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Comparison and assessment of ovarian follicular dynamics during the breeding and non-breeding season in Saanen goats

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Summary

We describe and compare the ovarian follicular dynamics and plasma progesterone concentrations in nulliparous Saanen does during the breeding and non-breeding seasons. Does were monitored daily using transrectal ovarian ultrasonography for a full oestrous cycle in the breeding (n = 19) and a 21-day period in the non-breeding season (n = 20). At two-day intervals, blood samples were taken to determine plasma progesterone by ELISA. The pattern of follicular growth was characterised by four and five follicular waves in both seasons. Although the number of follicular waves was similar in the two seasons, the number of codominant follicles (p=0.027), the diameter of the largest follicle of waves 1 (p=0.036) and 4 (p=0.031), the duration of waves 3 (p=0.029) and 4 (p=0.034), the diameter of the largest follicle (p=0.042), the sums of the durations of all waves (p=0.037), the interwave interval (p=0.026) and the follicle growth rate (p=0.044) were higher in the breeding than in the non-breeding season. In addition, although the follicular waves in both seasons were similar, the number of codominant follicles (p=0.027), the diameter of the largest follicle of waves 1 (p=0.036) and 4 (p=0.031), the duration of waves 3 (p=0.029) and 4 (p=0.034), the diameter of the largest follicle (p=0.042), the sums of the durations of all waves (p=0.037), the interwave interval (p=0.026) and the follicle growth rate (p=0.044) were higher in the breeding than in the non-breeding season. In addition, the number of follicles, the diameter of the largest follicle of waves 1 and 4, the duration of waves 3 and 4, the diameter of the largest follicle, the sums of the durations of all waves, the interwave interval and the follicle growth rate

dition, the numbers of small, medium and large follicles and the total number of all follicles of all waves differed between the seasons (p=0.037). In the breeding season, we found significant differences between the waves in the diameter of the largest follicles (p=0.042) and the duration of the waves (p=0.037); in the non-breeding season, no significant differences were found. Ovulatory follicles were most common in the fifth follicle wave. In the breeding season, the mean inter-oestrus and inter-ovulatory intervals and the numbers of ovulations after a synchronized oestrus with $PGF_{2\alpha}$ were 21.47 ± 0.19 days, 20.47 ± 0.19 days and 1.84 ± 0.86. The results indicate that follicular growth in Saanen goats during the breeding and non-breeding season is characterised by a wave-like pattern that differs between seasons.

nisation mit $PGF_{2\alpha}$ 21,47 ± 0,19 bzw. 20,47 ± 0,19 Tage; die Anzahl der Ovulationen betrug 1,84 ± 0,86. Die Ergebnisse zeigen, dass das Follikelwachstum bei Saanenziegen während und außerhalb der Fortpflanzungszeit durch ein unterschiedliches wellenförmiges Muster gekennzeichnet ist.

Abbreviations: CL = *corpus luteum*; FSH = follicle-stimulating hormone; LH = luteinizing hormone; P4 = progesterone

Introduction

An understanding of the hormonal and physiological events that control the reproductive cycle in goats is necessary for assisted reproductive techniques such as oestrus synchronization and embryo transfer (TENÓRIO FILHO et al., 2007). In most breeds, reproductive behaviour is usually seasonal (NOGUEIRA et al., 2015) and annual variations of the photoperiod regulate reproductive behaviour (FATET et al., 2011). The relationship between environmental factors and reproductive behaviour is always important for the control of reproduction in goat management.

In the last three decades, transrectal ultrasound has been used as a non-invasive method for the early diagnosis of pregnancy and the screening of ovarian structures in small ruminants (BARTLEWSKI et al., 2000; KANDIEL et al., 2010). However, there have been only limited comparisons of follicular growth and regression dynamics in goats in the breeding and non-breeding seasons. Using daily trans-rectal ultrasonography, GINTHER and KOT (1994) first reported that ovarian follicular development occurred in wave-like patterns in goats during the breeding season. They also found that four follicular waves usually occur during an oestrus cycle in the goats. The results have been confirmed by other studies in the breeding season (De CASTRO et al., 1998, 1999; GONZALEZ De BULNES et al., 1999; SIMÕES et al., 2006) and in the non-breeding season or anoestrous period (SCHWARZ and WIERZCHOŚ, 2002; CRUZ et al., 2005; KANDIEL et al., 2012; SARATH et al., 2012), at different latitudes and under different environmental conditions. Sonographic images of the ovaries show that follicles grow to a diameter of ≥5 mm within 5 days and increase in diameter by an average of 0.8–1.1 mm per day throughout the oestrous cycle (MEDAN et al., 2005; SIMÕES et al., 2006) and the anoestrous period (SCHWARZ and WIERZCHOŚ, 2002; CRUZ et al., 2005). NOGUEIRA et al. (2015) reported differences in ovarian follicular dynamics during the non-breeding and breeding season in Boer goats in a tropical environment. We now report the characterisation and comparison of the ovarian follicular dynamics and plasma concentration of progesterone during the breeding and the subsequent non-breeding season in Saanen goats.

Materials and methods

Animals

The study was carried out at the Research and Application Farm of the Veterinary Faculty of Uludag University in Bursa, Turkey (40° 11´ North, 29° 04´ East, altitude 155 m) and involved 39 nulliparous and non-pregnant Saanen does. The experiment was conducted in two consecutive seasons, on 19 does in the natural breeding season (October; mean ratio of darkness to daylight hours, 12.75:11.25; average ambient temperature 17.0 °C) and on 20 does in the following non-breeding season (April; mean ratio of darkness to daylight hours, 10.7:13.3; average ambient temperature 16.0 °C). At the onset of the experiment, the mean (±SEM) age of the does in the natural breeding season was 19.58 ± 0.64 months, the mean body weight 45.79 ± 1.66 kg and the mean body condition 2.95 ± 0.76 (on a scale of 1 to 5; MORAND-FEHR et al., 1989). In the non-breeding season, the does were 12.70 ± 0.47 months old, weighed 38.08 ± 4.36 kg and had a body condition score of 2.98 ± 0.26. The does were kept indoors in sand/hay-floored pens with access to outdoors in a 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). Clean water and mineral salt were provided *ad libitum* and no changes were made during the experiment. The experiment was approved by the Animal Ethics Committee of Uludag University (Reference No. B.30.2.ULU.0.8Z.00.00/09).

Synchronization of oestrus and ultrasonography

In the breeding season, an oestrous cycle was synchronized with two intramuscular injections of 125 µg cloprostenol (PGS) at a 10-day interval. In these goats, trans-rectal ovarian ultrasonographic examinations were performed once a day from 24 h after the sec-

ond cloprostenol injection until a complete oestrous cycle of normal length was recorded. A B-mode ultrasound scanner (RTU, Prosound 2) connected to a 7.5 MHz linear transducer (model UST-660-7.5), designed for human prostatic examination, was used to monitor ovarian follicular dynamics in all does. Oestrous behaviour, from 24 h after the second prostaglandin injection until the termination of apparent oestrus, was checked twice a day with two aproned bucks. The day of standing heat was indicated as day 0 of the interoestrous interval. After the second cloprostenol administration, the does were considered in oestrus when they were stopped and mounted by the teaser bucks and the date was defined as the onset of induced oestrus. The day of ovulation was defined by the disappearance of follicles ≥5mm between two successive observations and was also accepted as day 0 of the inter-ovulatory interval. The ovulation rate in each goat was determined by the number of *corpora lutea* in the ovaries 3 or 4 days after ovulation. In the non-breeding season, ultrasonographic examinations were performed once a day for a 21-day period after the identification of follicular wave emergence (NOGUEIRA et al., 2015). During the examination, the goats were restrained in the standing position. The probe was moistened with hydrosoluble contact gel and gently inserted into the *rectum*. 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 (SIMÕES et al., 2005). All ultrasonography evaluations were performed by the same person.

Follicular wave analysis

After freezing the image of each ovary on the screen, the maximum diameter of all follicles ≥2 mm in diameter with spherical or oval conformation and smooth walls and the *corpus luteum* (CL) were measured with the scanner´s electronic caliper system. The measurement was carried out once per day throughout the test period and the ovary map was drawn on paper and compared with that of the previous day. The follicles were classified according to their diameter as small (2.0–3.4 mm), medium (3.5–4.9 mm) and large (≥5.0 mm). Follicles with a diameter of 4 mm on the first day of measurement were assumed to have had a diameter of 3 mm on the previous day (GINTHER and KOT, 1994). The following characteristics of the follicular waves were determined for each doe, as described (MEDAN et al., 2005; SIMÕES et al., 2006):

Follicular wave: Determined as a decrease in the number of small follicles and an increase in the diameter of one or more follicles, which then regressed or ovulated.

Onset of follicular wave: The first observation of at least one follicle ≥3 mm diameter, then after 3 days a growing follicular wave resulted in a follicle ≥5 mm diameter.

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

End of 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): The growth rate of each dominant follicle, calculated by dividing the diameter of the follicle by the number of days between its appearance as a follicle ≥3 mm and its maximum size.

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

Interwave 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 goat.

Codominant follicles: The number of two or more follicles (≥5 mm) in the same follicle wave (FATET et al., 2011; NOGUEIRA et al., 2015).

The last wave for each ovary was classified as an ovulatory wave during the breeding season. Incomplete waves in the non-breeding season were ignored. When waves of the two ovaries overlapped by at least ¾, this was counted as a single wave. When more than one ovulatory wave was formed, only the wave with the longest duration was considered.

Blood samples and progesterone assays

During the 21-day periods in which follicular dynamics were monitored in the breeding and non-breeding season, blood samples were collected every two days from the jugular vein into tubes (BD Vacutainer) containing lithium heparin. The blood was centrifuged at 3000 g for 10 min and plasma was separated and stored frozen (-20 °C) until assayed for progesterone (P4) with a goat progesterone ELISA kit (SunRedBio SRB-T-86624), with absorption read by an ELISA reader (ELX808IU Ultra Microplate Reader). The standard range of the progesterone kit was 0.5–8.0 ng/ml. The inter- and intra-assay precisions were measured and the coefficients of variation were 2.7 and 4.6 %. Plasma P4 levels of ≥1 ng/ml were taken as an indication of ovulation (THIMONIER, 2000; MENCHACA and RUBIANES, 2001, 2002).

Statistical analysis

Statistical analysis was conducted with SPSS for Windows, Version 20. The distribution of data was determined by the Shapiro-Wilk test and an appropriate statistical method was chosen to analyse the parameter considered. Analysis of variance (ANOVA) was used to compare the effects of season on the number of follicular waves, of codominant follicles, of ovulations and of small (2.0–3.4 mm), medium (3.5–4.9 mm) and large follicles (≥5 mm) as well as the maximum diameter of the largest follicle in each wave, the duration of a wave, the interwave interval and the growth rate of the follicles. Differences in means between seasons were compared using a paired t-test. Analysis of variance with repeated measurements was used to compare the mean total number of small, medium and large follicles, the total number of follicles and the plasma concentrations of P4 from days 1 to 21 between goats in the two seasons. Tukey´s test was used for multiple comparisons. The numbers of follicular waves in the breeding and non-breeding season were compared by Fisher's exact test (3rd, 7th waves) and the Pearson chi-square test (4th, 5th, 6th waves) with the data expressed as percentages. Body condition scores in goats were compared with independent samples test between seasons (1st and 3rd quartile, 2.5 and 3.0). The results are given as mean (± SEM) and differences were considered significant when the p value was below 0.05.

Sources of supply:

BD Vacutainer®, Becton Dickinson, Plymouth, UK; cloprostenol, PGS, ALKE, Istanbul, Turkey; ELISA reader ELX808IU Ultra Microplate Reader, BIO-TEK Instruments, Winooski, USA; goat progesterone kit, SunRedBio SRB-T-86624, Shanghai, China; SPSS for Windows, Version 20, IBM, Armonk, USA; Ultrasound probe UST-660-7.5 Hitachi Aloka Medical, Ltd., Tokyo, Japan; Ultrasound Scanner RTU, Prosound 2, Hitachi Aloka Medical Ltd., Tokyo, Japan.

Results

None of the does showed oestrus behaviour and ovulation in the non-breeding season. All the does in the breeding season showed oestrus after the second cloprostenol administration. The average plasma

concentrations of P4 in the breeding season showed a nearly linear increase from approx. 1 mg/ml on day 0 to 7.68 mg/ml on day 15 and then declined linearly to 0.71 ng/ml on day 21. In contrast, average plasma P4 concentrations in the non-breeding season did not exceed 0.79 ng/ml. There was no difference in body condition scores between the seasons.

Although the mean number of codominant follicles was greater in the breeding season (p=0.027), the mean number of follicular waves in the 21-day observation period was similar between seasons (Tab. 1). The most common numbers of follicular waves in the does were four and five in both seasons (Tab. 1).

The characteristics of follicular waves during both seasons are summarized in Table 2. The diameter of the largest follicles measured in waves 2, 3, 5 and 6 were similar in the two seasons. However, the diameter of the largest follicle in waves 1 (p=0.036) and 4 (p=0.031) in the breeding season was greater than that of the largest follicle in waves 1 and 4 during the non-breeding season (Tab. 2). In the breeding season, there were significant differences between wave 1 and waves 2 (p=0.023) and 5 (p=0.033), between wave 2 and waves 4 (p=0.034), 5 (p=0.031) and 6 (p=0.036) and between wave 3 and waves 4 (p=0.029), 5 (p=0.032) and 6 (p=0.036) in terms of the mean large follicle (Tab. 2).

In the breeding season, the first follicular wave was shortest and wave 3 the longest (p=0.021). There were also significant differences between the duration of wave 1 and waves 2 (p=0.037) and 3 (p=0.021), between wave 2 and wave 3 (p=0.041) and between wave 3 and wave 5 (p=0.023) (Tab. 2). The mean duration of the various follicular waves in the non-breeding season was similar, although the mean duration of waves 3 (p=0.029) and 4 (p=0.034) was longer in the breeding season than that in the non-breeding season (Tab. 2). The diameter of the largest follicles of all waves (p=0.042), the mean duration of all waves (p=0.037), the inter-wave interval (p=0.026) and the growth rate (p=0.044) of follicles were greater in the breeding season than in the non-breeding season (Tab. 2).

The number of small, medium and large follicles and the total number of follicles during 21 days of trans-rectal ultrasound examinations are given in Figures 1a, b, c and d.

Tab. 1: The number of follicular waves during the breeding and non-breeding seasons in Saanen goats / Anzahl von Follikelwellen während und außerhalb der Fortpflanzungszeit von Saanenziegen

Parameters	Breeding season (n = 19)	Non-breeding season (n = 20)
Follicular waves (n)	4.84 ± 1.58	4.95 ± 0.27
Codominant follicles (n)	1.47 ± 0.27[a]	0.65 ± 0.99[b]
Goats with 3 waves, % (n)	0.0	10.0 (2/20) (p=0.487)*
Goats with 4 waves, % (n)	31.58 (6/19)	25.0 (5/20) (p=0.648)‡
Goats with 5 waves, % (n)	52.63 (10/19)	40.0 (8/20) (p=0.429)‡
Goats with 6 waves, % (n)	15.79 (3/19)	10.0 (2/20) (p=0.589)‡
Goats with 7 waves, % (n)	0.0	15.0 (3/20) (p=0.231)*

[a,b] values between seasons differ significantly (p=0.027) / [a,b] statistisch signifikanter Unterschied zwischen den Saisonen (p=0,027)
* Fisher's exact test,‡ Pearson chi-square test

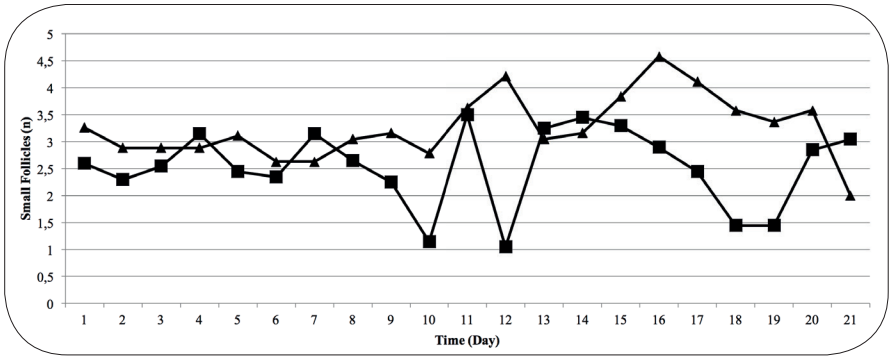

Abb. 1a: Daily changes in the number of small follicles (2.0–3.4 mm diameter) for 21 days in Saanen goats during the breeding (▲) and non-breeding seasons (■) / Änderungen der Anzahl kleiner Follikel (2,0–3,4 mm Durchmesser) während 21 Tagen bei Saanenziegen in (▲) und außerhalb (■) der Fortpflanzungssaison

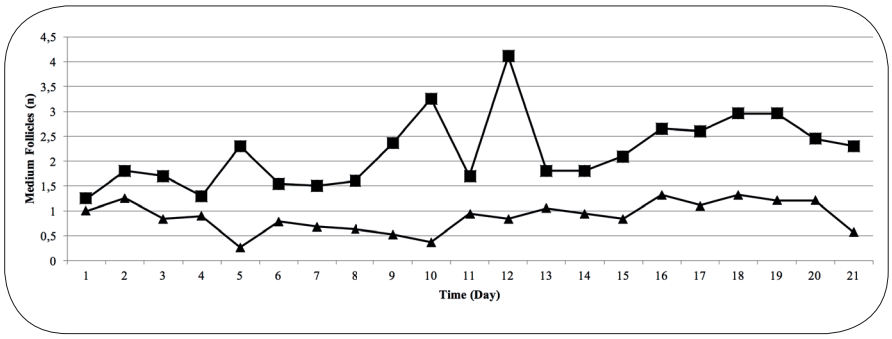

Abb. 1b: Daily changes in the number of medium follicles (3.5–4.9 mm diameter) for 21 days in Saanen goats during the breeding (▲) and non-breeding seasons (■) / Änderungen der Anzahl mittelgroßer Follikel (3,5–4,9 mm Durchmesser) während 21 Tagen bei Saanenziegen in (▲) und außerhalb (■) der Fortpflanzungssaison

Tab. 2: Characteristics of follicular waves during the breeding (n = 19) and non-breeding (n = 20) seasons in Saanen goats (mean ± SEM) / Charakteristika der Follikelwellen in Saanenziegen während (n = 19) und außerhalb der Fortpflanzungszeit (n = 20) (Mittelwerte ± Standardfehler)

Parameters	Breeding season	Non-breeding season
Average diameter (mm) of large follicles		
wave 1	5.51 ± 0.19^{aEF}	4.77 ± 0.11^{b} (p=0.036)
wave 2	4.48 ± 0.14^{C}	4.64 ± 0.12
wave 3	4.92 ± 0.16^{CF}	4.96 ± 0.16
wave 4	5.76 ± 0.25^{aDE}	5.09 ± 0.17^{b} (p=0.031)
wave 5	6.53 ± 0.23^{D}	4.92 ± 0.21
wave 6	6.75 ± 0.20^{DE}	5.50 ± 0.26
wave 7	0.0	5.07 ± 0.03
Duration of waves, days		
1	2.37 ± 0.34^{G}	3.10 ± 0.18
2	4.21 ± 0.46^{H}	3.65 ± 0.39
3	6.05 ± 0.59^{al}	3.80 ± 0.30^{b} (p=0.029)
4	4.68 ± 0.32^{aGHI}	3.50 ± 0.22^{b} (p=0.034)
5	3.77 ± 0.28^{GH}	3.15 ± 0.30
6	4.00 ± 1.15^{GHI}	3.60 ± 0.68
7	0.0	3.00 ± 0.58
Diameter (mm) of the largest follicle, all waves	6.85 ± 0.13^{a}	5.70 ± 0.12^{b} (p=0.042)
Duration of all waves (days)	4.24 ± 0.23^{a}	3.45 ± 0.12^{b} (p=0.037)
Interwave interval (days)	4.24 ± 0.23^{a}	3.11 ± 0.15^{b} (p=0.026)
Growth rate (mm/day)	1.00 ± 0.07^{a}	0.78 ± 0.36^{b} (p=0.044)

^{ab} different lower case superscript letters indicate significant differences between breeding and non-breeding seasons; ^{CDEF} different upper case superscript letters indicate significant differences between the mean of the largest follicles of the waves in the breeding season: 1st wave and 2nd (p=0.023), 5th (p=0.033); 2nd wave and 4th (p=0.034), 5th (p=0.031), 6th (p=0.036); 3rd wave and 4th (p=0.029), 5th (p=0.032), 6th (p=0.036); ^{GHI} different upper case superscript letters indicate significant differences between the mean duration of the waves in the breeding season: 1st wave and 2nd (p=0.037), 3rd (p=0.021); 2nd wave and 3rd (p=0.041); 3rd wave and 5th (p=0.023). / ^{ab} unterschiedliche hochgestellte Kleinbuchstaben zeigen statistisch signifikante Unterschiede zwischen den Saisonen an; ^{CDEF} unterschiedliche hochgestellte Großbuchstaben zeigen statistisch signifikante Unterschiede zwischen durchschnittlichen Durchmessern der größten Follikel der Wellen in der Fortpflanzungssaison an: 1. Welle zur 2. (p=0,023) und 5. Welle (p=0,033); 2. Welle zur 4. (p=0,034), 5. (p=0,031) und 6. Welle (p=0,036); 3. Welle zur 4. (p=0,029), 5. (p=0,032) und 6. Welle (p=0,036); ^{GHI} unterschiedliche hochgestellte Großbuchstaben zeigen statistisch signifikante Unterschiede zwischen der durchschnittlichen Dauer der Wellen in der Fortpflanzungssaison an: 1. Welle zur 2. (p=0,037) und 3. Welle (p=0,021); 2. Welle zur 3. Welle (p=0,041), 3. Welle zur 5.Welle (p=0,023).

The mean number of small, medium and large follicles and the total number of follicles differed between the seasons (p=0.037; Fig. 1a,b,c,d) and significant interactions were observed between all days and the breeding season for the number of medium follicles (p=0.030; Fig. 1b). However, the significant interactions between day and breeding season were only detected for the number of small follicles on days 16 (p=0.039) and 19–21(p=0.041) (Fig. 1a), for the number of large follicle on days 1–3 (p=0.036) and 5-6 (p=0.040; Fig. 1c) and for the number of total follicles on day 1 (p=0.043) (Fig. 1d). Table 3 presents some characteristics of the oestrous cycle in Saanen goats during the breeding season.

Discussion

We have compared the follicular dynamics and plasma progesterone concentrations during the breeding and the subsequent non-breeding season in Saanen goats. As reported (ORITA et al., 2000; SIMÕES et al., 2005), daily trans-rectal ultrasonography was an effective method for monitoring the ovarian follicular dynamics of goats. The number of waves varied between two and six, with four waves most common in the oestrus cycle (GINTHER and KOT, 1994; De CASTRO et al., 1999; SCHWARZ and WIERZCHOŚ, 2000; MENCHACA and RUBIANES, 2002; MEDAN et al., 2005; SIMÕES et al., 2006). Similarly, during the non-breeding season or anoestrus period a mean of four follicle waves occurred during the 21-day observation period (SCHWARZ and WIERZCHOŚ, 2000; CRUZ et al., 2005; NOGUEIRA et al., 2015). Although we found no significant difference between seasons, the mean number of follicular waves was greater in the non-breeding season (n = 4.95) than in the breeding season (n = 4.84; Tab. 1). The distribution of the number of follicle waves between seasons was similar to that reported by NOGUEIRA et al. (2015), who also found a higher number of follicle waves in the non-breeding season than in the breeding season. The findings are also consistent with those of others (MEDAN et al., 2005). We conclude that goats show a wave-like pattern of follicular development irrespective of season, as reported in cyclic (GINTHER and KOT, 1994; De CASTRO et al., 1999; MEDAN et al., 2003) and anoestrus (SCHWARZ and WIERZCHOŚ, 2002; CRUZ et al., 2005) goats.

We found significantly more codominant follicles in the breeding season than in the non-breeding season (p=0.027; Tab. 1). This agrees with NOGUEIRA et al. (2015). RUBIANES and MENCHACA (2003) and GONZALEZ-BULNES et al. (2005) also reported the presence of codominant follicles in follicular waves in goats. According to RUBIANES and MENCHACA (2003), the concept of codominant follicles in poly-ovulatory goats explains the presence of two large follicles in each wave. In our study, the ultrasound findings on the first day (Fig. 1a) showed more codominant follicles in the breeding season than in the non-breeding season, often resulting from the recruitment and selection of more small follicles. Endocrine and paracrine cells regulate the recruitment and selection of follicles by secreting gonadotropin and various growth factors (EVANS, 2003; HUNTER et al., 2004). In the early stages of follicular growth, gonadotropins such as LH and FSH are not required for follicular development but follicular growth at later stages is clearly dependent on gonadotropins, LH and FSH (HUNTER et al., 2004). In goats, the temporal relationship between the follicular dynamic and the hormonal profile has been demonstrated during the oestrus cycle (MEDAN et al., 2003, 2005). FSH is the main hormone controlling follicular growth in goats and its secretion is suppressed by inhibin and oestradiol secreted from growing follicles (MEDAN et al., 2005). When the negative-feedback effect of oestradiol on the hypothalamus-pituitary-ovarian axis is reduced, the number of co-dominant and ovulation follicles may be increased by enhancing the FSH concentration over the threshold level for longer (HUNTER et al., 2004). The development of follicles can also be influenced by insulin, insulin-like growth factors I and II and their binding proteins and environmental factors such as nutrition (HUNTER et al., 2004).

There were no significant differences between seasons in terms of the wave numbers (Tab. 1). We observed three to seven follicular waves within a 21-day period, most frequently five waves (Tab. 1), similar to the findings in other breeds of goat under different environmental conditions (GINTHER and KOT, 1994; De CASTRO et al., 1998, 1999; SCHWARZ and WIERZCHOŚ, 2002; MEDAN et al., 2003; SIMÕES et al., 2006). In Boer goats raised in the tropical environment,

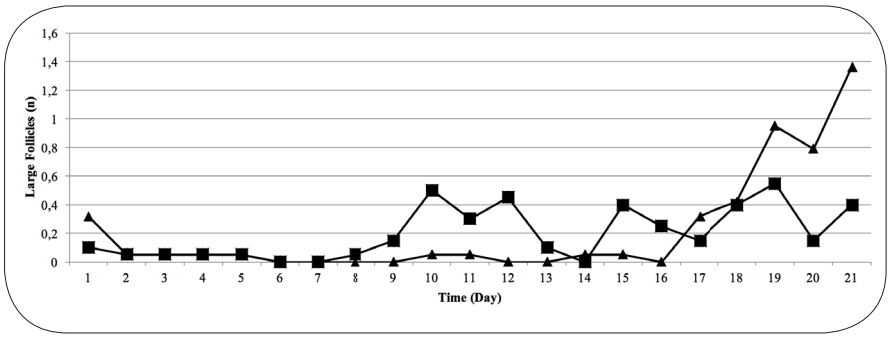

Abb. 1c: Daily changes in the number of large follicles (≥5.0 mm diameter) for 21 days in Saanen goats during the breeding (▲) and non-breeding seasons (■) / Änderungen der Anzahl großer Follikel (≥5,0 mm Durchmesser) während 21 Tagen bei Saanenziegen in (▲) und außerhalb (■) der Fortpflanzungssaison

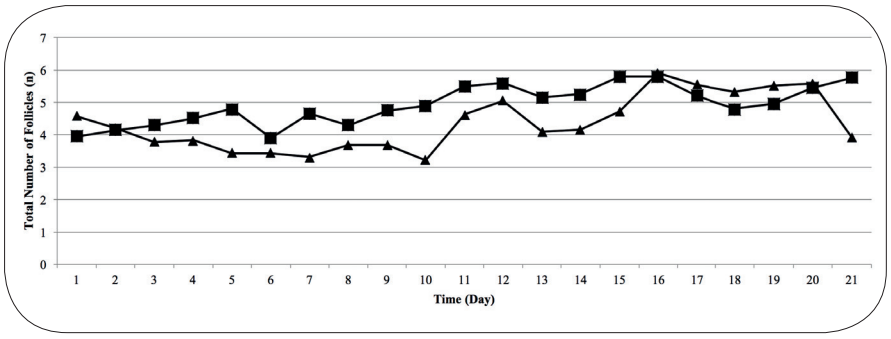

Abb. 1d: Daily changes in the number of total follicles (≥2 mm diameter) for 21 days in Saanen goats during the breeding (▲) and non-breeding seasons (■) / Änderungen der Gesamtzahl der Follikel während 21 Tagen bei Saanenziegen in (▲) und außerhalb (■) der Fortpflanzungssaison

Tab. 3: Some characteristics of the oestrous cycle of Saanen goats during the breeding season / Charakteristika des Östrus von Saanenziegen während der Fortpflanzungszeit

Variables	Mean ± SEM
Corpus luteum (CL) and ovulation after a synchronised oestrus with cloprostenol (n)	1.84 ± 0.86
Interovulatory interval (days)	20.47 ± 0.19
Interoestrus interval (days)	21.47 ± 0.19
Max. diameter of preovulatory follicle (mm)	6.65 ± 0.17
Emergence to ovulation of the ovulatory follicle (days)	4.21 ± 0.37
Growth rate of ovulatory wave (mm/day)	1.25 ± 0.16
Mean max. diameter of CL (mm)	11.62 ± 0.23
Lifespan of the CL observed by ultrasound (days)	15.26 ± 0.53
Mean max concentration of P4 (ng/mL)	7.68 ± 0.46
Day of max concentration of P4	16.89 ± 1.47

four waves in the breeding season and five waves in the anoestrus season were more frequent (NOGUEIRA et al., 2015). The difference could be related to the different breeds, to different environment and nutrition, to the age of the animals or to the frequency of release and amplitude of gonadotropic hormones between seasons.

The diameters of the largest follicles measured in waves 2, 3, 5 and 6 were similar in both seasons. However, the diameters of the largest follicle in waves 1 (p=0.036) and 4 (p=0.031) were significantly greater in the breeding season than in the non-breeding season (Tab. 2), which is in agreement with previous findings (GINTHER and KOT, 1994; SIMÕES et al., 2006). Similarly, NOGUEIRA et al. (2015) compared the largest follicle diameter of waves in the breeding and non-breeding season in Boer goats and reported that the largest follicle of waves 1 and 4 was larger than the largest follicles of waves 2 and 3. Although we found a significant difference between the diameters of the largest follicles within the waves during the breeding season (Tab. 2), the difference was not observed in the non-breeding season, which is in accordance with the findings of MEDAN et al. (2003), SIMÕES et al. (2006) and NOGUEIRA et al. (2015) in cyclic goats, although MEDAN et al. (2005) and MOHAMMADI et al. (2010) reported no difference between the diameters of the largest follicles among the waves during the breeding season. As in other ruminants, follicular growth in does is affected by endogenous progesterone (GONZALEZ-BULNES et al., 2005). Our observations support the negative effect of P4 on follicular growth (CHEMINEAU et al., 1988), which may be due to the differences in plasma levels of P4 at different stages of the oestrous cycle (GINTHER and KOT, 1994). This explanation is supported by the similarity in the largest follicle diameters during the non-breeding season.

We found almost identical durations of waves 1, 2, 5 and 6 in the two seasons but the third (p=0.029) and the fourth (p=0.034) waves were longer in the breeding season than in the non-breeding season (Tab. 2). Furthermore, wave 1 in the breeding season was significantly shorter than waves 2 (p=0.037) and 3 (p=0.021) and there were significant differences between the durations of waves 2 and 3 (p=0.041) and between waves 3 and 5 (p=0.023) (Tab. 2). Similar observations were reported by GINTHER and KOT (1994), De CASTRO et al. (1999) and NOGUEIRA et al. (2015). We found the fifth follicular wave in the breeding season to be the shortest (except for the first), probably because the fifth wave was the ovulatory wave in 52.63 % of the goats. The onset of luteolysis and an LH surge coincident with the development of the fourth (31.58 %) and fifth (52.63 %) waves may shorten the waves (De CASTRO et al., 1999; MENCHACA and RUBIANES, 2002). Our results differ from those of SIMÕES et al. (2006), who found similar wave durations during the breeding season in Serrana goats. The differences may relate to the effect of progesterone on follicular development during the oestrous cycle. High progesterone concentrations may reduce the time until the emergence of the next wave (TENÓRIO FILHO et al., 2007). During the non-breeding season, plasma progesterone concentrations were lower than 0.79 ng/ml and the duration of follicular waves was similar (Tab. 2), as noted by NOGUEIRA et al. (2015). The effect of gonadotropins on follicular development during the non-breeding season is the same as in the breeding season but the longer days in the non-breeding season reduce the secretion of melatonin in anoestrous sheep, thereby increasing the negative feedback of oestradiol (BARTLEWSKI et al., 2000), which inhibits the secretion of GnRH and reduces the pulse of LH secretion (FATET et al., 2011). Our findings in the non-breeding season suggest that this results from the small size and low growth rates of the follicles.

The largest follicle diameters were found in the breeding season (p=0.042; Tab. 2), in accordance with NOGUEIRA et al. (2015), who reported that the follicle diameter in anoestrus goats was maintained up to the ovulatory stage, although the follicle diameter was lower than in the breeding season. In the non-breeding season, the largest follicles were 6.6 mm in diameter in Anglo-Nubian goats and 6.8 mm in Saanen goats (CRUZ et al., 2005). There are reports of follicle diameters of 5.9 mm in non-ovulatory and 5.5 mm in ovulatory Shiba goats (ORITA et al., 2000), of 6.7 mm in non-ovulatory and 8.0 mm in ovulatory in Shiba goats (MEDAN et al., 2005), of 6.9 mm in Serrana goats (SIMÕES et al., 2007), of 7.3 mm in Boer x Feral does (BUKAR et al., 2012), of 7.3 mm in Beetal and of 6.9 mm in Teddy goats (RIAZ et al., 2013) during the breeding season, which are similar to the values we observed for in Saanen goats.

In agreement with the work of NOGUEIRA et al. (2015) in Boer goats, we found the average duration of all waves of goats in the breeding season to be longer than those of goats in the non-breeding season (p=0.037) and the average inter-wave interval was also longer (p=0.026; Tab. 2). This observation supports the idea that progesterone has a direct effect on both follicular turnover and the duration of the waves or the inter-wave interval. We observed consistently low plasma progesterone values in the non-breeding season, showing that the serum concentrations of progesterone, which are mediated by the control of LH, play an important part in the control of follicular turnover in the breeding season but not in the anoestrus period (MENCHACA and RUBIANES, 2002; RUBIANES and MENCHACA, 2003).

The average growth rate of follicles in goats is between 0.8 and 1.1 mm/day in the breeding season (GINTHER and KOT, 1994; De CASTRO et al., 1999; SCHWARZ and WIERZCHOŚ, 2000, 2002; CUETO et al., 2006; SIMÕES et al., 2006, 2007; NOGUEIRA et al., 2015) and from 0.61 to 0.76 mm/day in non-breed-

ing season (SCHWARZ and WIERZCHOŚ, 2002; NOGUEIRA et al., 2015). We found significantly more rapid follicle growth in the breeding season than in the non-breeding season (p=0.044; Tab. 2). This is in accordance with results in Boer goats (NOGUEIRA et al., 2015). The differences in the rates of growth of follicles between seasons might be caused by differences in LH secretion, as we and others have observed (GINTHER and KOT, 1994; NOGUEIRA et al., 2015).

We found significant differences in the numbers of small, medium and large follicles and the total number of follicles between goats in the breeding season and goats in the non-breeding season (p=0.037; Fig. 1a–d). The numbers of medium and large follicles in Boer goats did not vary between the breeding and non-breeding seasons: there were significant differences in the numbers of small and total follicles, although the causes are unknown (NOGUEIRA et al., 2015). Beetal goats have more small follicles than Teddy goats during the breeding season (RIAZ et al., 2013). Our (P4, 0.79 ng/ml) and previous work suggest that this may be due to the release of progesterone and gonadotropic hormones throughout the non-breeding season. High plasma P4 concentrations during the oestrous cycle suppress the basal secretion of LH, which is responsible for the changes in maturation of antral follicles until deviation. The dependence of the development of large antral follicles on FSH results in a decrease in the number of follicles (FATET et al., 2011), which might result in a reduced number of follicles in the non-breeding season, as we observed.

The inter-oestrous (21.47 ± 0 0.19 days) and inter-ovulatory (20.47 ± 0.19 days) intervals and the number of ovulations (1.84) after synchronized oestrus with cloprostenol in Saanen goats (Tab. 3) were similar to those observed in other breeds (SCHWARZ and WIERZCHOŚ, 2000; MENCHACA and RUBIANES, 2002; MEDAN et al., 2003, 2005; SIMÕES et al., 2006; BUKAR et al., 2012; RIAZ et al., 2013; NOGUEIRA et al., 2015) or in the same breed (De CASTRO et al., 1999). Our values for the mean maximum diameter of preovulatory follicles (6.65 mm) and the time of emergence to ovulation of the ovulatory follicle (4.21 days) were lower than those of SIMÕES and MASCARENHAS (2007) and NOGUEIRA et al. (2015) but our value for the growth rate of the ovulatory follicle (1.25 mm/day) was higher. The mean maximum diameter of preovulatory follicles reported by ORITA et al. (2000) in Shiba does during the breeding season was 5.5 mm, with MEDAN et al. (2005) giving a value of 8.0 mm. The mean maximum diameter of the CL (11.62 mm) that we found is similar to that of De CASTRO et al. (1999) and NOGUEIRA et al. (2015) but longer than that of SIMÕEA et al. (2007). The mean maximum progesterone concentration on the 16th day of the oestrous cycle in Saanen goats was similar to that reported by De CASTRO et al. (1999) and NOGUEIRA et al. (2015), who showed that the progesterone level started to decline on day 15 and reached basal levels on day 19 of the inter-ovulatory interval. There was a significant correlation between size of CL and plasma P4 levels in goats (SIMÕES et al., 2007).

Conclusions

There are seasonal differences in the follicular dynamics in the fourth and fifth waves in Saanen goats. The pattern of follicular dynamics is generally characteriswed by four waves in both seasons but the number of codominant follicles, the largest follicles of waves 1 and 4, the duration of waves 3 and 4, the diameter of the largest follicle, the duration of all waves, the inter-wave interval, the growth rate and the numbers of follicles of different sizes were significantly higher in the breeding season than in the non-breeding season. There were also differences in some other parameters. The results support the close relationship between follicle development and progesterone levels.

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Fazit für die Praxis:
Bei Saanenziegen erfolgt das Follikelwachstum während und außerhalb der Fortpflanzungssaison in meist vier Wellen. In der Fortpflanzungssaison sind aber die Follikel größer und auch die Zahl der kodominanten Follikel ist größer. Die Follikel können mittels transrektaler Sonografie untersucht werden.

References

BARTLEWSKI, P.M., BEARD, A.P., RAWLINGS, N.C. (2000): Ultrasonographic study of ovarian function during early pregnancy and after parturition in the ewe. Theriogenology **53**, 673–689.

BUKAR, M.M., YUSOFF, R., ARIFF, O.M., HARON, A.W., DHALIWAL, G.K., NAING, S.W., KHAN, M.A. (2012): Comparison of the patterns of antral follicular development between hormonally synchronized and natural estrous cycles of non-seasonal, polyestrous goats in the tropics. Reprod Biol **12**, 325–328.

CHEMINEAU, P., MARTIN, G.B., SAUMANDE, J., NORMANT, E. (1988): Seasonal and hormonal control of pulsatile LH secretion in the dairy goat *(Capra hircus)*. J Reprod Fertil **83**, 91–98.

CRUZ, J.F., RONDINA, D., FREITAS, V.J.F. (2005): Ovarian follicular dynamics during anoestrus in Anglo-Nubian and Saanen goats raised in tropical climate. Trop Anim Health Prod **37**, 395–402.

CUETO, M., GIBBONS, A., ALBERIO, R., TADDEO, H., GONZALEZ-BULNES, A. (2006): Timing of emergence of ovulatory follicles in polyovulatory goats. Anim Reprod Sci **91**, 275–284.

De CASTRO, T., RUBIANES, E., MENCHACA, A., RIVERO, A. (1998): Ultrasonic study of follicular dynamics during the estrous cycle in goats. Theriogenology **49**, 399 (Abstract).

De CASTRO, T., RUBIANES, E., MENCHACA, A., RIVERO, A. (1999): Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. Theriogenology **52**, 399–411.

EVANS, A.C.O. (2003): Characteristics of ovarian follicle development in domestic animals. Reprod Domest Anim **38**, 240–246.

FATET, A., PELLICER-RUBIO, M.T., LEBOEUF, B. (2011): Reproductive cycle of goats. Anim Reprod Sci **124**, 211–219.

GINTHER, O.J., KOT, K. (1994): Follicular dynamics during the ovulatory season in goats. Theriogenology **42**, 987–1001.

GONZALEZ De BULNES, A., SANTIAGO MORENO, J., GOMEZ-BRUNET, A., INSKEEP, E.K., TOWNSEND, E.C., LÓPEZ-SEBASTIÁN, A. (1999): Follicular dynamics during the oestrous cycle in dairy goats. Anim Sci **68**, 547–554.

GONZALEZ-BULNES, A., DÍAZ-DELFA, C., GARCIA-GARCIA, R.M., URRUTIA, B., CARRIZOSA, J.A., LOPEZ-SEBASTIAN, A. (2005): 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 **86**, 237–245.

HUNTER, M.G., ROBINSON, R.S., MANN, G.E., WEBB, R. (2004): Endocrine and paracrine control of follicular development and ovulation rate in farm species. Anim Reprod Sci **82–83**, 461–477.

KANDIEL, M.M.M., WATANABE, G., ABDEL-GHAFFAR, A.E., SOSA, G.A., ABOU-EL ROOS, M.E.A., EL-AZAB, A.E.S.I., LI, J.Y., MANABE, N., TAYA, K. (2010): Ovarian follicular dynamics and hormonal changes in goats during early pregnancy. J Reprod Dev **56**, 520–526.

KANDIEL, M.M.M., WATANABE, G., ABOU EL-ROOS, M.E.A., ABDEL-GHAFFAR, A.E., SOSA, G.A., EL AZAB, A.E.S.I., NAGAOKA, K., LI, J.Y., MANABE, N., TAYA, K. (2012): Follicular turnover and hormonal association in postpartum goats during early and late lactation. J Reprod Dev **58**, 61–68.

MEDAN, M.S., WATANABE, G., SASAKI, K., GROOME, N.P., SHARAWY, S., TAYA, K. (2005): Follicular and hormonal dynamics during the estrous cycle in goats. J Reprod Dev **51**, 455–463.

MEDAN, M.S., WATANABE, G., SASAKI, K., SHARAWY, S., GROOME, N.P., TAYA, K. (2003): Ovarian dynamics and their associations with peripheral concentration of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats. Biol Reprod **69**, 57–63.

MENCHACA, A., RUBIANES, E. (2001): Effect of high progesterone concentrations during the early luteal phase on the length of the ovulatory cycle of goats. Anim Reprod Sci **68**, 69–76.

MENCHACA, A., RUBIANES, E. (2002): Relation between progesterone concentrations during the early luteal phase and follicular dynamics in goats. Theriogenology **57**, 1411-1419.

MOHAMMADI, G., KOHRAM, H., GOORANINEJAD, S., YOUSEFI, A., MOTAGHEDI, A. (2010): Ovarian follicular dynamics during the interovulatory interval in Najdi goats. Afr J of Biotechnol **9**, 5236–5239.

MORAND-FEHR, P., HERVIEU, J., SANTUCC, P. (1989): Notation de l'etat corporel: a vos stylos. La Chevre **175**, 39–42.

NOGUEIRA, D.M., CAVALIERI, J., GUMMOW, B., PARKER, A.J. (2015): 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 Rum Res **125**, 93–100.

ORITA, J., TANAKA, T., KAMOMAE, H., KANEDA, Y. (2000): Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. J Reprod Dev **46**, 31–37.

RIAZ, H., AHMAD, N., YOUSUF, M.R. (2013): Ovarian follicular dynamics around estrus in Beetal and Teddy goats. Pak Vet J **33**, 120–122.

RUBIANES, E., MENCHACA, A. (2003): The pattern and manipulation of ovarian follicular growth in goats. Anim Reprod Sci **78**, 271–287.

SARATH, T., MEHROTRA, S., ARUNMOZHI, N., AGARWAL, S.K., HOQUE, M., MASHANKAR, U. (2012): Studies on follicular development and ovarian steroid profile in seasonal anestrus goats. Indian J Anim Reprod **33**, 6–8.

SCHWARZ, T., WIERZCHOŚ, E. (2000): Wzrost pęcherzyków jajnikowych w cyklu rujowym kóz. Medycyna Wet **56**, 194–197.

SCHWARZ, T., WIERZCHOŚ, E. (2002): Pęcherzyki jajnikowe kóz w okresie spoczynku plciowego. Medycyna Wet **58**, 620–622.

SIMÕES, J., ALMEIDA, J.C., BARIL, G., AZEVEDO, J., FONTES, P., MASCARENHAS, R. (2007): Assessment of luteal function by ultrasonographic appearance and measurement of corpora lutea in goats. Anim Reprod Sci **97**, 36–46.

SIMÕES, J., ALMEIDA, J.C., VALENTIM, R., BARIL, G., AZEVEDO, J., FONTES, P., MASCARENHAS, R. (2006): Follicular dynamics in Serrana goats. Anim Reprod Sci **95**, 16–26.

SIMÕES, J., MASCARENHAS, R. (2007): Origin and characterization of preovulatory follicles of hyperstimulated oestrous cycles in goats. Acta Vet Hung **55**, 259–266.

SIMÕES, J., POTES, J., AZEVEDO, J., ALMEIDA, J.C., FONTES, P., BARIL, G., MASCARENHAS, R. (2005): Morphometry of ovarian structures by transrectal ultrasonography in Serrana goats. Anim Reprod Sci **85**, 263–273.

TENÓRIO FILHO, F., SANTOS, M.H.B., CARRAZZONI, P.G., PAULA-LOPES, F.F., NEVES, J.P., BARTOLOMEU, C.C., LIMA, P.F., OLIVEIRA, M.A.L. (2007): Follicular dynamics in Anglo-Nubian goats using transrectal and transvaginal ultrasound. Small Rum Res **72**, 51–56.

THIMONIER, J. (2000): Détermination de l'état physiologique des femelles par analyse des niveaux de progesterone. INRA Prod Anim **13**, 177–183.