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Two Dimensional Trans-rectal Ultrasonographic Studies In Early Pregnant Murrah Buffaloes

GYAN SINGH[1], RAMESH KUMAR CHANDOLIA[2], RAVI DUTT[3], JASMER DALAL[4], ANIL SAINI[5] and RAKESH KUMAR MALIK[6]

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125004 (Haryana), India

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ABSTRACT

Two dimensional ultrasonographic studies were conducted in six pregnant Murrah buffaloes using 5.0–7.5 MHz frequency rectal probe twice a week from day 18 to 60 days followed by weekly interval up to 90 days post insemination. Two dimensional images of antenatal fetal organs particularly fetal stomach, eye, limbs, heart, umbilical cord, thorax, liver and placentomes were imaged and variations in size of placentome, stomach and crown rump length were measured. Fetal heart beat was measured from day 26 to 90 which decreased with advancement of gestation. From the present study it may be concluded that early pregnancy can be diagnosed ultrasonographically through trans-rectal approach by day 26 post insemination.

Key words: Early pregnancy diagnosis, fetal development, Murrah buffaloes, Two dimensional, Ultrasonography

Traditional methods for pregnancy diagnosis in large ruminants such as rectal palpation and udder enlargement are applicable only in late pregnancy. The application of real time B-mode ultrasonography in bovine reproduction especially follicular dynamics and pregnancy diagnosis has grown rapidly in the last decade. B-mode ultrasonography is a valuable alternate imaging system that can provide more accurate early information about pregnancy and reproductive disorders in comparison to traditional methods (Kahn, 1994).

Ultrasonography is non-invasive technique; therefore, repeated examinations of an animal's reproductive system can be performed without impairing its future breeding potential and fetal development. Accurate early pregnancy detection can be achieved easily and ultrasonography allows the estimation of fetal age, assessment of progression of fetal growth and diagnosis of pregnancy disorders. Moreover, fetal sex can be determined by ultrasonography in addition to early pregnancy diagnosis (Naikoo *et al.* 2013) and monitoring of fetal viability is a great advantage of ultrasonography (Medan, 2010). However, there are only few ultrasonographic studies monitoring the normal buffalo embryo/fetal development in the literature (Pawshe *et al.* 1994 and Fahmy., 2008). These studies describe only few characteristics of early embryo/fetal development in buffaloes compared to already established parameters in cattle and equines Therefore, the present study was designed for early pregnancy diagnosis alongwith assessment of fetal development in buffaloes using B-mode trans-rectal ultrasonography.

Present address: [1]Assistant Professor (vetgyan @rediffmail.com), Teaching Veterinary Clinical Complex, [2]Professor & Head (chandolia2003@yahoo.com), Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Sciences,; [3]Assistant Professor (raviduttvets @yahoo.co.in), [4,5]Ph.D Scholar (jasmer.sl.dalal@gmail.com, dranil0526m @gmail.com); [6]Professor (bkrkm@yahoo.com), Department of Veterinary Physiology and Biochemistry.

MATERIALS AND METHODS

The study was conducted on six pregnant Murrah buffaloes at Buffalo Farm situated at latitude of 29.1492º N, 75.7217º E, Department of Livestock Production Management, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana). All animals were kept in loose housing system under similar management conditions.

The study was conducted by using Toshiba Nemio-XG 3D ultrasound machines equipped with linear array trans-rectal-transducer (5.0 to 7.5 MHz). Pregnant buffaloes were scanned twice in a week using trans-rectal 2D probe from day 18 to 60, followed by weekly interval till day 90 of gestation.

Pixel values were measured in computer based "Adobe Photoshop" software. Minimum 3 to 4 areas for each image were selected to measure the mean pixel values. A particular area of image was selected and then clicked on "edit" and "histogram" to get the mean value. The ultrasound images recorded in the machine were reviewed in the scanner itself to re-examine the images in detail. First time appearance of fetal fluid, fetal heart beat, crown rump length (CRL), fetal parts development and placentomes were recorded.

The data collected were statistically analyzed by "ANOVA" for finding out average, standard deviation and standard error using computerized SPSS 16.0 software program. The measurements were plotted in graphs and tables.

RESULTS AND DISCUSSION

Foetal Fluid appearance

On day 18 post artificial insemination, a very small amount of anechoic fluid was observed in the uterine lumen in ipsilateral horn to CL (Fig. 1). On day 22, the amount of anechoic fluid in the uterine lumen increased and temporary intra-uterine compartment formation was recorded (Fig. 2). In contrast, Fricke (2002) reported that accuracy of pregnancy diagnosis based on fluid alone reached 100% by day 20 post insemination. The fetal fluid is visible in most species between days 18–22 by using different probes (Purohit, 2010). The accuracy of pregnancy diagnosis in dairy heifers was not greater than 50% before day 18 with 5.0 MHz transducer or before day 16 with 7.5 MHz transducer (Kastelic *et al.* 1988). However, Chaudhary and Purohit (2012) recorded pregnancy in cows on day 25.

Fetal heart beat

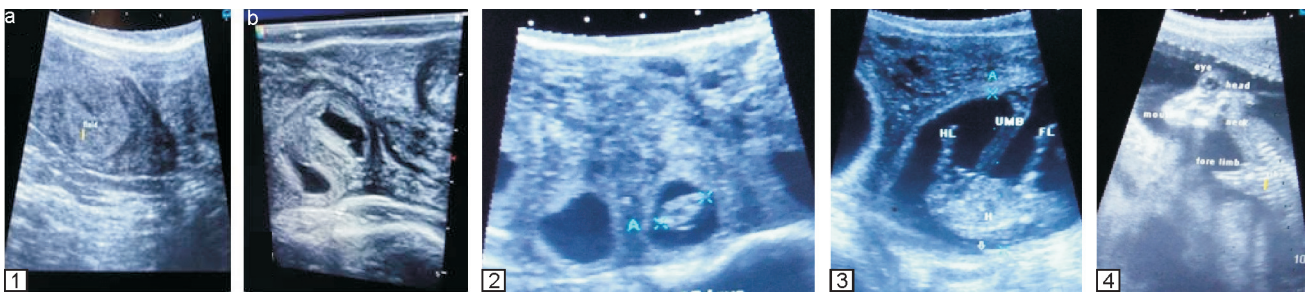
The fetal thorax was recognized by observing the vertebral column and rib cage in longitudinal plane. The fetal heart beats were used as the main criteria to assess the viability of the fetus. The fetal heart beat was observed within the cranial, cone-shaped thorax in all the buffaloes. Lumen of both the auricles and ventricles appeared as anechoic structure surrounded by hyper echoic wall. Fetal heart beat was first time recorded on day 26 post AI and was used as a confirmatory sign of fetal viability (Fig. 3). Curran *et al.* (1986) recorded fetal heart beat in bovines on 20.9 days (19–24 days) after breeding. Fricke and Lamb (2002) detected heart beat at day 22 of gestation period in cattle. In the present study, the fetus was seen as elongated arch shaped in 2D ultrasonography on day 26 of gestation as echoic structure with beating heart and the fetus surrounded by anechoic fluid. Purohit (2010) opined that fetal heart beat can be seen between day 24–30 and the fetus itself between days 25–30 in most of the species. Totey *et al.* (1991) reported that the embryonic vesicle was first observable between days 18 and 20 whereas fetal heart beat was detectable by day 22.6 post breeding. This difference may either be due to experience of sonologist or breed difference and in the current study the scheduled day of recording fell on day 26 post AI. Ingawale *et al.* (2012) in buffaloes recorded the fetal heart beat on day 28 after breeding and accuracy of diagnosis increased with advancement of pregnancy i.e. 85.71, 92.30 and 100% on days 21, day 28 and day 37 respectively. Similarly, Ferreira *et al*. (2011) observed fetal heart beats on day 25.27±3.58 of pregnancy in buffaloes.

In present study the highest mean value of heart beat (185.5±6.96) was recorded on day 54 of gestation period. It was significantly (P<0.05) higher compared to day 26 and 30 values. Further significant (P<0.05) increase was recorded on day 46, 50, 54, 58 and 81 as compared to day 26 value (Table 1). Similar findings were also reported by Kastelic *et al.* (1988) starting at day 25 of pregnancy and recorded the heart beat between 140 to 160 beats per minute as twinkling light. In contrast to present study, Pawshe *et al.* (1994; 2011) observed buffalo fetal heart beat (203.8±9.0 bpm) of the embryo on day 29.6±1.57 and the heart rate further decreased to 150 beats per minute on day 62. The variations in fetal heart beat may be either due to breed difference or expertise of the sonologist.

Table 1. Mean fetal heart beat/minute (Mean ±SE) recorded through trans-rectal ultrasonography *in utero* in Murrah buffaloes

Duration of gestation	Values (heart beat/minute)
26	163±6.03^{b}
30	147.66±3.40^{a}
34	163.16±4.88^{b}
38	162.83±3.01^{b}
42	175±3.93^{bcd}
46	181.33±4.16^{cd}
50	180.16±7.48^{cd}
54	185.5±6.96^{d}
58	179.17±4.37^{cd}
67	175.5±3.46^{bcd}
74	170.83±1.82^{bc}
81	176±3.19^{cd}
88	169.33±2.76^{bc}

Mean values with different superscripts (a, b, c, d) differ significantly (P<0.05).



Figs 1–4. **1.** Fluid in uterus on day 18 post insemination. 2. Fluid in uterus on day 22 post insemination. **3.** Recording of fetal heart beat on day 26 of gestation. **4.** Different fetal parts on day 67 of gestation

Appearance of fetal parts

In present study as per the schedule of ultrasonography differentiation of fore and hind limbs, amniotic membrane, differentiation of head and trunk, umbilical cord, eyes-mouth, fetal sex and neck were observed first time on day 34, 38, 42, 46, 50, 54, and 58, respectively. Ribs, tail, brain, stomach and fetus as a whole were visible on day 67 (Table 2). After day 67, fetus as a whole could not be observed and was visible in parts (Fig. 4). However, because of its size in relation to the image field of view, the fetus could be imaged *in to* after about 90 days using a 5.0 MHz linear array transducer. Similarly, Curran *et al.* (1986) and Fricke (2002) reported that bovine fetus can be visualized on day 20 after artificial insemination and fetal parts

Table 2: Ultrasonographic appearnce of foetal parts in days post insemination (Mean ±SE)

Foetal parts	days post insemination (Mean ±SE)	Remarks
Limbs	34	Pawshe *et al.* (1994) forelimbs and hind limbs differentiation on day 34.6±1.34 and 36.8±2.34, respectively.
		Pawshe *et al.* (2011) observed fore limbs, on day 34.6±1.34 and 33.00±1.67 respectively, and hind limbs on day 36.8±2.34 and 39.67±2.34 in buffalo and cattle.
		Kastelic *et al.* (1988) on day 40.
		Ferreira *et al.* (2011) on day 41.
Amniotic membrane	38	Curran *et al.* (1986) on day 29.5 (28-33day).
		Kastelic *et al.* (1988) on day 30 and 60.
		Pawshe *et al.* (1994) on day 33.4±1.64 post breeding.
Body of foetus	38	Ferreira *et al.* (2011) on day 47.33±4.9
Umbilical cord	38	Not reported in literature
Foetal eye ball and mouth	42	Pawshe *et al.* (2011) on day 38.2±2.39 and 46.67±2.07.
		Ferreira *et al.* (2011) eye by day 47.78±5.61.
		Curran *et al.* (1986) eye orbit on day 32.2 (29-33 day).
Genital tubercle	57	Curran *et al.* (1989) on day 55-60.
		Muller and Wittowski, 1986) on day day 50.
		Jost (1971) around day 50.
		Stroud (2006) at day 53-56 of gestation.
		Kahn (1990) on day 60.
		Ribadu and Nakao, (1999); Lamb and Fricke, (2004) between 60-85 days of gestation with 92-100% accuracy.
		Ferreira *et al.* (2011) on day 47.2±3.
		Groza *et al*. (2012) on day 55.
		Terzano (2012) between 57-59 days.
Ribs	67	Curran *et al.* (1986) around 52.8 days (51-55 days).
		Pawshe *et al.* (1994) in buffaloes on day 59.8±2.3.
		Pawshe *et al.* (2011) in cattle on day 56.60±2.10.
Tail	67	Groza *et al.* (2012) on day 75.
Stomach	67	Groza *et al.* (2012) on day 75.
		Ferreira *et al.* (2011) on day 59.17±4.36.
Brain	67	Ferreira *et al*. (2011) on day 57.5±1.2.
Crown rump length	26	Hughes and Davies (1989) - range of 6-11mm up to 84 days.
		Pawshe *et al.* (1994; 2011): 4.2±0.89 to 53.6±2.11 mm from day 19 to 62 in buffaloes.
Placentomes	74	Groza *et al.* (2012) on day 75.
		Curran *et al*. (1986) on day 35.2 (33-38 days).
Foetal fluid	18-22	Purohit (2010): 18-22 days
Foetal heart beats	26	Kastelic *et al.* (1988): 140 to 160 beats/min by day 25

throughout the gestation.

The limbs were differentiated into fore and hind limbs on day 34 of gestation. Our findings are in accordance with Pawshe *et al.* (1994) who reported forelimbs and hind limbs differentiation on day 34.6±1.34 and 36.8±2.34, respectively. In an another study Pawshe *et al.* (2011) observed fore limbs, on day 34.6±1.34 and 33.00±1.67 respectively, and hind limbs on day 36.8±2.34 and 39.67±2.34 in buffalo and cattle respectively. Some authors reported that fore and hind buds can be very easily visualized around day 40 (Kastelic *et al.* 1988) and day 41 (Ferreira *et al.* 2011).

The amniotic membrane was observed on day 38 of gestation whereas Curran *et al.* (1986) in buffaloes observed amnion on 29.5 days (28–33day) post breeding that is slightly earlier. Kastelic *et al.* (1988) in cattle observed the amniotic membrane between days 30 and 60 of pregnancy that is in agreement with current study. In this range, Pawshe *et al.* (1994) in bovines observed amnion on day 33.4±1.64 post breeding. These differences may either due to expertise of sonologist, species difference or schedule of ultrasonography coming after a gap of 4 days on day 38.

The body of fetus was differentiated into head and trunk on day 38 of gestation. In contrast to our results, Ferreira *et al.* (2011) observed the same on day 47.33±4.9 of gestation.

The umbilical cord was observed on day 38 of gestation as shown in Fig. 5. No evidence of first appearance of umbilical cord through trans-rectal ultrasonography has been reported in literature in cattle and buffalo so far. So, this finding might be a reference value.

The fetal eye ball and mouth were seen first time on 42 days of gestation period through trans-rectal ultrasonography. In buffalo and cattle, Pawshe *et al.* (2011) observed optic area on day 38.2±2.39 and 46.67±2.07 respectively whereas Ferreira *et al*. (2011) recorded developing eye by day 47.78±5.61 of pregnancy in buffaloes. Curran *et al.* (1986) in buffaloes observed eye orbit on 32.2 days (29–33day) after breeding that is slightly earlier. This difference may either be due to operator's expertise or breed difference.

In this study, the fetal sex differentiation on basis of location of genital tubercle behind the umbilical cord was recorded on day 57 of gestation (Fig. 6). The sex of fetus was first time observed by Curran *et al.* (1989) at 55–60 days of gestation by evaluating the morphology and location of genital tubercle through ultrasound or the scrotum and mammary gland of the male and female fetus, respectively (Muller and Wittowski, 1986). Similarly, Jost (1971) around day 50 post breeding reported that, male and female fetuses can be differentiated by the relative location of the genital tubercle and development of the genital swellings into the scrotum in male fetuses. Around 53–56 days of gestation, the genital tubercles migrate to their proper positions (Stroud, 2006). Accurate sex determination can be performed after day 60 whereas the gender of a male fetus was less difficult to determine than that of a female, based on the presence or absence of a scrotal pouch (Kahn, 1990). The sex determination is most practical between 60–85 days of gestation with 92–100% accuracy (Ribadu and Nakao, 1999; Lamb and Fricke, 2004). Ferreira *et al*. (2011) observed genital tubercle on 47.2±3 days of pregnancy. Groza *et al*. (2012) and Terzano (2012) recorded sex of fetus on day 55 and between 57–59 days of pregnancy in buffaloes which is in agreement to our findings.

The ribs were first observed on day 67 of gestation through trans-rectal ultrasonography (Fig. 4). Curran *et al.* (1986) observed ribs on 52.8 days (51–55 days) after breeding that is slightly earlier. These differences may either be due to expertise of operator, species difference or ultrasound machine. Pawshe *et al.* (1994) in buffaloes and Pawshe *et al.* (2011) in cattle recorded ribs and vertebrae on day 59.8±2.3 and 56.60±2.10, respectively.

The tail of the fetus was recognized on day 67 of gestation period through trans-rectal sonography. Groza *et al.* (2012) recorded tail on day 75 after insemination which is too later as compared to our findings.

The largest anechoic area in the fetal abdomen was the developing fluid-filled stomach further divided into compartments. The stomach compartments appeared anechoic. The fetal stomach was first time observed on day 67 and the mean diameter was recorded to be 2.55±0.13 mm. There was significant ($P<0.05$) increase in stomach diameter from day 67. After that due to compartmentization of stomach, it was difficult to scan the stomach as a whole after six months. In a study, Groza *et al*. (2012) recorded stomach on day 75 after insemination that is one week later than current study. Ferreira *et al.* (2011) observed stomach cavities on 59.17±4.36 days of pregnancy in buffaloes, which is slightly earlier to our findings.

Trans-rectal 2 D ultrasonography of gravid uterus revealed brain first time on day 67 of pregnancy. Ferreira *et al*. (2011) observed brain on 57.5±1.2 days of pregnancy which is slightly earlier than our findings.

Crown Rump Length

The distance between the top of the head and the rump of the fetus (crown-rump length, or CRL) is most frequently used in determining the age of the embryo (Kastelic *et al.* 1988). Crown rump length could be used to confirm early pregnancy diagnosis (Jones and Beal, 2003; Lamb and Fricke, 2004). In present study, CRL was recorded first time on day 26 of gestation period which increased non-significantly up to day 42 of gestation. The fetus was seen as elongated hyper-echogenic structure surrounded by hypo-echoic fluid in the lumen of uterus. A significant increase in diameter of CRL was recorded on day 46, 50, 54, 58 and 67 compared to day 26 value (Fig 5). A significant increase in CRL on day 49 of gestation was reported by Hughes and Davies (1989) that is in agreement with present study. Kastelic *et al.* (1988) observed that the fetal growth is more rapid after day 50 of pregnancy than at any preceding period which is in agreement with current investigation. After day 67, fetal CRL could not be measured due to large size of fetus that exceeded the size of the window of monitor.

However, Hughes and Davies (1989) measured CRL up to 84 day of gestation. This difference may be due to position of the fetus, expertise of operator, frequency of ultrasound machine or species variation. They recorded CRL 8.9 mm with the range of (6–11mm) on day 28 post AI which was slightly lower than the findings of present study (10.42±1.58mm on 26 day of gestation). Pawshe *et al.* (1994; 2011) recorded that CRL of the embryo increased from 4.2±0.89 to 53.6±2.11 mm from day 19 to day 62 in buffaloes. Pawshe *et al.* (2011) recorded CRL earlier than our recordings which may either due to species or breed difference. In a study Groza *et al*. (2012) recorded CRL on day 40 as 50 mm which on day 60 and 80 increased to 120 and 150 mm, respectively in buffaloes. They also recorded the CRL at later stage than current investigation and values of CRL were higher which may be due to position of the fetus, expertise of operator, ultrasound machine and species variation.

Placentomes

In the present study, ultrasonography proved as a useful technology in assessment of detailed structure of placentomes. Placentomes were clearly visible on the uterine wall and there was a great deal of variation in the size. Therefore, ultrasonography in the current study proved very useful in diagnosing the status of placentome. The measurement of placentome diameter could be a useful asset in diagnosing the abnormal pregnancies (Buczinski *et al*. 2007). Placentomes were observed as raised areas on the uterine wall on day 74 of pregnancy and this is in accordance with Groza *et al.* (2012) who observed placentome on day 75 after AI. In contrast to present study, Curran *et al*. (1986) observed placentome on 35.2 days (33–38days) after breeding in cattle that is appreciably earlier than the present study. In contrast to present study, Purohit (2010) reported that the cotyledons were visible between days 30–40 of pregnancy and placentomes of 5mm in length may be visible beginning on day 35. In a study, Kastelic *et al*. (1988) reported the size of placentomes around 20mm on large surface of the gravid uterine horn on day 60 of gestation. This difference may either be due to expertise of sonologist, species difference or ultrasound machine. The mean values of placentome diameter on day 74, 81 and 88 differed non-significantly (P>0.05; Table 3). Similar to present finding, Ferreira *et al.* (2011) could not observe the placentomes until the 70th day of gestation in buffaloes.

Table 3. Average diameter of placentomes (Mean ±SE) on various days of gestation in Murrah buffaloes recorded through trans-rectal ultrasonography

Duration of pregnancy	Diameter of placentomes (mm)
74 day	8.46±0.18^{a}
81 day	10.01±0.15^{a}
88 day	10.83±0.18^{a}

Mean values with same superscripts differ non-significantly (P>0.05)

From the present study it is concluded that transrectal approach is best to diagnose early pregnancy about day 26 post AI in Murrah buffaloes. It is also the best suited approach to study the early fetal development upto 90 days post AI.

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