PLASMA PROGESTERONE PROFILE DURING GESTATION AND PERIPARTUM PERIOD IN CORRIEDALE SHEEP

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ABSTRACT

Fifty Corriedale ewes aged between 3.5-4.5 years, weighing 35-40 kg and maintained at Sheep Research Station, Srinagar under semi-intensive type of housing system were used. The ewes were tupped after proper estrus detection and the blood samples were taken fortnightly starting from day of tupping till 15 days post-tupping. The average gestational length was 149 ± 2 days. Plasma progesterone was estimated by Enzyme Immune Assay (EIA) using ELISA kit. On the day of tupping the mean plasma progesterone concentration was 0.65 ± 0.13 ng/ml and increased significantly ($P<0.05$) to a mean concentration of 4.0 ± 0.87 ng/ml at days 18.23 ± 0.78 of gestation. The progesterone concentration increased gradually up to days 76-90 followed by another significant rise ($P<0.05$) to 9.16 ± 0.79 ng/ml at days 91-105 and reached to a peak value of 14.06 ± 1.59 ng/ml at days 121-135 of gestation. A non-significant decline ($P>0.05$) in the progesterone concentration was observed during last two weeks of gestation and it reached below 1.0 ng/ml (0.35 ng/ml) 1-7 days after lambing. The estimation of plasma progesterone at day 18 after breeding served as a good means of pregnancy diagnosis with a sensitivity, specificity and accuracy of 100, 83.3 and 98 per cent respectively. The accuracy of the technique increased to 100 per cent at days 46-60 of gestation and remained constant till lambing. The discriminating value of progesterone for pregnancy was e" 1.75 ng/ml. It was concluded that estimation of plasma progesterone at an early stage of gestation could be applied at farm level for pregnancy diagnosis in sheep with a high accuracy.

Key words: Plasma Progesterone Profile, Gestation, Pregnancy diagnosis, Corriedale sheep

INTRODUCTION

Pregnancy and ovarian activity in sheep and goats could be monitored by determining changes in their blood or milk progesterone concentrations (Tyrel *et al.*, 1980). Plasma progesterone assay has been used for early pregnancy diagnosis in many species including sheep (Boscos *et al.*, 2003; Karen *et al.*, 2003). Early embryonic losses in sheep might occur due to peripheral progesterone insufficiency (Cumming *et al.*, 1971). Reproductive and production losses in the form of abortions, stillbirths and production of weak lambs can be reduced by separating the ewe flock into pregnant and non-pregnant groups (Wani *et al.*, 1998). Non-pregnant ewes can be sold thereby reducing feed expenses, while non-pregnant lambs can be marketed at higher price than they would bring as mature ewes (Gearhart *et al.*, 1988). Profitable and scientific sheep farming demands the accurate, rapid and safe methods of pregnancy diagnosis to minimize the production losses. Therefore, the present study was undertaken to characterize progesterone profile during gestation and peripartum period and to use progesterone assay as a means of early pregnancy diagnosis in Corriedale sheep.

MATERIALS AND METHODS

The study was carried out at Sheep Research Station of the University at Srinagar. From the main Corriedale flock of the station, fifty (50) ewes were

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selected for the study. The average weight of the ewes at the time of breeding was 35-40 kg, whereas their average age ranged between 3.5 and 4.5 years. The ewes were maintained under iso-managerial conditions, under a semi-intensive type of housing system. The study was started in the month of September-October, which is the season of breeding in sheep in Kashmir valley. The estrus was detected in ewes using Draminiski estrus detector (a waterproof device that measures electrical resistance of vaginal mucus. It consists of an inbuilt probe fitted with two electrodes, an LCD display and works on 9 volt battery). It works on the principle that during estrus the vaginal mucus has a minimum electrical resistance that can be measured by it. After estrus detection the ewes found to be in estrus were exposed to fertile Corriedale rams painted with coloured grease on their briskets under pen mating system. The day of tupping was recorded as Day 0 of pregnancy. The actual stage of gestation was confirmed by comparing with the lambing records and/or noticeable abortions. At lambing the weight of both dams and lambs were recorded. On the basis of body weight the ewes were divided into two groups *viz.*, Group A and B comprising of ewes of less than 35 kg and more than 35 kg, respectively. Correlation between the male and female lamb weights with the plasma progesterone profile of their mothers under respective groups was also studied.

Blood collection : Five ml of jugular blood was collected from the experimental ewes on days 0, 7 18, 30, 45, 60, 75, 90, 105, 120, 135, 150 post-tupping and 1-7 and 8-15 days post-lambing in sterilized heparinised test-tubes and transported to laboratory on ice. Soon after the collection, the blood was centrifuged at 3000 rpm for 15 minutes. The plasma was collected in sterilized plastic tubes and kept at -20ÚC until assayed for progesterone.

Progesterone estimation : Plasma progesterone concentrations were determined using competitive ELISA using Progesterone estimation kit (*EIAgen PROGESTERONE, Ref LI.4010.K, Adaltis Italia S.P.A).*

After comparing the progesterone results with the lambing records and/or noticeable abortions, sensitivity, specificity, prediction values (+PV and –PV) and the accuracy of the test were calculated as per Hanzen *et al.* (2000).

The indices were determined as:

Sensitivity (Se %): Accuracy of detecting pregnant ewes = (a/a+d) x100

Specificity (Sp %): Accuracy of detecting non pregnant ewes = (c/b+c) x 100

Positive prediction value (+PV %): probability of the presence of pregnancy in an animal diagnosed pregnant = (a/a+b) x100

Negative prediction value (-PV %): probability of absence of pregnancy in an animal diagnosed non-pregnant = (c/c+d) x100

Accuracy (%) = (a+c/a+b+c+d) x100 -Where a =No. of correct positive diagnoses, b=No. of false positive diagnoses, c=No. of correct negative diagnoses and d= No. of false negative diagnoses.

The statistical analysis of data was done as per Senedecor and Cocharan (1994).

RESULTS AND DISCUSSION

The average progesterone concentration at days 18.23±0.78 was 4.0±0.48 ng/ml in pregnant animals which differed significantly (P<0.05) from the progesterone level at day 0 (0.65 ± 0.13 ng/ml) and the level dropped to less than 1.0 ng/ml in the ewes that returned to estrus at days 16-30 (18.23±0.78) post tupping. The discriminating value of progesterone assay for pregnancy diagnosis was e"1.75 ng/ml when progesterone estimation was compared with lambing records and/or noticeable abortions. The average gestational length of the ewes was 149 ± 2 days.

With the advancing gestational stages the plasma progesterone level showed an increasing trend. The progesterone level increased significantly (P<0.05) to 9.1 ± 0.79 ng/ml at days 91-105 (99.66 ± 2.84) of gestation. This continuous increase in progesterone level might be due to synthesis and secretion from extra-ovarian source like placenta, which is additive and sufficient to maintain pregnancy, excluding that released from corpus luteum (Riera (1984). The placental progesterone appeared in dominating concentrations from days 87.83±1.27 of gestation, though it appeared

from the progesterone profile that placenta might be well functional at days 31-45 of gestation. It was conceived based on the findings that at days 31-45 the mean progesterone concentration (4.5±0.53 ng/ml) was more than the peak progesterone concentration during the luteal phase of estrous cycle (3.25±0.34 ng/ml). These findings were similar to Mukasa and Viviani (1992). The significant increase ($P<0.05$) in the progesterone concentration with the increase in the gestational stage appears to be due to sufficient growth of placental progesterone secreting tissue. The progesterone level reached to the peak level (14.06 ±1.59) during days 121-135 of gestation. A gradual and non-significant decrease in progesterone level (13.33±2.43) recorded during the last two weeks of gestation (140.67 ± 2.33 days) was in agreement with the reports of Mukasa and Viviani (1992) and Ranilla *et al.* (1994). This gradual decline in plasma progesterone concentration might be explained by a dramatic rise in the fetal cortisol levels during last 20-25 days of gestation, which occurs due to stressful conditions like hypoxia, hypercapnia, changes in blood pressure and blood glucose to which the fetus is exposed (Wood, 1999). The increased cortisol level activates the placental enzyme 17-α hydroxylase, which hydroxylates progesterone into estrogen in the placenta via androstendione (Liggins, 1982). After lambing the plasma progesterone levels decreased to basal levels of less than 1 ng/ml. The progesterone profile during the gestation of Corriedale ewes was similar to that reported by Mukasa and Viviani (1992), but was higher than the reports of Ranilla *et al.* (1994) in Churra and Merino sheep and Ranilla *et al.* (1997) in Assaf ewes. Estimation of plasma progesterone for pregnancy diagnosis in sheep on day 18 post-breeding using EIA and RIA has been reported (Gvozdic and Ivkov, 1994; Zarkawi *et al.*, 1999).

Out of 50 ewes 44 were pregnant, with 41 lambing successfully at term, one aborted at about 3 months and two suffered from stillbirths. The average weight of ewes and lambs in group A differed significantly ($P=0.03$ and $P<0.01$) from the average weight of ewes and lambs belonging to group B, respectively. However, the average progesterone concentration of group A ewes differed non-significantly from that of group B. No correlation was found between the birth weight of lambs and the progesterone production at any stage of gestation. Basset and Thorburn (1973) and Ranilla *et al.* (1994) reported a strong correlation, while as Mukasa and Viviani (1992) reported a low correlation between the progesterone production and birth weight of lambs. There was no correlation between progesterone production and the sex of lambs. Similar observation has been reported by Kalkan *et al.* (1996).

The accuracy of detecting pregnancy (100 %) and non-pregnancy (83.3 %) was same for days 15-30 and 31-45 of gestation. This observation is in line with the findings of Amezcua-Moreno (1988), Gvozdic and Ivkov (1994), Zarkawi (1997), Zarkawi *et al.* (1999) and Karen *et al.* (2003). Engeland *et al.* (1997) reported a sensitivity and specificity of 92 and 100 per cent respectively by using Radio Immuno Assay (RIA), while by using ELISA the corresponding values recorded were 82 and 88 per cent at day 20 after breeding. Boscos *et al.* (2003) also reported lower accuracy for pregnancy diagnosis (91.4%), while the predictive values for pregnancy and non-pregnancy were 98.3 and 85.3 per cent, respectively at day 19 after breeding. In contrast the present study showed an accuracy of 98 per cent, prediction values of 97.7 and 100 per cent for positive and negative diagnosis, respectively at days 18.23±0.78. There was no false negative case, but one false positive case was present at days 15-30 and 31-45. The false positive cases might be due to early embryonic deaths, uterine and/or ovarian pathological conditions like pyometra and hydrometra or luteal cysts (Ishwar, 1995). The measurement of progesterone concentration is a reliable indicator of the functional corpus luteum only and not always the pregnancy, which is the limitation of this assay. The disparity in the results may be due to the difference in the sensitivity and superiority of the kits for progesterone assay or the breed of the experimental animal used. The results of this study showed that estimation of plasma progesterone using ELISA at day 18 after breeding is a reliable means of pregnancy diagnosis with a sensitivity, specificity and accuracy of 100, 83.3 and 98 percent, respectively. The false positive diagnosis associated with the assay due to uterine and/ or ovarian pathological conditions or early embryonic deaths are a limitation to the assay.

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