EARLY PREGNANCY DIAGNOSIS BY TRANSRECTAL ULTRASONOGRAPHY IN MEHSANA BUFFALOES (*Bubalus bubalis*)

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ABSTRACT

The aim of this study was to detect early pregnancy and early embryonic mortality using transrectal ultrasonography in Mehsana buffaloes. The study was carried out on 18 postpartum anestrus Mehsana buffaloes of a dairy farm during breeding season, using different estrus synchronization protocols, followed by fixed time AI. Ultrasound examinations were performed using a real time B-mode ultrasound scanner (ALOKA SSD - 500) equipped with 5 MHz linear array transducer on day 26 and 40 post AI. Ultrasound scanning on day 26 post AI revealed visualization of embryonic vesicle around the conceptus. The embryo proper was observed in nine out of 13 animals (69.23%) on day 26 and all 12 conceived animals (100%) on day 40 post AI. The amniotic and allantoic vesicles were observed as non-echogenic cavities closely surrounding the embryo proper on day 40 post AI. Ovarian corpus luteum and foetal heart beats were observed in conceived buffaloes on both 26 and 40 days post AI. Early embryonic death occurred in one buffalo each between days 26 and 40 and between day 40 and 60 post AI. The overall diagnostic accuracy of ultrasonography for early pregnancy was recorded as 94.44% and 100% on day 26 and day 40, respectively. Plasma progesterone concentrations ranged from 3.00-6.02 ng/ml with an average of 4.42±0.52 ng/ml in pregnant animals and from 0.97-1.91 ng/ml with an average of 1.22±0.06 ng/ml in nonpregnant animals on day 20 post AI. It was concluded that ultrasound scanning is a useful tool in detection of early pregnancy, early non-pregnancy and early embryonic mortality in buffaloes.

Keywords: ultrasonography, early pregnancy diagnosis, Mehsana buffaloes

INTRODUCTION

Livestock production in general and milk production in particular play an important role in our national economy and thus dairying is one of the most important agricultural activities in India. Buffaloes are the major contributor to milk production in our country, especially in Gujarat state. Conditions like early embryonic mortality, early fetal death and unobserved abortions due to the tropical environment lead to an increased

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anestrus period and thereby increase the costly calving interval. To overcome such problems, reproductive ultrasonography may serve as a better tool.

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. Ultrasonographic observation of the postpartum changes in female reproductive tract, early pregnancy diagnosis by reproductive ultrasonography and detection of early embryonic mortality are some of the efficient means of improving domestic animal reproduction and production and help in fulfilling the basic necessities of man in the day-to-day world.

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). The present study was conducted to detect early pregnancy and early embryonic mortality by transrectal ultrasonography in oestrus induced postpartum anestrus Mehsana buffaloes.

MATERIALS AND METHODS

A total of 18 postpartum anestrus Mehsana buffaloes of a private dairy farm at Vadodara, Gujarat were subjected to different estrus synchronization protocols and fixed time AI with good quality frozen semen. The buffaloes not returning to estrus were subjected to ultrasonographic scanning for early pregnancy diagnosis on days 26 and 40 post AI and finally pregnancy was confirmed by rectal examination on day 60 post AI.

The ultrasound examinations were performed using a transrectal B-mode ultrasound scanner (ALOKA SSD - 500, SN - M10408. Japan) equipped with a 5 MHz linear array transducer designed for intra-rectal placement. The scanning of uterine horns was carried out on their dorsal and lateral surfaces. Ovaries were also scanned for the presence of a corpus luteum and/or any other palpable structure. The images displayed were thoroughly examined and frozen on the screen and the hard copies (sonograms) were taken using a video graphic thermal printer (Sony, UP-895 MDW, Japan).

Blood samples were collected twice from all the buffaloes for estimation of plasma levels of progesterone, i.e. on the day of AI and on day 20 post AI. Plasma progesterone concentrations were estimated by employing the standard radio-immuno-assay (RIA) technique of Kubasic *et al.* (1984). Labeled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech - SAS, Marseille Cedex, 9, France. The sensitivity of assay was 0.1 ng/ml. Intra-assay coefficient of variation was 5.4 percent and inter-assay variation was 9.1 percent. Cross reactivity of the antibody with progesterone, 17α-dihydroprogesterone and 20α-hydroxyprogesterone was 100, 0.13 and 0.96 percent, respectively. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated based on findings of ultrasound scanning on different days (day 26 and 40 post AI) and those of plasma progesterone concentrations on day 20 post AI in relation to findings of pregnancy diagnosis by rectal palpation on day 60 post AI.

RESULTS AND DISCUSSION

The results of ultrasound scanning and progesterone assay were correlated with the findings of palpation per rectum on day 60 post AI with respect to accuracy and reliability of these two tests. Although the sensitivity on days 26 and 40 and the specificity on day 40 of ultrasound scanning was 100 percent, the specificity was lower on day 26 being 80 percent. On day 26 one animal was incorrectly diagnosed pregnant. However, the progesterone assay revealed two animals having been diagnosed incorrectly pregnant. The sensitivity, specificity, positive predictive value, negative predictive value and overall diagnostic accuracy of progesterone assay were comparatively lower as compared to ultrasound scanning results on days 26 and 40 (Table 1).

Developmental profile of embryonic vesicle and embryo

The scanning of recently bred buffaloes through B-mode transrectal ultrasonography on day 26 allowed the visualization of non-echogenic embryonic vesicle around the conceptus (Figure 1). The embryo proper was observed on day 26 post AI, however, it was observed in only nine out of 13 (69.23%) buffaloes. Thereafter, the embryo proper was observed in all 12 conceiving animals (100%) on day 40 post AI (Figure 2). The amniotic and allantoic vesicles were observed as non-echogenic cavities closely surrounding the embryo proper on day 40 post AI. These cavities were surrounded by large anechoic area i.e. embryonic fluid. The scanning of ovaries revealed the presence of a pregnancy corpus luteum in conceiving buffaloes on both the days i.e. day 26 and 40 post AI. The heartbeats were observed as a pulsatile flickering

Table 1. Accuracy of ultrasonographic and P_4 assay for early pregnancy diagnosis in Mehsana buffaloes on different days after insemination.

| Diagnostic results/Predictive values	Days of ultrasound scanning		Plasma P4 assay by RIA[†]
	26	**40**	
Diagnosis pregnant correct (a)	13	12	11
Diagnosis pregnant incorrect (b)	1	0	2
Diagnosis nonpregnant correct (c)	4	6	4
Diagnosis nonpregnant incorrect (d)	0	0	1
Sensitivity (%) 100 x a /(a + d)	100%	100%	91.66 %
Specificity (%) 100 x c /(c + b)	80%	100%	66.66 %
Positive predictive value (%) 100 x a /(a + b)	92.85%	100%	84.61 %
Negative predictive value (%) 100 x c /(c + d)	100%	100%	80.00%
Overall diagnostic accuracy (%) 100 x (a + c)/(a + b + c + d)	94.44%	100%	83.33%

(†) RIA performed at day 20 post-breeding.
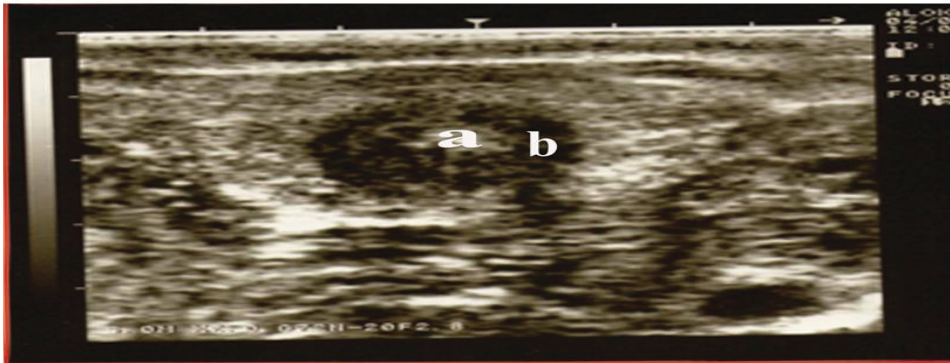
Figure 1. Sonogram on day 26 post-insemination.
　　　a) Conceptus and
　　　b) Fluid filled embryonic vesicle
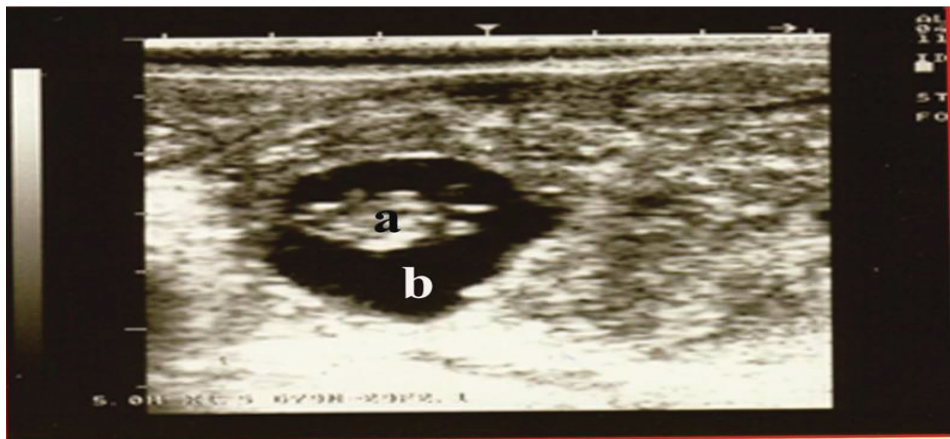

Figure 2. Sonogram on day 40 post-insemination.
　　　a) Embryo proper and
　　　b) Fluid filled embryonic vesicle

at a faster rate in the centre of the embryo proper within the embryonic vesicle on day 26 and 40 post AI. However, heartbeats were observed in only 10 out of 13 animals on day 26 post AI and in all 12 animals on day 40 post AI. The mean heart rate was 174.60±0.81 beats per minute on day 26 post AI, which decreased to 165.28±1.4 on day 40 post AI. These findings were in accordance with the findings of many workers (Rosiles *et al.,* 2005 and Agarwal *et al.,* 2007).

In the present study it was possible to clearly visualize the embryonic vesicle in all animals and embryo proper in nine out of 13 animals on day 26 post AI. Moreover, both the embryonic vesicle and the embryo proper were detected in all pregnant animals on day 40 post AI. Thus, ultrasonography facilitates diagnosis of all nonpregnant animals at an early stage (day 26 post AI) and is really more advantageous than pregnancy diagnosis by rectal palpation method, wherein 100 percent reliable results cannot be obtained at such an early stage of pregnancy. Agarwal *et al.* (2007); Ali and Fahmy (2008) reported similar findings in buffaloes. The results of the present study showed that transrectal ultrasound scanning of buffaloes on day 26 post AI was less accurate than on day 40 post AI.

The sensitivity of ultrasound scanning was 100 percent on days 26 and 40 post AI (Table 1). The specificity of ultrasound scanning was 80.00 and 100 percent on days 26 and 40 post AI, respectively. The positive predictive value (PPV) of the ultrasound scanning was 92.85 and 100 percent on day 26 and 40 post AI, respectively. The negative predictive value (NPV) of the ultrasound scanning was 100 percent on both the days i.e. day 26 and 40 post AI. The overall diagnostic accuracy in the present study was recorded as 94.44 and 100 percent on days 26 and 40, respectively. Similar findings were recorded by Bhosreker and Hangarge

(2000); Rosiles *et al.* (2005). They observed 97.90 and 100 percent accuracy in detecting pregnancy through ultrasonography, respectively. The possible explanation for higher overall diagnostic accuracy in the present study might be because ultrasound scanning was carried out on days 26 and 40 post AI with proper restraining of the animals.

Early embryonic mortality and/or early fetal death

In one animal, ultrasound scanning revealed the presence of a conceptus inside the fluid filled embryonic vesicle on day 26 post AI. However, ultrasonography on day 40 post AI revealed the same animal as nonpregnant as evident by the absence of a conceptus and resorption of embryonic fluid; suggestive of early embryonic death between days 26 and 40 post AI. In one buffalo, ultrasound scanning revealed presence of a conceptus and an embryonic vesicle on both the occasions, i.e. days 26 and 40 post AI. Later on, palpation per rectum at day 60 post AI revealed the animal as nonpregnant, suggestive of early embryonic or fetal death between days 40 and 60 post AI. Similar findings were recorded by Campanile *et al.* (2005); Vecchio *et al.* (2007).

Hormone profile

In the present study, plasma progesterone concentrations ranged from 3.00-6.02 ng/ml with an average of 4.42±0.52 ng/ml in pregnant animals on day 20 post AI. In nonpregnant animals plasma progesterone concentrations ranged from 0.97-1.91 ng/ml with an average of 1.22±0.06 ng/ml on day 20 post AI. These findings were in close accordance with the findings of Muhammad *et al.* (2000).

The findings of the present study indicate that ultrasound scanning of freshly bred animals is helpful in detection of early pregnancy, early

nonpregnancy and early embryonic deaths (EED) in buffaloes. The detection of early pregnancy in buffaloes may be accomplished on or after day 26 post AI through ultrasound scanning with positive predictive value and accuracy over 92 percent and the resul ts are instantly available.

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