ASSESSMENT OF FETAL AGE BY MONITORING FETAL GROWTH USING TRANSABDOMINAL ULTRASOUND IN NELLORE EWES

[1]K. JYOTHI*, [2]G. SUDHA, [3]K. MOULI KRISHNA, [4]A. SAHADEV, [5]M. NARAYANSWAMY, [6]SUGUNA RAO AND [7]M. A. KSHAMA

Veterinary Clinical Complex, Veterinary College, Hebbal, Bangalore,
*E-mail: jyothik100@gmail.com (*Corresponding Author)*

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ABSTRACT

Nellore Brown ewes (n=20) were subjected to short day estrus synchronization protocol and transabdominal ultrasonography was conducted weekly once from day 8 onwards until parturition. Pregnancy was determined after observation of fluid accumulation in uterine horns on day 22. The embryo was observed on day 29 in all ewes, head and trunk demarcation, fore and hind limb buds on day 36, heartbeat, umbilical cord, C shaped placentomes on day 43, ossification of vertebral column, rib cage, orbits, long bones of limbs, genital tubercle, rumen, brain ventricles on day 50, the four chambers of heart on day 57, diaphragm, eye lens, stomach chambers, major blood vessels on day 64, Kidneys, liver, urinary bladder, intestinal masses and lungs with bronchi on day 71, the trachea, jugular vein on day 99, the kidneys took bean shape with demarcation of cortex and medulla, placentomes hollowed out with hyperechoic borders and gastric chambers were observed on day 113. The time of organization of embryo, ossification of fetal bones and echogenicity of placentomes might help in judging the fetal age.

Key words: Nellore Brown ewes, Estrus Synchronization, Transabdominal ultrasonography, embryo, placentomes

INTRODUCTION

Fetal age estimation in sheep provides valuable information for late gestation management. Accurate mating date is usually unknown in most of the sheep flocks. Real time ultrasonography is useful in determining the duration of pregnancy. The suboptimal fetal growth detection in sheep will be useful for early detection and treatment of metabolic disorders, for correction of fetal growth retardation, for prevention of periparturient obstetrical problems and to avoid birth of weak lambs with reduced viability (Valasi *et al*., 2017).

MATERIALS AND METHODS

Nellore Brown ewes (n=20) aged 1-4 years reared under semi intensive conditions, with standard management conditions fed with greens, concentrates and adlib fresh water and salt licks were selected for the study. The ewes were subjected to estrus synchronization protocol for seven days using vaginal sponges and 300 IU of eCG (Folligon, Intervet International, Boxmeer, Netherlands) and 75 µg of $PGF_{2\alpha}$ (Cloprostenol; Pragma, Intas Pharmaceutica Limited, Matoda, Ahmedabad) intra muscularly at sponge withdrawal. Upon sponge removal the synchronized ewes were kept with a ram, color painted and each day ram was replaced with a new one with changed color paint at brisket region. The next day of receiving mating mark was treated as day 1 of pregnancy. Ultrasonography was conducted using a real time B mode scanning (Aloka SSD 500, Aloka Co Ltd, Japan) equipped with 5 MHz convex transducer on mated ewes. Serial transabdominal ultrasonographic examinations were carried out on weekly basis starting from day 8 of mating. Once embryonic vesicle was identified the pregnancy was confirmed and scanning continued weekly until end of parturition.

1 Assistant Professor, VGO; 3 Associate Dean, College of Veterinary Science, Proddatur, AP; 2 Associate Professor, 4 Professor, VGO, 5 Professor, Veterinary Physiology, 6 Professor, Veterinary Pathology, 7 Associate Professor

RESULTS AND DISCUSSION

A total of 387 ultrasound examinations were performed on 20 pregnant ewes starting on day 8 until parturition. Normal embryonic and fetal development shall help to supervise pregnancy for understanding and predicting the consequences of adverse conditions at various stages of pregnancy (Valasi *et al.,* 2017).

Accumulation of fluids in uterine horns was observed in many ewes by day 22. Imaging the fluid accumulation or embryonic vesicle characterized by the division of uterine lumen in many anechoic areas has helped to diagnose the early pregnancy. Contrary to the present findings, many authors (Karen *et al.,* 2009; Airina *et al.,* 2011; Kumar *et al.,* 2015; Valasi *et al.,* 2017; Santos *et al.,* 2018) observed embryonic vesicle earlier, while others recorded between days 25 - 28 of gestation (Padilla Rivas, 2005; Suguna *et al.,* 2008; Jones *et al.,* 2016). The variations might be due to effect of age, fasting and handling of the animals (Karen *et al.,* 2004), breed and probe differences (Suguna *et al.,* 2008) and interval of examination.

In this study embryo was observed for the first time on day 22 in one ewe and by day 29 in all other ewes as comma shaped embryo with amnion in some ewes. These findings were in agreement with Ali and Hayder

(2007), Kumar *et al.* (2015), and Valasi *et al.* (2017) who have first detected embryo between day 24-27. However, Melia *et al.* (2018) detected embryo earlier than this study (day 20) in goats. The variations might be due to type of transducer, method of ultrasonography used, frequency of scanning, breed, litter size and skill of technician (Fridlund *et al.,* 2013). Likewise, amnion was detected on day 28 and day 29 by Ali and Hayder (2007) and Valasi *et al.* (2017), respectively.

In the present study, placentomes were first observed as echogenic densities on day 22, nodule like convex, elevated structures on day 29 and 'C' like on day 36. These findings are in convergence with reports of Valasi *et al.* (2017). The first detection of placentomes between days 27 – 32 has been reported by many authors (Doize *et al.,* 1997; Kaufuss *et al.,* 1998; Kuru *et al.,* 2018). However, Kasiksi *et al.* (2011) and Santos *et al.* (2018) observed placentome earlier than present study, i.e. on day 25 and 21 in sheep, respectively.

On day 36, the embryo was demarcated into head and trunk in this study while, fore and hind limb buds were observed and movements of embryo also noticed in few ewes. These findings are in agreement with Ali and Hayder (2007) and Valasi *et al.* (2017). Anwar *et al.* (2008) also reported leg buds appearance on 36-40 days of gestation in ewes. On the contrary, Kumar *et al.* (2015) reported organization of fetal body on day 42 later than recorded in this study and Santos *et al.* (2018) in 4th week, earlier than that observed in this study.

In the present investigation, heart movements in the chest region and umbilical cord was first observed on day 36 and on day 43 in all fetuses. However, Padilla – Rivas *et al.* (2005), Karen *et al.* (2009) and Kumar *et al.* (2015) detected heart beat earlier than this study (19-24 days). While detection of umbilical cord was between 37-39 days by Kumar *et al.* (2015) and Valasi *et al.* (2017) which was in agreement with present study.

The orbits, mandible and nasal bone ossification was noticed on day 43 but ossification of head bones was not clearly visible until day 50 post conception. Present findings were in corroboration with Ali and Hayder (2007) and Kumar *et al.* (2015) who reported skull ossification between 42 – 48 days of gestation and differed from Valasi *et al.* (2017) who reported mandible and orbit walls ossification on day 36 and head bones ossification on day 56.

The fetus showed active fore and hind limb movements and more ossification of fore limbs on day 43, in this study. Karen *et al.* (2009) found fetal motility on day 38 post conception, however, Kumar *et al.* (2015) noticed the movements on day 48 along with chewing like movements, which were observed on day 92 in this study.

Identifying multiple fetuses with real time ultrasonography is a clear advantage over other techniques. In this study, three ewes carried twins and this was first identified on day 36 and clearly evident by day 43. Twins could be demarcated up to day 71, afterwards occasionally only detected depending upon the position of fetuses. At 70 days and beyond, additional fetuses may lie beyond the depth of the 5 MHz linear array transducer (Dawson, 2002). These findings were in parallel with Medan *et al.* (2004) and Airina *et al.* (2011) who declared that fetal counting was possible between days 35 - 70 of gestation. In this study, 5-7 weeks of gestation was the ideal period for differentiation between singles and twins (Suguna *et al.,* 2008) but Kasiksi *et al.* (2011) detected fetal numbers accurately at day 25 after insemination.

On day 50, ossification of skull, vertebral column, rib cage, long bones of hind and fore limbs, split hooves, genital tubercle, rumen, brain ventricles were recorded in this study. These findings were in consistent with Ali and Hayder (2007), Kumar *et al.* (2015) and Valasi *et al.* (2017). However, Anwar *et al.* (2008) detected vertebral column (day 45 -50) earlier than this study.

In this study, four chambers of heart on day 57, diaphragm, fore stomachs, aorta, posterior vena cava on day 64 and liver, intestinal loupes, lungs with bronchi on day 71, were observed. These findings differed with other authors who reported diaphragm, aorta and intestines on day 56 (Valasi *et al.,* 2017), stomach and liver on day 76 (Kumar *et al.,* 2015), intestinal loops on 12th week (Santos *et al.,* 2018). The time at which the maternal fetal anatomical structures identified may assist in both the estimation of gestational age and evaluation of embryonic and fetal development. The different timings of events that were described as compared with present study findings might have resulted from differences in the image quality, ultrasound equipment used, technique for obtaining images, frequency of examination or evaluation period and experience of the operator (Santos *et al.,* 2018).

Kidneys were first identified on day 71 in present study as elongated leaf like and which took bean shape by day 113 with clear demarcation of cortex and medulla. Ali and Hayder (2007) reported first visualization of kidney between 62 – 76 days (73.2 ± 6.3) and Valasi *et al.* (2017) estimated kidney length after day 77. Kumar *et al.* (2015) identified kidney and internal organs on day 76, however Santos *et al.* (2018) detected kidneys in 6th week of gestation itself.

Until day 85 the placentomes were uniformly hyper echoic, later placentome border became hyperechoic and in between hypoechoic in texture. The echogenecity of placentomes recorded in this study throughout pregnancy

were similar to that of Ali and Hayder (2007) in Ossimi sheep. These changes in the placentome may be attributed to increased placentome perfusion and permeability of tissues as the gestation advanced.

Variations between studies could be explained by the fact that different breeds of sheep studied (Kumar *et al.,* 2015) as well as many managemental systems prevailing in the world particularly of pregnant ewes and effects of individual differences. Differences between authors might be the effects of inability to establish precise timing of ovulation as well as fertilization. The blastocyst hatching appears to happen between 6th and 10th day after mating leading to variation of two to three days between individuals. Further, this may lead to variations in development of embryos during the embryonic life but in later gestation, after 40 – 50 days, they decrease and would be of little importance in estimation of gestational age of fetuses (Valasi *et al.,* 2017).

CONCLUSION

It may be concluded from this study that the time of embryo organization, ossification of fetal bones, fetal organs and echogenicity of placentomes might help in judging the fetal age.

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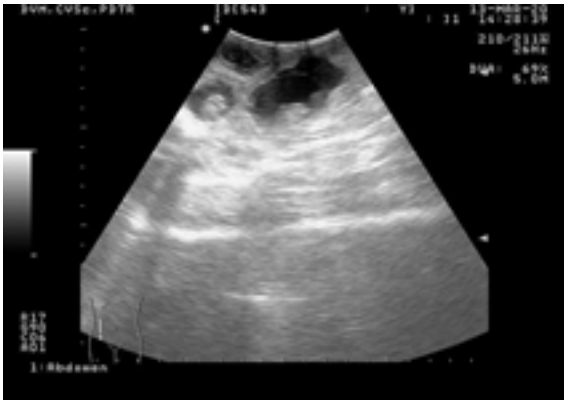

Fig 1: Twins (arrows) on day 36 of gestion in Nellore Brown ewes

Fig 2: Fetal heart (white arrow), rumen (arrow head), kidneys (black arrow) and intestinal mass (yellow arrow) on day 99 of gestation