

*Original Research***Accuracy of Real-Time B-Mode Trans-Rectal Ultrasonography for Early Pregnancy Diagnosis in Crossbred Ewes****Vinay Yadav^{1*}, R. K. Chandolia², Amarjeet Bisla³, Gitesh Saini¹, Ravi Dutt⁴ and L. C. Ranga⁵**

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Abstract

The concept of doubling the farmer's income in India can materialize early when recommended animal husbandry practices are adopted with a practical approach by the sheep farmers. One such practical and very useful technique is ultrasonography (USG) for early pregnancy diagnosis in ewes. In India, Sheep industry is mainly confined to small and marginal poor farmers with unavailability of cost effective technological aids to diagnose pregnancy early at the field level. The present study evaluates the sensitivity and specificity of trans-rectal USG to diagnose early pregnancy. Results of USG carried twice on day 25 and 45 post-mating on 60 crossbred ewes compared with the actual lambing data retrospectively. The accuracy, sensitivity, specificity, positive as well as negative predictive values for these tests were 91.7, 100, 78.3, 88.1, 100 per cent, respectively on day 25, and 100% on day 45 post-mating. Embryonic/fetal heart was also assessed using 6.0 MHz trans-rectal probe for confirming the pregnancy with viable embryo/fetus. The embryonic/fetal heart rate at day 25 (130.9 ± 0.86) and 45 (127.3 ± 0.81) post-mating were found to be significantly different ($p < 0.01$) with decreasing trend with the advancement of age. The relative difference in specificity of technique on day 25 and 45 post-mating was assumed to be due to embryonic/fetal losses confirmed by absence of heartbeat on day 45 post-mating. The study results indicate high accuracy of USG for pregnancy diagnosis as early on day 25 post-mating in sheep, and multiple assessments during the gestation period can be a means to detect embryonic/fetal losses.

Key words: Embryonic heart rate, Pregnancy diagnosis, Sheep, Trans-rectal B-mode USG**How to cite:** Yadav, V., Chandolia, R., Bisla, A., Saini, G., Dutt, R., & Ranga, L. (2019). Accuracy of Real-Time B-Mode Trans-Rectal Ultrasonography for Early Pregnancy Diagnosis in Crossbred Ewes. *International Journal of Livestock Research*, 9(10), 170-178. doi: 10.5455/ijlr.20190821041723

Introduction

The sheep animal husbandry is a source of economic returns for small and marginal farmers in India. The seasonality in reproductive behavior of sheep requires early pregnancy diagnosis for improving the reproductive efficiency in sheep herds. This in turn could be a means of separation of non-pregnant ewes from pregnant ones for their early marketing to reduce feed expenses and to fetch better market price at the earliest (Gearhart *et al.*, 1988). This shall also help to minimize the reproductive and productive losses in terms of abortion, stillbirths and birth of debilitated lambs (Wani *et al.*, 1998) by way of better management practices. Moreover, adoption of intensive sheep management by application of emerging assisted reproductive techniques like artificial insemination; and estrus synchronization out of breeding season necessitates accurate and early pregnancy diagnosis in sheep industry (Tavana *et al.*, 2014). Towards this goal the prevalent methods of pregnancy diagnosis like early pregnancy factor, progesterone assay, pregnancy associated glycoproteins (PAGs), and estrone sulphate are too laborious, invasive and require laboratory facilities which are impractical at field conditions (Goel and Agrawal, 1992).

The more accurate diagnosis of early pregnancy and other reproductive disorders could be made possible via use of diagnostic USG (Purohit, 2010). The integration of diagnostic USG in the small ruminants breeding practices could be advantageous for farmers, veterinarians and researchers (Jones and Reed, 2017). Real time B-mode trans-rectal USG has been recommended as an early mean of pregnancy diagnosis in sheep due to non-invasiveness, simplicity and rapid practical approach (Buckrell *et al.*, 1986; Cunningham and Marsh, 1997). Buckrell (1988) and Gracia *et al.* (1993) observed that the early pregnancy diagnosis in small ruminants could be done by detecting uterine changes, embryonic vesicle, embryo proper and heart rate for fetal viability.

The presence of uterus in the pelvic cavity during first 20-30 days of gestation in ewes makes the trans-rectal approach to be well suited for pregnancy diagnosis as compared to trans-abdominal approach, which also requires shaving of ventral part of body (Buckrell, 1988; Garcia *et al.*, 1993; Scherick and Inskeep, 1993; Amer, 2008). The Ovine pregnancy could be diagnosed at an earliest day 17-19 of gestation based on presence of anechoic intrauterine fluid by trans-rectal USG (5 MHz) while the embryo proper could be imaged between days 21 to 34 of gestation with placentomes visualization on day 26-28 (Buckrell *et al.* 1986; Garcia *et al.*, 1993).

Valasi *et al.* (2017) suggested that multiple observations during the gestation period using USG makes the management practices at sheep farms suitable as per gestation stage. Moraes *et al.* (2008) and Valasi *et al.* (2017) also detected early pregnancy in sheep on the basis of intrauterine fluid on day 14 and 21, embryos

on day 23 and 25, and heart rate on day 25 and 26. The objectives of the present study were to determine the earliest accurate diagnosis of pregnancy in crossbred ewes by means of Real time B-mode trans-rectal USG using a 6.0 MHz transducer and to test the sensitivity, specificity and accuracy of trans-rectal USG on day 25 and 45 post mating in comparison to lambing data.

Materials and Methods

A total of 60 crossbred ewes (Nali × Rambouillet) aged between 3-5 years, weighing 34-45 kg maintained at Central Sheep Breeding Farm, Hisar, Haryana, India were part of the present study. The institute is located at latitude 29° N and longitude 75° E with average elevation of 215 m from the sea level. The institute is located at the place where mainly continental climatic conditions are present with a significant annual variation in the temperature (summers and winters; 7-42 °C).

The animals were kept in-door at night and had access to natural grazing area for most of the day. Water and mineral licks were available ad libitum with animals reared under iso-managerial conditions. The ewes were subjected to natural mating and pregnancy diagnosis of the ewes was done using real time B-mode trans-rectal USG (6.0 MHz) on day 25 and 45 post-mating.

The food and water were not withheld from ewes before USG. During the examination, each ewe was placed in dorsal recumbency. A portable Scanner (Sonoscape S6, Portable USG machine, China) with a 6.0 MHz rectal transducer was used. The transducer was coated liberally with ultrasonic coupling gel consisting of carboxymethylcellulose. The transducer/probe was inserted into the rectum, 15 to 20 cm deep, to be able to scan the uterine horns. The surface of the transducer was oriented first towards the right ileum to scan the bladder and thereafter it was rotated 120° to 180° clockwise or counterclockwise across the uterine horns to scan the entire pelvic region in each ewe (Schrick and Inskeep, 1993). The entire procedure took approximately 5 minutes to complete.

The pregnancy was diagnosed, using one or more of following criteria: luminised and fluid filled uterine horns, embryonic vesicles, embryo proper, fetal pole or body of the fetus, embryonic/fetal heartbeats and placentomes, depending on the status of pregnancy. Embryonic/fetal heart rate was also calculated both on day 25 and 45 post-mating. Ultrasonic results of day 25 post mating were recorded and then compared with results of trans-rectal USG at day 45-post mating and with actual lambing records. The accuracy, sensitivity, specificity, positive and negative predictive values were calculated as described by Taverne *et al.* (1985); Buckrell *et al.* (1986), and Gearhart *et al.* (1988) to assess the performance of the USG technique. Student's T-test was used to compare the fetal heart rate values at two-time interval.

Results and Discussion

According to the lambing data obtained from the farm, 37 ewes lambed out of 60 underwent natural mating. The results of ultrasonic examination on day 25 and 45 post mating were compared with actual lambing records and the criteria of assessment of performance are presented in Table 1.

Table 1: Real time B-mode trans-rectal USG findings on day 25 and 45 post mating in crossbred ewes

Parameter	Day 25 post mating (n=60)	Day 45 Post mating (n=60)
Diagnosed pregnant correct/TP (a)	37	37
Diagnosed pregnant incorrect/FN (b)	0	0
Diagnosed non-pregnant correct/TN (c)	18	23
Diagnosed non-pregnant incorrect/FP (d)	5	0
Diagnosed pregnant/TP+FP (a+d)	42	37
Diagnosed non-pregnant/FN+TN (b+c)	18	23
Total pregnant/TP+FN (a+b)	37	37
Total non-pregnant/FP+TN (c+d)	23	23
Total number of animals (e=a+b+c+d)	60	60

(TP, True positives; FN, False negatives; TN, True negatives; FP, False positives)

The recommendation of withholding of food and water 12 h prior to USG examination for better imaging (Buckrell, 1988) was not applicable in the present study similar to that observed by Dinc *et al.* (2001). Urinary bladder was the point of reference during trans-rectal USG.



Fig. 1: Pregnancy diagnosis on day 25 post-mating using trans-rectal USG (6.0 MHz) depicting fetal fluids (Anechoic) and embryo proper (Hypoechoic)



Fig. 2: Pregnancy diagnosis on day 45 post-mating using trans-rectal USG (6.0 MHz) depicting fetal fluids (Anechoic) and embryo proper (Hypoechoic)

The majority of pregnancies were detected based on presence of anechoic fluid *in utero*, embryonic vesicle and embryo proper both on day 25 (Fig.1) and 45 (Fig.2) post mating. The presence of anechoic fluid in the lumen of uterus was considered the first and foremost reliable indication of pregnancy (Gearhart *et al.*, 1988; Gonzalez-Bulnes *et al.*, 1998). The findings of present study were in accordance with Buckrell,

(1988); Meinecke-Tillman and Meinecke, (2007) and Gonzalez-Bulnes *et al.* (2010), who found that imaging of embryo proper, heartbeat of embryo and placentomes might be possible from day 18, 18-23 and 26-28 days of gestation onwards in sheep, respectively. The variation in the time of the detection of embryo proper, embryonic heartbeat, placentomes might be due to differences in frequency of scanning, breed, litter size, and skill of the operator (Fridlund *et al.*, 2013).

From the results of the Table 1, the characteristics i.e. sensitivity, specificity, accuracy, positive and negative predictive value (PPV and NPV) of the data was calculated for Real time B-mode trans-rectal USG on day 25 and 45 (Table 2). The combined sensitivity and specificity (Table 3) was calculated from results of Table 2 as per Parikh *et al.* (2008).

The sensitivity, specificity, PPV, NPV and accuracy of pregnancy diagnosis on day 25-post mating were lesser than on day 45 in the present study. These findings were consistent with other studies suggesting that with advancement in the gestation period the accuracy of pregnancy diagnosis increased via trans-rectal USG (Taverne *et al.*, 1985; Buckrell, 1988; Schrick and Indeeep, 1993; Meinecke-Tillman and Meinecke, 2007 and Ganaie *et al.*, 2009).

Table 2: Characteristics of Real time B-mode trans-rectal USG on day 25 and 45 post mating in crossbred ewes

Parameter	Day 25 post mating (n=60)	Day 45 post mating (n=60)
Sensitivity $(a/a+b) \times 100$	100	100
Specificity $(c/c+d) \times 100$	78.3	100
PPV $(a/a+d) \times 100$	88.1	100
NPV $(c/b+c) \times 100$	100	100
Accuracy $(a+c/e)$	91.7	100

The sensitivity, specificity, NPV and PPV with accuracy on day 25 and 45 post-mating were observed to be higher in the present study as compared to previous studies *viz.* Dinc *et al.* (2001); Ganaie *et al.* (2009). However Gearath *et al.* (1988) observed 100 % ability of diagnosing a non-pregnant animal but the conditional probability of pregnancy diagnosis was much lower even upto day 50 of gestation using 5.0 MHz trans-rectal transducer. The findings of Buckrell *et al.* (1986) supported the results of present study with good sensitivity and specificity of pregnancy diagnosis during 25-30 days of gestation. The overestimation of pregnancy on day 25 post-mating were consistent with the findings of Schrick and Indeeep (1993) with the absence of heart beat and embryonic vesicle on day 45 post-mating indicating the embryonic wastage. The reason for occurrence of more false positives might be due to early diagnosis of pregnancy or embryonic death (Fowler and Wilkins, 1984; Ley, 1985; Buckrell, 1988). The findings of the present study

indicated that early pregnancy diagnosis could also be a mean of assessment of late embryonic deaths occurring post maternal recognition of pregnancy (MRP).

Table 3: Combined sensitivity and specificity of Real time B-mode trans-rectal USG on day 25 and 45 post mating

Parameter	Value
Combined Sensitivity $\{1 - (1 - \text{sensitivity at day 25}) \times (1 - \text{sensitivity at day 45})\} \times 100$	100
Combined Specificity $\{1 - (1 - \text{specificity at day 25}) \times (1 - \text{specificity at day 45})\} \times 100$	100

The observed 100% combined sensitivity and specificity of trans-rectal USG on day 25 as well as 45 post mating in together were suggestive of multiple assessment of ewes during gestation. The multiple assessment of gestation in ewes via trans-rectal or trans-abdominal USG was also suggested by Buckrell (1988) and Kahn (1992). The multiple assessment of pregnancy in ewes using USG could be a mean of detection of embryonic viability, fetal number count, late embryonic or fetal wastage and impending dystocia (Buckrell, 1988 and Kahn, 1992).

The embryonic/fetal heart rate (beats per min) was also calculated using 6.0 MHz trans-rectal transducer (Fig. 3 and 4) and the values were found to be significantly different ($P < 0.01$) between day 25 and 45 post mating with decreasing trend i.e. 130.9 ± 0.86 on day 25 to 127.3 ± 0.81 beats per min on day 45 post mating (Fig 5). The findings of the present study regarding embryonic/fetal heart rate were observed to be different from the other studies where higher ovine fetal heart rates were observed by other workers (Aiumlamai *et al.*, 1992; Godfrey *et al.*, 2010). The decrease in fetal heart rate as fetal age increases was consistent with other reports in sheep (Aiumlamai *et al.*, 1992; Godfrey *et al.*, 2010). However, the difference in embryonic/fetal heart rate can be attributed by breed difference and by other climatic factors.

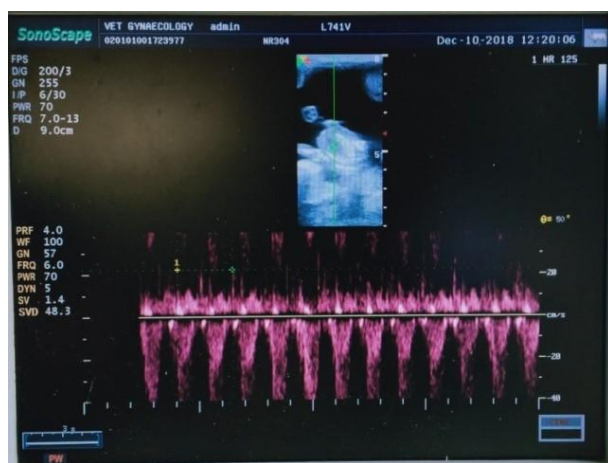


Fig. 3: Embryonic heart rate via Real time B-mode trans-rectal USG (6.0 MHz) on day 25 post-mating



Fig. 4: Fetal heart rate via Real time B-mode trans-rectal USG (6.0 MHz) on day 45 post-mating

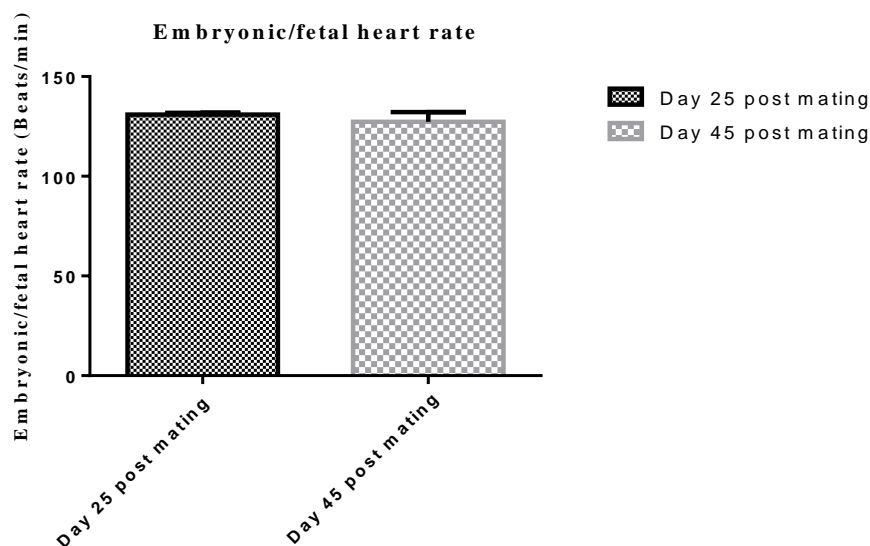


Fig. 5: Embryonic/fetal heart rate (beats/min) on day 25 and 45 post mating in crossbred ewes detected via Real time B-mode trans-rectal USG

Conclusion

It has been concluded that early pregnancy diagnosis could be done using Real time B-mode trans-rectal USG on day 25 post mating with high accuracy and good sensitivity and specificity. The multiple assessment of gestation via trans-rectal USG in sheep could solve the various mysteries and dilemmas of late embryonic and fetal losses in sheep. The early pregnancy diagnosis using trans-rectal USG could be a mean to increase the efficacy of various assisted reproductive techniques like estrus synchronization for improving reproductive efficiency in sheep.

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Conflict of Interest

The authors declare no conflict of interest.

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