

Prediction of Gestational Age and Differential Diagnosis of Pregnancy and Uterine Pathology Using Ultrasonography in Buffalo

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Abstract

Ultrasonography is gaining importance as a diagnostic tool in large animal theriogenology in recent years in India. It can be used with a greater accuracy for confirmation of pregnancy and ruling out uterine pathology. The present study described the application of ultrasonography for early pregnancy diagnosis and prediction of gestational age in buffaloes under the organized farm set up. A total of fifty parous Murrah buffaloes at 27 to 53 days gestation were included in the study. The diameter of amniotic vesicles, crown-rump lengths of developing embryo were determined during confirmation of pregnancy using B mode trans-rectal ultrasonography at different days of gestation (days 31 to 53). Attachment of embryo to the uterine wall, development of amniotic vesicle, appearance of limb buds and differentiation of body structures of developing embryo and early fetus were observed. A strong positive correlation observed between the days of gestation and amniotic vesicle diameter ($r=0.807$, $p = 0.001$) and days of gestation with crown-rump length ($r=0.816$, $p= 0.001$). As incidental findings, two cases of embryonic death based on the presence of empty amniotic vesicle, detachment of chorio-allantoic membranes and changes in amniotic fluid appearance and embryonic degeneration were recorded. Additionally, a case of pyometra

and mucometra were also diagnosed during the study. The prediction equation based on the ultrasonographic attributes would be useful in determination of gestational age and confirmation of early pregnancy in buffaloes.

Key words: Buffalo, ultrasonography, early pregnancy, amniotic vesicle, embryonic death

Early and accurate pregnancy diagnosis is crucial for effective reproductive management of buffaloes to minimize the calving to conception interval (Karen *et al.*, 2007). In recent years, ultrasonography has gained significant popularity as a diagnostic tool in large animal theriogenology. Sonographic examination allows the detection of embryonic structures as early as 25-30 days post-breeding in buffaloes and also provides valuable information on the viability of the conceptus (Ibrahim *et al.*, 2018). It allows visualization of fetal heartbeat, placental development, and assessment of fetal conformation indicating its well-being during development (Gupta *et al.*, 2016). It also helps in identifying potential pregnancy complications or fetal abnormalities, enabling appropriate interventions or adjustments in management practices. Use of B-mode real-time ultrasonography in the diagnosis of pregnancy in buffaloes are mostly based on the observation of irregular-shaped anechogenic structures in the uterus as early as day 28 post-breeding, similar to the dairy cattle (Chaffaux *et al.*, 1982). Early pregnancy diagnosis allows for early identification of non-pregnant buffaloes, enabling the farmer to take prompt actions for re-breeding or culling, thus reducing the non-productive

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period, minimizing the cost associated with maintaining non-pregnant animals in the herd and optimizing the utilization of resources such as feed and space (Kamboh *et al.*, 2018).

Among the available methods of pregnancy diagnosis, trans-rectal palpation is the oldest and commonly used methods for large ruminants. The sensitivity at days 31-35 is significantly low (37.5%), which improved to 93.8% at 45-60 days (Karen *et al.*, 2011). Due to low accuracy before 45 days and risk of causing early embryonic death due to rough genital manipulation (Karen *et al.*, 2007), pregnancy diagnosis through rectal palpation is usually preferred after 3-4 months in the field. Transrectal ultrasonography has relatively greater sensitivity (44.4%) at early pregnancy, during days 19 to 24; however, it increases to 100% by day 31 post breeding. Meanwhile, its specificity ranges from 92.5% to 100% between days 19 and 55 (Karen *et al.*, 2007). The earliest time point of achieving 100% sensitivity in gestational diagnosis is based on the detection of heartbeat (Ferreira *et al.*, 2011). Moreover, gestational age can be determined using transrectal ultrasonography between days 35 and 91 days of gestation (Brownlie *et al.*, 2016). The pregnancy associated glycoprotein based methods are often used for the diagnosis of pregnancy (Dufour *et al.*, 2017), although accuracy varies for test to test and difficult to predict the gestation period (Brownlie *et al.*, 2016). Compared to all the available clinical and laboratory methods, USG offers distinct advantages, being a non-invasive, safe, well-suited for routine clinical set up (Humble *et al.*, 2016) and provides real-time imaging for assessment of pregnancy and favors in the decision-making for course of treatment if found non-pregnant. Besides pregnancy, it is also valuable for differentiating uterine pathology, such as pyometra and mucometra (Kumar and Purohit, 2009). Despite efforts having been made to diagnose early pregnancy in buffaloes using ultrasonography, there is a limited amount of research available on the prediction of gestational age in these animals. In this perspective, present study was designed to document the sonographic attributes of early pregnancy in buffaloes for prediction of gestational age and to differentiate viable pregnancy

from embryonic mortality and uterine pathology.

Materials and Methods

The present study comprised of the pregnancy records of a total of 50 parous Murrah buffaloes, in the age group of 4-9 years maintained at cattle and buffalo farm, Livestock Production and Management section, Indian Veterinary Research Institute, Izatnagar. All the buffaloes were presented for pregnancy diagnosis randomly during the days 27 to 53 of post breeding. The ultrasonographic examination was performed using a portable color Doppler ultrasound scanner (EXAGO-ECM, France) equipped with a 7.5 MHz linear array transducer as described by Patra *et al.* (2023).

The amniotic vesicle diameter (AVD) and long axis of embryo proper or crown-rump length (CRL) of developing conceptus were measured in 44 pregnant buffaloes at four defined windows, days 31-38 (n=12), days 39-42 (n=12), days 43-47(n=11) and days 48-53 (n=9). This measurement was taken by drawing a straight line from the fetal crown to the base of the tail, as seen on the ultrasound scanner display, after freezing the image and using the built-in calipers. Additionally, the study recorded cases (n=5) of twinning, fetal death, pyometra and mucometra complicated with cystic ovaries. The study was conducted in compliance with the guidelines set by the Institute Animal Ethics Committee (IAEC).

Statistical analysis

The mean AVD, CRL at four different windows were compared using one-way Anova. The correlation between AVD, CRL and days of gestation was estimated using Pearson's correlation and a regression equation was derived from the best fitted model. Graph pad prism software 6.0 was used for data analysis.

Results and Discussion

Present study documented the sonographic attributes of early pregnancy diagnosis in buffalo based on the appearance developing embryos, presence of fluid in the gravid uterine horn, appearance of amniotic membranes and vesicle. The earliest pregnancy diagnosis was

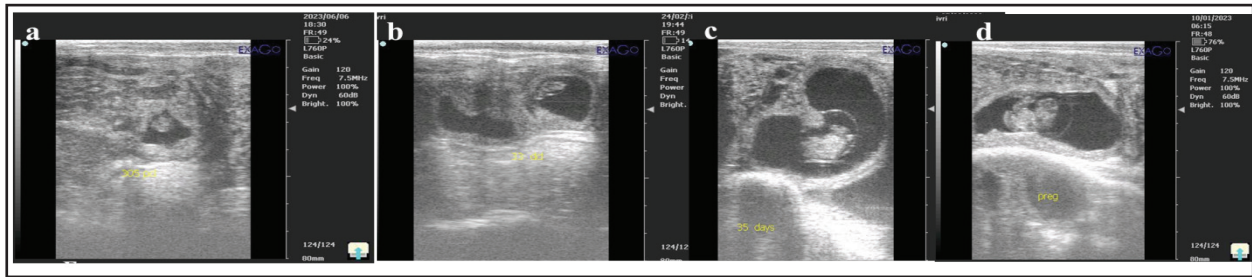


Fig 1. Ultrasonographic images of buffalo conceptus. a) 27 days b) 33 days c) 35 days d) 45 days

Table I. Amniotic vesicle diameter (AVD) and crown-rump length (CRL) in relation to days of gestation in buffalo.

Days of gestation	AVD (mm)	CRL(mm)
31-38 (n=12)	15.87±1.09 ^a	12.56±0.60 ^a
39-42 (n=12)	21.19±0.84 ^b	20.46 ±1.05 ^b
43-47 (n=11)	29.53±1.93 ^c	24.93±1.40 ^c
48-53 (n=9)	37.20±1.23 ^d	31.52±0.84 ^d
P value	<0.0001	<0.0001

done by day 27 based on the appearance of close proximity of developing embryos to the uterine wall without distinct appearance of amniotic membranes and vesicle (Fig.1a). In a similar study, Glatzel *et al.* (2000) identified pregnancy in water buffalo heifers on day 25 post breeding based on the presence of fluid in the apex of the uterine horn and the presence of a corpus luteum. However, by day 33 the amnion, which surrounds the embryo became discernible and the visibility of the structure improved further by the day 35 and the attachment of the embryo to the uterine lining was prominently observed (Fig.b,c). The average diameter of amniotic

vesicle (AV) and CRL increased along the progression of gestation (Table I). The AVD and CRL showed a significant increase in size relative to days of gestation till day 53 ($P<0.0001$).

A strong positive correlation was observed between days of gestation and AVD ($r=0.807$, $p<0.001$). Similarly CRL also shown strong positive correlation with days of gestation ($r=0.816$, $p<0.001$). Furthermore, there was a notable positive correlation between AVD and CRL ($r=0.917$, $p<0.001$, Fig.2). These results were aligned with the previous report (Ali and Fahmy, 2008), which also stated a strong positive correlation between CRL and AVD during early gestation. However, it is important to note that as pregnancy advances, CRL and AVD measurements become less accessible due to their increased length, making it challenging to efficiently visualize them on an ultrasound screen (Ali and Fahmy, 2008).

The pregnancy was confirmed by the appearance of a non-echogenic circular region of different sizes within the echogenic uterine horn. This area represented the fluid-filled

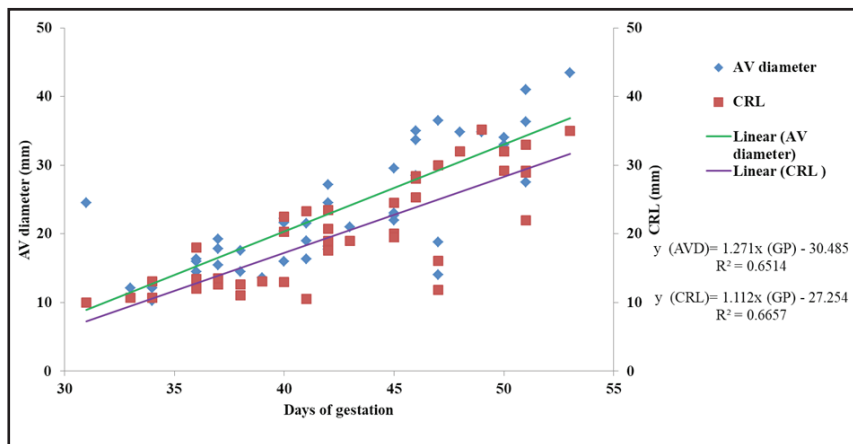


Fig 2. Amniotic vesicle diameter and crown rump length at different days of gestation

allantoic cavity known as the embryonic vesicle (Pieterse *et al.*, 1990). Furthermore, the presence of an embryo inside the amniotic vesicle was verified by observing a rhythmic pulsation in a white echogenic area, indicating the presence of heartbeats. The identification of embryonic heartbeats is crucial for its viability in ultrasonographic pregnancy diagnosis and it enables a clear distinction between the embryo and the uterine wall (Ferreira *et al.*, 2011).

In two cases, embryonic death was confirmed, of which in one case of twin pregnancy, one of the amniotic vesicles was empty (Fig.3a) and in second case the detachment of the chorio-allantoic membranes from the endometrium was evident. The appearance of amniotic fluid changed to partially echogenic, and various degrees of embryonic degeneration were also observed (Fig.3b). The absence of a heartbeat in the degrading embryonic mass along with membrane detachment or disorganization, decreased intrauterine fluid, and the presence of echoic floating structures, including remnants of the conceptus confirmed its mortality (López-Gatius and García-Ispuerto, 2010; Lokavya *et al.*, 2020). The identification of embryonic loss holds equal importance to that of detecting live embryos. Trans-rectal palpation makes it difficult to detect early foetal membrane detachment and degenerating conceptus. Although we have not estimated the diagnostic precision of USG for pregnancy diagnosis, a study conducted by Al-Eknaah and Al-Seaf (2014) reported 95% sensitivity in detecting early embryonic death. Therefore, incorporating ultrasonography into routine diagnosis enables us to efficiently identify late embryonic and early fetal loss in

buffaloes, ultimately improving the overall reproductive efficiency of the herd.

Besides pregnancy, a number of uterine pathologies were recorded in two buffaloes. During rectal examination of one buffalo, it was observed that both uterine horns were equally distended with a doughy consistency although there was no evidence of vaginal discharge. On sonographic examination it was cleared that the distended uterine lumen contains bountiful of echogenic materials and the distended uterus had thickened endometrium wall (Fig.4a) and the right ovary displayed a functional corpus luteum (CL) measuring 16.3 mm. Based on the sonographic attributes correlating clinical history and gynecological findings the condition was diagnosed as pyometra (Kumar and Purohit, 2009). Ultrasonography not only provides the accurate diagnosis in pyometra but also provide an overview of pathological changes of the endometrium correlating the clinical severity (Sasidharan *et al.*, 2022).

In another case, uterine distension of a buffalo was observed during rectal examination. Later, on sonographic examination it was confirmed as case of mucometra based on the presence of thin-walled uterus, appearing as a white streak, filled with anechoic fluid mixed with substantial amount of echogenic particles inside it (Fig. 4c). No CL was observed in either ovaries, and a follicle measuring 19.65 mm was identified on the left ovary (Fig.4d). The typical finding in cases of long-standing cystic ovarian disease complicated to mucometra or hydro-metra in bovines is previously reported (Dutt *et al.*, 2019). In scenarios requiring the differentiation between mucometra and pregnancy,

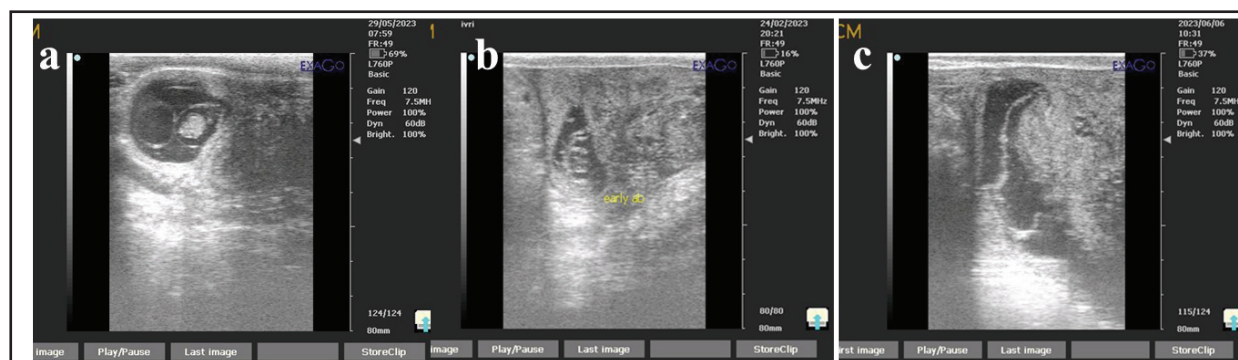


Fig 3. a) Absence of embryo in one of the amniotic vesicle b) Degenerated embryo c) Detached fetal membrane

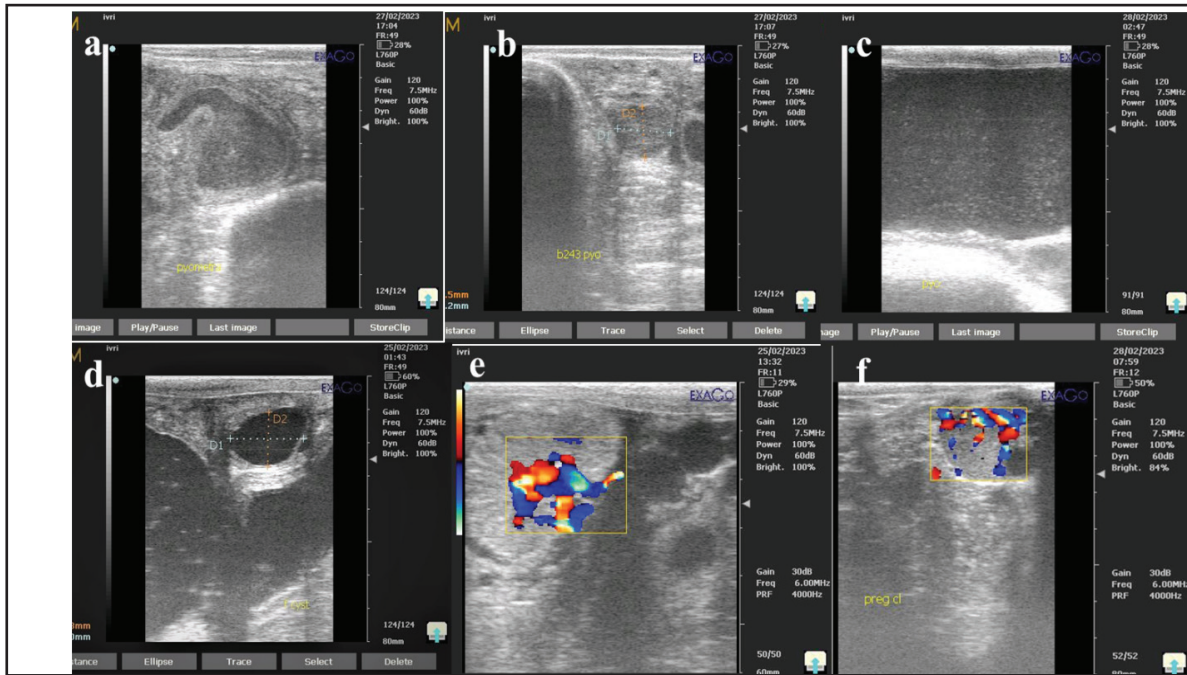


Fig 4. a) Echogenic contents in pyometra uterus b) Presence of CL in the ovary in pyometra c) Distended uterine lumen with scattered echogenic particles floating in mucometra d) Presence of follicular cyst in mucometra e) Blood flow to placentome f) Pregnancy corpus luteum

the presence of placentome and corpus luteum and its vascular characteristics can be assessed using Doppler ultrasonography (Fig.4e,f). This technique offers the potential to monitor the advancement of embryo development and its viability over time (Awasthi *et al.*, 2016).

Conclusions

From this study, it can be concluded that ultrasonographic attributes can be effectively used for the diagnosis of pregnancy in buffaloes as early as 27-33 days and for differential diagnosis of uterine pathology. In addition, prediction of gestational age can be estimated measuring AVD and CRL from 31-53 days in buffalo. Early diagnosis of non-pregnant buffaloes and buffaloes with uterine pathology would help in institution of therapeutic approaches with greater chances of recovery and breeding within short span. Thus, adoption of ultrasonography technique in routine breeding operation could be useful in augmenting reproductive efficiency in the buffaloes under organized farm set up.

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Conflict of interest

The authors declare no conflict of interest among them-selves.

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