



## Transrectal Ultrasonographic Monitoring of Early Pregnancy in Graded Murrah Buffalo Cows (*Bubalus bubalis*)

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### Abstract

The objective of the present study was to evaluate the accuracy of transrectal ultrasonography as a method of pregnancy diagnosis at an early stage in graded murrah buffaloes. 30 inseminated buffaloes were utilized and pregnancy diagnosis was commenced on day 20, 25 and 30 post breeding. Positive diagnosis of pregnancy was based on the presence of an anechoic round area of varying size in the lumen of an echogenic uterine lumen representing the fluid filled allantoic cavity termed as the embryonic vesicle. The presence of an embryo within the embryonic vesicle was confirmed by observing an echogenic area with rhythmic pulsation representing heartbeats. The average lengths of embryonic vesicle on day 20, 25 and 30 were  $0.415 \pm 0.05$ ,  $1.116 \pm 0.107$  and  $1.85 \pm 0.068$  cm, respectively and the average lengths of embryo on day 25 and 30 were  $0.45 \pm 0.064$  and  $1.05 \pm 0.086$  cm, respectively. Sensitivity was lower on day 20 and 25 being 50 and 75%, respectively when compared to day 30 which was 100%. The overall accuracy increased as the pregnancy advances and was observed to be 100% from day 30 onwards. It was confirmed that early pregnancy diagnosis using ultrasonography can be done effectively from day 25 post insemination.

**Keywords:** Ultrasonography, transrectal, murrah buffalo, early pregnancy, accuracy

Early diagnosis of pregnancy is an important aspect for both the reproductive management and the profitability of dairy herds. Early identification of non pregnant

dairy animals post breeding can improve reproductive efficiency and pregnancy rate by decreasing the interval between artificial insemination services and increasing artificial insemination service rate. Ultrasound pregnancy diagnosis is a reliable method of determining the presence of a conceptus and viability of an embryo, which is essential to increase the profitability of the animal (Tiwari *et al.* 2002). In 1990's, various workers started transrectal ultrasonography in buffaloes to visualize the embryonic vesicle and embryo proper in pregnant buffalo cows between 19 and 22 days post insemination (Pawshe *et al.* 1994). Ultrasound is a less invasive technique for early pregnancy diagnosis and may minimize the rare incidence of palpation induced abortions. In buffaloes, it is most commonly used to determine pregnancy, fetal age, sex as well as ovarian activity (Ali and Fahmy, 2008). Transrectal ultrasound can be used as early as 25 days after insemination but is more typically applied after day 30 (Fricke, 2002).

**Materials and Methods**

A total of 30 Graded Murrah she buffaloes aged 4 to 8 years, between 2nd to 6th lactation and weighing 400-500 kg were selected at Buffalo Research Station and some of the buffalo cows presented to college hospital were utilized in the present study. All the experimental animals showing estrus signs were examined per rectally and scanned using transrectal ultrasonography and were artificially inseminated with good quality frozen thawed semen. All the inseminated buffaloes which did not return to estrus by day 20 were subjected to pregnancy diagnosis using ultrasound.

**Technique:** All the experimental buffaloes subjected to ultrasonographic examination were properly restrained to avoid image distortions. The faecal material was removed from the rectum before introducing the transducer. A coupling gel was applied on the transducer which was kept in a condom to obtain better contact with rectal wall. The transducer was positioned in close proximity to the dorsal surface of the uterine horn (Curran *et al.* 1986, Boyd *et al.* 1988) and moved sideways and lengthwise to scan the contents of both uterine horns (Hanzen and Delsaux 1987). At each examination, an attempt was made to record the first detection of embryonic vesicle and embryo. Pregnancy confirmation was based on the presence of an anechoic round area of varying size in the lumen of an echogenic uterine horn representing the fluid filled allantoic cavity termed as embryonic vesicle (Pieterse *et al.* 1990).

Pregnant	Non-pregnant
Diagnosis pregnant correct (a)	Diagnosis non-pregnant correct (c)
Diagnosis pregnant incorrect (b)	Diagnosis non-pregnant incorrect (d)

Number of pregnant animals = a+d

Number of non pregnant animals = b+c

Sensitivity =  $a / (a+d) \times 100$   
 Specificity =  $c / (c+b) \times 100$   
 Positive predictive value =  $a / (a+b) \times 100$   
 Negative predictive value =  $c / (c+d) \times 100$   
 Overall diagnostic accuracy =  $a+c / (a+b+c+d) \times 100$ .

**Statistical Analysis**

The means and standard errors of the size of embryonic vesicles and embryos at different stages of gestation were calculated and are presented.

**Results and Discussion**

Out of 30 buffaloes scanned on day 20, 4 (50%) were diagnosed as pregnant based on the presence of anechoic embryonic vesicle (Fig. 1) which was spherical in outline within the uterine lumen.

**Table 1: Percentage of occurrence and measurements of embryonic vesicle and embryo at different stages of gestation (Mean ± SE)**

Sl. No.	Days of gestation	Embryonic vesicle	Size of embryonic vesicle (cm)	Embryo	Size of embryo (cm)
1.	20	4/8 (50%)	0.415 ± 0.05	—	—
2.	25	6/8 (75%)	1.116 ± 0.107	4/6 (66.66%)	0.45 ± 0.064
3.	30	8/8 (100%)	1.85 ± 0.068	8/8 (100%)	1.05 ± 0.086

The remaining 26 (86.67%) inseminated buffaloes were considered as non-pregnant as there was no observable presence of embryonic vesicle. Ultrasound scanning performed on day 25, revealed presence of increased size of embryonic vesicle and compartmentalization (Fig. 2) of the above four animals and two more buffaloes had also shown the presence of embryonic vesicle, thus leading to a total of 6 (75%) as pregnant and those which did not show embryonic vesicle were treated as non pregnant (24). Similarly, ultrasound scanning performed for the third occasion on day 30 (Fig. 3), in 8 (8/8; 100%) buffaloes, the embryonic vesicle visualized with the presence of an echogenic structure within the vesicle and diagnosed as pregnant while remaining 22 were diagnosed as non pregnant as these buffaloes did not visualize neither anechoic embryonic vesicle nor echogenic embryo in the uterine lumen. The embryo proper was clearly visualized as an echogenic structure within the uterine lumen on day 30 of gestation. The mean lengths of embryonic vesicle and embryo proper on different

stages of gestation were presented (Table. 1). Accuracy in terms of sensitivity, specificity, positive and negative predictive values were presented (Table. 2)

**Table 2: Overall accuracy of early pregnancy diagnosis using ultrasonography (n=30)**

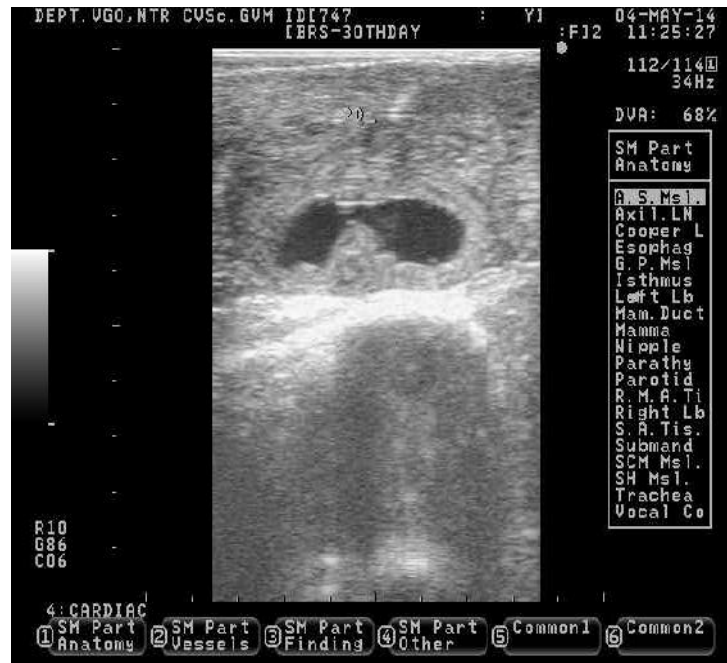
Sl. No.	Particulars	Days after insemination		
		Day 20	Day 25	Day 30
1	Diagnosis pregnant correct (a)	4	6	8
2	Diagnosis pregnant incorrect (b)	4	0	0
3	Diagnosis non-pregnant correct (c)	18	22	22
4	Diagnosis non-pregnant incorrect (d)	4	2	0
5	Sensitivity (Se ; %) $100xa/(a+d)$	50.0	75.0	100
6	Specificity (Sp ; %) $100xc/(c+b)$	81.81	100	100
7	Positive predictive value (PPV ; %) $100xa/(a+b)$	50.0	100	100
8	Negative predictive value (NPV ; %) $100xc/(c+d)$	81.81	91.66	100
9	Overall diagnostic accuracy	73.33	93.33	100

In the present study, the percent pregnant animals increased from 4 on day 20 to 8 on day 30, based on the visualization of embryonic vesicle and embryo.



**Fig. 1: Spherical embryonic vesicle**

The clear visualization could be due to development of early stage of embryo. The remaining 22 buffaloes did not show any evidence of pregnancy even at day 45-60 on rectal examination. Further it was observed that all 8 were found pregnant on rectal examination at day 45-60 post insemination. Sometimes the vesicle appeared as two anechoic areas around the embryo.

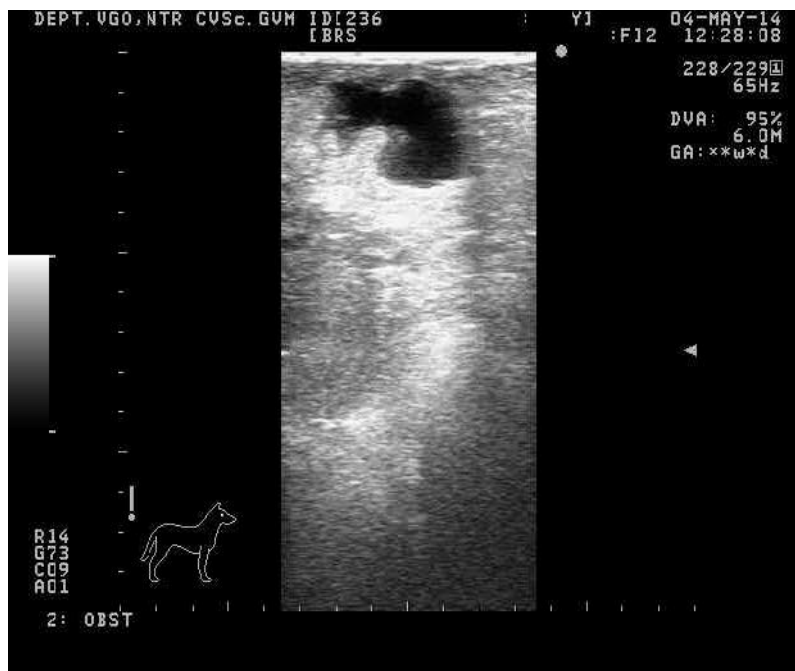


**Fig. 2: Elongation of embryonic vesicle**

Similar findings were reported by Curran *et al.* (1986) and Pawshe *et al.* (1994). However, Bhosrekar and Hungarge (2000) and Glatzel *et al.* (2000) reported the visualization of embryonic vesicle by day 30 and 25 respectively. The mean length of embryonic vesicle and embryo on different stages of gestation were calculated (Table. 1). These findings were in close agreement to the findings of Rosiles *et al.* (2005). On the contrary, these were higher than those reported by Bhosrekar and Hangarge (2000). Based on the above observations, the sensitivity and specificity for early pregnancy diagnosis through ultrasound on day 20, 25 and 30 of gestation were calculated (Table. 2). Both negative and positive predictive values improved as the pregnancy advanced from day 20 to 30.

The reported detection of embryonic vesicle on day 20 with 50 per cent sensitivity was in close agreement with Karen *et al.* (2007) and Ingawale *et al.* (2012) who reported that as 44.4 and 40.00 per cent, respectively. However, the present finding was much

less to the sensitivity reported by Nakhshi *et al.* (2010) and Awasthi *et al.* (2011), who reported 75 and 100 per cent respectively. The 75.00 per cent sensitivity in detecting the embryonic vesicle and embryo observed in this study on day 25 was higher than the observations of Nakhshi *et al.* (2010) and Ingawale *et al.* (2012), who reported 66.7 and 70.00 percent respectively. The difference could be due to the great difficulty in early identification of the embryo in the present study was due to its position, which might be very close to or in contact with the uterine wall (Ferreira *et al.*, 2012) in buffaloes.



**Fig. 3: Embryo proper within embryonic vesicle**

The same difficulty was also expressed in cattle by Kahn (1994), who considered that signs of pregnancy in bovine ultrasound are positive only when embryonic vesicle, embryo together with its heartbeats could be detected. In buffaloes the 100 percent accuracy, sensitivity and specificity of ultrasound detection of pregnancy at day 30 post insemination observed in this study was in total agreement with Bhosrekar and Hungarge (2000), Karen *et al.* (2007), Nakhshi *et al.* (2010), Ingawale *et al.* (2012) and Naikoo *et al.* (2013). The results observed in this study for sensitivity, specificity, positive and negative predictive values were in total agreement with the findings of Moharrami *et al.* (2013), who observed the accuracy of pregnancy diagnosis using ultrasound in cows.

Based on the results of the present study, 4 buffaloes pregnant on day 20, 6 on day 25 and 8 on day 30 and the same were confirmed by rectal examination at day 45-60 post insemination. From these observations, it was inferred that maximum sensitivity and specificity were recorded with best time at 30 days post insemination by using transrectal ultrasound with 7.5 MHz linear array transducer in buffaloes. In conclusion, embryonic vesicle and embryo were 100% evident in Graded Murrah buffaloes after day 30 post insemination using ultrasonography.

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