EARLY PREGNANCY DIAGNOSIS IN CORRIEDALE SHEEP BY REALTIME B-MODE ULTRASONOGRAPHY

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ABSTRACT

Fifty Corriedale ewes were scanned in dorsal recumbency for pregnancy diagnosis using real-time B-mode ultrasonic equipment provided with a 3.5 MHz sector array transducer at 15 days interval starting from 15 days post tupping upto lambing through trans-abdominal approach. The accuracy, sensitivity, specificity and the prediction values of the technique were evaluated at the different stages of gestation, by comparing the ultrasonic results with lambing records and noticeable abortions. An accuracy of 68% and a sensitivity of 70.4% were observed at Day 27.0 ± 0.83 (15-30), which increased significantly ($P<0.05$) to 100% at day 63.2 ± 0.75 (day 61-75) of gestation. It was found that presence of echoic embryo within an anechoic fetal fluid, fetal cardiac activity, placentomes and developing fetal skeleton were the reliable indicators of pregnancy.

Key words: Early pregnancy diagnosis, Sheep, B-mode ultrasonography

INTRODUCTION

Early pregnancy diagnosis ensures economic rearing of sheep by an early culling or rebreeding of barren ewes (Goel, A.K and Agarwal, K.P, 1992) and there by feed expenses could be reduced and a high market price fetched by the salvage of non-pregnant ewe lambs than they would bring as mature ewes (Gearhart *et al.*, 1988). Reproductive losses in the form of abortions and still births would be reduced by providing a proper care to separated pregnant ewes from non-pregnant ones (Wani *et al.*, 1998). The commonest method for pregnancy diagnosis used in veterinary practice is the realtime B-mode ultrasonography (Karen *et al.*, 2001). However, a systematic work on real time B-mode ultrasonographic pregnancy diagnosis at various stages of gestation in sheep is meagre. The aim of our study was to diagnose pregnancy at an early stage by trans-abdominal real-time B-mode ultrasonography and to detect the gross visible changes occurring in the early conceptus and pregnant uterus for confirmation of pregnancy in corrieadale sheep.

MATERIALS AND METHODS

Fifty Corriedale ewes from the main breeding flock of the Sheep Research Station, Shuhama Srinagar, aged 3.5-4.5 years, weighing 35-40 Kg, subjected to standard immunoprophylactic and anthelmentic regimens and maintained under isomanagerial conditions were used in the study.

The study was started in the month of September-October, which is the breeding season in sheep under the temperate conditions of Kashmir valley. The estrus was detected in ewes using Draminiski estrus detector. This is a waterproof device that measures vaginal mucus resistance. The equipment consists of a 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. The estrus detected by the equipment was confirmed by parading a teaser ram. After estrus detection the ewes found to be in estrus were exposed to fertile corriedale rams smeared with

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coloured grease on their briskets. The ewes tupped were recorded based on the presence of colour marks on their backs and rump. The day of tupping was treated as Day 0 of pregnancy.

All the ewes were put to pregnancy diagnosis using trans-abdominal approach of realtime B-mode ultrasonography. For this an ultrasonic equipment (*Sonalisa 32, ultrasound Scanner, Larsen and Toubro Limited*) equipped with a 3.5MHz sector array probe was used. The lower abdominal region of the ewes was shaved before submitting them for scanning. The scanning procedure was started from Day 15 and repeated on Days 31, 46, 61 and 76 of gestation post tupping. The ewes were scanned in dorsal recombency restrained by the help of two attendants. An ultrasonic jelly was applied on the shaved area and the trans-abdominal probe was directed through the jelly to scan the lower abdominal regions of the ewes. The urinary bladder was taken as reference point to locate the uterus during the scanning process.

The ewes were diagnosed as pregnant based on the visualization of an enlarged uterine lumen with amniotic fluid, which appeared as an anechoic area near the anechoic zone of urine filled bladder. Pregnancy was also diagnosed on the basis of visualization of echoic embryo within the amniotic cavity, placentomes, and fetal skeleton.

Based on the lambing records and /or noticeable abortions, the sensitivity, specificity, positive and negative prediction values and the accuracy of the technique were calculated at different stages of gestation. The indices were determined as per the method described by Hanzen *et al.* (2000).

Accuracy = (a+c/a+b+c+d) x100

Sensitivity (Se %) = (a/a+d) x100

Specificity (Sp %) = (c/b+c) x 100

Positive prediction value (+PV %) = (a/a+b) x100

Negative prediction value (-PV %) = (c/c+d) x100

-where a= number of correct positive diagnosis, b= number of false positive diagnosis, c= number of correct negative diagnosis and d= number of false negative diagnosis.

The results were statistically analyzed by one way analysis of variance as per the methods described by Senedecor and Cochran (1994).

RESULTS AND DISCUSSION

Comparing the scanning results of the 50 animals studied with the lambing records, confirmed pregnancy and non-pregnancy were found in 44 and 6 ewes, respectively. Out of 44 pregnant ewes, 31 were correctly diagnosed as pregnant at day 15-30 (27.0 ± 0.83) of gestation, whereas 3 ewes were diagnosed as non-pregnant of the total 6 non-pregnant ewes during the same stage of gestation. The Se, Sp, +PV, -PV and the accuracy of the technique at day 15-30 was 70.4, 50.0, 91.2, 18.7 and 68 per cent, respectively and all the values reached to 100 per cent at day 63.2 (61-75) and thereafter remained constant till the last stage of gestation (day 136- lambing) [Table].These sensitivity and accuracy values at day 31-45 were significantly higher (P<0.05) than those at day 15-30. The sensitivity and accuracy (100%) at day 61-75 were significantly higher (P<0.05) compared to the values at earlier scanning stages. All the ewes could be correctly diagnosed for pregnancy and non-pregnancy from day 61-75 (63.2± 0.75) till the last stage of gestation studied (day 136-lambing).

The ewes were diagnosed as pregnant at day 15-30 post tupping based on the visualization of an enlarged uterine lumen that appeared as an anechoic area near

Fig. 1: 41 days old Pregnancy; Echoic embryo (arrow) visible within amniotic fluid

Fig. 2: 64 Days Pregnancy; C-shaped placentomes (Within circles)

Fig. 3: 75 Days Pregnancy; Head, neck and ribs (vertical arrow) visible

the urinary bladder. Fetal heart beat was also an indicator of early pregnancy and fetal viability, which was detected at an earliest at 28 day of pregnancy. During the gestational stage 31-45 (36.0 ± 0.46 day), the pregnancy was confirmed based on the visualization of an echoic embryo within the anechoic amniotic fluid. Similarly at day 46-60 of gestation, presence of an echoic embryo and placentomes served as the basis of pregnancy diagnosis. (Fig.1 and 2). After day 60, the fetal skeleton became visible and appeared as hyper echoic areas. (Fig. 3).

Table 1 : Sensitivity, Specificity, Prediction values (+PV, -PV) and Accuracy of trans- abdominal B-mode ultrasonography for Pregnancy diagnosis in Corriedale sheep.

Stage of gestation Accuracy% (days)	No. of ewes	Average Gestational age (days)	a	b	c	d	Se %	Sp %	+PV %	-PV %	
15-30	50	27.0±0.83	31	3	3	13	70.4[a]	50.0	91.2	18.7	68.0[a]
31-45	50	36.0±0.46	35	2	4	9	79.5[b]	66.6	94.6	30.7	78.0[b]
46-60	50	51.5±0.56	42	1	5	2	95.4[c]	83.3	97.7	71.4	94.0[c]
61-75	50	63.2±0.75	44	0	6	0	100[d]	100	100	100	100[d]
76-90	50	83.3±0.95	44	0	6	0	100[d]	100	100	100	100[d]
91-105	50	95.5±0.99	44	0	6	0	100[d]	100	100	100	100[d]
106-120	50	107.4±1.62	44	0	6	0	100[d]	100	100	100	100[d]
121-135	50	129.0±1.84	44	0	6	0	100[d]	100	100	100	100[d]
136-lambing	50	139.2±1.41	44	0	6	0	100[d]	100	100	100	100[d]
Over all	50	80.23±1.04	41.33	0.66	5.33	2.66	93.9	88.87	98.16	80.08	93.33

*The values with different superscripts within a column differ significantly (P<0.05)

Pregnancy has been detected in sheep before day 30 of gestation by using realtime B-mode ultrsonography (White *et al.*, 1984; Taverne *et al.*, 1985; Davey, 1986). The lower accuracy and false negative diagnoses at day 15-30 might be attributed to the intrapelvic location of the conceptus in the first month of pregnancy and the smaller size of conceptus that becomes difficult to visualize via trans-abdominal approach. During early pregnancy, the ovine conceptus has a filamentous structure with a small amount of fluid occupying both the uterine horns and the body of uterus (Buckrel *et al.*, 1986). As the gestation progresses the developing conceptus and the placentomes become ultrasonically visible thereby helped in diagnosing pregnancy more accurately. The rapid growth of conceptus after Day 30 that makes it more visible ultrsonographically may be the reason for significant increase in the sensitivity and specificity of the technique. The reason for the false positive diagnosis may be attributed to pyometra, hydrometra and early embryonic deaths which are highest between days 3-26 of pregnancy (Michels *et al.*, 1998).

The overall sensitivity (98.8%) during day 46-105 of gestation in the present study was similar to the reports of Fowler and Wilkins (1984); white *et al.* (1984) and Davey (1986). The results resembled with Tasal *et al.* (2006) who observed accuracies of 79.73%, 87.11% and 98.84%, respectively at days 36-46, 46-60 and 61-81 of gestation in Akraman Merino and Awassi breeds of sheep. Kahn *et al.* (1993) reported accuracy a sensitivity of 95% between days 22 and 90 post insemination. Kaulfuss *et al.* (1996) reported an accuracy of 80% by post insemination day 21 and 100% by day 60 in German Merino mutton sheep which is in agreement with the present findings. However a higher accuracy (87.1%) was reported by Yotov (2005) at day 27 post insemination in Satra Zagora dairy sheep breed, using 5MHz trans-abdominal probe.

Garcia *et al.* (1993) reported an accuracy of 85% at day 32 post tupping. Much higher accuracy (98%)

reported by Quintella *et al.* (1999) at day 23 post insemination using B-mode ultrasound might be due to use of trans-rectal approach. The variation in the accuracy of early pregnancy diagnosis in sheep using ultrasonic method might be attributed to the difference in the approach, frequency of transducer used and the experience of the investigator (Fowler and Wilkins, 1984; Karen *et al.*, 2001).

It is revealed from this study that trans-abdominal of real-time B-mode ultrsonography is a reliable, safe and practical means of early pregnancy diagnosis in corriedale sheep, with an accuracy of 68.0 % at days 27.0 ± 0.83, 94.0 % at days 51.5 ± 0.56 and reached to 100% at days 63.2 ± 0.75 of gestation. The visualization of conceptus, placentomes and detection of fetal cardiac activity were the most accurate criteria for diagnosis of early pregnancy.

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