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SHORT COMMUNICATION

Hormonal Profile of Kari Ewes with Variable Gestation Length

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A B S T R A C T

A breeding trial under controlled farm conditions was carried out to estimate serum estrogen and progesterone spectrum over the entire gestation length in Kari ewes belonging to three subtypes. Kari is an exceptional breed within ovine species that gestates in variable length of period (85-154 days). Ewes were bred through hand mating, using rams from respective subtype based on the history of pregnancy duration (A:<110, B:111-130 and C:131-150 days). Upon lambing, the gestation length (GL) of ewes within each subtype was found at variance. Blood-serum sampling was carried out weekly, commencing after mating and continued until lambing. Samples collected from open ewes were discarded upon pregnancy confirmation examined through ultrasonography. The serum was analyzed for progesterone and estrogen profiles. The maximum progesterone concentration (24.2 ng/ml) observed in group C, during the 19th week of its gestation. Advancement in gestation boosted the peripheral blood progesterone concentration but in an undulating pattern. Serum progesterone at the start of pregnancy was at lower (2.1 ng/ml), reached at its peak (24.2 ng/ml) towards the end of gestation and dropped to (12.2 ng/ml) right after lambing. Maximum estrogen level (43.86 ng/ml) was observed on in group C, followed by B. Group A had the lower estrogen level with a minimal undulation throughout the gestation length. Estrogen was found fluctuating across the entire gestation period in ewes irrespective of their gestation length, which surged up towards the pregnancy termination.

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INTRODUCTION

Progesterone is one of the major hormones secreted before and during pregnancy from the *corpus luteum*, and later in pregnancy from the placenta. Among the major functions includes inhibiting FSH and LH and prolactin secretion. It does cause uterine growth and increases its sensitivity to oxytocin for facilitating birth, stimulating ductal system in the udder and softening the ligament of the pelvic girdle. Progesterone has a similar secretion pattern as of estrogen. In addition to slight suppressing the dam's immune system and muscular contractions for preventing foetus expulsion, it stimulates growth and glandular activity of the uterus. Being steroid in nature it is transferred to the foetus and is used for triggering foetal hormones at the foetal adrenal glands. Elevated progesterone concentrations during pregnancy, is one of the indicators for pregnancy diagnosis. This might be due to a functional corpus luteum during pregnancy or supplementary secretion from placenta, which occurs post-conception in sheep **(**Refsal *et al*., 1991).

Under field conditions, pregnancy is verified using the method of no return into succeeding oestrous. Research evidences suggest that these are poor indicator for pregnancy diagnosis (Senger, 1994). Increased belly size and palpation of foetal skeleton (skull) at the later stage of pregnancy do confirm successful conception.

Blood, urine and milk have successfully been used for pregnancy diagnosis is sheep (Ishwar, 1995). Efficiency of such diagnosis using progesterone may be as high as 90%. Progesterone concentration above 10 ng/ml in milk between 22 and 26 days after breeding may be classified as positive (pregnant). Single and multiple fetus also affect concentration of progesterone. Manalu and Sumaryadi (1998) reported that in Javanese thin tail sheep, progesterone concentration averaged 2.89±0.27 ng/ml in non-pregnant ewes and was more in ewes carrying multiple fetus as compared to those carrying

single fetus (18.56±1.55 vs 12.02±1.22 ng/ml). Differences were however, non-significant for first four weeks but became highly significant (P<0.01) from week 8 reaching to maximum levels of 23.24 for single and 37.24 ng/ml for multiple fetus pregnancies.

Kari is a newly discovered fine-wool, thin tail, mini breed, gestates in variable length of period (84-154 days). It dwells in the foot-hills of Hindukush situated at upper Chitral, Pakistan (Ahmad and Khan, 2010). Determination of progesterone levels during different physiological stages in animals is considered one of the important parameters of carrying the fetus (Dipyaman *et al*., 2011; Elgindy, 2011). Being the new discovery, the estrogen and progesterone profile in Kari ewes exhibiting variations in its gestation length was attempted in the current study. The article focuses on exploring weekly spectrum of oestrogen and progesterone hormones over the entire gestational length in Kari ewes groups exhibiting variable gestation length.

MATERIALS AND METHODS

Location and experiment plan: The experiment was carried out at Civil Veterinary Dispensary Shoghoor, Chitral, Pakistan. The village is situated at about 1800 meter a.s.l., located 15 km in the North-east of Chitral town, lies at the lower terminal of Karimabad valley, which is the narrow and steep valley. Animals grazed on the meadows situated on the mountains tops, and the mountain flanks and post-harvest fields in seasons around summer. During the entire winter, animals are stalled indoor and fed stored hay.

Animals were purchased at the advent of winter and were kept inside in the house during entire winter, fed Lucerne hay plus wheat straw daily with ample access to water and mineral licking blocks (Molasses Blocks). During summer, partial grazing was practiced on the mountain flanks and post-harvest fields. A total of 29 ewes and three rams, from all three subtypes i.e., small, medium and large were subjected to the trial. Ewes were kept for five months separate from the rams to confirm that all the ewes are non-carrier of fetus. Before initiation of the research the ewes dewormed and vaccinated against enterotoxaemia, contagious caprine pleuropneumonia and PPR diseases. All ewes were hand mated, and only seventeen conceived on the first attempt, which entered in to this trial. Two died in the course of the study whose data were discarded.

Blood sampling collection: Blood samples (5 ml) were collected from the jugular vein at weekly intervals for the determination of serum progesterone and estrogen throughout the gestation length. Samples were immediately centrifuged at +4ºC, 3000 x g for 15 min and serum was stored at -20ºC until analysis. A trail under controlled breeding program was performed at Chitral to determine the spectrum of estrogen and progesterone. Based on the gestation length of the ewes, they were grouped in to three groups i.e., A (<110), B (111-130) and C (131-150 days).

Hormonal analysis: Estrogen (Estradiol, E2) and progesterone, were estimated quantitatively using ELISA kit (Bio Check, Inc837 Cowan Rd. Burlingame, CA94010). The progesterone was determined using ELISA kit (Bio Check, Inc 323 Vintage Park Dr. Foster City, CA94404). The concentrations of standard progesterone samples were used to construct a standard curve ranging from 0.1 to 20 ng/ml. For progesterone and estrogen assay, the respective minimal detectable concentration were 0.15 ng/ml and 50.00 pg/ml. Data were analyzed frothier means with their scatter in terms of standard error.

Statistical analysis: Single factor analysis of variance was conducted for weekly data pertaining to estrogen and progesterone hormones on individual ewes (18 weeks x 2 hormones=36 analyses) to test the differences among three pregnancy groups and probability of rejecting the null hypothesis (of equal means) was presented.

RESULTS AND DISCUSSION

Weekly serum profile for progesterone across gestation for the three groups is plotted in Fig. 1) and their mean differences (F values) in Table 1. Mean progesterone concentrations had an increasing trend with the gestation progress. Progesterone level was higher in group C during the entire gestation length followed by the group B, except during week-1, -9 and -11. However, a slight drop was observed in week-13. The difference however, was very prominent during the early 8 weeks of gestation. Onwards from week-12 the difference continued widening until the termination of the respective pregnancies. Maximum progesterone concentration i.e., 24.2 ng/ml was observed in group C, on 19th week of gestation.

Advancement in gestation boosted the peripheral blood progesterone pool throughout the period but in undulating pattern. Serum progesterone at the start of pregnancy was at the lower level (2.1 ng/ml), reached at its peak (24.2 ng/ml) towards the end of gestation and dropped to lowest level (12.2 ng/ml) after lambing. Ewe with shorter gestation length had a lower profile with comparatively least fluctuation.

Mean serum estrogen in Kari ewes throughout gestation are plotted in Fig. 2, and the F value ANOVA in Table 1. Mean estrogen concentration was at the higher plane for group B and C than A. At the start of gestation, A and B has similar concentration but higher than A. However, at later stages, an increasing, although fluctuating, trend was observed in mean concentration for group C and A, contrary to B which remained almost flat during the entire gestation length. Estrogen level towards the termination showed a slight surge in group C whereas a drop was observed in group A.

Regulation of both progesterone and estrogen hormones secretion throughout gestation was complex; however, the amplitude of their secretory concentrations was to some extent related to the length of gestation. Progesterone is a steroid hormone secreted by the CL and later in gestation by the placenta, mainly responsible for maintenance of pregnancy. Blood secretory profile in Kari ewes was consistent with that recorded by workers **(**Boly *et al*., 1993). Progesterone concentration in ewes reaches a higher level on 6th day after ovulation, which drop in non-

Table 1: Probabilities* for variation in mean serum concentration of progesterone (ng/ml) and estrogen (pg/ml) in Kari ewes at different weeks of their gestation length

Week of Gestation	Progesterone	Estrogen
1	0.1338	0.0032
2	0.0000	0.0001
3	0.0001	0.0118
4	0.0000	0.0000
5	0.0000	0.0001
6	0.0058	0.0097
7	0.0000	0.0000
8	0.0003	0.0000
9	0.0508	0.0002
10	0.0476	0.0008
11	0.2025	0.0025
12	0.0278	0.0060
13	0.0070	0.0006
14	0.0714	0.0128
15	0.0098	0.0458
16	0.0436	0.0205
17	0.0004	0.0111
18	0.0000	0.0034
19		
20		
21		
22		

*Probability of rejecting the null hypothesis of equal means.

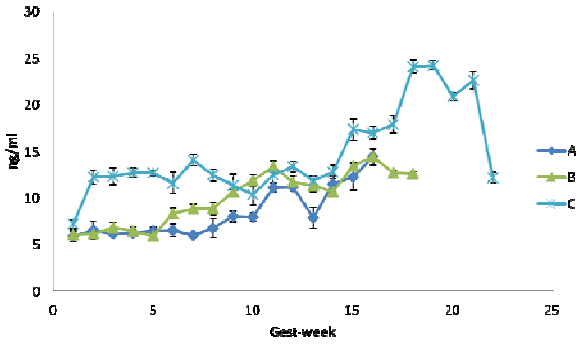

Fig. 1: Weekly serum progesterone concentrations in A, B and C group of Kari sheep across their entire gestation length.

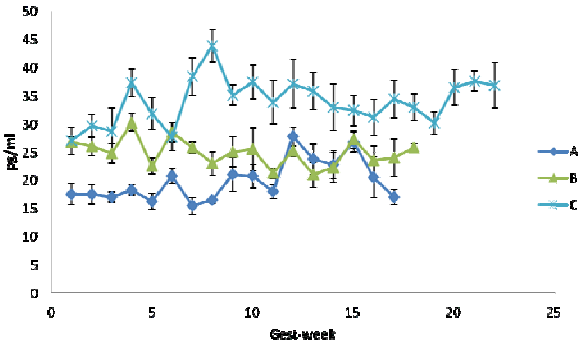

Fig. 2: Weekly serum estrogen concentrations in A, B and C group of Kari sheep across their entire gestation length.

pregnant ewes on day 16th and remain sustained in pregnant ewes (Strmsnik *et al*., 2002). The increase in progesterone at the later stage i.e., after 50 days of gestation, is attributed to additional secretion from extra-ovarian sources (placenta). Changes in estrogen secretion in Kari were consistent with the findings of Spencer *et al*. (2004).

The fluctuation of estrogen and progesterone was less pronounced in Group A, and was comparatively more erratic in the other groups. Variation in Progesterone concentration is affected by season, age of the animal, ovulation rates and the analytical method (Mitchell *et al*., 1999) in addition to stress factor (Dobson *et al*., 1999). This peculiar hormonal profile in Kari ewes could be attributed to aseasonal behaviour of ovarian cyclicity. The comparative profile for progesterone concentration after week-8 could serve as a possible indicator for predicting gestation length despite the fact that many factors that control the fluctuation of various pregnancy hormones are known (Bazer *et al*., 2012). More intensive studies may reveal the reasons for different gestation lengths on Kari sheep.

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