

## Seasonal Variations of Progesterone Level and Characteristics of Breeding Season and Anoestrus Period on Carpathian Goats

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**Abstract:** Reproductive seasonality is a major limitation in goat production on temperate and subtropical regions. The objective of this study was the characterization of the seasonal variations of progesterone level, length of oestrus cycles, breeding season and anoestrus period on Carpathian goats. The breeding season on Carpathian goats was observed between late Septembers to early March with peak of reproductive activities on November to February when day length was short. In breeding season the Carpathian goats presents the cyclic variations of progesterone concentrations. The anoestrus period starts in late March and finish in middle September.

**Key words:** Carpathian goats, ELISA tests, seasonality of reproduction, progesterone concentrations, seasonal anoestrus, Romania

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### INTRODUCTION

The goat breeding systems in Romania are organized according to the requirements of breeding technologies and from 1,094,000 goats of which 759,000 are reproduction goats. The Carpathian breed represents 90% of the breed structure in Romania. It is well adapted to the weather conditions but it represents a low milk production potential in comparison with Saanen and Alpine goats from EU countries (Zamfirescu *et al.*, 2009). Reproductive seasonality is a major limitation in goat production on temperate and subtropical regions (Delgadillo *et al.*, 2000). Various treatments have been proposed to control this phenomenon including male effect or photoperiod (Chemineau *et al.*, 1999). Seasonality is under photoperiodic control with long days inhibiting sexual activity and short days stimulating it (Bissonnette, 1941; Chemineau *et al.*, 1982; Maeda *et al.*, 1988; Valencia *et al.*, 1990; Maria *et al.*, 1990). In goats, reproductive patterns reflect the expression of a self-sustained endogenous rhythm which is synchronized or induced by photoperiod (Bondurant *et al.*, 1981). However, other environmental stimuli such as availability of food and social interactions (Restall, 1992; Restall *et al.*, 1995; Walkden-Brown *et al.*, 1993;

Mani *et al.*, 1996) should not be disregarded as potential regulators of seasonality of reproduction. The objective of this study was the characterization of the seasonal variations of progesterone level, length of oestrus cycles, breeding season and anoestrus period on Carpathian goats.

### MATERIALS AND METHODS

**Location and animals:** The studies were conducted at the experimental farm of ANCC Capirom and the biochemical determinations of progesterone concentrations were realized at the Ovidius University, Laboratory of Cellular and Molecular Biology. The Carpathian goats (n = 40) with body weights between 25-30 kg and age of 2-3 years were utilised in this study. In winter the goats constantly received 500 g/day/animal of ground barley and sunflower meal with free access to hay and water. The rest of the year the animals were fed with grassland feed and supplemented with 500 g corn grains day<sup>-1</sup>, received constantly in the morning and in the evening before and after the grassland feed. Vitamins were added as supplement powder to ration whereas minerals were given as sucking stones. The groups of goats were maintained in an intensive breeding system in closed and semi-

opened shelters which respect the farm norms (space/animal 1.5-2 m/goat, normal temperature conditions, 18-30°C in summer and 6°C in winter, <75% humidity). Goats were isolated from males during the experiment in different rooms, being separated by visual, olfactory and auditory stimuli coming from males.

**Determination of progesterone concentrations by ELISA**

**Method:** The plasma samples recovered from the experimental animals (n = 40) for determination of progesterone concentrations were processed between January 5th and November 9th, 2010. Seasonal variations in the cyclic ovulatory activity were studied by the analysis of plasmatic progesterone levels in blood samples collected twice a week at 9 o'clock in the morning in every Monday and Thursday of the week. The blood was recovered by jugular venipuncture with vacutainers (Ref. 360211, BD, Diagnostic, UK) and vacutests (Vacuum tubes, Italy, Li-heparin 95IU, 4mL, Ref. 12010). The blood samples were centrifuged at 1500 rot min<sup>-1</sup>, for 10 min. The plasma samples were stocked at -20°C. The progesterone concentrations were determinates by ELISA Method with DRG tests (DRG Instruments GmbH, Ref EIA-1561, human use, Germany). The reason to use the human ELISA tests was the sill of sensitivity very low of veterinary ELISA tests to detect the values very small of progesterone concentrations which characterized the anoestrus period. After take the advice of manufacturer was tested and adapted a method in the laboratory using the double sample for worked with human tests can be reading by the Magellan soft of ELISA System. The analytical sensitivity was calculated from the mean minus two standard deviations of twenty replicate analyses of standard 0 and was found to be 0.045 ng mL<sup>-1</sup>. The cross reactivity of the assay for progesterone was 100%. Cross reactivity with other steroids was <1.0%. The precision was determinate by the intra and inter assay variations. For three different progesterone standards (low: 0.5 ng mL<sup>-1</sup>; medium: 4.67 ng mL<sup>-1</sup> and high: 10.80 ng mL<sup>-1</sup>) intra and inter assay precision (CV%) were 5.4; 6.99 and 6.86 and 9.96; 4.34 and 5.59, respectively. The percent of recovery for progesterone has been calculated by multiplication of the ratio of the measurements and the expected values with 100 and was between 97.8-112% in function of measured and expected concentration. The percent of linearity was 92% for a 1:2 dilution of samples. The range of the assay is between 0-40 ng mL<sup>-1</sup>.

**Length of estrus cycle, reproduction season and anoestrus period:** The length of oestrus cycle was measured by the interval between two successive

expressions of oestrus. In function of progesterone concentrations observed were calculated the length of reproduction season and anoestrus period. The reproduction season was characterized by the cyclic variations of progesterone concentrations on goats. For appreciate the beginning of anoestrus period were considered minim successive three values of progesterone concentrations down to basal level (<1 ng mL<sup>-1</sup>) and the end of anoestrus season was considered the last value of progesterone level (<1 ng mL<sup>-1</sup>) before the goats to manifest the cyclic sexual activity.

**Statistical methods:** The plasma progesterone level, oestrus cycle duration, the number of oestrus cycles, lengths of breeding season and anoestrus were worked using analysis of variance (mixed model ANOVA/ANCOVA of GLM). Analysis of data was graphic represented (histograms, boxplot and simple linear regression) using the Smith's Statistical Package (SSP). Means with different superscript which are significant different was calculated with t-test (paired t-test for means of populations) with Statext v 1.4.2 package. We also examined the multiple linear regression between progesterone concentrations and normal oestrus cycles lengths, breeding season and anoestrus period lengths with Statext v 1.4.2 package.

**RESULTS AND DISCUSSION**

**Oestrus cycle:** The statistical measurements showed an effect of the season on oestrus cycles durations for entire of the year (p<0.001) and on number of oestrus cycles (p<0.05).

In breeding season the means of short and normal oestrus cycles on Carpathian goats were 13.99±0.96 days (range 12.3-14.8 days, Fig. 1) and 17.7±0.6 days (range

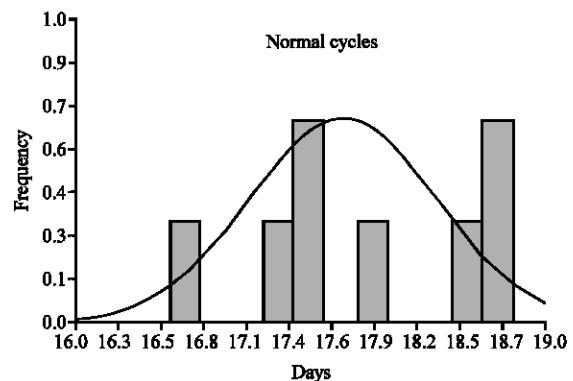


Fig. 1: The normal oestrus cycles duration (days) on Carpathian goats

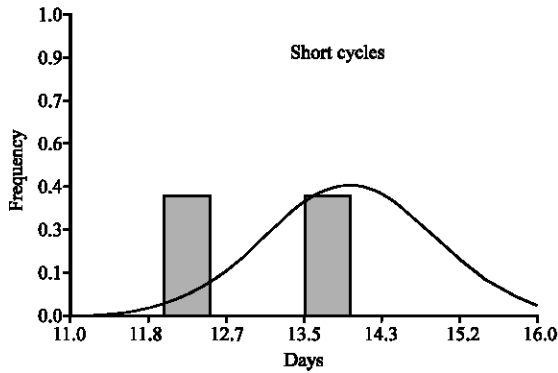


Fig. 2: The short oestrus cycles duration (days) on Carpathian goats

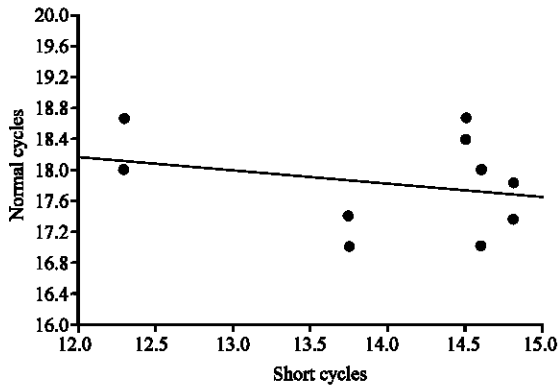


Fig. 3: Simple regression between oestrus cycles on Carpathian goat

16.75-18.66 days, Fig. 2). The mean of number for normal oestrus cycles was  $6.26 \pm 1.55$  (range 4-10 cycles) and for short oestrus cycles was  $4.6 \pm 1.07$  (range 3-6 cycles). Figure 3 shows the simple regression between normal oestrus cycles and short oestrus cycles on goats and the coefficient of regression for the independent variable was linear which shown a positive correlation between independent variable and dependent variable ( $Y = 0.829$ ).

**Seasonal variations of progesterone level on Carpathian goats:** Table 1 shows the progesterone levels (Means $\pm$ SEM) on throughout the study period. The statistical measurements showed an effect of the season on progesterone levels for entire of the year ( $p < 0.05$ ).

The progesterone concentrations on Carpathian goats between January and February were over than basal level and after than in March the progesterone level decreases. Towards on the end of spring and in early-middle summer was observed a significant decreasing of the progesterone level down to basal level.

On middle-late spring the progesterone concentrations down to basal level  $< 1 \text{ ng mL}^{-1}$  which

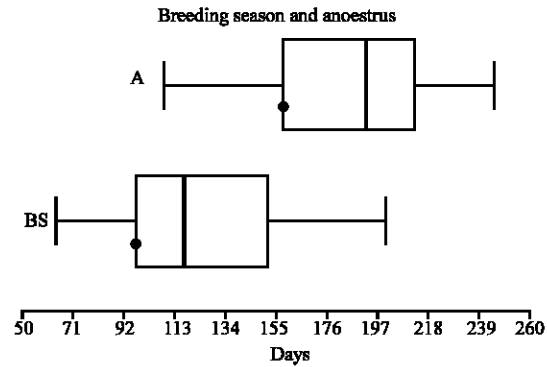


Fig. 4: The length of breeding season and anoestrus period (days) on Carpathian goats; BS: Breeding Season and A: Anoestrus period

Table 1: Progesterone concentrations on Carpathian goats

Months	Number	Means $\pm$ SEM	CV (%)
January	320	4.31 $\pm$ 0.24 <sup>a</sup>	72
February	320	4.18 $\pm$ 0.27 <sup>a</sup>	81
March	360	2.49 $\pm$ 0.22 <sup>d</sup>	119
April	360	0.45 $\pm$ 0.05 <sup>e</sup>	149
May	360	0.34 $\pm$ 0.02 <sup>e</sup>	79
June	320	0.77 $\pm$ 0.10 <sup>b</sup>	159
July	320	0.91 $\pm$ 0.13 <sup>b</sup>	181
August	360	1.16 $\pm$ 0.14 <sup>c</sup>	158
September	320	1.86 $\pm$ 0.21 <sup>b</sup>	144
October	360	3.68 $\pm$ 0.22 <sup>d</sup>	80
November	160	4.38 $\pm$ 0.72	92

<sup>a-d</sup>Means ( $\pm$ SE) with different superscript are significant different ( $p < 0.05$ )

characterized the anoestrus period. The progesterone concentrations increase up to basal level on late Summer. In September, the concentration of progesterone increased because the goats present the progesterone level up to  $1 \text{ ng mL}^{-1}$ . In September the goats manifests the cyclic sexual activity because begins the reproduction season. For October and November, the progesterone concentrations increased significant and all experimentally goats presents the cyclic sexual activity.

**Length of breeding season and anoestrus period:** The statistical measurements showed an effect of the season on length of breeding season and anoestrus period for entire of the year ( $p < 0.001$ ). The lengths of breeding season and anoestrus period on Carpathian goats were shown in Fig. 4.

The means ( $\pm$ SEM) of length of breeding season and anoestrus period for Carpathian goats were  $122 \pm 33.4$  days (range 64-200 days) and  $187 \pm 33.4$  days (range 109-245 days).

**Correlations:** Multiple linear regressions shown a positive correlation between progesterone concentrations (dependent variable), normal oestrus cycles length, breeding season and anoestrus period lengths

(independent variables). Coefficients of correlation for independent variables ( $R = 0.2443, 0.0461$  and  $0.2708$ ) determinate the value of dependent variable ( $Y = 0.8909$ ).

**Oestrus cycle:** The mean duration of the goat oestrous cycle varied in function of breed. For Carpathian goats during the breeding season recorded 75% normal cycles with 17-25 days and 25% were short cycles with 7-16 days. Another studies made on Alpine breed's presents the similar results with the results about the duration oestrous cycle on goats. The breeding season on Alpine goats recorded 77% cycles with normal duration between 17-25 days, 14% had short cycles with 8 days in average and 9% had long cycles with 39 days in average.

In breeding season, the means of short and normal oestrus cycles on Carpathian goats were  $13.99 \pm 0.96$  days and  $17.7 \pm 0.6$  days. Akusu and Ajala (2000) reported the mean of oestrus cycle length as  $18.3 \pm 1.6$  days on West African Dwarf goats. In Damascus goats, the length of oestrous cycle was reported as  $21.2 \pm 1.5$  days (Zarkawi and Soukouti, 2001). The length of oestrus cycle on Abadeh goats is  $19.7 \pm 1.1$  days (Emady *et al.*, 2006). The normal oestrus cycle duration between 17-25 days on goats was reported by researchers (Chemineau *et al.*, 1982; Pineda, 1989; Greyling, 2000; Khanum *et al.*, 2000).

The breeding season on Carpathian goats have a number of cycles per animal varied between 3 and 10 cycles. The mean of oestrus cycles number on Carpathian goats with normal oestrus cycles was  $6.26 \pm 1.55$  and for goats with short oestrus cycles was  $4.6 \pm 1.07$ . The studies made on another breeds presents the similar results with our results about the number of oestrus cycles. The Damascus goats had an extended breeding season with the number of cycles per animal varied between 7 and 10 cycles (Papachristoforou *et al.*, 2000). During the course of the breeding season, females can undergo several oestrous cycles successively and the number of successive cycles is dependent on the length of the breeding season and the breed of goat (Fatet *et al.*, 2010).

**Seasonal variations of progesterone level of Carpathian goats and the breeding season and the anoestrus period:**

Reproduction in goats has differences in seasonality between breeds and locations. Increasing duration of days starting from Winter to Spring reduces the reproductive activity to Carpathian goats. The progesterone level was lowest beginning with March to August. Decreasing duration of days starting from Summer to Autumn had the inverse effects, stimulated the progesterone level beginning with September. The progesterone level was the highest values between

October to February when the goats present the cyclic sexual activity. Assessment of progesterone levels during different physiological stages on goats is considered the most important parameter to determine the ovarian activity (Zarkawi and Soukouti, 2001). Progesterone concentrations play a pivotal role in determining reproduction efficiency (Mmbengwa *et al.*, 2009). The progesterone profile has been study for reproductive physiology of various animals including some breeds of goats like: Nubian (Fleming *et al.*, 1990), Dutch White (Van De Wiel *et al.*, 1991), Boer (Greyling and van Niekerk, 1991), Angora (Ozsar *et al.*, 1991), Saanen (De Castro *et al.*, 1999), Murciana-Granadina (De Bulnes *et al.*, 1999) and Damascus (Khadiga *et al.*, 2005).

The length of the breeding season on goats was different in function of factors like: photoperiod, climate, breed, physiological stage, presence of male, breeding system. Two distinct periods was observed for entire of the year in temperate regions: a period of anoestrus without oestrous behaviour and ovulations and the breeding period with oestrous behaviour and cyclic ovarian activity.

The breeding season on Carpathian goats was observed between late Septembers to early March (about 7 months) with peak of reproductive activities on November to February when day length was short. In breeding season on Carpathian goats presents the cyclic variations of progesterone concentrations. On middle-late Spring the progesterone concentrations on Carpathian goats down to basal level  $< 1 \text{ ng mL}^{-1}$  which characterized the anoestrus period. This period starts in late March and finish in middle September.

These results were in conformity with previous literature regarding the length of the breeding season on adult goats (Hafez, 1952; Trodahl *et al.*, 1981). On Damascus goats, the last ovulatory cycles of the season were recorded in February or March and the animals entered into seasonal anoestrus until the following Autumn (Mavrogenis, 1988).

The anoestrus period exhibit by Damascus goats during August as indicated by very low concentrations of progesterone was followed by a sharp increase of these values in September and the conclusion for this was the breeding season started in September (Zarkawi and Al-Masri, 2002). In temperate regions the breeding period was observed in the Autumn and Winter. In France, the breeding season starts in September when day length is declining and persists until March (Bodin *et al.*, 2007). In Australia, goats have a period with spontaneous ovulatory activity, from April to August with a peak in June (Restall, 1992). In the Alpine and local goats bred in

subtropical Mexico, the breeding season begins in the early Autumn and ends in the late Winter (Delgadillo *et al.*, 2004).

### CONCLUSION

In this study researchers can say that the Carpathian goats present seasonal variations of progesterone level like other breeds for temperate climates and the shorter days from Summer to Autumn were stimulants for reproductive activity and the longer days from Winter to Spring reduce or inhibit it. Because the photoperiod is the primary environmental element used to regulate reproduction the next studies will be about the effect of artificial treatments of photoperiod on sexual activity at Carpathian goats. These studies realized or future were required to understand various aspects of reproductive life of goats in function of breed and how can be modulated in economical interests.

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