ABSTRACT

Use of ultrasonography for early pregnancy diagnosis as well as studying various reproductive disorders in bovines is a recent development. Real-time ultrasonic transrectal scanning of the uterus appears to offer rapid, safe, accurate and practical method of early pregnancy diagnosis. The main principle of early pregnancy diagnosis is to detect open (non-pregnant) buffaloes as early as possible and rebreed the nonpregnant animals by adopting suitable treatment to maintain optimum calving interval. In the present study, it is observed that due to the confirmatory signs of pregnancy and non-pregnancy at 37 days of gestation by ultrasonography proves this technique to be highly accurate for early pregnancy diagnosis at this stage for field use with 100% accuracy.

Keywords: buffaloes, Bubalus bubalis, pregnancy, ultrasonography, diagnosis, uterus

INTRODUCTION

The use of real-time, B-mode diagnostic ultrasound has been increasing as an imaging modality in dealing with the bovine reproduction cycle and its concurrent disorders and pregnancy diagnosis. The reproductive performance and productive efficiency of the animals are directly related to each other. Diagnosis of pregnancy at an early stage is essential for effective reproductive management. Ultrasonography has been found to be the most reliable method for early pregnancy diagnosis in buffaloes among other methods of pregnancy diagnosis. In this method, presence of the conceptus and viability of embryo can be detected (Tiwari et al., 2002). This will not only helpful in ruling cases of early embryonic mortality but also for diagnosing different reproductive problems during scanning. This is essential to take appropriate remedial measures.

The purpose of early pregnancy diagnosis is to identify all “open animals”: so that these animals can be reinseminated and become pregnant as early as possible before they are designated as reproductive culls. This will helpful for achieving an optimum calving-to-conception interval of 85-110 days as well as an ideal calving interval of 14-16 months in buffaloes. Early pregnancy diagnosis will also help to improve the reproductive efficiency as well as to develop strategies for adopting suitable remedial measures for non-pregnant animals. Therefore, the present study was undertaken to assess the earlier day for detection of early pregnancy in buffaloes by ultrasonography.
MATERIALS AND METHODS

Housing and management of experimental buffaloes

Four groups of 24 buffaloes were housed in a loose housing barn. They were kept indoors, and there was no open paddock in the barn. Each lot had 26 resting places (1.2 X 2 m) on one side and a manure alley with Delta Master™ manure scraper (Delaval AB, Sweden) on the other hand side positioned towards the feed rack. The feed rack had 26 standing places (each 1.2 m wide) and was without any locking arrangement. Each lot had one automatic concentrate feeding station (AFS) and nine valve-controlled automatic water bowls. Concentrate feeding was done at milking, and milking was controlled with the Alpro™ system (Delval AB, Sweden) where a central processor received the milking and concentrate feeding data of all the buffaloes in the herd. Manure scrapers were turned on twice in the morning and afternoon before milking, while the workers raked down the dung from the cubicles. A micro sprinkler was fitted above each buffalo’s standing place and was turned on between 12.00 to 14.00 h every day. Buffaloes also received a shower before being milked twice a day, at 7.00 and 17.00 h in a tandem parlor. Temperatures were recorded every fourth hour. Indoor and outdoor temperature ranged between 19 and 36°C.

Feeding

The ordinary routine was to place roughages for ad lib feeding in the barn three times a day. The leftovers were marginal, and very little feed was left in the troughs before the morning cleaning. The roughages fed during the experiment consisted of fresh, cut and chopped sugarcane, alfalfa, napier grass, green maize and jowar straw, which were chopped and transported to the barn in tractor trolley and dispensed manually into the feed troughs. A pre-calculated quantity of concentrate mixture was fed to each buffalo based on milk yield, body weight and pregnancy status. Concentrate was fed through the automatic concentrate feeding station (AFS) in the barn. If the pre-calculated amount was not consumed, the residual was transferred to the next feeding. Residual amounts at the end of a 24 h period were transferred to the next 24 h period. During milking, an in-parlor feeding (IPF) system supplied a fixed amount of concentrates. The buffaloes were provided mineral mixture according to their milk production and body weight.

Modus oprendi of ultrasonography

Early pregnancy diagnosis was attempted in 72 buffaloes on days 21, 28 and 37 day post insemination. The machine (SD-900 Aloka Co. Ltd., Japan) with 7.5 MHz rectal transducer was used to scan the pregnant uterus. The buffalo was restrained in standing position in travis. The buffalo was backracked by evacuating the rectum. The contact jelly was applied over the transducer before insertion. After backracing, the transducer was inserted per-rectally and uterus was visualized by moving transducer from left to right and vice versa. The rectal probe was positioned in close proximity to the dorsal surface of the uterine horn, ipsilateral to the ovary containing CL. After initial orientation, ipsilateral uterine horn was scanned on its dorsal and then on lateral surface for signs of pregnancy.

Positive diagnosis of pregnancy was based on the presence of a non-echogenic round area of varying size in the lumen of an echogenic uterine horn representing the fluid filled allantoic cavity termed the embryonic vesicle (Pieterse et al., 1990). The presence of an embryo within the
embryonic vesicle was confirmed by observing an echogenic (white) area with rhythmic pulsation representing heartbeats (Pierson and Ginther, 1984). Correct diagnosis in this study was defined as either (1) an animal diagnosed pregnant with ultrasound examination and subsequently confirmed pregnant during palpation per rectum on day 60 post-service, or (2) an animal diagnosed non-pregnant with ultrasound examination and subsequently confirmed non-pregnant during palpation per rectum or returned to estrus at a later date. An incorrect diagnosis was defined as either (1) an animal diagnosed pregnant with ultrasound examination and subsequently confirmed non-pregnant during palpation per rectum or returned to estrus at a later date, or (2) an animal diagnosed non-pregnant with ultrasound examination and subsequently confirmed pregnant during palpation per rectum.

Diagnostic accuracy was defined as the percentage of correct diagnoses out of the total number of ultrasound examinations. Sensitivity of the method was defined as the percentage of animals found pregnant by ultrasound scanning out of the total number of animals found pregnant by palpation per rectum. Specificity was defined as the percentage of non-pregnant animals diagnosed non-pregnant by ultrasound scanning and later confirmed non-pregnant by rectal palpation or returned to estrus at a later date. The positive predictive value was defined as the percentage of actual pregnant animals out of the total number of animals diagnosed pregnant through ultrasound scanning. The negative predictive value was defined as the percentage of actual non-pregnant animals out of the total number of animals diagnosed non-pregnant through ultrasound scanning (Badtram et al., 1991).

Statistical analysis

The data pertaining to uterine involution, ovarian activity, post-partum exhibition of oestrus, early pregnancy diagnosis, conception rate and blood biochemical profiles in the six different groups were suitably tabulated and analyzed following statistical methods described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Early pregnancy diagnosis through ultrasound scanning on day 21 of gestation

Seventy-two buffaloes were scanned by trans-rectal ultrasonography on day 21 after insemination. Out of these eight buffaloes were diagnosed as pregnant and 64 as non-pregnant. The buffaloes which were diagnosed pregnant showed thin elongated anechoic areas of 3-4 mm in length. These areas indicated areas of fluid accumulation and were found in the horn, ipsilateral to the corpus luteum. An embryonic vesicle could be noticed with hypoechoic endometrium indicating increased circulation. It was not possible to locate an embryo and heart beats in all cases. These observations regarding early pregnancy diagnosis corroborate Pawashe et al. (1994) and Rane et al. (2002) who observed the presence of the embryonic vesicle as early as day 19.0±1.69 and day 24, respectively in buffaloes. However, Glatzel et al. (2000) detected pregnancy as early as the 25th day after breeding in water buffalo heifers by detection of fluid in the apex of the uterine horn and the presence of CL.

Early pregnancy diagnosis through ultrasound scanning on day 28 of gestation

Seventy-two buffaloes were scanned on day 28 post insemination. Out of them, 14 were
diagnosed pregnant and 58 were diagnosed non pregnant. The buffaloes which were diagnosed pregnant showed expansion of the embryonic vesicle with a diameter of 6-8 mm. In 14 buffaloes, an embryo was detected in the embryonic vesicle and in four buffaloes heartbeats were observed. The most peculiar characteristic of the image during this stage of gestation was compartmentalization of the embryonic vesicle. These compartments were seen as hypoechoic images of the fluid sac interrupted in places by hyperechoic folds projecting in the hypoechoic lumen. This created a pseudo-ampullar image as observed in cattle by Kahn (1989). Typically two to three anechoic sections were visible through the chorionic vesicle. Similar observations regarding compartmentalization of the embryonic vesicle was reported in buffaloes by Bhosrekar and Hangare (2000). An embryo was noticed in one of these compartments in all the cases. The echogenicity of the embryo was little more intense than that of the neighbouring endometrium. The embryo was seen projecting from the wall into the anechoic uterine lumen. A flickering echo of the heartbeats confirmed the presence of live embryo. A few millimeters away from the embryo a very thin, arched, hyperechoic amniotic vesicle surrounded the embryo was observed in all pregnant buffaloes. Limb buds were also clearly seen. A total 72 buffaloes were scanned during this stage. Out of them, 20 were diagnosed as pregnant. In all these buffaloes, embryos along with heartbeats were observed. These results were further confirmed with per-rectal examination at 60 days after insemination. The observations regarding presence of amnion and limb buds corroborate those reported by Pawshe et al. (1994); Rane et al. (2002); Mali (2006) in buffaloes.

**Early pregnancy diagnosis through ultrasound scanning on day 37 of gestation**

This period is most suitable for the confirmation of early pregnancy as the vesicle is further expanded and compartmentalization was observed. The compartments were seen as hypoechoic images of the fluid sac interrupted in places by hyperechoic folds projecting in the hyperechoic lumen. A flicking echo of the heartbeats confirmed the presence of live embryo. A few millimeters away from the embryo a very thin, arched, hyperechoic amniotic vesicle surrounded the embryo was observed in all pregnant buffaloes. Limb buds were also clearly seen. A total 72 buffaloes were scanned during this stage. Out of them, 20 were diagnosed as pregnant. In all these buffaloes, embryos along with heartbeats were observed. These results were further confirmed with per-rectal examination at 60 days after insemination. The observations regarding presence of amnion and limb buds corroborate those reported by Pawshe et al. (1994); Rane et al. (2002); Mali (2006) in buffaloes.

**Accuracy of early pregnancy diagnosis through ultrasound scanning**

In the scanning the buffaloes which were confirmed pregnant ultrasonographically as well as by rectal palpation were classified as “diagnosis pregnant correct” (a). The buffaloes diagnosed pregnant ultrasonographically but confirmed non-pregnant by rectal palpation were classified as “diagnosis pregnant incorrect” (b). The buffaloes confirmed non-pregnant ultrasonographically as well as by rectal palpation were classified “diagnosis non pregnant correct” (c). The buffaloes diagnosed non-pregnant ultrasonographically but confirmed pregnant by rectal palpation were classified as “diagnosis non-pregnant incorrect”
These observations were used to calculate sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of pregnancy.

A total of 72 buffaloes were scanned for early pregnancy diagnosis from 21 to 37 days of gestation. This period was divided in three stages (day 21, day 28 and day 37) depending upon the stage of gestation. These buffaloes scanned in the respective stage of gestation by ultrasonography and confirmed with rectal palpation at 60 days after insemination. The data regarding these observations for the respective stage of gestation are represented in Table 1.

The above table reveals that the overall percentage of accuracy of early pregnancy diagnosis was 85.71%, 92.30% and 100% on days 21, 28 and 37 of gestation in buffaloes. The data regarding sensitivity, specificity, positive predictive value, negative predictive value and percent accuracy for the respective stage of gestation are presented in Table 2.

Although the specificity was 100 percent in all the three stages (21, 28 and 37) of ultrasound scanning, the sensitivity was lower on day 21 than on day 28, being 40 and 70 per cent, respectively.

Although positive predictive value of early pregnancy was 100 percent in all three stages (21, 28 and 37 day) of ultrasound scanning, the negative predictive value of early pregnancy diagnosis was lower on day 21 than on day 28, being 84.21 and 90.62 percent, respectively. Whereas, sensitivity, specificity, positive and negative predictive value were 100 percent on day 37 of gestation. Awasthi

Table 1. Accuracy of early pregnancy diagnosis in different stages of gestation.

<table>
<thead>
<tr>
<th>Stage of gestation</th>
<th>No. of scans</th>
<th>USG P</th>
<th>USG NP</th>
<th>Rectal P</th>
<th>Rectal NP</th>
<th>Overall accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>On day 21</td>
<td>72</td>
<td>8</td>
<td>64</td>
<td>20</td>
<td>52</td>
<td>85.71%</td>
</tr>
<tr>
<td>On day 28</td>
<td>72</td>
<td>14</td>
<td>58</td>
<td>20</td>
<td>52</td>
<td>92.30%</td>
</tr>
<tr>
<td>On day 37</td>
<td>72</td>
<td>20</td>
<td>52</td>
<td>20</td>
<td>52</td>
<td>100%</td>
</tr>
</tbody>
</table>

P- Pregnant, NP- Non-pregnant

Table 2. Overall accuracy percentage of early pregnancy diagnosis by ultrasonography.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>On day 21</th>
<th>On day 28</th>
<th>On day 37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis pregnant correct (a)</td>
<td>8</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>Diagnosis pregnant incorrect (b)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diagnosis non-pregnant correct (c)</td>
<td>64</td>
<td>58</td>
<td>52</td>
</tr>
<tr>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>12</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity (100x a/(a+d))</td>
<td>40</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (100 c/(c+b))</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value (100x a/(a+b))</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (100x c/(c+d))</td>
<td>84.21</td>
<td>90.62</td>
<td>100</td>
</tr>
<tr>
<td>Overall accuracy percentage</td>
<td>85.71</td>
<td>92.30</td>
<td>100</td>
</tr>
</tbody>
</table>
(2004) who observed 87.5 percent specificity and 93.33 percent positive predictive value which were lower than results of present study whereas the negative predictive was slightly higher (95.45 per cent) than results of present study on day 28 of gestation in Surti buffaloes. Mali (2006) reported 100 percent specificity, positive predictive value and overall accuracy, which were higher than the present study, below 25 days of gestation in buffaloes. The variation in the findings depends upon a variety of factors including type of ultrasound used (sector or linear), frequency of transducer selected, age and parity animals, stage of gestation at which examination was carried out and the operator’s skill.

CONCLUSION

Strictly adhering to the principles of calculation, in the present study, it can be emphatically said that the confirmatory signs

Figure 1. Initiation of compartmentalization and embryo with vesicle.
of pregnancy and non-pregnancy at 37 days of gestation by ultrasonography proves this technique to be highly accurate for early pregnancy diagnosis at this stage for field use with 100% accuracy.

REFERENCES


