

# SEASONAL VARIATIONS IN THE ACTIVITY OF CORPUS LUTEUM PERIODICUM ET GRAVIDITAS OF EWES

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*Plasma progesterone (P) levels were determined on the 5th, 8th, 14th and 16th day of the estrous cycle of nonpregnant and pregnant Tsigai ewes, during the breeding season as well as during anoestrus. Estrus was synchronized using intravaginal pessaires impregnated with 60 mg MAP for 14 days and 600 IU PMSG i/m at the removal of pessaires. P levels were significantly lower ( $p < 0.05$  or  $p < 0.01$ ) in the ewes during anoestrus, compared to the same category of ewes in the breeding seasons (pregnant/pregnant; nonpregnant/nonpregnant). Differences in the P levels between pregnant and nonpregnant Tsigai ewes during one seasonal period became apparent at the 16th day postcoitum.*

**Key words:** progesterone, estrous cycle, breeding season, anoestrus pregnancy, ewe.

## INTRODUCTION

Numerous studies have demonstrated that photoperiod is the primary environmental cue controlling seasonal breeding in the ewe (Robinson, 1959; Ducker et al., 1970, 1980a; Legan & Karsch, 1980). Changes in photoperiod control the annual reproductive cycle in the ewe by modulating the inhibitory actions of estradiol on tonic LH secretion (Goodman & Karsch, 1980). Whereas estradiol is critical for the events leading to seasonal breeding, many investigators began to attribute a major role to progesterone in models for the estrous cycle (Baird & McNailly, 1981; Karsch, 1980).

There are many methods for synchronization on estrus and ovulation in ewes (Colas, 1975; Killian, 1985; Lopez et al., 1988; Scaramuzzi et al., 1984; Pearce & Oldham, 1984; Foster et al., 1984). Regardless of the methods of synchronization, progesterone pretreatment is essential for subsequent luteal function (Colas, 1975; McLeod & Haresign, 1984; Gilbert et al., 1988). Killian et al. (1985) showed that there were no differences in duration of the estrous cycles synchronized either in the breeding season or in anestrus, while progesterone secretion was higher in anestrus.

Knowing that peak ovarian activity occurs about the middle of the breeding season (Robertson, 1977), this work was focused on the following

questions: 1) are there any differences in progesterone levels between corpus luteum periodicum formed in the breeding season and in anestrus (after induction of estrus), 2) are there any differences in progesterone levels in pregnant ewes in different seasonal periods (breeding season/anoestrus), 3) what is the earliest time when pregnant and nonpregnant ewes can be separated using the progesterone test?

#### MATERIALS AND METHODS

*Breeding season.* Eight multiparous ewes of the Tsigai breed were selected using a marked ram fitted with a device that prevented copulation. All ewes were in estrous, which was evident by their willingness to stand when mounted by the ram. Venous blood for progesterone assay was collected on the 5th, 8th, 14th and 16th day of the estrous cycle. At the next estrus those ewes were mated (introduction of ram for 24 h) and plasma progesterone was measured at the same time intervals. Ewes with plasma progesterone concentrations of  $> 1.59$  nmol/l on the 16th and 21st day after mating were considered to have an active corpus luteum and were classified as pregnant (6 of 8 ewes) (Robertson & Sarda, 1971; Thimonier et al., 1977; Langford, 1982).

*Anestrous season.* Ten multiparous ewes of the Tsigai breed were treated with intravaginal pessaries impregnated with 60 mg MAP for 14 days (Veramix, UpJohn) and 600 IU PMSG at the time of the pessary removal. 48 h after removal of the pessaries a fertile ram was introduced for 24 h. Venous blood for progesterone assay was collected on the 5th, 8th, 14th, 16th and 21st day of the induced estrous cycle, and ewes were separated into two groups (5 pregnant and 5 nonpregnant ewes), on the basis of high progesterone concentration ( $> 1.59$  nmol/l) on the 16th and 21st day postcoitum.

*Progesterone assay.* Plasma progesterone concentration was determined using commercial radioimmunoassay (RIA) kits (INEP-ZEMUN). Progesterone concentration was directly measured using a gamma-counter (GAMMACHEM 4800), and a spline function as the calculation method.

*Statistical analysis.* Two-way analysis of variance (Wiener, 1985) was used as the statistical method for determination of differences between plasma progesterone concentrations in different categories of ewes (pregnant and nonpregnant) in different seasonal periods (breeding season/anoestrus).

#### RESULTS AND DISCUSSION

Plasma progesterone concentrations during the estrous cycle of pregnant and nonpregnant Tsigai ewes in the breeding season and anoestrus are presented in Table 1. Our results showed that progesterone concentrations in the same category of ewes (pregnant or nonpregnant) were significantly lower during the anoestrus season. Only at the 14th day of

the induced estrous cycle, at the peak of luteal activity, did the progesterone concentration reach the level of estrous cycles of the breeding season, and even that concentration was somewhat lower than in the natural estrous cycle ( $5.74 \pm 1.35$ ;  $8.86 \pm 2.23$ , NS). Plasma progesterone concentrations during the natural estrous cycle of nonpregnant Tsigai ewes were within the range determined by other authors for different breeds (Yuthasastrakosol et al., 1975; Pearce & Oldham, 1985; Henricks & Mayer, 1977; Fitzgerald et al., 1985; Killian et al., 1985).

Table 1. Plasma progesterone concentrations ( $X \pm SE$  nmol/l) in pregnant and nonpregnant Tsigai ewes during the breeding season and anestrus

days	breeding season		anestrus	
	nonpregnant	pregnant	nonpregnant	pregnant
5.	$4.85 \pm 0.94^A$	$4.72 \pm 0.75^B$	$1.42 \pm 0.21^A$	$1.59 \pm 0.40^B$
8.	$7.74 \pm 1.35^C$	$9.32 \pm 1.03^D$	$3.19 \pm 0.44^C$	$5.01 \pm 0.12^D$
14.	$8.86 \pm 2.23$	$9.72 \pm 1.56^E$	$5.74 \pm 1.35$	$5.18 \pm 1.15^E$
16.	$0.29 \pm 0.03^G$	$9.34 \pm 1.60^{GF}$	$2.01 \pm 0.54^H$	$5.29 \pm 0.85^{HF}$

A, B, C, D, G —  $p < 0.01$

E, F, H —  $p < 0.05$

values with the same letter are statistically different

Luteal progesterone secretion is slightly higher at midcycle in breeds of ewes with higher ovulation rates than in those with low ovulation rates (Bindon et al., 1979; Quirke et al., 1979), and lower progesterone levels in anestrus ewes could originate from the small number of ovulated follicles. Evers (1988), using PMSG in a superovulation program found that, during the winter, superovulatory treatment yielded poorer ovarian response, regarding the number of corpora lutea; Killian et al. (1985), used short progestogen treatment and 750 IU PMSG and found that progesterone level was higher in anestrus ewes after synchronization than in the controls. Our results indicate that low plasma progesterone in synchronized estrous cycles in anestrus ewes derives from an inadequate dose of PMSG.

Early pregnancy diagnosis is essential for successful reproduction programs. Popovski et al., (1989) observed that RIA of progesterone could be used as a diagnostic method as early as the 18th day after insemination. Robertson & Sarda (1971), Thimonier et al., (1977) as well as Langford (1982) stated that ewes with progesterone levels above 1.59 nmol/l (0.5 ng/ml) on the 18th day after insemination should be considered pregnant. Our results showed that differences in plasma progesterone concentrations between pregnant and nonpregnant ewes during the same season are not evident until 16 days after mating.

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## SEZONSKE RAZLIKE U AKTIVNOSTI CORPUS LUTEUM PERIODICUM ET GRAVIDITAS OVACA

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### SADRŽAJ

Koncentracija progesterona (P) u krvnoj plazmi gravidnih i negradivnih ovaca rase Čigaja određivana je 5, 8, 14. i 16. dana estrusnog ciklusa u sezoni parenja i u toku anestrusa. U toku perioda anestrije indukcija i sinhronizacija estrusa je sprovedena aplikacijom intravaginalnih sundera natopljenih sa 60 mg MAP 14 dana i 600 IU PMSG (i/m) na dan uklanjanja sundera. Koncentracija P je statistički značajno ( $p < 0.05$ ) do vrlo značajno ( $p < 0.01$ ) niža u toku anestrusa, poredeći iste kategorije ovaca (gravidne/ /gravidne; negravidne/negravidne). Razlike u nivou P između gravidnih i negravidnih ovaca u istom sezonskom periodu nalaze se 16. dana od parenja.

