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ABSTRACT

The present experiment was planned to conduct pregnancy diagnosis by ultrasonography to detect pregnant or non-pregnant buffaloes as early as possible. Ultrasonographic scanning was carried out in 39 buffaloes which were apparently healthy and were kept under uniform managemental conditions. The animals were divided in four groups depending upon expected stage of gestation. In group I, (Below 25 days post breeding) all three buffaloes were correctly diagnosed pregnant between 18-24 days of gestation post breeding. In group II, (25 to 35 days post breeding) six buffaloes were confirmed pregnant on the basis of compartmentalization and embryo proper. In group III, (36 to 45 days post breeding) twelve buffaloes were confirmed pregnant with presence of amniotic vesicles and limb buds. In group IV, (46 to 60 days post breeding) seven buffaloes were confirmed pregnant and ossification of foetus was noted. The average crown rump length (CRL) of buffalo embryos in group II, III and IV was 0.92 ± 0.06 , 1.89 ± 0.05 and 4.04 ± 0.74 cm, respectively. The average Body Diameter (BD) of fetuses in group III and IV was 0.73 ± 0.03 and 1.19 ± 0.16 cm, respectively. The average Bi-Parietal Diameter (BPD) of fetuses from group IV was 1.00 ± 0.03 cm. It was concluded that the signs of pregnancy and non-pregnancy at day 25-35 of gestation can be confirmed using ultrasonography as a suitable diagnostic tool for field use. CRL, BD and BPD can be used as diagnostic tool for ageing of embryo and foetus in buffaloes.

Key words: Buffalo, Early pregnancy diagnosis, Ultrasonography.

The assessment of reproductive performance is dependent on fertility of the animal. An early diagnosis of pregnancy is essential for reproductive management as well as economic production in buffaloes. The purpose of pregnancy diagnosis is to identify all 'open buffaloes' so that these can be re-inseminated and conceived as early as possible. The use of real-time, B-mode diagnostic ultrasound has been increasing as an imaging modality in bovine reproduction, as it become more apparent that its use can produce solutions to a number of unanswered questions in dealing with the bovine reproduction cycle and its concurrent disorders and pregnancy diagnosis. Therefore, the present study was undertaken to assess the use of linear-array, real-time, B-mode ultrasound for detection of early pregnancy as well as confirmation of non-pregnancy in buffaloes.

MATERIALS AND METHODS

The present study was carried out in the Department of Animal Reproduction, Bombay Veterinary College Parel, Mumbai. A total of 39 buffaloes with known breeding history were selected from commercial buffalo dairy farms located in Western suburbs of Greater Mumbai for early pregnancy diagnosis by real time B-mode transrectal ultrasonography. The buffaloes were categorized in four different groups on the basis of days postmating/insemination as Group I, below 25 days post breeding (n=03); Group II, 25 to 35 days post breeding (n =11); Group III, 36 to 45 days post breeding (n = 15) and Group IV, 46 to 60 days post breeding (n = 10). A real time, B-mode, portable ultrasonography machine (SSD-500 Aloka, Co. LTD. Japan) equipped with 5 MHz linear-array, rectal transducer was used for scanning of the uterus. Buffaloes were restrained in standing position for scanning

in the travis. Since, the scanning was for a short duration, no special medication was required for restraining. Animals were back-racked for evacuating the rectum prior to scanning. For a clear visualization of the images, care was taken to avoid direct light on the monitor. During scanning the relevant image was frozen and measurements of embryo (CRL, BD and BPD) were taken by electric caliper. All the relevant pictures were printed by using electronic printer (Sony, UP-895 MDW, Japan). Pregnancy status was confirmed by rectal palpation between days 45-60 following natural service in all buffaloes, which were scanned by ultrasonography. Statistical analysis was conducted as described by Snecedor and Cochran (1994).

RESULTS AND DISCUSSION

Ultrasonographic examinations were carried out in four groups between 18-60 days of post-breeding in buffaloes. In first group (below 25 days post breeding), three buffaloes were scanned for pregnancy diagnosis by ultrasonography. All of these three buffaloes were diagnosed as pregnant correctly. The buffaloes, which were diagnosed pregnant, showed thin, slightly elongated anechoic area inside the uterine lumen. Initiation of compartmentalization as well as embryonic heart-beat were noticed in two buffaloes at 23-24 days post breeding along with hypoechoic endometrium indicating increased circulation as per observations by Kahn (1994); however in buffalo, which was 18 days post breeding, initiation of compartmentalization and embryonic heart-beat was not seen (Fig. 1). These observations regarding early pregnancy diagnosis corroborated with Pawshe et al. (1994) and Rane et al. (2002) who observed presence of embryonic vesicle and embryo as early as day 19.0 ± 1.69 and day 24 post breeding, respectively. However, in the

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Fig.1: Early Blastocyst on 18th day

Fig.2: Embryo on 29th day of gestation

present study embryonic heart-beats were detected much earlier on day 24 than the findings by other research workers, (Pawshe *et al.*,1994 and Glaztel *et al.*, 2000), who detected heart-beat of embryo as late as day 29.6 \pm 1.57 and day 28 post breeding in buffaloes, respectively which could be attributed to old generation of machine used by them.

In group II (day 25-30 post breeding), 11 buffaloes were scanned by trans-rectal ultrasonography. Amongst them, six buffaloes were diagnosed as pregnant and five were diagnosed as non-pregnant. The most peculiar characteristic of the image during this stage of gestation was compartmentalization of embryonic vesicle (Fig. 2 and 3). A similar observation regarding compartmentalization of embryonic vesicle was reported by Bhosrekar and Hangare (2000). Typically, 2 to 3 anechoic sections were visible through the chorionic vesicle. Similar observations regarding visualization of allantois were reported by Pawshe et al. (1994) and Rane et al. (2002) in buffaloes. An embryo, which was little more intense than that of the neighboring endometrium, was noticed in one of these compartments in all the cases. A flickering echo of the heart-beats confirmed the presence of embryo. A typical thin but hyper-echoic membrane was observed many times representing the allantoic membrane. The average CRL of embryo of four buffaloes out of six from day 25 to 35 was 0.92 ± 0.06 (range 0.72 to 1.05 cm). No report could be traced to compare the present results in the scanned literature regarding measurement of CRL in buffalo embryos. The average measurements of CRL for the embryos during present study are in concurrence with those reported in cows by Pierson and Ginther (1984) and Rathod (2002).

In group III (day 36-45 post breeding), 15 buffaloes were scanned during this stage by ultrasonography. Out of them, 12 buffaloes were confirmed pregnant whereas 3 were confirmed nonpregnant. The buffaloes, which were diagnosed pregnant, showed further very clear expansion of the hypoechoic embryonic vesicle with multiple compartments, with embryo in one of the compartments (Fig. 4). Limb buds and heart-beats were also clearly seen. The observations regarding presence of amnion and limb buds corroborated with those reported by Pawshe *et al.* (1994) and Singh *et al.* (2018) in buffaloes and Rathod (2002) and Patil (2003) in cows. The average CRL and BD of embryos from day 36 to 45 in 11 buffaloes was 1.89 ± 0.05 cm and 0.73 ± 0.03 cm, respectively. However, observations on CRL and BD Fig.3: Embryo on 31st day of gestation

Fig.4: Embryo on 44th day of gestation

specific to buffaloes were not available in the literature cited for comparison. In cows, Pierson and Ginther (1984) reported CRL and Rathod (2002) reported CRL and BD, which is more than that the findings recorded in the present study.

In group IV (day 46-60 post breeding), ten buffaloes were scanned by ultrasonography and seven were diagnosed pregnant whereas three buffaloes were diagnosed as non-pregnant. During this period the outline of the fetus with its head, extremities and umbilical cord became visible. Foetal movements, ribs and pelvic bones were visible during this stage. The compartmentalization observed in the initial stages became less prominent. Flickering echo at the umbilical attachment to the abdominal wall was also clearly visible. Similar observations regarding foetal movements and visibility of bones at this stage was reported by Pawshe et al. (1994) in buffaloes and Kahn (1994) in cows. The average CRL and BD of foetuses at this stage in 7 buffalo fetii were $4.04 \pm$ 0.74 and 1.19 ± 0.16 cm whereas, measurements of BPD was possible only in four buffalo fetii with average of 1.00 ± 0.03 cm. Pawshe *et al.* (1994) reported slightly higher CRL on day 62 post-insemination. This could be due to the fact that in the present study, the average of CRL from day 46-60 was assessed. In the literature scanned no specific reference regarding measurement of BD and BPD in buffalo fetii could be found to compare the present results. However, in cows Rathod (2002) reported CRL, BD and BPD, while White et al. (1985) reported BPD, which is more than the present findings. The variation in CRL, BD and BPD could also be attributed due to species variation.

Confirmatory signs of pregnancy and nonpregnancy at day 25-35 post- mating/insemination make ultrasonography a suitable diagnostic tool for field use at this stage of gestation. Though initial cost of ultrasonography is high but it is better diagnostic tool for pregnancy diagnosis at field level as it gives instant results and the recurring cost per scan is also negligible.

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