sponges (0.89 ± 0.23, 2.83 ± 0.40 ng/ml, respectively, P<0.05).

Among the six days of sponges application, the highest mean plasma E_2 concentration was detected on the fourth day (67.36 ± 7.75 pg/ml) and there was a statistical difference between the fourth day and the first three days (P<0.05). Plasma E_2 concentrations decreased from 53.90 ± 5.18 to 34.19 ± 3.44 pg/ml during the first four days after removal of the sponges, then an increase in E_2 concentration (42.27 ± 6.46 pg/ml) was observed again with the emergence of new follicle wave on the last day of the study period.

DISCUSSION

The use of eCG and $PGF_{2\alpha}$ in conjunction with a short-term intravaginal progestogen treatment (6 days), regardless of GnRH treatment, was found to be an efficient protocol for estrus induction and synchronization in the non-lactating Saanen goats during transitional period. The administration of 0.004 mg buserelin acetate (GnRH) 36 or 24 h after sponge withdrawal had no significant effect on estrus behavior and ultrasonography findings. In the present study, there were no significant differences among the three groups in term of estrous response rate, within 72 h after progestogen withdrawal. In small ruminants, estrus synchronization is normally defined as acceptable when 90% or more of treated goats come into estrus within observation period (Wildeus, 2000). In the present study, 93.1% of all goats exhibited estrous during the 72 h observation period following the cessation of treatment. This result was within the range of 89.5-100%, reported by other researchers (Fonseca et al., 2005; Dogan et al., 2008a, 2016) following treatment with FGA or MAP intravaginal sponges for 6 days in combination with eCG and $PGF_{2\alpha}$ 24 h prior to sponge removal, in various goat breeds and different seasons. Similar estrous response rate has been reported (Fonseca et al., 2005; Fonseca and Torres, 2005; Salvador et al., 2005; Dogan et al., 2008a, 2016) after long (9, 11, 13-day) progestagen protocols in goats,

without GnRH. Similar to the present study, Medan et al. (2002), reported no significant differences in estrus response in Baladi goats treated with norgestomet with or without GnRH (10.5 μg buserelin acetate) 24 h after ear implant removal during the non-breeding season. Kridli et al (2003) reported that 60% of ewes showed estrus after treatment with FGA sponges for 12 days either with or without GnRH administration 28 h after sponge removal. Considering our overall estrus rate, it can be concluded that short-term intravaginal progestogen treatment with eCG and $PGF_{2\alpha}$ is effective in the induction and synchronization of estrus in Saanen goats, during the transition period.

In the current study, the mean interval from progestagen withdrawal to estrus and the duration of estrus were 27.48 ± 2.71 h and 33.66 ± 2.85 h, respectively, with no significant difference among groups (Table 1). Similarly, Pierson et al. (2003) who used 50 μg GnRH 24 h after sponge withdrawal reported no significant effect on the onset of estrus in goats during the breeding and non-breeding season. These observations are similar to those reported by Fonseca et al. (2005), Fonseca and Torres (2005), Valentim et al. (2006) and Dogan et al. (2008a, 2016) after various synchronization protocols. In a previous study, Kridli et al. (2003), reported that GnRH administration, 28 h after the end of 12-day FGA sponge treatment, decreased (42.0 v.s 58.0 h; $P<0.05$) the interval from cessation of treatment to estrus. eCG stimulates follicular development enhancing the recruitment of small follicle in the ovary (Fatet et al., 2011). Therefore in goats, eCG given at the end of progestagen treatment reduces the interval to onset of estrus (Dogan et al., 2008b). Also, high eCG doses enhance estrogen concentration and induce LH surge; therefore, it reduces the time from progestagen removal to the onset of estrus (Dogan et al., 2004).

The duration of estrus was longer in the present study when compared with previous study that reported estrous duration of 14.7 and 17.3 h in Alpine and Saanen goats, respectively (Fonseca and Torres, 2005). In untreated goats, the duration of estrus is about 36 h but varies from 24-96 h (Fatet et al., 2011) depending on breed, age (Fonseca and Torres, 2005), season (Pierson et al., 2003), parity (Simões et al., 2008), the size of the dominant follicle, E_2 concentrations (Medan et al., 2003, 2005) and the presence of a male (Dogan et al., 2016).

In a previous study, Riaz et al. (2012) reported that the use of GnRH at the time of mating did not improve the reproductive performance of goats. Sponge removal and treatment with eCG indirectly initiated the endogenous GnRH peak which resulted in the LH surge, as suggested by Pierson et al. (2003). On the contrary, as observed in this study, administration of low dose eCG may not synchronize LH increase together with follicle development in goats coming to early and late estrus. This may also be related to the absence of large follicles at the time of administration of GnRH. In addition, ovulation occurring on average 48 hours after removal of the sponge may indicate that GnRH administration was performed too early.

In the current study, the mean overall intervals from sponge removal to the onset of estrus and to ovulation were 21.51 ± 2.37 h and 48.05 ± 1.24 h, respectively, with no significant difference among groups (Table 1). These results are supported by the earlier work in which compared with intravaginal progestogen plus eCG and $PG_{2\alpha}$. (Fonseca et al., 2005; Valentim et al., 2006) or double $PG_{2\alpha}$ alone (Simões et al., 2008). Nevertheless, these results are not in agreement with Riaz et al. (2012) who found interval from onset estrus to ovulation 31.2 and 36.0 h after Ovsynch and double $PGF_{2\alpha}$ treatment groups, respectively.

The GnRH agonist used in this trial had no effect on the timing of ovulation compared to $Group_1$ (no-GnRH). This is in agreement with the results of Cavalcanti et al. (2012), who applied a 6-day MAP-sponges treatment with 300 IU eCG and 37.5 μg d-cloprostenol 24 hours before sponge removal and 0.025 mg of a synthetic GnRH analogue (lecirelin) 24 h after sponge withdrawal and reported no significant difference in the interval from sponge removal to the onset of estrus in non-treated and GnRH-treated ewes (59.1 and 58.4 h, respectively; $P>0.05$).

In this study, no differences were observed in mean concentrations of plasma E_2 for Group1 compared with the other two Groups during the 13 days of the experiment. Additionally, GnRH injection 36 and 24 hours after sponge withdrawal did not change the concentration of P_4 and E_2 during and following ovulation. Similarly, Balaro et al. (2016) applied GnRH injection at 36 and 24 hours after sponge removal reported that, in ewes, plasma concentrations of P_4 and E_2 could not find any difference. Anyway, a direct comparison of ovulation time and hormone concentration in nulliparous and multiparous goats between studies is difficult due to differences in breed, hormonal milieu, age, nutrition status and synchronization protocols.

The percentage of goats ovulating of 93.10% (27/29), regardless of the application time of GnRH, showed the efficiency of the synchronization protocol used in this study (Table 1). Likewise, using the same protocols as in the present study Balaro et al. (2016) reported no significant difference in the percentage of ovulating (96.5%) between Santa Inês ewes treated with GnRH (0.025 mg, lecirelin) 36 and 24 hours after removal of the sponges. Similarly, using 20 mg FGA sponge for 6 days with 300 IU eCG and 30 μg of d-cloprostenol, Cavalcanti et al. (2012), reported 100 and 90 % the mean number of ovulations in ewes non-treated or treated GnRH (25 μg, licerelin) 24 h after sponge removal, respectively, during breeding season, and no significant difference between groups were also observed. Pierson et al. (2003) reported that the administration of GnRH results in an earlier and more regular formation of the LH peak, and shortens the time between the withdrawal of intravaginal sponges and the ovulation, and ultimately increases the mean number of ovulations per goat, but this was not observed in the present study.

The mean number of ovulations per doe, as observed by transrectal ovarian ultrasonography in the present trial, was not different between GnRH-treated and non-treated groups (Table 1); this finding was similar to that observed in other (Medan et al., 2003, 2005; Simões et al., 2006; Nogueira et al., 2015) or in the same goat breeds (de Castro et al., 1999) without exogenous GnRH administration. However, the mean number of ovulations per doe was higher to that reported by Riaz et al. (2012**),** (1.6 ± 0.2) in Beetal and Dwarf does, after using Ovsynch protocol. The differences in the results of these studies could be due to differences in dose of hormones, genetics, breed, age, nutrition and other environmental and management factors.

According to Cavalcanti et al. (2012), the ovulation response to GnRH injection might depend on the time of treatment and size of ovulatory follicle. In this study, though, the largest and second largest follicle diameter was similar in both the GnRH-treated goats and the untreated goats (Table 1).). According to previous studies, the largest follicle diameter is between 6.6-6.8 mm in acyclic (Cruz et al., 2005) and 5.9-8.0 in cyclic (Orita et al., 2000; Medan et al., 2005; Simões et al., 2006; Riaz et al., 2013) goats, which was similar to observed in Saanen in the our study. On the contrary, after using Ovsynch and double $PGF_{2\alpha}$ protocol in goats Riaz et al (2012) reported that ovulatory follicle diameter was 7.1 and 7.0 mm, respectively. Nogueira et al. (2015) reported that, in Boer goats, the largest follicle diameter was 6.7 mm in the non-breeding season and 7.8 mm in the breeding season.

As a consequence, six-day progesterone priming, in combination with eCG and $PGF_{2\alpha}$ can be used successfully for estrous induction and synchronization in Saanen goats during the transition period, but the administration 0.004 mg of GnRH 24 or 36 h after sponge removal had no effect on estrous behaviors, ovarian structures and plasma P_4 and E_2 concentrations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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