# EARLY PREGNANCY DIAGNOSIS IN WATER BUFFALOES USING TRANSRECTAL ULTRASONOGRAPHY *

**M. K. AWASTHI[1], ABHISHEK KHARE[2], F.S. KAVANI[3], G. M. SIDDIQUE[4], and A.J. DHAMI[5]**

*Department of Animal Reproduction, Gynaecology & Obstetrics*

*College of Veterinary Sciences & Animal Husbandry, Anand Agricultural University, Anand, 388 001 (Gujarat)*

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## ABSTRACT

The objective of present study was to evaluate the accuracy of simplified technique of pregnancy diagnosis at an early stage using transrectal ultrasonography (TRUS) in milch water buffaloes. The diagnosis of pregnancy was primarily based on visualization of embryonic vesicle and observation of embryo was compromised with detection of embryonic vesicle for determination of pregnancy. The uterine horn ipsilateral to ovary bearing corpus luteum (CL) was scanned through serial ultrasound scanning on Days 23, 25, 28 and 30 post-service in freshly bred Mehsana buffaloes (n=40) for pregnancy diagnosis. Sensitivity and negative predictive value of early pregnancy diagnosis was 100% on each day of TRUS, but the specificity was lower on Days 23 and 25 than Days 28 and 30. Specificity (93.3%), positive predictive value (96.1%) and accuracy (97.5%) were maximum and similar on Days 28 and 30 post-service. The embryo was first observed on 26.33 ± 0.52 (mean ± SE) days post-service, while the heartbeats were first detected on 29.25 ± 0.36 days post-service; however, embryo could be visualized in 17 out of 25 pregnant animals. The mean length and diameter of embryonic vesicle were recorded as 15.5 ± 0.94 and 6.5 ± 0.55 mm on Day 23, which increased to 24.1 ± 1.07 and 9.6 ± 0.77 mm, respectively, on Day 30 post-service. It is concluded that detection of early pregnancy in buffaloes may be accomplished on and after Day 28 post-service through TRUS with positive predictive value and diagnostic accuracy over 96% and observation of embryo may be compromised with detection of embryonic vesicle for confirmation of pregnancy during early stage.

**Keywords:** Water buffaloes; Ultrasonography; Early pregnancy diagnosis

A novel way to reduce calving to conception interval is the diagnosis of pregnancy at an early stage and early detection of those animals that have not conceived after service (Pieterse *et al.*, 1990). Transrectal ultrasonography (TRUS) has been successfully used for early pregnancy diagnosis in cattle (Bonato *et al.*, 1990; Totey *et al.*, 1991; Nation *et al.*, 2003) and buffaloes (Pawshe *et al.*, 1994; Glatzel *et al.*, 2000; Bhosrekar and Hangare, 2000). The uptake of such a technique might be improved if an indirect form of pregnancy detection, namely the observation of embryonic vesicle could be substituted for the requirement to observe an embryo (Nation *et al.*, 2003). Nevertheless, the observation of embryonic vesicle would take substantially less time than detection of embryo and would require less interference with the uterine horns (Nation *et al.*, 2003).

The technique of TRUS may be further simplified by scanning only one uterine horn, which is ipsilateral to ovary bearing CL for detection of embryonic vesicle and/ or embryo rather than scanning both horns due to the fact that transuterine migration of embryo hardly occurs in cattle (Noakes, 2001) and information on transuterine migration of embryo is probably not available for buffaloes. In perspective, the objective of the present study was to evaluate the accuracy of simplified technique of TRUS for pregnancy diagnosis in water buffaloes between 23 and 30 days after service.

Freshly bred Mehsana buffaloes (n=40), 4 to 7 years of age, between second and fourth lactation, and weighing 490 to 660 Kg were selected at Livestock

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1. Corresponding Author, Associate Professor, Deptt. of AR, Gynaecology & Obstetrics, College of Veterinary Science & Animal Husbandry, PB No 6, Anjora, Durg (CG) 491 001, E-mail: mkawasthidurg@indiatimes.com
2. MVSc Scholar,
3. Professor & Head,
4. Professor & Head, Deptt. of AR, Gynaecology & Obstetrics, College of Veterinary Science & Animal Husbandry, SDAU, Sardar Krushinagar, 385 506, (Gujarat)
5. Research Scientist, Livestock Research Station, AAU, Anand, 388 001 (Gujarat)

Research Station, SD Agricultural University, Sardar Krushinagar, Gujarat for pregnancy diagnosis through TRUS at early stages. These animals were stall-fed and provided with balanced ration, mineral supplementation and seasonal green fodder. The experiment was conducted during breeding season from September to April. Estrus buffaloes were naturally served and those that did not return to estrus 23 days after service were selected for serial examinations through ultrasound scanner. Their reproductive tract was palpated per rectum for confirmation of pregnancy between 60 and 70 days post-service.

The uterus of each animal was scanned using a real-time B-mode ultrasound scanner (Sigma-110 Master-Vetson, Kontron Medical, SAS, France) equipped with a 6.5 MHz convex linear array transducer designed for intra-rectal placement. After evacuating the faecal matter, the location of CL on ovary was determined. Transducer was placed in rectum and positioned in close proximity to the dorsal surface of uterine horn ipsilateral to ovary bearing CL. After initial orientation, uterine horn was scanned on its dorsal and lateral surface for signs of pregnancy. Positive diagnosis of pregnancy was based on the detection of a anechoic elongated area of varying size representing the fluid filled allantoic cavity, the embryonic vesicle (Pieterse *et al.*, 1990). The presence of embryo within the embryonic vesicle was considered a confirmatory sign of pregnancy by observing an echogenic (white) area with rhythmic pulsation representing heartbeats (Ginther, 1995). However, no additional efforts were made to detect embryo in each animal showing presence of embryonic vesicle. The length and diameter of embryonic vesicle were measured using a built in caliper system. Hard copy (sonogram) was taken using videographic thermal printer (Sony, UP-895 MD, Sony Corporation, Japan).

Correct diagnosis in this study was defined either as (1) an animal diagnosed pregnant with TRUS and subsequently confirmed pregnant during palpation per rectum, or (2) an animal diagnosed non-pregnant with TRUS and subsequently confirmed non-pregnant during palpation per rectum or returned to estrus at a later date. An incorrect diagnosis was defined either as (1) an animal diagnosed pregnant with TRUS 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 TRUS and subsequently confirmed pregnant during palpation per rectum (Pieterse *et al.*, 1990).

Diagnostic accuracy was defined as the percentage of correct diagnosis out of total number of ultrasound examinations. Sensitivity of the method was defined as percentage of animals found pregnant by ultrasound scanning out of 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, which was later confirmed non-pregnant by palpation per rectum or returned to estrus at a later date. The positive predictive value (PPV) was defined as the percentage of actual pregnant animals out of total number of animals diagnosed pregnant through TRUS. The negative predictive value (NPV) was defined as the percentage of actual non-pregnant animals out of total number of animals diagnosed non-pregnant through TRUS (Badtram *et al.*, 1991). The sensitivity, specificity, PPV, NPV and diagnostic accuracy of early pregnancy diagnosis by ultrasound scanning was calculated as stated above in terms of percentage.

Sensitivity and NPV were 100% on each day of TRUS; however, the specificity was lower on Day 23 and 25 being 53.3 and 73.3%, respectively compared to Days 28 and 30 being 93.3%. Similar observations were recorded for PPV, which increased from 78.1% on Day 23 to 96.1% on Days 28 and 30. Likewise, diagnostic accuracy showed similar trend to that of PPV increasing from 80% on Day 23 to 97.5% on Days 28 and 30 post-service. The present findings are consistent with earlier reports in cows (Pieterse *et al.*, 1990; Bonato *et al.*, 1990; Muller *et al.*, 1999) and in buffaloes (Glatzel *et al.*, 2000).

The scanning of freshly bred buffaloes by real-time ultrasound allowed the visualization of embryonic vesicle and embryo The embryo was first observed on Day 25 in 6 animals and on Day 28 in 17 animals with an average of 26.33 ± 0.52 days after service. Embryo could be detected in 17 out of 25 confirmed pregnant animals (68%) up to Day 30 post-service. The heartbeats were observed as flickering at a brisk rate in the image within the embryonic vesicle between Days 28 and 30 with an average of 29.25 ± 0.36 days post-service. The mean length and diameter of embryonic vesicle were recorded as 15.5 ± 0.94 and 6.5 ± 0.55 mm on Day 23, which increased to 24.1 ± 1.07 and 9.6 ± 0.77 mm, respectively, on Day 30 post-service.

In the present study, embryonic vesicle was detected from Day 23 onwards in uterine horn ipsilateral to ovary bearing CL in all animals, which were later, confirmed pregnant by palpation per rectum. The present

observation suggests that scanning of uterine horn ipsilateral to ovary bearing CL is sufficient enough for detection of embryonic vesicle for confirmation of pregnancy at early stages. Remarkably, NPV was 100% on Days 23 and 25 post-service indicating that accuracy of diagnosing non-pregnant animals was superior to that of pregnant animals during these days. The sensitivity, specificity and accuracy are direct measures of efficacy of technique and will not change with variation in fertility in the herd (Chauffax *et al.*, 1986; Pieterse *et al.*, 1990). Cent percent sensitivity on each day of TRUS suggests that simplified technique of ultrasonography is a reliable method of detecting pregnancy at early stage in water buffaloes.

Six cases of false negative diagnosis were reported in 30 cows due to location of the uterus cranial to pelvic inlet when ultrasound scanning was performed between Days 27 and 31 post-service (Szenci *et al.*, 1998). In the present study, however, the uterus was located well within the pelvic cavity in operator's reach and visualized through all the sides with transducer of higher frequency (6.5 MHz) in all animals. Therefore, the chances of error in locating the embryonic vesicle were negligible. It is worth mentioning here that the location of uterus remains in pelvic cavity during early stages of pregnancy between Days 23 and 30 in multiparous Mehsana buffaloes in contrast to uteri of multiparous crossbred and indigenous cows in which location of uterus is highly variable owing to enlargement after repeated pregnancies.

The results of present study showed that transrectal ultrasound scanning for pregnancy in of buffaloes on Days 23 and 25 post-service was less accurate than Days 28 and 30. This could be attributed to embryonic mortality during early stage of pregnancy. The present observation approximates with early reports in cows on these days (Pieterse *et al.*, 1990). Pregnancy was detected as early as 19-21 days in 12 out of 26 water buffaloes (Pawshe *et al.*, 1994); however the accuracy was less. since early pregnancy diagnosis was confounded by the presence of intrauterine fluid in non bred heifers on day 18 and 20, no attempts were made to scan the animals for early pregnancy diagnosis before Day 23 in the present study.

The embryo could be detected in 17 animals only out of 25 confirmed pregnant animals based on visualization of embryonic vesicle up to Day 30 in the present study. The identification of embryonic vesicle was more efficient process than the time required in finding an embryo as small as 6 mm in convoluted uterine horn in cattle (Nation *et al.*, 2003) and by using this simplified method of early pregnancy diagnosis these authors recorded PPV as 92% compared to observation of embryo and palpation per rectum 13-week post-service in cattle. The embryonic vesicle was oblong or resembled bean shape in all animals on Day 28 and 30 of pregnancy in the present study, which is consistent with earlier report in buffaloes (Bhosrekar and Hangare, 2000).

The measurement of size of embryonic vesicle on Days 23 to 25 approximated to earlier findings in cows (Curran *et al.*, 1986). Mean length of embryonic vesicle was nearly similar as reported in buffaloes; however, mean diameter was comparatively lesser than reported earlier on Day 30 of pregnancy (Bhosrekar and Hangare, 2000). This variation in measurement of diameter of embryonic vesicle may be attributed to the breed of the buffalo. The timing in detection of embryo and heartbeats was similar to previous report in buffaloes (Pawshe *et al.*, 1994).

In conclusion, pregnancy may be detected in water buffaloes through TRUS on or after Day 28 post-service and visualization of embryo may be compromised with detection of embryonic vesicle for confirmation of pregnancy during early stage. The simplified method of pregnancy detection provided a rapid gain in efficiency during early stages.

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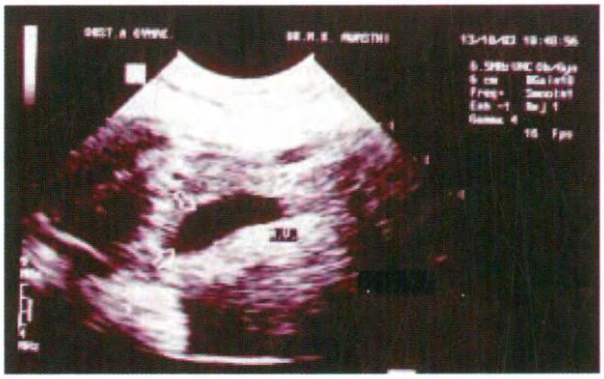

*Figure 1. Arrow showing embryonic vesicle on Day 25 of pregnancy in Mehsana buffalo*

*Figure 2 Arrow showing presence of embryo within embryonic vesicle Day 30 of pregnancy in Mehsana buffalo*

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