APPLICATIONS OF TRANSABDOMINAL ULTRASONOGRAPHY IN BOVINE REPRODUCTION: A REVIEW

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ABSTRACT

The non-invasive nature of ultrasonography makes it an important clinical and research tool in bovine reproduction and obstetrics. Ultrasound techniques are becoming increasingly important in animal reproduction and obstetrics and these techniques are helpful in evaluation of various disease conditions of uterus during advance pregnancy in bovines. Accordingly, understanding the use of ultrasound technology is of utmost importance in animal sciences, since ultrasound examinations are now a routine part of diagnostic workups in reproduction and obstetrics. Up to now, most of studies were conducted to assess the status of fetal and uterine structures by trans-rectal ultrasonography, but sometimes it was found to be inefficient in judging the complete status of fetal and uterine structures during mid and advance pregnancy when the fetus is located deep into the abdominal cavity. So, in modern times transabdominal ultrasonography is getting popularity over trans-rectal ultrasonography in bovines during mid and advance gestation to determine the complete status of pregnant animals. The new information that has been generated by use of transabdominal ultrasonography has thrown light on diagnostic and research aspects and created new areas for research. Transabdominal ultrasonographic examination of the reproductive tract can provide useful information related to fetal viability, diagnosis of mid to late term pregnancies, gestational age, assessment of fetus and fetal organs, placentomes, fetal fluids, uterine wall, umbilicus, and identification of developmental abnormalities. To best of my knowledge this is the first review of applications of transabdominal ultrasonography in bovine reproduction because literature related to transabdominal ultrasonography in bovine reproduction and obstetrics is very scanty.

Keywords: *Bubalus bubalis*, buffaloes, transabdominal ultrasonography, bovine, reproduction

INTRODUCTION

One of the greatest supremacy of ultrasonography is that it is totally non-invasive and so repeated evaluation of an animal's reproductive tract can be performed without having any ill effect on the fetus (Ribadu *et al*., 1999). In bovines, ultrasonography is an important diagnostic aid

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for examining the female reproductive system, pregnancy diagnosis (Kahn, 2004) and estimation of gestational age in cows (Bergamaschi *et al*., 2004; Kohan-Ghadr *et al*., 2008; Hunnam *et al*., 2009; Adeyinka *et al*., 2014; Lawrence *et al*., 2016). Ultrasonographic evaluation of the pregnancy can be performed either trans-rectally or trans-abdominally. In bovines, the trans-rectal ultrasonography is preferred during the first trimester of gestation. Previously**,** ultrasonography was applied trans-rectally during early gestation in cows for measurement of crown rump length (Kahn, 1989; Lawrence *et al*., 2016), head length (Lazim *et al*., 2016), diameter of the trunk (Lazim *et al*., 2016), uterine diameter (Lazim *et al*., 2016); Lawrence *et al*., 2016), placentomes length and size (Hussein, 2013; Adeyinka *et al*., 2014; Lawrence *et al*., 2016), intercostal space (Kahn, 2004). But it is not feasible to scan each and every part of fetus by trans-rectal ultrasonography during mid and late gestation in bovines as the pregnant uterus incline deeper into the abdomen and it becomes difficult to examine all parts of the fetus (Bertolini *et al*., 2002). Pregnancy diagnosis in groups of obese, large, deep-bellied, multiparous animals and beef cattle using either palpation per rectum or transrectal ultrasonography can also be inaccurate, with the operator commonly dependent on fremitus as the primary positive indicator of mid to late-pregnancy (Noakes *et al*., 2001). The trans-abdominal approach is, therefore, preferred during mid and late gestation in bovines for evaluation of disease conditions of uterus.

The transabdominal ultrasonography has become a widespread ancillary tool for assessing the fetus and its uterine adnexa in many veterinary species (England, 1998; Bucca, 2006; Ward *et al*., 2006) and has been studied more extensively in human pregnancies (Lerner, 2004; Devoe, 2008). This review aims to recapitulate the significant contributions made by trans-abdominal ultrasonography in the past and update the more recent developments in diagnostic ultrasonography with particular reference to bovine reproduction. We hope that such a focused review will be useful, specifically to those associated with bovine reproduction.

Procedure

Site of scanning

The animals should be restrained in chute for transabdominal ultrasonography. Generally, no sedation to animals is required for ultrasonographic scanning. The ventral part of the right abdomen should be shaved in caudo-cranial direction from the udder to the end of the milk vein cranially. This area extends to the horizontal line 15 cm dorsal to the stifle on the right and to the linea alba on the left (Buczinski, 2009). For better diagnostic images, shaving of the ventral and lower lateral (mostly right side) abdominal area on both side of udder (Figure 1) is essential prior to transabdominal ultrasonography.

Probe/Transducers

In the past various frequencies have been used for transabdominal ultrasonography (Buckzinski *et al*., 2007b). The accessibility of various organs and parts of the fetal body depends on the orientation of transducer with respect to fetus and position of the fetus. Owing to the large size of the maternal abdomen and the fetus, a low-frequency probe (less than 5 MHz) is required to have a deep penetration (Reef *et al*., 2007). Transabdominal ultrasonography, using 2.0 to 5.0 MHz 2D convex transducer is useful in generating detailed images of bovine fetal and uterine anatomy and these images are of quality which is

not dissimilar to those which have been reported for smaller species. The relatively small surface area and arc shaped beam of convex transducers, in comparison with linear transducers, enhance their use in transabdominal ultrasound with easy skin positioning and a wide field of view (Hunnam *et al*., 2009).

Applications of transabdominal ultrasonography

Diagnosis of mid to late term pregnancies

Accurate pregnancy diagnosis is vital in achieving efficient reproductive management of a dairy or beef herd. Commonly used pregnancy diagnosis techniques are manual palpation per rectum and trans-rectal ultrasonography. Over the period between approximately day 35 to 90 of gestation (from recorded mating date), the sensitivity, specificity for pregnancy diagnosis by transrectal ultrasound are typically above 90% with increasing gestational age and transducer frequency resulting in improved accuracy (Szenci *et al*., 1995). Pregnancy testing *via* transrectal ultrasound becomes more difficult after first trimester of gestation, as the majority of structures required for a positive diagnosis of pregnancy are beyond the reach of ultrasonologist. Pregnancy at that stage can therefore be diagnosed by transabdominal ultrasonography.

Transabdominal ultrasonography is a reliable procedure for determination of pregnancy and fetal viability especially for cows between fourth and sixth months of gestation (Aziz, 2013). Results of the study by Aziz (2013) showed that determination of pregnancy and fetal viability obtained are significantly faster using transabdominal ultrasonography than rectal palpation. The accuracy, sensitivity and specificity of rectal palpation are 67.4%, 84.8% and 48.8% for detection of pregnancy, and 33.3%, 90.4% and 1.9% for determination of fetal viability, respectively. While these three parameters for transabdominal ultrasonography are 100% for both pregnancy and fetal viability determination.

Assessment of placentomes

Transabdominal ultrasonography is useful in measuring size, shape and morphology of placentomes. The placentomes are easily recognized by their specific aspect of an echogenic ovale to elliptic structure with a dimension of a chicken-egg (Kohn-Gadr *et al*., 2008; Devender *et al*., 2016). They are discrete, raised, arcuate areas distributed on the uterine wall and are easily observed surrounded by anechoic-to-hypoechoic uterine fluids (Figure 2) in normal advanced pregnant bovines and appears well demarcated and without any signs of degeneration. Length of placentomes at <90 and >250 days of gestation in cows reported is 17.87±2.26 and 75.92±7.76 mm, height of placentomes is 13.37±1.12 and 36.50±3.92 mm respectively (Lazim *et al*., 2016).

Transabdominal ultrasonography is also useful in evaluating morphological changes which can be observed either at the apex (Figure 3) or throughout the parenchyma of placentomes (Figure 4) in uterine torsion cases in bovines.

Fetal fluid evaluation

With the help of transabdominal ultrasonography, it could be possible to assess the condition of fetal fluids in bovines by measuring pixel values. The amniotic fluid appears as hypoechoic with various amounts of echoic particles in normal pregnant buffaloes (Figure 5) (Jonker, 2004). Amniotic and allantoic fetal fluids are separated by a thin echoic membrane which is the amniotic membrane (Figure 5). Mean

pixel values of fetal fluids are found increased in uterine torsion in buffaloes (Figure 6). So, with the help of transabdominal ultrasonography it could be possible to assess the prognosis of disease conditions of advanced pregnancy by measuring mean pixel values of fetal fluids.

Status of fetus and its internal structures

Status of bovines fetus (live or dead) can be successfully evaluated with the help of transabdominal ultrasonography by closely monitoring the fetal heart beats. Moreover, other internal details of fetus which include the abdominal organs and thoracic organs can be studied by taking transabdominal ultrasonographic images. Transabdominal ultrasonography is very useful in advanced pregnancy and uterine torsion cases in bovines where it was very difficult to assess the status of fetus manually.

Transabdominal ultrasonography using 2.0 to 5.0 MHz 2D convex transducer produce detailed images of bovine fetal and uterine anatomy, and these images were of quality that is not dissimilar to those which have been reported for smaller species. Therefore, it is inferred that this frequency is very useful in late pregnancy for diagnostic point of view. It is observed that fetal structures were easy to image in normal advanced pregnant buffaloes as compared to uterine torsion buffaloes. However, the accessibility of various organs and parts of the fetal body depend on the orientation of transducer with respect to position of the fetus (Devender *et al*., 2016).

Because of large size of fetus, it is not possible to observe the whole bovine fetus in single ultrasonographic view in contrast to ovine fetuses (Buczinski *et al.*, 2007a). Therefore, the assessment of specific fetal parts is limited. The fetal thorax is identified by observing the fetal heart beats (Figure 7). The costal reverberations due to the bone or soft tissue interactions can also be seen when the thorax is observed in longitudinal plane (Figure 8). The abdominal organs can be easily identified if the ultrasonologist is used to bovine ultrasonography (Figure 9).

Transabdominal ultrasonography in the past proved useful in imaging fetal organs like liver (Figure 10), spleen, lungs, heart, stomach (Figure 11), kidney, eyeball, head, and limbs in normal advanced pregnant and uterine torsion affected buffaloes. The fetal liver is examined towards the right side of the fetus from the fifth intercostal space to the region caudal to the last rib of fetus by using 2D convex transducer applied transabdominally caudal and just lateral to the naval (umbilicus) of the dam. The largest anechoic area in the fetal abdomen is developing fluid-filled rumen subdivided into compartments, and cranioventral to the rumen is echogenic reticulum (Figure 11). So, transabdominal ultrasonography in recent times is proving to be useful in assessing the antenatal development of fetal organs.

Because of the position of the fetus, it is difficult to systematically assess the fetal organs. To date, the ultrasonographic observation of the fetal organs may be of interest in the detection of congenital anomalies and has proved useful in past in two Holstein cows in late pregnancy having prolonged gestation for pathologic findings in the fetuses (Buczinski *et al*., 2007b).

Fetal heart rate

Transabdominal Doppler technique is helpful for recording of fetal heart rate (FHR) in the bovine fetus (Jonker *et al*., 1994). During the last 2 weeks of gestation recordings are made once or twice per week in eight cows by placing 1.5 MHz Doppler transducer on the right ventral abdominal

wall. The overall mean baseline fetal heart rate is 105±1.5 beats per minute (bpm) with a range of 90 to 125 bpm. The alternation of bovine fetal heart rate patterns indicates existence of different fetal behavioural states.

Size and status of umbilicus

The umbilical cord is identified by its characteristic sonographic images showing four echo-poor tubes representing the paired umbilical arteries and veins. Umbilical vessels in umbilical cord are visible in a quadrilateral arrangement in cross-section (Figure 12 and 13), whereas, in the longitudinal view (Figure 14), only one to two umbilical vessels are visible. The width of umbilical cord measured by transabdominal ultrasonography at <90 and >250 days of gestation in cows is (7.80±0.42) and (16.41±0.41) mm, respectively (Lazim *et al*., 2016). Umbilical cord width is correlated significantly with gestational age in cows, and it increases significantly until 210 d of gestation (Lazim *et al*., 2016). This finding agreed with results that published by Hunnam *et al*. (2009) who found a significant association between gestational age and umbilical diameter that measured via transcutaneous ultrasound during the gestation age between 73 and 190 days. Transabdominal ultrasonography is also useful in uterine torsion cases in buffaloes in which umbilical cord is found constricted in comparison to normal advanced pregnant buffaloes.

Changes in uterine wall

Transabdominal ultrasonography is useful for measuring thickness of uterine layer in normal advanced pregnant bovines (Figure 15) and in disease conditions of uterus. Uterine layer appears as a hyperechoic layer over the anechoic fetal fluids. Inflammatory changes, if any can be (Figure 16) observed in uterine wall in uterine torsion affected buffaloes during late gestation with the help of transabdominal ultrasonography.

DISCUSSION

Transabdominal scanning has been criticized in the past on the basis of a perceived need for special skin preparation and apparatus, the assumption that the cow or buffalo will kick in response to scanning over the abdominal area and the notion that the bovine abdomen is too large to allow detailed examination (Hunnam *et al*., 2009). However, in recent times it is proving to be a wonderful tool in bovine reproduction and obstetrics despite having aforesaid limitations. Images of the conceptus and reproductive tissues could be obtained using the transabdominal technique and are of a quality that are not dissimilar to those which have been reported for smaller species (i.e. sheep, red deer and pig), allowing ready differentiation of the conceptus and pregnant uterus from non-reproductive structures (Hunnam *et al*., 2009). Furthermore, many of the fetal structures that are difficult to visualise via transrectal ultrasonography in animals at second and third trimester of gestation are readily accessible through transabdominal examination. Acoustic shadowing, or areas of low-amplitude echoes created by bony structures such as the vertebrae and ribs, are readily visible in the transabdominal scans (Hunnam *et al*., 2009). The bones of the fore- and hindlimbs, including the humerus, femur, radius, tibia, ulna, fibula, metatarsus and metacarpus and cartilage of the developing hooves, are also clearly visible (Hunnam *et al*., 2009). Likewise, the rumen could be seen as a large, fluid-filled structure, with the fetal reticulum appearing as echogenic areas. The

Figure 1. Site and probe positioning for transabdominal ultrasonography in buffaloes.

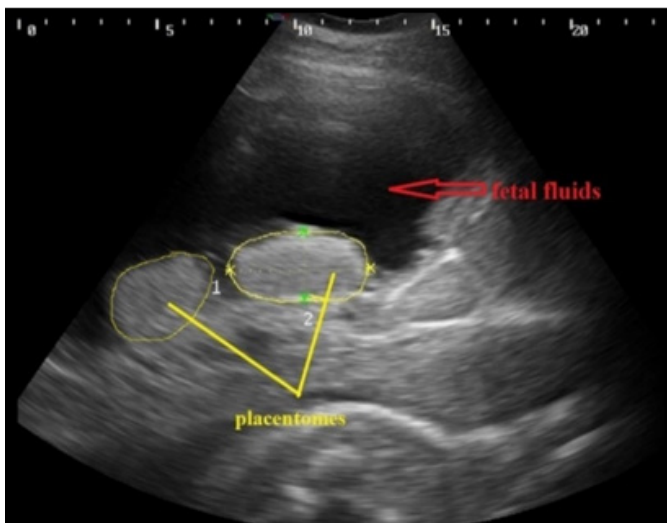

Figure 2. Transabdominal sonographic image of placentomes (marked by yellow arrows) in normal advanced pregnant buffalo.

Figure 3. Sonographic image of placentomes (showing degenerative changes at the apex) in buffalo suffering from uterine torsion.

Figure 4. Sonographic image of placentome (showing the changes throughout the parenchyma) in buffalo suffering from uterine torsion.

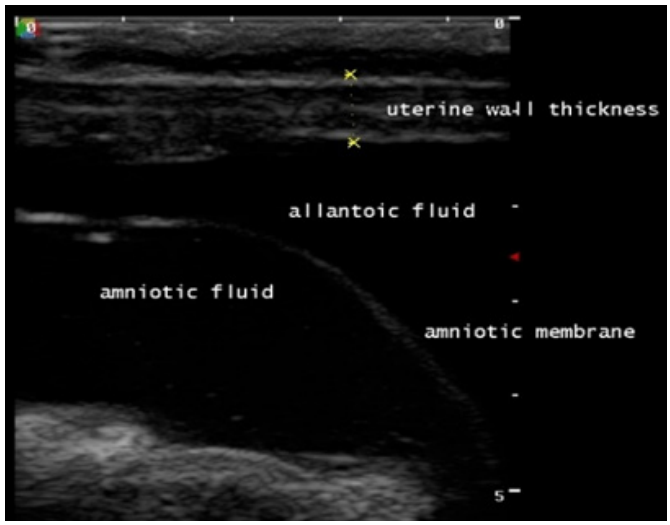

Figure 5. Sonographic image showing the fetal fluids of normal advanced pregnant buffalo.

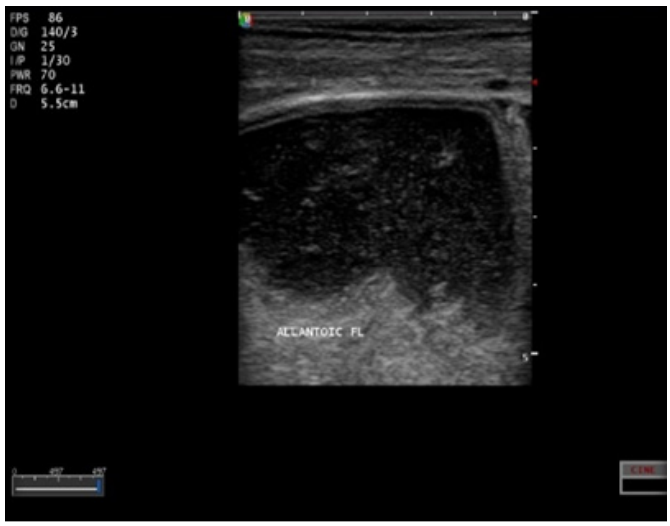

Figure 6. Image showing allantoic fluid in uterine torsion affected buffaloes.

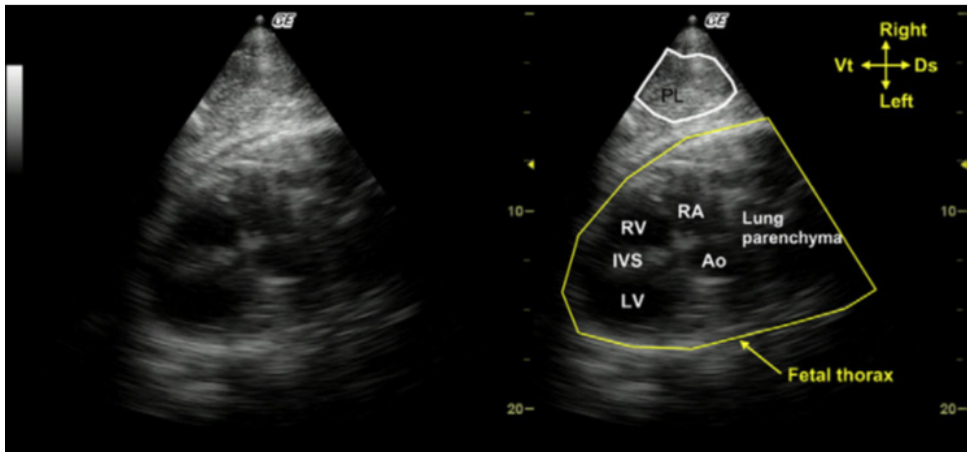

Figure 7. The fetal heart and lung observation during transabdominal ultrasonography.

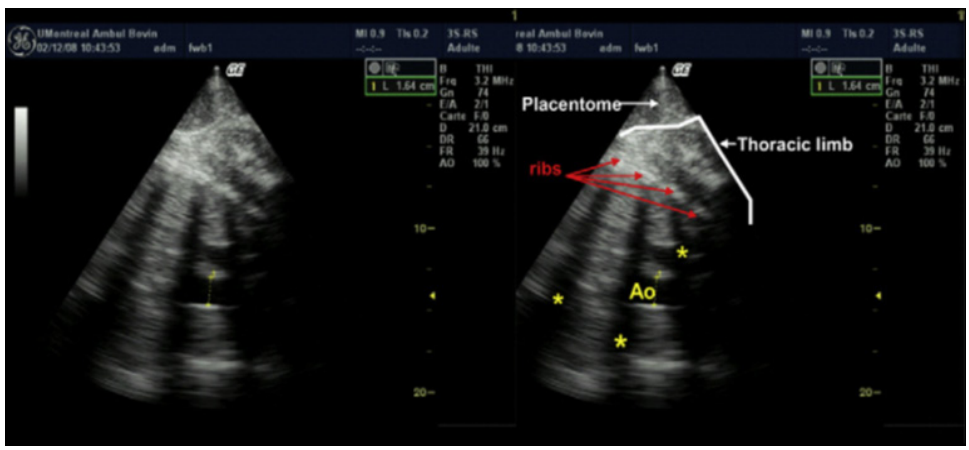

Figure 8. Observation of placentome, thoracic limbs, fetal ribs and fetal aorta during tranabdominal ultrasonography of pregnant animals.

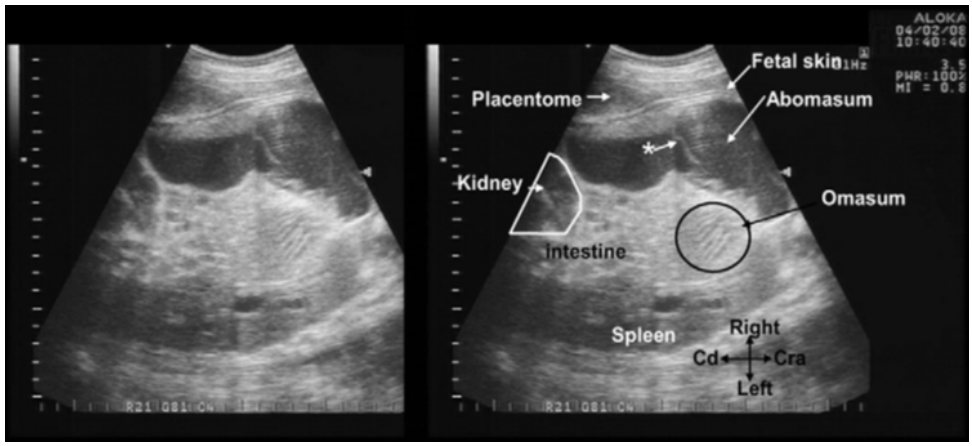

Figure 9. Transabdominal ultrasonographic images of various abdominal organs of fetus.

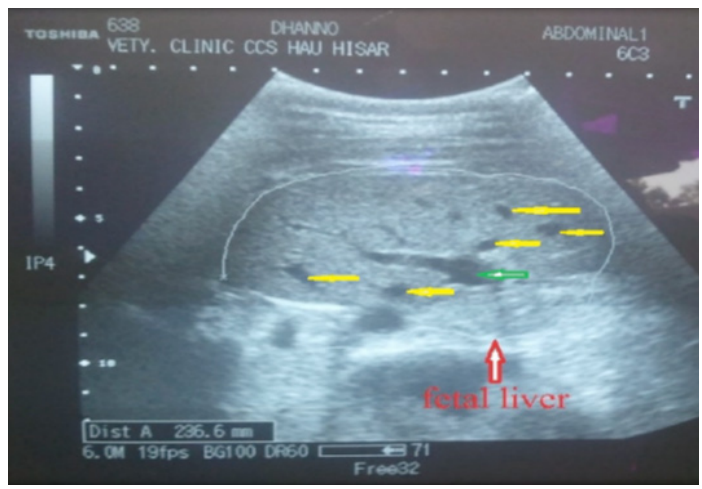

Figure 10. Transabdominal sonographic image of fetal liver in normal advanced pregnant buffalo.

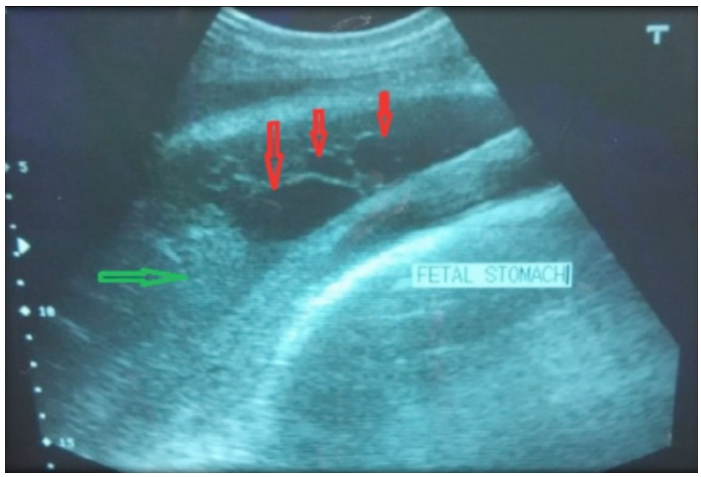

Figure 11. Transabdominal sonographic image of fetal stomach in normal advanced pregnant buffalo.

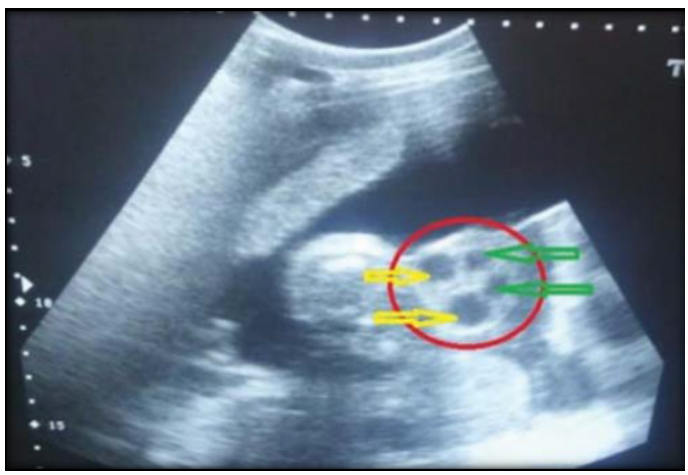

Figure 12. Transabdominal sonographic cross-sectional image of umbilical cord (umbilical arteries marked by yellow arrow and umbilical veins marked by green arrows) in normal advanced pregnant buffalo.

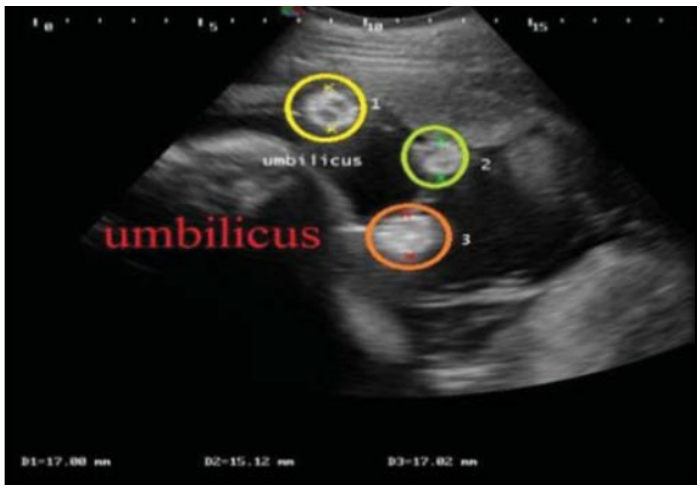

Figure 13. Transabdominal sonographic cross-sectional image of umbilicus in uterine torsion buffalo.

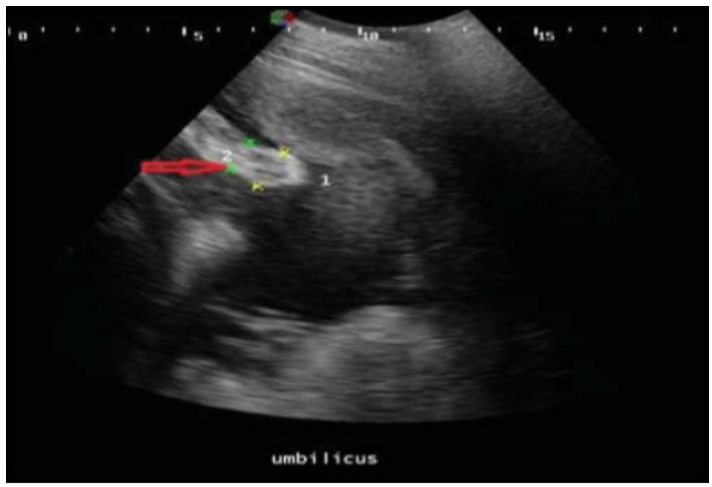

Figure 14. Transabdominal sonographic longitudinal view of umbilicus (marked by red arrow) in uterine torsion buffalo.

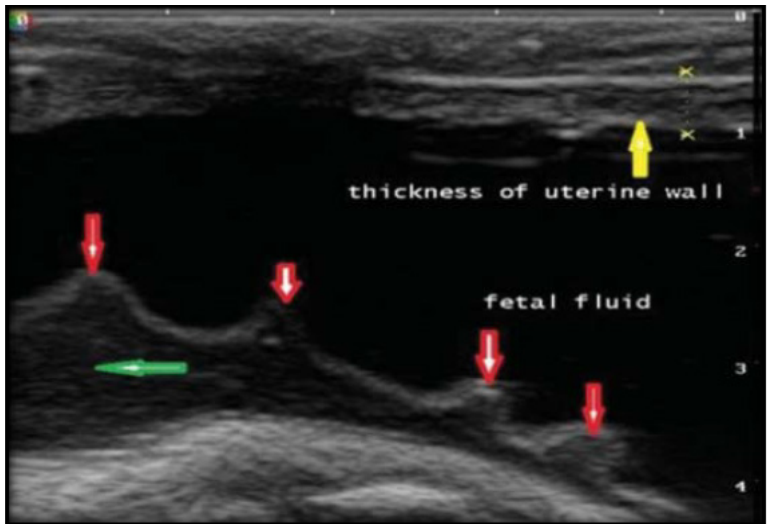

Figure 15. Sonographic image of thickness of uterine wall (marked by yellow arrow) in normal advanced pregnant buffalo.

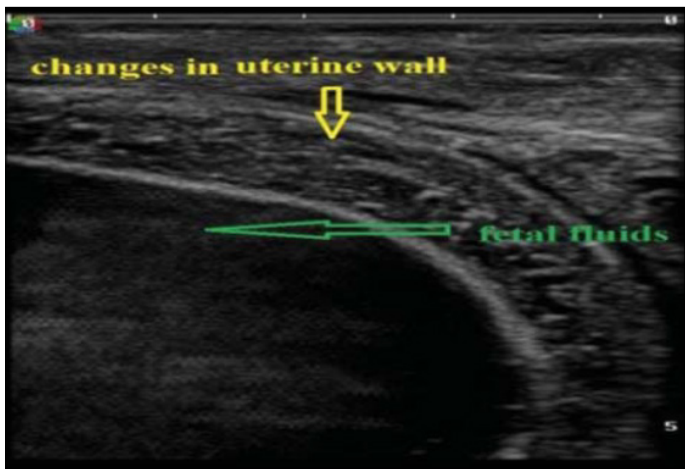

Figure 16. Sonographic image of uterine wall in uterine torsion affected buffaloes.

chambers and beating of the heart could be readily measured to assess compromised pregnancies. Fetal ultrasonography is helpful in monitoring prolonged pregnancy, accurate diagnosis and to evaluate fetal viability (Buczinski *et al*., 2007b). The non-fetal parts of the conceptus are a valuable aid in the diagnosis of pregnancy if the fetus itself cannot be seen (Hunnam *et al*., 2009). Shape, size and morphology of placentomes and umbilicus can be assessed in normal and compromised advanced pregnant bovines.

CONCLUSION

As far as pregnancy diagnosis and other gynaecological consideration, per-rectal ultrsonography is most reliable and practical method for diagnostic point of view during the first trimester of gestation in bovines. However, when the fetus decends in abdominal cavity during mid and advance gestation, transabdominal ultrasonography is empirical method for fetal and uterine measurements. With a good knowledge of bovine anatomy and ultrasonography, the trans-abdominal ultrasonography of the fetomaternal unit can be performed and important information can be obtained in complicated advance pregnancy in bovines. However, the repeatability of the ultrasonographic findings still needs to be achieved.

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