

Ovarian follicle dynamics and hormonal changes during early pregnancy in Saanen goats

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Citation: Dogan I, Toker MB, Alcay S, Udum Kucuksen D (2020): Ovarian follicle dynamics and hormonal changes during early pregnancy in Saanen goats. Vet Med-Czech 65, 8–17.

Abstract: This study is aimed at describing the ovarian follicular dynamics, *corpora lutea* and their hormonal control during early pregnancy in Saanen goats. The ovaries of pregnant goats ($n = 10$) were monitored daily for follicles (≥ 2 mm) and *corpora lutea* by transrectal ultrasound over the 35 days after mating. Blood samples were collected by jugular venipuncture at the time of ultrasonography and were analysed for the concentrations of oestradiol (E_2) and progesterone (P_4), inhibin, FSH (follicle-stimulating hormone) and LH (luteinizing hormone). The pattern of follicular growth was characterised by five ($n = 2$; 20 %), six ($n = 5$; 50 %) and seven ($n = 3$; 30 %) follicular waves during early pregnancy. The first *corpora lutea* (size 6.8 ± 0.4 mm, mean + SEM) were detected at 5.0 ± 0.3 days after the mating with a maximum diameter (12.2 ± 0.3 mm) observed on day 24.0 ± 1.1 of pregnancy. The dominant follicle diameter of waves 1 and 4, and the duration of waves 1–4 and 7 were maximal during the early pregnancy period ($P < 0.05$). In addition, the inter-wave interval of the 5 waves was significantly ($P < 0.05$) longer compared to the six and seven inter-wave intervals (7.0 ± 0.1 , 5.8 ± 0.1 and 5.0 ± 0.2 days, respectively). Although the number of small and medium-sized follicles did not differ with the days post-breeding, the number of large-sized follicles in the same period significantly decreased between 16 and 26 days and was negatively correlated with the period of pregnancy ($P < 0.05$). The plasma LH and E_2 concentrations were negatively correlated with the P_4 concentration. The inhibin concentrations were negatively correlated with FSH, but positively correlated with the number of large-sized follicles. These results indicate that ovarian follicular growth during the first 35 days of pregnancy in goats was characterised by a wave-like pattern and there was a close relationship between the number of FSH peaks and the number of follicular waves.

Keywords: *corpus luteum*; follicles; goat; hormonal dynamic; ultrasonography

Real-time ultrasonographic scanning was initially used as a non-invasive method for the detection of ovarian structures and early pregnancies in farm animals such as cattle (Pierson and Ginther 1984). This method was then adapted to small ruminants with the development of appropriate ultrasonic

probes, and, thus, monitoring ovarian structures and diagnosing early pregnancy became easier (Bartlewski et al. 2000; Tenorio Filho et al. 2007; Kandiel et al. 2010). Transrectal ovarian ultrasonography is the most practical and reliable tool for monitoring the development of the follicle and

Supported by the Uludag University Scientific Research Projects Unit, Bursa, Turkey, (BAP) (Project No. BUAP (V)-2016/1).

<https://doi.org/10.17221/65/2019-VETMED>

corpus luteum (CL) in the ovary in goats compared to laparoscopy or laparotomy (Simoes et al. 2005). By using daily transrectal ultrasonography, Ginther and Kot (1994) first reported that ovarian follicular development occurred in wave-like patterns in cyclic goats, which was later confirmed by other researchers (de Castro et al. 1998; de Castro et al. 1999; Simoes et al. 2006). Similarly, follicular development in anoestrus (Cruz et al. 2005; Nogueira et al. 2015) and postpartum (Kandiel et al. 2012) and transition (Schwarz and Wierzchos 2004) period were observed with a wave-like pattern in goats. A follicle wave consists of a synchronous growth of a small follicle cohort from which at least one large preovulatory follicle continues growing through three stages, recruitment, selection and dominance, whereas other follicles regress (Evans 2003; Hunter et al. 2004; Fatet et al. 2011), but the dominant follicle phenomena in goats are less pronounced than in cattle (Ginther and Kot 1994; de Castro et al. 1999; Orita et al. 2000; Schwarz and Wierzchos 2000). The number of follicular waves during the inter-ovulatory interval in cyclic goats varies between two and six, but the four wave numbers are the most dominant (Ginther and Kot 1994; Schwarz and Wierzchos 2000; Menchaca and Rubianes 2002; Medan et al. 2005; Simoes et al. 2006; Mohammadi et al. 2010; Nogueira et al. 2015). Similarly, there were usually four follicular waves in acyclic, anoestrous goats during a 21-day observation period (Schwarz and Wierzchos 2002; Cruz et al. 2005; Nogueira et al. 2015), in different goat breeds and environmental conditions. Ultrasonography images of the ovaries have shown that the growth of the follicles with a diameter of ≥ 5 mm at approximately five day intervals occurred in an orderly pattern and growing an average of 1.0 mm per day in the cyclic (Medan et al. 2005; Simoes et al. 2006) or acyclic (Schwarz and Wierzchos 2002; Cruz et al. 2005) goats. In goats, follicular waves emerge after an increase in serum FSH concentrations (Schwarz and Wierzchos 2000; Menchaca and Rubianes 2002). Furthermore, there is a positive relationship between the increase in oestradiol and inhibin concentrations and the development of the largest follicle in a wave (de Castro et al. 1999; Medan et al. 2003). As in other ruminants, follicular growth in goats is affected by endogenous progesterone (Gonzalez-Bulnes et al. 2005). However, the number of studies examining the follicular dynamics and hormonal changes

during the early pregnancy period in goats is very limited (Kandiel et al. 2010; Schwarz and Wierzchos 2010). Therefore, the objective of the current study was to use daily transrectal ultrasound imaging of the ovaries to identify the patterns of the follicular dynamics and *corpus luteum* growth during the early pregnancy in Saanen goats and to correlate the follicular waves with changes in plasma concentrations of the gonadotropic and ovarian hormones.

MATERIAL AND METHODS

Animals

The study was carried out at the Research and Application Farm of the Veterinary Faculty at Uludag University, located in Bursa (latitude $40^{\circ} 11' N$, longitude $29^{\circ} 04' E$, altitude 155 m a.s.l.), Turkey. Ten multiparous Saanen does were used during the physiological breeding period from October to November. At the onset of the experiment, the does were 38.00 ± 3.64 months old (\pm SEM) and weighing 57.06 ± 1.80 kg with a body condition score of 3.20 ± 0.82 (evaluated on a scale of 1 to 5; Morand-Fehr et al. 1989). The does were kept indoor in sand/hay-floored pens with access to the outdoors in a sheltered paddock under the conditions of natural daylight and temperatures and were fed dry oat hay supplemented with commercial pellets (18% crude protein; 1 000 g/goat/day). Clean water and mineral salts were provided *ad libitum* and no changes were made during the experiment. The experiment was approved by the Animal Ethics Committee of Uludag University (References No. B.30.2.ULU.0.8Z.00.00/09).

Experimental design

The does were synchronised with 20 mg FGA (fluorogestone acetate; Esponjavit, Hipra, Spain) vaginal sponges for 11 days and 400 IU of eCG (equine chorionic gonadotrophin; Chrono-Gest, Intervet, The Netherlands) at the sponge withdrawal. A teaser buck was used for detection of oestrus every 24 h from the day of the sponge removal and each doe within the same oestrus period naturally mated at least twice with fertile bucks on the following day. The last mating day of each doe was

determined as the starting day (0 Day) of pregnancy. Blood samples (10 ml) were collected each day from Day 1 to Day 35 of pregnancy by jugular venipuncture, using heparinised vacutainer tubes (BD Vacutainer®; Becton Dickinson, Plymouth, UK), then centrifugation was performed at $2000 \times g$ for 10 min at 4 °C, and the plasma was separated and stored at -20 °C for the hormone assay. Later, the hormonal data were compared for the relationship between the characteristics of the follicular waves and the plasma concentrations of P₄, E₂, FSH, LH and inhibin.

Ultrasonographic examination

All the does were given a daily ultrasound examination from Day 1 to Day 35 post-mating using a real-time B-mode ultrasound scanner (Prosound 2; Hitachi Aloka Medical, Ltd., Tokyo, Japan), connected with a transrectal 7.5 MHz linear array probe (model UST-660-7.5), that was designed for human prostatic examination. During the examination, the goats were restrained in the standing position. The probe was moistened with a hydrosoluble contact gel and then gently inserted into the rectum. In brief, after the probe 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 described by Orita et al. (2000), Simoes et al. (2005) and Kandiel et al. (2010) in goats. After the sonographic images of the left and right ovaries were taken, a complete screening of each ovary was performed and recorded in the B mode of the real-time transrectal ultrasonography (RTU) equipment and, later, measurements were made on the frozen images. All the follicles ≥ 2 mm in diameter with a spherical or oval conformation and smooth walls and the *corpus luteum* (CL) were measured using the built-in electronic calliper system, and the ovary map was drawn on a sheet of paper and compared to the previous day (Kandiel et al. 2010). The ultrasonography evaluations were always performed by the same person.

Follicular wave analysis

The following characteristics of the follicular waves were determined per each doe, as described below (Medan et al. 2005; Simoes et al. 2006).

Follicular wave. Determined as a decrease in the growing number of small follicles until (post) deviation and an increase in the diameter of at least one follicle, and then regressed or ovulated.

Onset of the follicular wave. The first observation of at least one follicle with a diameter ≥ 3 mm, in the following days a growing follicular wave resulted in a follicle ≥ 5 mm diameter. The follicles measured with a diameter of 4 mm for the first time were thought to have a diameter of 3 mm in the previous day (Ginther and Kot 1994; de Castro et al. 1999; Cruz et al. 2005), which was identified as the day of the wave emergence (Kandiel et al. 2012).

Day of the maximum follicular diameter. The first day of each wave when the dominant follicle (≥ 5 mm) reached the largest diameter.

End of the follicular wave. The day when the number of follicles (< 3 mm) increased and the number of follicles (≥ 3 mm) decreased by the same rate.

Growth rate (mm/day). Calculated for each dominant follicle by dividing the number of days between its appearance as a follicle ≥ 3 mm and its maximum size.

Duration of the wave. The number of days between the onset and the end of a follicular wave.

Inter-wave interval. The number of days between the onsets of two sequential follicular waves.

Total follicle number. The total number of follicles (≥ 2 mm) per day in each doe.

The follicular waves were collected for both ovaries on each day and then combined so that a single observation in each goat was used during the early pregnancy (e.g., the total number of ovarian follicles, the number of follicular waves, the end and onset of the follicular wave, the day when the dominant follicle reached the maximum diameter). The follicular waves of both ovaries in a goat were combined when at least three-fourths of the duration of a wave occurred at the same time as the other wave and two different waves in two different ovaries were considered as a single wave (Simoes et al. 2006). The first wave at the beginning of the early pregnancy period was defined as Wave 1, and the other waves were numbered sequentially. The last follicular waves that were not completed during the early pregnancy were ignored.

After the oestrus synchronisation, all the follicles of ≥ 2 mm diameter with an echo-free black spherical or oval shape and smooth walls were screened daily by RTU and their diameters were measured (Simoes et al. 2006). The follicles were classified

<https://doi.org/10.17221/65/2019-VETMED>

as small (2.0–3.4 mm), medium (3.5–4.9 mm), and large (≥ 5.0 mm), and determined daily according to Ginther and Kot (1994). At the same time, the total number of follicles was calculated every day for each doe.

Corpus luteum analysis

The *corpus luteum* was detected as spheres or ellipsoids with echodense and echolucent imaging slightly less than the echo-genic ovarian stroma (Orita et al. 2000). The maximum diameter of the *corpus luteum* in each ovary was measured daily by the scanner's electronic calliper system. The central CL cavity was not subtracted from the total diameter of the CL. Also, the first day of detection, the daily growth rate, and the day of maximum diameter were recorded for each CL.

Hormone analysis

The plasma progesterone (P_4 , SRB-T-86624), oestradiol (E_2 , SRB-T-87401), follicle stimulating hormone (FSH, MBS-263518), luteinizing hormone (LH, MBS-265390) and inhibin beta A (MBS-015096) concentrations were measured by ELISA (ELX808IU Ultra Microplate Reader; Bio-Tek Instruments Inc., Winooski, VT, USA) system using commercial goat kits. The standard range of the P_4 , E_2 , FSH, LH and inhibin kits used were 0.05–8.0 ng/ml, 1–300 pg/ml, 100–1.56 mIU/ml, 100–1.56 ng/ml and 31.2–1 000 pg/ml, respectively. The inter-assay coefficients of variation (CV_s) for P_4 , E_2 , FSH, LH and inhibin were 2.7, 2.7, ≤ 12 , ≤ 12 and $\leq 15\%$, respectively, and the intra-assay CV_s were 4.6, 4.6, ≤ 8 , ≤ 8 and $\leq 15\%$, respectively. The sensitivities of the P_4 , E_2 , FSH, LH and inhibin assays were 0.048 ng/ml, 0.925 pg/ml, 0.5 mIU/ml, 0.5 ng/ml and 5.0 pg/ml, respectively. The plasma P_4 levels ($1 \geq$ ng/ml) were recorded as an indication of ovulation (Menchaca and Rubianes 2002).

Statistical analysis

SPSS for Windows, Version 20 was used to conduct the statistical analyses. The numbers of follicular waves were statistically analysed using a chi-square test and the data were ex-

pressed as percentages. ANOVA (analysis of variance) was used to analyse the number of small (2.0–3.4 mm), medium (3.5–4.9 mm), and large follicles (≥ 5 mm), the maximum diameter of the largest follicle in each wave, the duration of a wave, the inter-wave interval, and the growth rate of the follicles. The relationship between the CL size and the day was analysed by ANOVA test. Tukey's test was used in multiple comparisons. The relationship between the follicle development or the CL size and the plasma hormone concentrations was assessed using Pearson's correlation test. Pearson's correlation test was also correlated with the follicles or the hormones and the number of pregnancy days; with the inhibin and the FSH; with the LH and E_2 or P_4 . The results are given as a mean (\pm SEM) and the differences were considered significant when $P < 0.05$.

RESULTS

Based on the definition of the follicular waves used in the study, three different wave patterns were observed in the Saanen goats. These patterns included either five ($n = 2$; 20%), six ($n = 5$; 50%) or even ($n = 3$; 30%) follicular waves over the 35 days after mating and the mean number of follicular waves in the pregnant goats was 6.1 ± 0.2 . There were significant ($P < 0.05$) differences in the inter-wave intervals among the five, six and seven wave groups (5.0 ± 0.2 , 5.8 ± 0.1 and 7.0 ± 0.1 days, respectively).

The characteristics of the follicular waves recorded during the study period are given in Table 1. There were significant ($P < 0.05$) differences between the 1st, 2nd, 3rd and 4th waves and the 7th wave in terms of the mean wave duration. Within the seven follicular wave patterns recorded in the study, only the mean diameters of the largest follicles were significantly ($P < 0.05$) different between Waves 1 and 4 (Table 1). Although the number of small and medium-sized follicles did not differ with the day post-breeding, the number of large-sized follicles significantly ($P < 0.05$) decreased between 16 and 26 days (Figure 1). The number of large-sized follicles also had a negative correlation between 16 and 26 days of the pregnancy ($r = -0.36$, $P < 0.05$).

The *corpora lutea* ($n = 16$) were 6.8 ± 0.4 mm at first detection (Day 5.0 ± 0.3) and were maximal in diameter (12.2 ± 0.3 mm) on Day 24.0 ± 1.1 .

Table 1. Mean \pm SEM characteristics of follicular waves during early pregnancy in Saanen goats ($n = 10$)

Follicular wave (n)	Duration of wave (days) mean \pm SEM	Maximum follicular diameter (mm) mean \pm SEM	Growth rate (mm/days) mean \pm SEM
First	5.90 \pm 0.18 ^a	6.20 \pm 0.15 ^a	1.28 \pm 0.13
Second	5.90 \pm 0.48 ^a	5.80 \pm 0.30 ^{ab}	1.16 \pm 0.17
Third	6.20 \pm 0.33 ^a	5.50 \pm 0.21 ^{ab}	1.35 \pm 0.15
Fourth	6.00 \pm 0.21 ^a	5.00 \pm 0.22 ^b	1.20 \pm 0.13
Fifth	5.60 \pm 0.45 ^{ab}	5.30 \pm 0.13 ^{ab}	1.44 \pm 0.18
Sixth	5.38 \pm 0.26 ^{ab}	5.75 \pm 0.37 ^{ab}	1.14 \pm 0.14
Seventh	3.66 \pm 0.67 ^b	5.17 \pm 0.33 ^{ab}	1.66 \pm 0.67
Mean	5.52 \pm 0.33	5.73 \pm 0.50	1.29 \pm 0.07

^{a,b}Values with different superscripts in the same parameters are significantly different ($P < 0.05$)

The daily mean luteal diameter per pregnant goat increased steadily until Day 10 ($P < 0.05$) and remained high thereafter until the end of the study period.

Figure 2 presents the daily changes in the plasma E_2 and P_4 concentrations in the goats with 5, 6 and 7 wave patterns during the 35-day observation period, respectively. The plasma E_2 concentration in the goats showed a postovulatory peak (78.59 ± 0.47 ng/ml) on day 3 post-natural mating concomitant with the largest follicles of the first wave reaching their maximum diameters. During the study period, the oestradiol concentrations remained low for the remaining days of early preg-

nancy and negatively correlated with the days after the natural mating ($r = -0.26$, $P < 0.01$). The plasma P_4 concentration increased slightly for nine days after mating and reached its maximal concentration by Day 10 and remained high in concentration until the end of the study period. At the same time, the P_4 concentration showed a positive correlation with the CL diameters ($r = 0.53$, $P < 0.01$), but showed a negative correlation with E_2 ($r = -0.17$, $P < 0.01$) during the study period. There is a close relationship between the number of follicular waves and the FSH peaks in the goats ($P < 0.05$). In addition, the plasma inhibin concentration showed a negative correlation with both the FSH ($r = -0.35$,

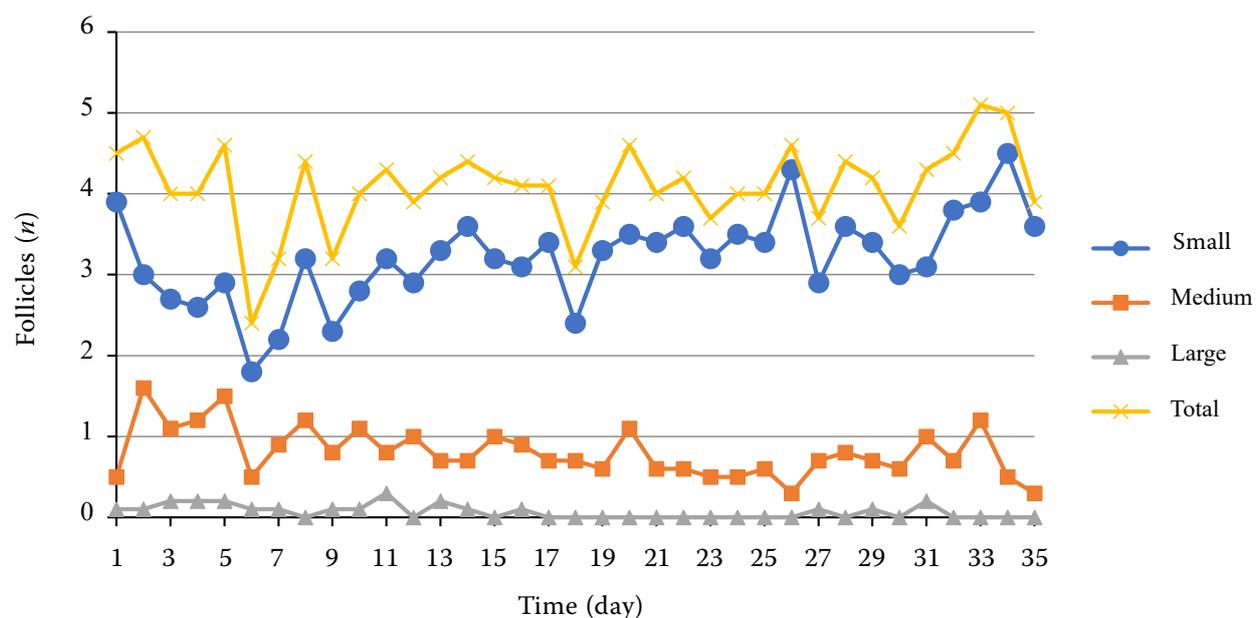


Figure 1. Daily changes in the number of small (2.0–3.4 mm), medium (3.5–4.9 mm), large (≥ 5.0 mm) and total follicles (≥ 2 mm) for 35 days in pregnant goats ($n = 10$). Values presented are mean \pm SEM ($n = 10$). The number of large-sized follicles were a significant ($P < 0.05$) difference between 16–26 days during early pregnancy

<https://doi.org/10.17221/65/2019-VETMED>

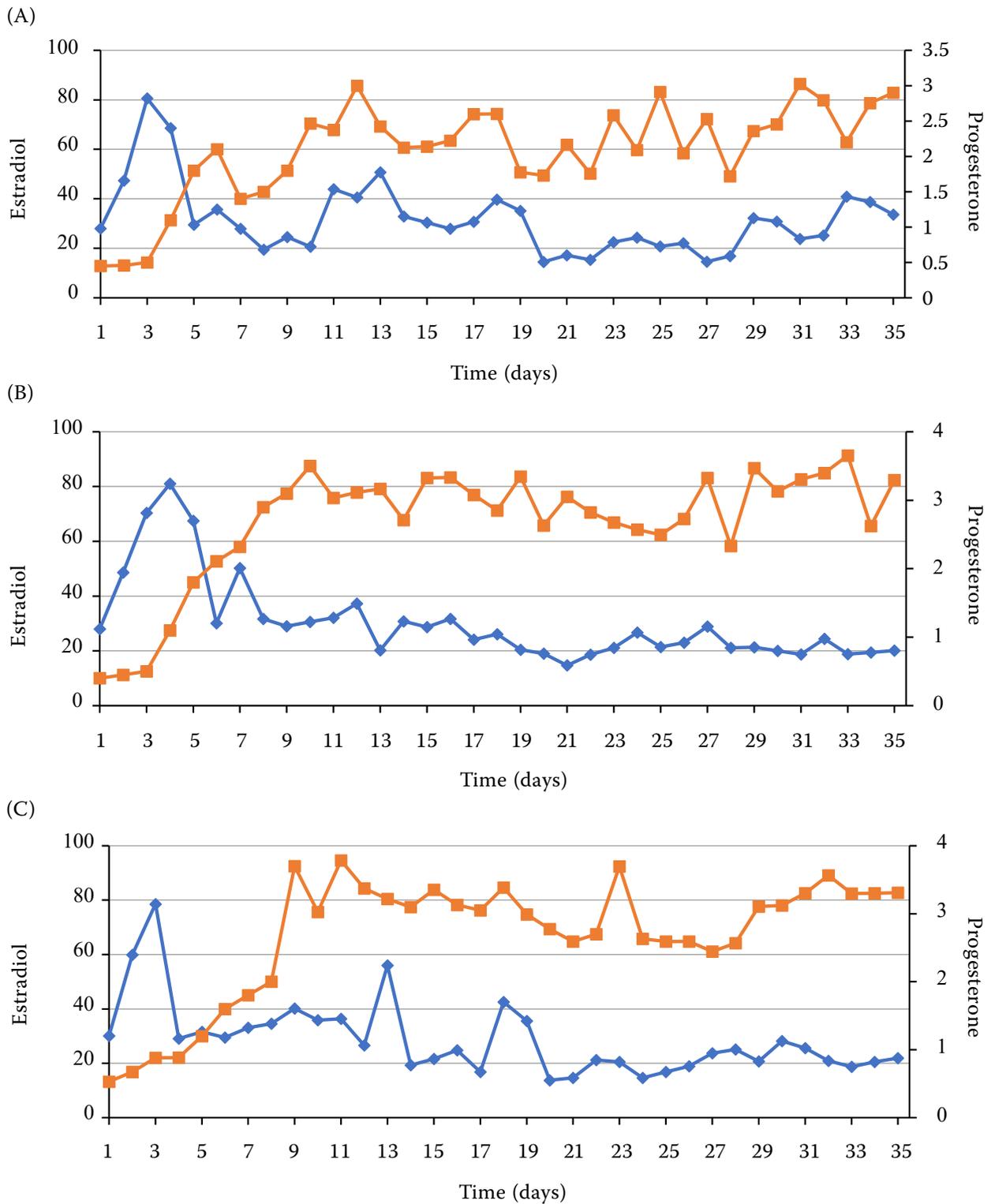


Figure 2. A representative pattern of plasma concentrations of E₂ (pg/ml, ■) and P₄ (ng/ml, ■, C) in a goat with five (A), six (B) and seven (C) waves during the early pregnancy

$P < 0.01$) and the days ($r = -0.44$, $P < 0.01$) during the same study periods. The plasma LH concentrations were low throughout the study; they

positively correlated with the E₂ concentrations ($r = 0.41$, $P < 0.05$), but negatively correlated with the P₄ concentrations ($r = -0.48$, $P < 0.01$).

DISCUSSION

In this study, the general hormonal structure responsible for the follicular development and follicular dynamics is demonstrated in the ovaries during the early pregnancy period in the Saanen goats. The results of daily trans-rectal ultrasonography indicated that until the 35th day of pregnancy, the goat ovaries were characterised by a wave-like pattern of follicular development, which was also reported in previous studies (Kandiel et al. 2010; Schwarz and Wierzchos 2010). Similarly, researchers who examined the follicular dynamics of goats in the breeding season (Ginther and Kot 1994; de Castro et al. 1999; Schwarz and Wierzchos 2000; Menchaca and Rubianes 2002; Medan et al. 2005; Simoes et al. 2006) or the anoestrus period (Schwarz and Wierzchos 2000; Cruz et al. 2005) reported that the growth phase of the follicles showed a wave-like fashion.

The current study indicated that up to the 35th day of pregnancy, the number of the waves varies from five (20%) to seven (30%) waves, with a higher incidence of six waves in the Saanen goats (50%). However, Kandiel et al. (2010) reported that the number of waves varied between seven (12.5%) and nine (37.5%) changes in the same period and the most frequent eight waves (50%) occurred in pregnant Shiba goats. The reason for this difference is unclear; it may be attributed to differences such as the breed, nutrition, season, different hormonal structure between the individuals and the physiological status of the females. These findings are concomitant with earlier findings in pregnant goats (Kandiel et al. 2010) and indicated that the follicular waves occur regularly due to the FSH peaks during the early pregnancy period in the goat. In the current study, there was a close relationship between the number of follicular waves and the FSH peaks, as previously recorded in cyclic (Medan et al. 2003), pregnant (Kandiel et al. 2010) and postpartum period (Kandiel et al. 2012) goats. A peak in the plasma FSH concentrations was followed by emergence of a new wave and increased plasma inhibin concentrations that caused the FSH to decrease in the current and previous studies (Medan et al. 2003; Medan et al. 2005; Fatet et al. 2011; Kandiel et al. 2012). Conversely, plasma inhibin concentrations may be low in the early period of pregnancy due to the decrease in the number of the large follicles.

Taking these results in mind, it could be concluded that pregnant goats are characterised by a wave-like pattern of follicular development, the same as during the cyclic or acyclic period in goats (Ginther and Kot 1994; de Castro et al. 1999; Schwarz and Wierzchos 2002; Medan et al. 2003; Cruz et al. 2005).

In this study, when the diameters of the largest follicles of the waves were compared, there was only a significant difference between waves 1 and 4 during the observation period (Table 1). On the other hand, the first postovulatory waves largest follicle diameter was numerically larger than the follicle diameters of the following waves, which is in agreement with previous findings (Ginther and Kot 1994; Menchaca and Rubianes 2002; Simoes et al. 2006; Nogueira et al. 2015). The reason for this difference may be due to the differences in the P₄ plasmatic concentrations between the stages of the oestrous cycle (Ginther and Kot 1994). Similarly, Kandiel et al. (2010) compared the largest follicle diameter of the waves over the 35 days after mating in pregnant goats and reported that the largest follicle of the first wave was larger diameter than for all the other waves. This is the result of low progesterone secretion from the newly developed CL as proposed by Simoes et al. (2007) and Arashiro et al. (2010) and may be due to the regularity of the pulsatile LH release and the amplitude of the LH release during the postovulatory period and then the high P₄ concentration suppresses the LH release suggested by Simoes and Mascarenhas (2007) and Fatet et al. (2011).

Considering the wave duration in the study, the durations of the waves within the 35-day period was almost the same, on the other hand, the duration of the seventh wave was significantly shorter than the durations of the first four waves (Table 1), which is constant with some previous studies (Orita et al. 2000). As observed in this study, the increased concentrations of progesterone after the first wave (ovulatory wave) have a lasting effect on the subsequent waves and also prevent the preovulatory LH peak. Consequently, progesterone concentrations have a sustained effect on the wave durations. These findings are in agreement with earlier findings in cyclic goats (Medan et al. 2003; Nogueira et al. 2015) and indicated that the durations of second and third waves are similar, except for the durations of postovulatory (wave 1) and preovulatory waves.

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In this study, when different wave numbers were analysed in the pregnant goats after mating, the mean inter-wave interval of seventh wave was shorter than the other waves, which was also similar to the results obtained by other researchers (Cruz et al. 2005; Medan et al. 2005; Simoes et al. 2006) in cyclic goats. On the contrary, our results are not in agreement with Kandiel et al. (2010) who reported that there was no significant difference between the inter-wave interval among the seventh, eighth and ninth wave in the goats during the early pregnancy period. As previously reported on the effect of the plasma P_4 concentrations on the follicular development (de Castro et al. 1999), this different turnover speed of waves may be associated with the different endocrine environments during the early pregnancy of goats.

As reported in this and other studies (Kandiel et al. 2010), high P_4 concentrations, which suppress the growth of large follicles during pregnancy, could delay the interval to the emergence of the following wave and ultimately result in the lower secretion of E_2 .

In our study, the inter-wave intervals in the pregnant goats varied according to the number of follicular waves and were previously confirmed in cyclic (de Castro et al. 1999; Medan et al. 2003) and pregnant (Kandiel et al. 2010) goats.

Researchers have reported that the average daily growth rate of the follicles is between 0.8–1.1 (mm/day) in cyclic (Ginther and Kot 1994; de Castro et al. 1999; Schwarz and Wierzchos 2000; Schwarz and Wierzchos 2002; Cueto et al. 2006; Simoes et al. 2006; Nogueira et al. 2015) and 0.61–0.76 (mm/day) in acyclic (Schwarz and Wierzchos 2002; Nogueira et al. 2015) goats. The daily growth rates of the largest follicles were not different between the developing follicular waves (Table 1) and the mean daily growth of the largest follicles was 1.29 ± 0.07 (mm/days), which were similar to those earlier reported by researchers.

Considering different size follicles in our study, the pregnancy in the goats changed the ovaries' follicle activity (Figure 1). The numbers of large-sized follicles, especially between 16 and 26 days, decreased during the early pregnancy. On the other hand, no changes were observed in the numbers of small and medium-sized follicles in the same period, which were in agreement with some previous results recorded in pregnant Shiba goats (Kandiel et al. 2010). This difference in the numbers

of the large-sized follicles could have been caused by the high plasma P_4 concentrations that suppressed the basal secretion of the LH, as observed in this and other studies (Kandiel et al. 2010; Fatet et al. 2011). Furthermore, the LH is responsible for the final maturation changes of the antral follicles (Medan et al. 2003) therefore, a reduction in the number of large follicles during the pregnancy period may occur.

The first day of detection (5.00 ± 0.33 day) and diameter (6.8 ± 0.4 mm) of the CL recorded in this study is similar to those reported by Simoes et al. (2005). On the other hand, Orita et al. (2000), who used transrectal ultrasonography in goats, reported that the first day of detection and the diameter of the CL were 3.0 ± 1.6 days and 6.1 ± 1.3 mm, respectively. This difference could be due to the identification of the individual *corpus luteum* and the differences in the technologies used or the personnel.

The data presented in this study showed that ultrasonography can be reliably used in pregnant goats to study the ovarian structures (follicle and CL) and the growth of the ovarian follicles is characterised by a wave-like pattern until the 35th day of pregnancy. In pregnant goats, the number of follicular waves during the observation period ranged from five to seven with six waves being predominant. The identification and diameter of the CL post-breeding using the RTU were determined to be 5.0 ± 0.3 days and 6.8 ± 0.4 mm, respectively. During the early pregnancy period in our study, there was a significant difference between the largest follicles of waves 1 and 4, the duration of waves 1–4 and 7, the mean inter-wave interval of the 5th, 6th and 7th waves and the number of the largest sized follicle.

There is a relationship between the number of the follicle waves and the peaks of the plasma FSH. The number of the large sized follicles, influenced by the plasma inhibin concentrations, decreased over 35 days of the pregnancy period. Conversely, the regulatory effect of the inhibin on the FSH secretion, and the progesterone on the LH was continued during early pregnancy period in the Saanen goats.

Conflict of interest

The authors declare no conflict of interest.

<https://doi.org/10.17221/65/2019-VETMED>

REFERENCES

- Arashiro EKN, Viana JHM, Fonseca JFD, Camargo LSDA, Fernandes CAD, Brandao FZ. Luteal dynamics in goats: morphological and endocrine features. *R Bras Zootec.* 2010 Sep;39(9):1937-42.
- Bartlewski PM, Beard AP, Rawlings NC. Ultrasonographic study of ovarian function during early pregnancy and after parturition in the ewe. *Theriogenology.* 2000 Feb;53(3):673-89.
- Cruz JF, Rondina D, Freitas VJE. Ovarian follicular dynamics during anoestrus in Anglo-Nubian and Saanen goats raised in tropical climate. *Trop Anim Health Prod.* 2005 Jul;37(5):395-402.
- Cueto M, Gibbons A, Alberio R, Taddeo H, Gonzalez-Bulnes A. Timing of emergence of ovulatory follicles in polyovulatory goats. *Anim Reprod Sci.* 2006 Feb;91(3-4):275-84.
- de Castro T, Rubianes E, Menchaca A, Rivero A. Ultrasonic study of follicular dynamics during the estrous cycle in goats. *Theriogenology.* 1998 Jan;49(1):399.
- de Castro T, Rubianes E, Menchaca A, Rivero A. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. *Theriogenology.* 1999 Aug;52(3):399-411.
- Evans ACO. Characteristics of ovarian follicle development in domestic animals. *Reprod Domest Anim.* 2003 Aug;38(4):240-6.
- Fatet A, Pellicer-Rubio MT, Leboeuf B. Reproductive cycle of goats. *Anim Reprod Sci.* 2011 Apr;124(3-4):211-9.
- Ginther OJ, Kot K. Follicular dynamics during the ovulatory season in goats. *Theriogenology.* 1994 Nov;42(6):987-1001.
- Gonzalez-Bulnes A, Diaz-Delfa C, Garcia-Garcia RM, Urrutia B, Carrizosa JA, Lopez-Sebastian A. Origin and fate of preovulatory follicles after induced luteolysis at different stages of the luteal phase of the oestrous cycle in goats. *Anim Reprod Sci.* 2005 Apr;86(3-4):237-45.
- Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Reprod Sci.* 2004 Jul;82-83:461-77.
- Kandiel MMM, Watanabe G, Abdel-Ghaffar AE, Sosa GM, Abou-El Roos MEA, El-Azab AESI, Li JY, Manabe N, Taya K. Ovarian follicular dynamics and hormonal changes in goats during early pregnancy. *J Reprod Dev.* 2010 Oct;56(5):520-6.
- Kandiel MMM, Watanabe G, Abou El-Roos MEA, Abdel-Ghaffar AE, Sosa GA, El Azab AESI, Nagaoka K, Li JY, Manabe N, Taya K. Follicular turnover and hormonal association in postpartum goats during early and late lactation. *J Reprod Dev.* 2012 Oct;58(1):61-8.
- Medan MS, Watanabe G, Sasaki K, Sharawy S, Groome NP, Taya K. Ovarian dynamics and their association with peripheral concentration of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats. *Biol Reprod.* 2003 Jul;69(1):57-63.
- Medan MS, Watanabe G, Sasaki K, Groome NP, Sharawy S, Taya K. Follicular and hormonal dynamics during the estrous cycle in goats. *J Reprod Dev.* 2005 Aug;51(4):455-63.
- Menchaca A, Rubianes E. Relation between progesterone concentrations during the early luteal phase and follicular dynamics in goats. *Theriogenology.* 2002 Mar;57(5):1411-9.
- Mohammadi G, Kohram H, Gooraninejad S, Yousefi A, Motaghedi A. Ovarian follicular dynamics during the interovulatory interval in Najdi goats. *Afr J Mycol Biotechnol.* 2010 Aug;9(32):5236-9.
- Morand-Fehr P, Hervieu J, Santucci P. Body condition rating: Start writing [Notation de l'état corporel: a vos stylos]. *La Chèvre.* 1989;175:39-42. French.
- Nogueira DM, Cavalieri J, Gummow B, Parker AJ. Comparison of follicular dynamics and hormone profiles in Boer goats examined during the breeding and non-breeding seasons in the tropics of Queensland, Australia. *Small Rumin Res.* 2015 Apr;125:93-100.
- Orita J, Tanaka T, Kamomae H, Kaneda Y. Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. *J Reprod Dev.* 2000 Jan;46(1):31-7.
- Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. *Theriogenology.* 1984 Mar;21(3):495-504.
- Schwarz T, Wierzchos E. Wzrost pecherzykow jajnikowych w cyklu rujowym kóz [Growth of the ovary follicles in the oestrus cycle in goats]. *Med Weter.* 2000;56(3):194-7. Polish.
- Schwarz T, Wierzchos E. Pecherzyki jajnikowe kóz w okresie spoczynku plciowego [Ovarian follicles in goats during anoestrus]. *Med Weter.* 2002 Jan;58(8):620-2. Polish.
- Schwarz T, Wierzchos E. Wzrost pecherzyków jajnikowych kóz w okresach przejściowych między sezonem rozrodowym i spoczynkiem plciowym [Ovarian follicle growth in goats during the transition from breeding season to anoestrus, and from anoestrus to breeding season]. *Med Weter.* 2004 Aug;60(8):877-9. Polish.
- Schwarz T, Wierzchos E. The distribution of corpora lutea and ovarian follicular development in pregnant goats. *Reprod Biol.* 2010 Mar;10(1):53-66.
- Simoës J, Potes J, Azevedo J, Almeida JC, Fontes P, Baril G, Mascarenhas R. Morphometry of ovarian structures by transrectal ultrasonography in Serrana goats. *Anim Reprod Sci.* 2005 Feb;85(3-4):263-73.

<https://doi.org/10.17221/65/2019-VETMED>

- Simoes J, Almeida JC, Valentim R, Baril G, Azevedo J, Fontes P, Mascarenhas R. Follicular dynamics in Serrana goats. *Anim Reprod Sci*. 2006 Sep;95(1-2):16-26.
- Simoes J, Almeida JC, Baril G, Azevedo J, Fontes P, Mascarenhas R. Assessment of luteal function by ultrasonographic appearance and measurement of corpora lutea in goats. *Anim Reprod Sci*. 2007 Jan;97(1-2):36-46.
- Simoes J, Mascarenhas R. Origin and characterization of preovulatory follicles of hyperstimulated oestrous cycles in goats. *Acta Veterinaria Hungarica*. 2007 May; 55(2):259-66.
- Tenorio Filho F, Santos MHB, Carrazzoni PG, Paula-Lopes FF, Neves JP, Bartolomeu CC, Lima PE, Oliveira MAL. Follicular dynamics in Anglo-Nubian goats using transrectal and transvaginal ultrasound. *Small Ruminant Res*. 2007 Sep;72(1):51-6.

Received: May 27, 2019

Accepted: December 10, 2019