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Original Research Article

Comparison of hCG and GnRH for Synchronization of the Follicular Wave in Saanen Goats During the Breeding Season

Key words

estrus synchronization;
follicle;
hCG;
GnRH;
goat

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Abstract: This study aimed to compare the effects of a single dose of GnRH or hCG administered at the beginning of the short-term oestrus synchronization protocol on ≥4 mm diameter follicles in Saanen goats during the breeding season. The goats estrus cycles were synchronized with intravaginal sponges containing 60 mg of medroxyprogesterone acetate for five days. Additionally, 1 ml of physiological saline solution (control-group; n=30), 0.004 mg of buserelin acetate (GnRH-group; n=31) or 150 IU of hCG (hCG-group; n=31) were injected intramuscularly to the goats during the sponges insertion. Transrectal ultrasonographic examination was performed immediately before and 24 h after intravaginal sponge application into all goats and follicles with a diameter of ≥4 mm in the ovaries were counted. Blood samples were collected on the same days to determine serum progesterone (P4) and estradiol (E2) concentrations. At the first ultrasonographic examination, the percentages of ≥4 mm diameter follicles were 56.66% (17/30), 54.83% (17/31) and 70.96% (22/31) in the control, GnRH and hCG groups, respectively. The percentage of goats with reduced follicle diameters 24 h later was 29.41% (5/17), 52.94 (9/17) and 59.09% (13/22) in the same groups, respectively. The mean regression rates of follicle diameters between days 0 and 1 in each group were significantly different (P<0.05). Serum E2 concentrations were significantly different (P<0.05) between days 0 and 1 in hCG group. There were no differences in serum E2, P4 concentrations and mean regression rates in follicle diameters between days 0 and 1 for all groups. As a result, a significant relationship between the administration of hCG or GnRH and the reduction of large follicle diameters could not be established.

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Introduction

Goat breeds located in temperate and subtropical latitudes generally show seasonal breeding behavior (1), resulting in seasonal fluctuations in the availability of goat products such as milk and meat. Controlling the seasonality of goat reproduction is a way to overcome this problem. Nowadays, assisted reproductive technologies such as artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) allow faster genetic progress on farms by increasing the number of offspring from animals with high genetic merit, also creating a great opportunity for farmers and industry (2, 3). The induction and synchronization of estrus using exogenous hormones in goats is the most important reproductive management tool for the application of these techniques (1-4). The success of these technologies depends on the developmental stage of the follicles in the ovaries at the time of application of exogenous hormones (3, 5). Hence, the synchronization of the follicular wave is important to achieve high fertility rates in both AI and MOET. Because of their ease of use, and availability, progestogen sponges such as medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA) have been the most commonly used exogenous hormones in goats (4-7). The target of traditional long-term protocols developed with a focus on the lifespan of the corpus luteum is to

reduce the secretion of luteinizing hormone (LH) that prevents the occurrence of estrus, the preovulatory LH surge, and ovulation, and to allow them to occur after progestagen removal (6, 7). Nevertheless, in long-term protocols, serum progesterone concentrations decrease to subluteal levels 6 days after treatment and remain at subluteal level until days 10-14 when the sponge is withdrawn (8). Subluteal progesterone concentrations cannot adequately suppress LH, leading to the development of abnormal and permanent follicles and adversely affect the fertility of oocytes, and the function of the corpus luteum (6, 8-10). Currently, data based on the dynamics and regulation of follicular development have led to the design of new follicle-focused protocols (8). New short-term protocols based on 5-7 days of progesterone administration have provided better control of preovulatory events and ovulation, resulting in similar or higher pregnancy rates (3, 11). In short-term protocols subluteal progesterone levels do not occur and the quality of preovulatory follicles, appearing 5 to 6 days after the emergence of a new follicle wave, is not affected (4, 8, 11-13). Our hypothesis was to examine whether hCG or GnRH administered at the beginning of the short-term estrus synchronization protocol will cause regression of all large follicles present on the ovaries at administration. Therefore, the objective of the present study was to compare the effect of a single dose of GnRH or hCG administered at the start of the short-term estrus synchronization protocol on the diameter of all ≥4 mm follicles in Saanen goats, during the breeding season.

Materials and methods

Animal management

This study was conducted at the Research and Application Farm of the Veterinary Faculty, University of Bursa Uludag, Bursa (latitude 40° 11` N, longitude 29° 04` E, altitude 155 m), Turkey. A total of 92 clinically healthy, free of reproductive disorders, and non-lactating, multiparous Saanen does were used during the breeding season in the region. The does were kept outdoors in a sheltered paddock under natural photoperiod and temperature conditions and were fed dry grain wheat hay (1500 g/doe/day) supplemented with commercial pellets (18% crude protein; 800 g/doe/day, 2800 Kcal). No additional feed supplement was given to the goats during the study. The goats were provided ad libitum with clean drinking water and mineralized salt.

Estrus synchronization and hCG or GnRH treatment

Estrus was synchronized in all goats by the insertion of an intravaginal sponge impregnated with 60 mg medroxyprogesterone acetate (MAP, Esponjavet, Hipra, Spain) for 5 days. The insertion day of intravaginal sponges was considered as day 0 of the study period. Subsequently all goats were divided into three groups according to their age, body weight, and body condition score (scale 0 to 5, according to the model proposed by Morand-Fehr et al (14). In groups 1 to 3, the age averaged 37.00 ± 5.36, 37.90 ± 4.90, and 36.70 ± 3.24 months, body weights averaged 50.25 ± 3.04, 50.58 ± 2.92, and 50.94 ± 2.17 kg, and the body condition score averaged 3.09 ± 0.18, 3.10 ± 0.19, and 3.05 ± 0.14, respectively; these parameters were not statistically different between the groups. Does in the control group (n=30), hCG group (n=31) and GnRH group (n=31) received i.m.1 ml of physiological saline solution (0.9% NaCl), 150 IU of hCG (hCG, Chorulon, MSD, Netherlands), or 0.004 mg of buserelin acetate (GnRH, Buserin, Alke, Turkey), respectively, as soon as intravaginal sponges were placed.

Ovarian ultrasonography examination

All examinations to determine ovarian structures in goats were performed on a real-time transrectal ultrasonographic scanner (RTU, Prosound 2, Hitachi Aloka Medical, Ltd., Tokyo, Japan) with a 7.5 MHz linear transducer (model UST-660) and by the same operator. Before ultrasonic examination, the goat was placed in a standing position in a raised narrow wooden box, after which the hydro-soluble contact gel was applied, and the transducer was gently guided into the rectum. Briefly, after imaging the urinary bladder and the uterus horns on the monitor, the transducer was rotated 45°-90° clockwise or counterclockwise to observe both ovaries (15). Ultrasound examination of all goats was performed just prior to sponge insertion on day 0 and repeated on the following day. The number and size of all follicles equal or greater than 4 mm in diameter in both ovaries were recorded. After freezing the image of each ovary on the screen, the follicle diameter was measured using the built-in electronic caliper system and each ovary schematic map was drawn on a sheet of paper. The diameters of the follicles were characterized as large (i.e. ≥ 4mm) on day 0 and day 1. Ovarian data were then combined for both ovaries of each doe.

Hormonal analysis

Blood samples (8 ml) were collected from all goats by jugular venipuncture into vacuum blood tubes (Ref. Hp. 0013, Hema & Lab. Ankara, Turkey) just before sponge insertion and 24 h later. The tubes were immediately placed on an ice pack, transported to the laboratory, and then centrifuged at 4 °C for 10 min at 1500 x g. After centrifugation, serum was transferred to 1.5 ml micro-tubes and stored at -20 °C until assayed for progesterone (P4) and estradiol (E2). The concentrations of P4 (SRB-T-86624) and E2 (SRB-T-87401) in the blood serum were determined with commercial ELISA kits and the results were read by the ELISA reader (ELX-808IU Ultra Microplate Reader, BioTek, USA) according to the manufacturer's instructions. The sensitivity of the P4 and E2 assay were 0.048 ng/ml and 0.925 pg/ml, respectively. The mean intra- and inter-assay coefficients of variation were <10% and <12% for P4 and E2, respectively.

Table 1: Percentage of Saanen goats with large (≥4mm diameter) follicles before (Day 0) and 24 h after (Day 1) at the intravaginal sponge insertion in the Control, GnRH and hCG groups. All goats were synchronized using intravaginal sponges, and divided into three groups according to the treatment with either GnRH, or hCG (Day 0) or left untreated (control) group

	Control Group	GnRH Group	hCG Group	Total
Percentage of goats with large follicles at on Day 0	56.67% (17/30)	54.84% (17/31)	70.97% (22/31)	60.87% (56/92)
Mean number of large follicles in goats	1.18±0.10	1.18±0.10	1.18±0.11	1.17±0.06
Percentage of goats with regression of large follicles on Day 1	29.41% (5/17)	52.94% (9/17)	59.09% (13/22)	48.21% (27/56)

Table 2: Regression rate of large (≥4 mm diameter) follicles and serum E2 and P4 concentrations before (Day 0) and after 24 h (Day 1) after intravaginal sponge insertion in Saanen goats in the Control, GnRH and hCG groups. All goats were synchronized using intravaginal sponges, and divided into three groups according to the treatment with either GnRH, or hCG (Day 0) or left untreated (control) group

	Large follicles (≥ 4mm)			E2 concentrations (pg/ml)		P4 concentrations (ng/ml)	
	Day 0 (mm)	Day 1 (mm)	Regression rate (mm)	Day 0	Day 1	Day 0	Day 1
Control Group(n=5)	4.73±0.34^{a}	3.50±0.33^{b}	1.37±0.37	29.57±4.11	21.49±2.99	2.29±0.15	2.85±0.40
GnRH Group(n=9)	4.52±0.19^{a}	3.26±0.24^{b}	1.10±0.33	38.29±4.57	27.59±4.59	1.59±0.23	1.66±0.43
hCG Group(n=13)	4.41±0.09^{a}	3.44±0.23^{b}	0.87±0.30	37.95±5.45^{x}	19.19±4.71^{y}	1.67±0.18	1.53±0.28
Total(n=27)	4.51±0.98	3.38±0.14	1.04±0.20	36.51±3.11	22.42±2.81	1.76±0.12	1.82±0.22

$^{a, b, x, y}$ Values for each parameter in the same row with different superscripts differ significantly (p<0.05)

Statistical analysis

SPSS for Windows, Version 20 was used to analyze the study data. Results were presented as mean (± SEM) and differences were considered significant when the p value was below 0.05. The normality of the distribution data was tested by Shapiro-Wilk test. Kruskal-Wallis test was chosen to analyze the findings. After that, Mann-Whitney U test was used to discover the statistical differences between the groups.

Results

Ovarian structures

The first ultrasonographic examination (day 0) performed in all goats just before the insertion of intravaginal sponges showed that 60.87 % (56/92) of the goats had follicles ≥4 mm in diameter (Table 1). In detail, these ratios were 56.67% (17/30) in the control, 54.84% (17/31) in GnRH and 70.97% (22/31) in hCG groups, respectively. In the ultrasonographic examination performed 24 h after the intravaginal sponge application, it was observed that ≥4 mm follicle diameters were decreased in 29.41% (5/17) of goats in the control, 52.94% (9/17) in GnRH and 59.09% (13/22) in hCG groups, respectively. The mean rates of ≥4 mm follicles diameter reduction were significantly different for each group between days 0 and 1 (P<0.05), but not between the groups.

Serum P4 and E2 profile

Serum progesterone concentrations at the time of sponge insertion (day 0) revealed that 83 of 92 (90.21%) goats had a functional corpus luteum (> 1 ng/ml). Serum E2 concentrations were significantly different between days 0 and 1 in hCG group (P<0.05), but not in control and GnRH groups (Table 2). There was no difference in serum E2 and P4 concentrations between days 0 and 1 for all groups.

Discussion

This study showed that the insertion of sponges containing medroxyprogesterone acetate caused the regression of ≥4 mm follicles in 29.41% (5/17) of the goats in the control group. According to the results of ultrasonographic measurement, an average reduction of the diameters by 1.37 mm in of the initially ≥4 mm follicles was observed in approximately a third of these goats after 24 h (P<0.05), while the diameters of similar follicles did not change in the remaining goats in the group. In addition, there was no difference between days 0 and 1 in serum P4 and E2 concentrations in the control group. In the present trial, the mean diameter reduction of the large follicles in the control group was 1.37 mm (mm/day), which is higher compared with those previously reported in Shiba goats (0.8-0.9 mm/day) (16) and in Serrana goats (0.79 mm/day) (17). In addition, in these studies, no significant differences were observed in the regression rates of dominant follicles between follicular

waves in natural estrous cycles in goats, except for the ovulatory wave. The higher regression rate observed in our study, suggests that this difference might be probably due to fluctuation in naturally occurring plasma P4 concentrations on different days of the estrous cycle. Unlike other studies, this difference may also be due to the exogenous administration of a progesterone analogue (medroxyprogesterone acetate). In goats synchronized with different methods, plasma P4 concentration peaked on day 10 of the estrous cycle and remained high until day 15, and then declined rapidly (15, 16, 18).

In addition, high plasma P4 concentrations in goats caused that the third and fourth wave emerge earlier compared to the first and second wave of the estrous cycle (16-18), what supports our study. Furthermore, the high regression rate found in our study also supports the negative effect of high plasma/serum P4 concentrations on follicular growth, as observed in Saanen (18) and Shiba goats (16), and as claimed by other researchers (16, 18, 19), due to the variation in plasma P4 concentrations between the middle and the onset or end of the estrous cycle in cycling goats, depending on the developmental stage of the corpus luteum. A positive correlation was observed between high plasma P4 concentrations and regression rates of large follicles in P4-treated goats, and high plasma P4 concentrations were considered as an accelerating factor in follicular turnover (6, 8, 12). As a result, it can be claimed that in 29.41% of the goats in the control group a new follicle wave emerged. Daily transrectal ultrasonographic examination results in goats showed that follicles on the ovaries developed in wave-like patterns throughout an interovulatory interval (15) and the first wave emerged just after ovulation (Day 0) (16-19). This could also be a reason why most of the studies have focused on the manipulation of the first wave, which emerges on the ovulation day of the previous cycle, as it is very difficult to predict when other waves would emerge (6, 8). Therefore, our study, as in researchers' studies, has been designed to regress large follicles on the ovaries with the insertion of intravaginal devices containing progesterone or its analogues, thus creating a new follicle wave after the withdrawal of intravaginal devices (Day 0 Protocol) (8, 20). Also, it has been reported that a new wave of follicles does not emerge unless the large follicles on the ovaries regress (5, 8, 21). In a previous study, Año-Perello et al (20) reported that 81.1% (30/37) of large follicles regressed 24 h after insertion of a controlled internal drug releasing device (CIDR) in Segureña meat ewes, and plasma E2 concentrations decreased significantly between days 0 and 1. Despite using the same synchronization protocol in our study, no change in serum E2 concentrations was observed between days 0 and 1 in the control group. This difference may be due to the negative relationship between high serum/plasma P4 concentrations and the development of large follicles and E2 synthesis. As previously reported, plasma progesterone concentrations increase rapidly within the first 48 h after insertion of a progesterone or progestogen-containing intravaginal device, reaching a peak after 3 days and then gradually decrease (22). Similarly, Rubianes and Manchaca (6) reported that large follicles that grow in the mid-late luteal phase do not synthesize high levels of estradiol-17β due to the high P4 concentrations produced by the corpus luteum. This variation may be due to natural progesterone, breed, age, nutrition and development stage of follicles (2, 6, 8). Our results support previous studies showing that exposure to high levels of exogenous progesterone or progestogens affects the development of large follicles and, as a result, increases follicular turnover (2, 7).

In the current study, administration of 0.004 mg buserelin acetate (GnRH analogue) at the time of sponge insertion resulted in regression of large follicles in 52.94% (9/17) goats in the GnRH group. An average reduction of 1.10 mm in the diameters of larger follicles was observed in these goats. No significant change in serum P4 and E2 concentrations was observed between days 0 and 1. Unlike our study results, Año-Perello et al (20) reported significant differences in the percentages of sheep with regression of large follicles 24 h after CIDR insertion after treatment with GnRH (50 µg gonadorelin, 100%) or without GnRH (81.1%) during the breeding season. Furthermore, they also observed a significant decrease in E2 concentration between days 0 and 1 in GnRH-treated ewes. In the present trial, serum E2 concentrations did not decrease from day 0 to day 1 in the GnRH group, suggesting that possibly large follicles were not fully responsive to GnRH and yet not dependent on LH, possibly due to high serum P4 concentrations. Similarly, it has been reported that administration of GnRH 24 h after sponge withdrawal does not improve the time of ovulation or pregnancy rate in estrus synchronization in ewes (23) and also did not change plasma P4 and E2 concentrations in goats (4). On the other hand, Eppleston et al (24) reported that administration of GnRH at the end of progestogen treatment induces preovulatory secretion of LH by the anterior pituitary gland within 1-4 hours which ovulation improve in ewes. In conclusion, the result of this experiment supported that regression rate of large follicles in goats in the GnRH group was probably associated with high serum P4 concentration, but not dependent on GnRH administration. Our observation was similar to data from other researchers (5, 18, 19).

In the current study, administration 150 IU of hCG i.m. at the time of the sponge insertion resulted in regression of large follicles in 59.09% (13/22) goats, with an average reduction of 0.87 mm in large follicle diameters between days 0 and 1 ($P<0.05$). E2 concentrations were significantly different between both days ($P<0.05$). hCG, a powerful luteotropic agent is a glycoprotein hormone that has a similar chemical structure to LH, binds to the same receptor, and has a longer half-life and rapid absorption than LH (25). hCG shows its effect on follicular growth by binding directly to LH receptors on granulosa cells in large follicles that emerge in the luteal phase of the estrous cycle (5). As observed in our study, hCG administration in the luteal phase may cause faster atresia or luteinization of the large follicles and so,

decrease estradiol-17β production. Early findings in goats (5, 16, 18) showed that estradiol-17β was mostly produced by the largest follicle of the wave and E2 produced by other follicles contributed less to the plasma E2 concentrations. Therefore, the absence of such an increase in E2 concentrations on day 1 in our study may reflect a lower production of E2 in all or some of the large follicles. In contrast, injection of hCG in the presence of preovulatory follicles caused a sharp increase in LH, which resulted in luteinization or ovulation but no effect on the fertility rate in goats (26).

Our results indicated that the insertion of a MAP-loaded intravaginal sponge, regardless of the GnRH and hCG treatment caused the regression of large follicles in 48.21% (27/56) of all the treated goats. The regression of all gonadotropin-dependent follicles was complete in about half of these goats, and as a result, a new follicular wave was initiated in these goats. Regardless of GnRH or hCG administration, our results support previous studies indicating that high serum P4 concentrations affect the development of large follicles and cause faster follicular turnover. Similarly, administration of a single dose of GnRH or hCG at sponge insertion did not affect the regression rate of large follicles and serum P4 and E2 concentrations.

Conclusions

In conclusion, a significant relationship between the administration of hCG or GnRH and the reduction of large follicles diameters or the serum concentrations of P4 and E2 could not be established.

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Ethical approval: All of the methods and management procedures in this study were evaluated and approved by the Animal Experiments Local Ethics Committee of the Uludag University (approval reference number: B.30.2.ULU.08Z.00.00).

Conflict of interest: The authors declare no conflict of interest.

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Primerjava hCG in GnRH za sinhronizacijo folikularnega vala pri kozah Saanen med sezono parjenja

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Izvleček: Namen te študije je bil primerjati učinke enkratnega odmerka GnRH ali hCG danega na začetku kratkotrajnega protokola za sinhronizacijo estrusa na folikle s premerom ≥ 4 mm pri kozah pasme Saanen med sezono parjenja. Cikle estrusa pri kozah smo pet dni sinhronizirali z intravaginalnimi gobicami z vsebnostjo 60 mg medroksi progesteron acetata. Poleg tega smo jim v času vstavljanja gobic intramuskularno aplicirali 1 ml fiziološke fiziološke raztopine (kontrolna skupina; n=30), 0,004 mg buserelin acetata (skupina GnRH; n=31) ali 150 IU hCG (hCG-skupina; n=31). Neposredno pred in 24 ur po intravaginalni uporabi gobice smo pri vseh kozah opravili transrektalni ultrazvočni pregled in prešteli jajčne folikle s premerom ≥ 4 mm. Ob istih dnevih smo odvzeli tudi vzorce krvi za določitev serumskih koncentracij progesterona (P4) in estradiola (E2). Ob prvem ultrazvočnem pregledu so bili odstotki foliklov s premerom ≥ 4 mm 56,66 % (17/30) v kontrolni, 54,83 % (17/31) v GnRH in 70,96 % (22/31) v hCG skupini. Odstotek koz z zmanjšanim premerom foliklov 24 ur pozneje je bil 29,41 % (5/17) v kontrolni, 52,94 % (9/17) v GnRH in 59,09 % (13/22) v hCG skupini. Povprečna stopnja regresije premerov foliklov med dnem 0 in 1 se je pomembno razlikovala (P<0,05) v vseh skupinah. Koncentracija E2 v serumu se je med dnem 0 in 1 pomembno razlikovala (P<0,05) v skupini hCG. V serumskih koncentracijah E2 in P4 ter povprečni stopnji regresije v premeru foliklov med dnevi 0 in 1 ni bilo razlik v nobeni skupini. Posledično ni bilo mogoče potrditi povezave med dajanjem hCG ali GnRH ter zmanjšanjem premera velikih foliklov.

Ključne besede: inhronizacija estrusa; folikel; hCG; GnRH; koza