

MONITORING OF EMBRYONIC AND FETAL DEVELOPMENT USING REAL TIME 'B' MODE ULTRASOUND IN BUFFALO (*Bubalus bubalis*)

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ABSTRACT

The present study was conducted in buffaloes which were maintained at experimental animal sheds of Animal Reproduction Division, IVRI, Izatnagar. All the experimental buffaloes (n=6) were observed for onset of estrus using a teaser bull and visual signs of estrus followed by rectal examination twice daily. Animals detected in estrus were inseminated using frozen semen of Murrah buffalo. The inseminated buffaloes were allowed for real time B-mode ultrasonographic examination at 10 days interval starting from day 20 to 120 of pregnancy. Monitoring of embryonic development was carried out by viewing embryonic vesicle diameter (EVD), Crown Rump length (CRL), embryonic heartbeat, skull and placentome through ultrasonography. The mean diameter of EV was 0.52±0.12, 1.56±0.16, 2.94±0.07, 3.80±0.14 and 6.37±0.15 cm on day 20, 30, 40, 50 and 60 of pregnancy, respectively. EVD increased with advancement of pregnancy and reached around 6.37±0.15 cm on day 60 of pregnancy. The CRL was 0.73±0.05, 1.45±0.55, 2.13±0.05 and 3.24±0.10 cm on day 30, 40, 50 and 60 of pregnancy, respectively. The mean heart rate was 197.33±4.60, 173.16±1.55, 162.33±1.20, 155.33±1.60, 152.16±2.58 and

146.66±2.40 per minute on days 50, 60, 70, 80, 90 and 120 of pregnancy, respectively. The heart rate was significantly (P<0.01) higher on day 50 of pregnancy, thereafter, it decreased with advancement of pregnancy. The mean diameter of skull was 2.33±0.18, 2.90±0.12, 3.52±0.19 and 4.58±0.22 cm on days 60, 70, 80 and 90 of pregnancy, respectively. The skull diameter increased with the advancement of pregnancy. The accessibility of skull or head was very difficult as the

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pregnancy advanced into mid of the second trimester. The mean diameter of placentome increased with the advancement of pregnancy i.e. 0.38±0.01, 0.64±0.14, 1.05±0.07 and 1.26±0.08 cm on days 70, 80, 90 and 120 of pregnancy, respectively. The present study indicated use of Real time B mode transrectal ultrasonography in buffalo for early diagnosis of pregnancy, embryonic development and its viability by viewing embryonic vesicle, embryo, embryonic heart beat and other structures.

Keywords: Buffalo, Embryo, Fetal development, Pregnancy diagnosis, Ultrasonography

INTRODUCTION

Early pregnancy diagnosis and determination of embryonic and fetal viability and their development are of considerable importance in improving reproductive efficiency in buffalo. Conditions like early embryonic mortality, early fetal death and unobserved abortions lead to an increased anestrus period and thereby increase the costly calving interval. To overcome such problems, reproductive ultrasonography may serve as a better tool as the use of ultrasonography has been increasing as an imaging modality in bovine reproduction. Its use can provide solutions to a number of unanswered questions in dealing with the bovine reproductive cycle and its concurrent disorders, including early pregnancy diagnosis (Naikoo *et al.*, 2013). Real time B mode ultrasonography is a non-invasive, accurate and rapid alternative for diagnosing pregnancy and studying the development of the conceptus in livestock. The objective of the present experiment is to study embryonic development and its monitoring using real time B-mode ultrasonography in buffalo.

MATERIALS AND METHODS

The study was conducted on adult female buffalo (n=6) of 5-8 years of age with

fairly good body condition. The animals were maintained at experimental animal sheds of Animal Reproduction Division, IVRI, Izatnagar (UP), India. All the experimental buffaloes were observed for onset of estrus using a teaser bull and visual signs of estrus followed by rectal examination twice daily. The day of standing estrus was designated as day 0 of that cycle. Animals detected in estrus were inseminated using frozen semen of Murrah buffalo. The inseminated buffaloes were allowed to real time B-mode ultrasonographic examination at 10 days interval starting from day 20 to 120 of pregnancy. Monitoring of embryonic development was carried out by viewing embryonic vesicle, embryo proper, embryonic heart beat and skull and placentome diameter through ultrasonography. The uterus was examined by guiding the probe slowly over the dorsal surface of the uterine horns. The location and dimensions of resolvable (>2mm), non - echogenic (black) areas of discrete structure within the uterus lumen were recorded. These structures were assumed to represent the fluid filled early conceptus *i.e.*, embryonic vesicle. The presence of embryo was confirmed based on defined intrauterine echogenic (white) structure. Subsequent viability and development of embryo was assessed on follow up ultrasonic observation. After posterior part of uterus was viewed, the

transducer was rotated 45° to 90° clockwise and counter clockwise to locate the ovaries and the diameter of CL was also measured.

RESULTS AND DISCUSSION

In the present experiment, Real time B-mode ultrasound (6.0 MHz) was used to detect and monitor the early conceptus, its growth and anatomical features in 6 buffaloes. Earliest diagnosis of pregnancy in buffalo was possible on day 19 by viewing non-echogenic structure i.e. embryonic vesicle in the uterine horn ipsilateral to the ovary bearing corpus luteum. Rapid changes were observed in the scanning image pattern of the pregnant uterus and embryo over the initial weeks of pregnancy. Between days 19 and 25, confirmation of pregnancy was based on the demonstration of more echogenic structure. Rapid development of amniotic vesicle was observed during 40 and 50 days of pregnancy. This contributed more vacuolated appearance of uterus. The embryo was visualized as an echogenic structure of 2 to 3 cm length in the amniotic sac. At this stage, the anatomical structure of the fetus could be distinguished clearly.

Observations were made in respect to luteal development and changes in the conceptus viz. development of embryonic vesicle (Fig. 1), crown- rump length (CRL) (Fig.2), heartbeat, skull (Fig. 3), thoracic cavity, vertebral column and placentome and different body parts of fetus from day 20 post service onwards. The time of embryo organization into head, body and limbs was detected. On each ultrasonographic examination, attempts were made to measure the following fetometric parameters: diameter

of the embryonic vesicle (EVD, the widest diameter), crown-rump length (CRL, a straight line between the fetal crown and the origin of tail), heart rate (HR), skull diameter (SLD) and placentome diameter (PLD), maximum diameter while it was in a cross section.

Embryonic vesicle diameter (EVD)

The detection of the embryonic vesicle (the amnion containing the conceptus and the amniotic fluid) were recorded on day 20 onwards in the present study. The embryonic vesicle identified as non-echogenic structure (black) through ultrasound scanning. In the present study, the EVD and CRL were accessible only during the first 60 days of gestation, as by the progress of pregnancy, it became too long to be presented efficiently on the screen. The mean diameter of EVD was 0.52 ± 0.12 , 1.56 ± 0.16 , 2.94 ± 0.07 , 3.80 ± 0.14 and 6.37 ± 0.15 on day 20 (Fig. 1 & 2), 30, 40, 50 and 60 of pregnancy, respectively. The mean diameter of EVD was significantly ($P<0.01$) greater on days 30, 40, 50 and 60 of pregnancy. The result of present study is in agreement with findings of other workers as reported in buffalo (Pawshe *et al.*, 1994; Ali and Fahmy, 2008) and cattle (Rosiles *et al.*, 2005). Embryonic vesicle diameter has been reported to increase up to 30 mm on day 35 of pregnancy in cattle (Chaffaux *et al.*, 1986). Pawshe *et al.* (1994) reported detection of the embryonic vesicle on day 19 ± 2.1 post service with diameter of 4.2 mm which increased 53.6 ± 2.1 mm on day 62 of pregnancy in buffalo. The findings of EVD on day 40 was higher as compared to Rosiles *et al.* (2005) who reported diameter of embryonic vesicle as 2.50 ± 0.36 cm on day 40 of pregnancy in cattle

which might be due to rapid development of buffalo embryo than the cattle (Karaivanov *et al.*, 1987). Furthermore, in the present study, as pregnancy advances the EVD also increased rapidly and reached around 6.37 ± 0.15 cm on day 60 of pregnancy.

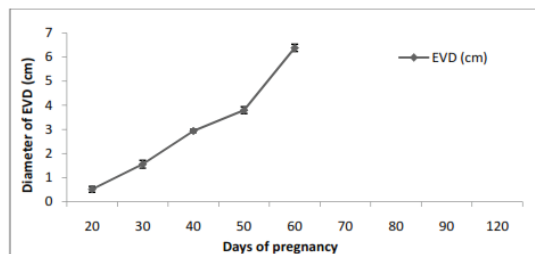


Fig.1. Embryonic vesicle diameter (EVD) during different days of pregnancy in buffalo

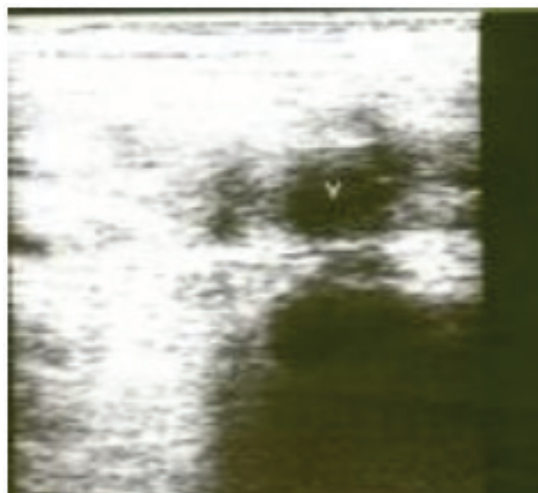


Fig. 2. Ultrasound image showing embryonic vesicle on day 21 of pregnancy in buffalo

Crown-rump length (CRL)

The CRL was 0.73 ± 0.05 , 1.45 ± 0.55 , 2.13 ± 0.05 and 3.24 ± 0.10 cm on day 30, 40, 50

and 60 of pregnancy, respectively. The CRL was significantly ($P < 0.01$) higher on day 40, 50 and 60 as compared to day 30 of pregnancy. The result of present study is accordance with Ali and Fahmy, (2008) who have observed the crown rump length as 0.5 -1.2, 1.5- 2.0, 2.0- 3.0 and 3.0 -3.2 cm on day 30 (Fig. 3 & 4), 40, 50 and 60 of pregnancy in buffalo. The findings is also in concurrence with Pierson and Ginther (1984) who have reported the CRL 1.0, 2.0 and 3.2cm on day 30, 40 and 50 in cattle, respectively.

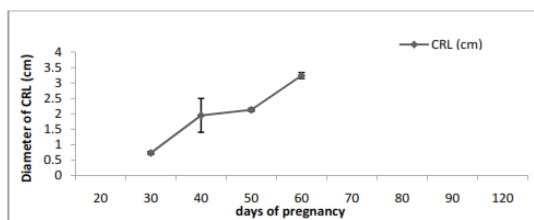


Fig. 3. Crown-rump length (CRL) during different days of pregnancy in buffalo

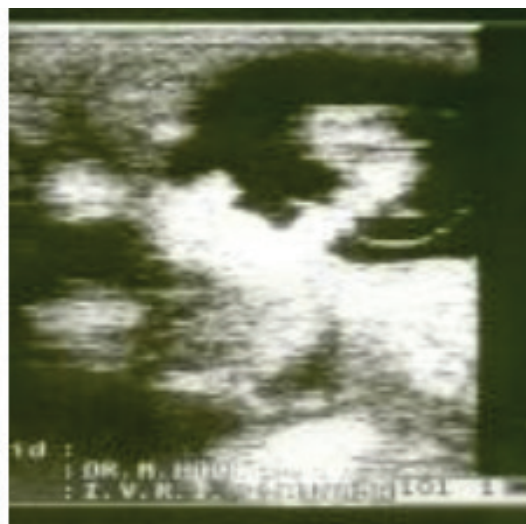


Fig. 4. Ultrasound image showing Crown rump length on day 30 of pregnancy in buffalo

Heart Rate (HR)

Heart of the embryo was detected by day 40, however, heart beat could be counted by day 50 of pregnancy onwards. However, Pawshe *et al.* (1994) reported that the heartbeat of the embryo proper could be detected on day 29.6 ± 1.57 of pregnancy in buffalo which due to efficiency in interpretation which might vary from lab to lab and type of ultrasonography machine etc. The mean heart rate (HR) was 197.33 ± 4.60 , 173.16 ± 1.55 , 162.33 ± 1.20 , 155.33 ± 1.60 , 152.16 ± 2.58 and 146.66 ± 2.40 /min on days 50, 60, 70, 80, 90 and 120 of pregnancy (Fig.5), respectively. The heart rate was significantly ($P < 0.01$) higher on day 50 of pregnancy, thereafter, it reduced as pregnancy advances. The heart rate in the present study is comparable with Pawshe *et al.* (1994) who reported heart rate was 203.8 ± 9.0 beats per minute on day 29 and decreased to 150 beats per minute on day 62 of pregnancy in buffalo.

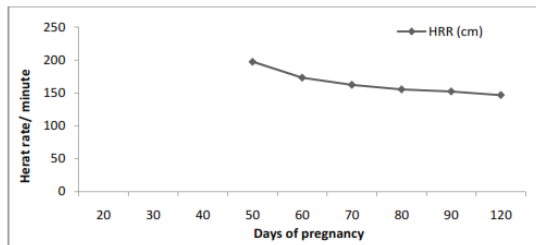


Fig. 5. Heart Rate (HR) during different days of pregnancy in buffalo

Skull Diameter (SLD)

The mean diameter of skull was 2.33 ± 0.18 , 2.90 ± 0.12 , 3.52 ± 0.19 and 4.58 ± 0.22 cm on days 60, 70, 80 and 90 of pregnancy, respectively (Fig. 6 & 7). The mean diameter of skull was significantly ($P < 0.05$) higher on days 80 and 90 of

pregnancy. The skull diameter increased with the advancement of pregnancy. The results of present findings are comparable with findings of Ali and Fahmy, (2008) who reported the skull diameter was 1.5, 2.0, 2.5 and 3.8 cm on 8th, 10th, 12th and 14th week of gestation in buffalo. The accessibility of skull or head was difficult as the pregnancy advances in to mid of the second trimester.

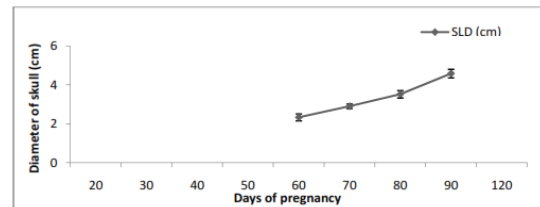


Fig. 6. Skull Diameter (SLD) during different days of pregnancy in buffalo

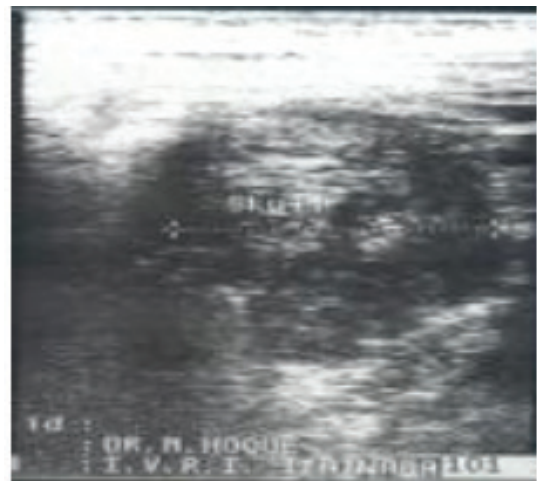


Fig. 7. Ultrasound image showing fetal head on day 30 of pregnancy in buffalo

Placentome diameter (PLD)

The mean diameter of placentome was 0.38 ± 0.01 , 0.64 ± 0.14 , 1.05 ± 0.07 and 1.26 ± 0.08 cm on days 70 (Fig. 8 & 9), 80, 90

and 120 of pregnancy, respectively. The mean diameter of placentome was significantly ($P<0.01$) greater on day 80, 90 and 120 of pregnancy. The results of present study are in accordance with Ali and Fahmy (2008) who observed that the PLD as 0.5, 0.5-1.0 and 1.5-3.0 on 10th, 12th and 14th week of gestation in buffalo, respectively. The placentome grew slowly and reached around 4 cm during 5th month of pregnancy and its diameter rapidly increased 6 -7 cm during the last trimester of pregnancy in buffalo (Ali and Fahmy, 2008).

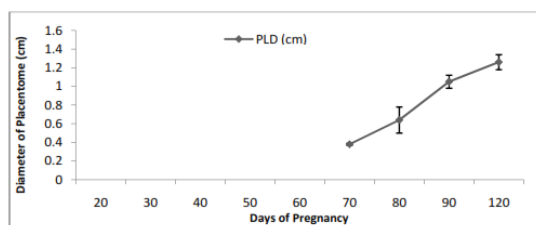


Fig. 8. Placentome diameter (PLD) during different days of pregnancy in buffalo

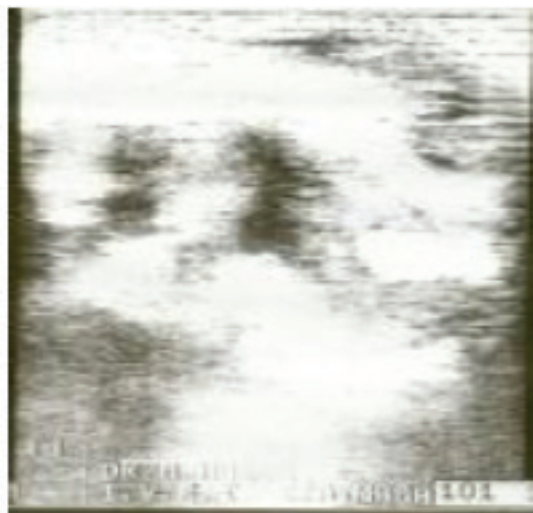


Fig. 9. Ultrasound image showing placentome on day 70 of pregnancy in buffalo

Diameter of Corpus luteum

The CL diameter was 1.36 ± 0.017 , 1.40 ± 0.027 , 1.45 ± 0.027 and 1.47 ± 0.054 cm on days 20, 30, 40 and 50 of pregnancy, respectively. There was no significant difference over the diameter of CL during different days of pregnancy in buffalo. These results are in concurrence with findings of Taylor and Rajamahendran, (1990). Further, it has been reported that diameter of CL was achieved its maximum 9.2 \pm 1.7 days post breeding and there was no significance difference between pregnant and non-pregnant cows as far as the size of the CL is concerned (Taylor and Rajamahendran, 1990).

CONCLUSION

It was concluded that ultrasonography can be used for effective and accurate diagnosis of early pregnancy, gestational stage, embryonic viability and fetal development and there is a lack of accurate methods for the evaluation of bovine fetal growth in the last trimester of gestation in buffaloes.

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