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Fetal age determination in Murrah buffaloes from days 22 through 60 with ultrasonography

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ABSTRACT

Murrah heifers (n,20) that were inseminated at spontaneous or induced estrus and maintained mature corpus luteum up to day 22 post-insemination, were subjected to serial transrectal real-time ultrasound scanning of fetus, every alternate day from day 22 through 60 to correlate fetal measurements with gestational age. Mean length of the embryo increased from 3.07±0.04 mm on day 22 to 60.55±0.58 mm on day 60 post-insemination. The heart beat, amnion, optic area, limb buds, tail bud, optic lens, umbilical cord, split hooves, fetal movements and genital tubercle were first visualized on mean post-insemination day 27.1, 30.4, 32.4, 34.8, 36.7±0.21, 40.8, 40.9, 42.7, 45.0 and 49.8, respectively. Fetal growth curve best fitted with the Gompertz model. It was then used to ascertain gestational age of 74 pregnant buffaloes for validation and its accuracy, determined from farm AI records, was ±1 day in 67.6% buffaloes and ±2 days in 87.8% buffaloes. The information generated in the present study can be used to predict the gestational age of buffalo fetus more accurately.

Key words: Buffalo, C R length, Fetal age, Ultrasonography

Early diagnosis of pregnancy is desirable to rebreed the non-pregnant animal at the earliest opportunity whereas accurate identification of gestational age helps in appropriate management of animal around parturition. While maintenance of reproductive records is the easiest way, it is almost never practiced by buffalo farmers. Veterinary diagnosis with palpation per rectum allows only rough assessment of the fetal age. Transrectal real-time ultrasonography offers several advantages over manual palpation including early pregnancy diagnosis, determination of gestational age, twinning and fetal viability. Owing to large scale sale and purchase of buffaloes in the region, correct date of breeding and thus gestational stage is not known in many animals that hamper correct management of pregnant animals during gestation as well as at parturition. Studies carried out on bovine fetometry revealed that crown rump length has the highest correlation with gestational age (Curran *et al*. 1986, Kahn 1989). However, only few reports are available on development of the buffalo fetus with variable findings either due to frequency of examination or frequency of the ultrasound transducer used in the study (Pawshe *et al*. 1994, Ali and Fahmy 2008). Therefore, to generate the reference curve for buffalo fetal development, present study was carried out on pregnant heifers of known gestational age and then validated on pregnant buffaloes with recorded insemination dates for determining accuracy of the advocated model.

MATERIALS AND METHODS

Animals and ultrasonography

The experiment was carried out in 2 phases: first to develop a reference curve/equation and second to validate the same for determining gestational age in buffaloes. In the first phase, 20 nulliparous Murrah buffalo heifers about 36 months old and weighing approximately 350 kg were included. These heifers had conceived to inseminations made either at spontaneous or OvSynch/prostaglandin induced oestrus when a large (>10 mm) ovarian follicle was detected on ultrasound examination together with cervical relaxation and uterine tone. Repeat examinations, made 24 h later, confirmed ovulations. Subsequently, serial transrectal ultrasonographic examinations were made on alternate days between day 22 and day 60 post-insemination. The uterine cavity, particularly the horn ipsilateral to the ovary bearing corpus luteum, was scanned to visualize the fetus in its entirety in sequential cross-sectional, frontal and/or sagital planes. In early stages, the embryo proper was defined as a distinct echogenic spot within the nonechogenic vesicle. Long axis of embryo proper during early stages or its crown-

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rump length (CRL) were measured through a straight line between the fetal crown and the base of tail appearing on the display of ultrasound scanner after freezing the image using inbuilt calipers. The earliest days of detection of various structures, viz. heart beat, amnion, optic area, limb buds, tail bud, optic lens, umbilical cord, split hooves, fetal movements and genital tubercle were also recorded. Sex of the fetus was confirmed at calving so that fetal CR measurement data could also be analyzed with respect to fetal sex. All ultrasound examinations were done by a single operator with B mode ultrasound scanner equipped with an intraoperative 7.0 MHz microconvex multifrequency transducer. The measurements for different days of gestation for all 20 heifers were pooled and mean values were used to plot a reference graph for subsequent application.

In the second phase, 74 buffaloes in early pregnancy were scanned on single occasion for determination of gestational age based on the model developed in the first phase. The gestational age thus determined was then matched with the actual insemination date from farm AI records to ascertain accuracy of the reference equation.

Data analyses

The ultrasound measurement data of the fetal crown rump length were presented as mean (±SE). In the present study non-linear mechanistic dynamic growth models were used to derive the equation for fetal age determination based on CR length.

RESULTS AND DISCUSSION

The embryo proper could be visualized as an echogenic structure enclosed within the embryonic vesicle. On day 22 of gestation, embryo proper was visible only in 7 heifers. On day 24, it could be spotted in 15 heifers, while it was consistently visible in all 20 heifers from day 26 onwards. Mean length of the embryo proper increased progressively from 3.07±0.04 mm on day 22 to 60.55±0.58 mm on day 60 post-insemination. Fetal size first recorded on day 22 of gestation in pregnant heifers was 3.07±0.04 mm which was smaller as compared to 4.2±0.89 mm reported by Pawshe *et al*. (1994) on day 19 of examination in pluriparous Murrah buffaloes. Although, we used a higher frequency transducer (7.0MHz) for better clarity of structures as compared to 5.0 MHz used in the earlier study (Pawshe *et al*. 1994), yet we could identify only 7/20 fetuses on later stage (day 22) and of the size smaller to previous report. Parity of the dams could have also influenced the early fetus size. Yet, the size of fetus on day 60 was larger (60.55±0.58 mm) in the present study as compared to 53.6±2.11 mm observed on day 62 by Pawshe *et al*. (1994). Glatzel *et al*. (2000) reported that the embryo proper was firstly observed by day 28 of examination in Murrah buffaloes. In another report (Ali and Fahmy 2008), data were presented at weekly intervals and at later stages, hence not critically comparable. In case of bovine, a crown rump length of 66.1 mm was reported on day 60 of gestation (Curran *et al*. 1986).

At term, 8 male and 12 female calves were born. When fetal CR length was compared between male and female fetus, it was consistently longer for male fetus as compared to female fetus, though statistically the values were not different. The growth curves of male and female fetuses between day 22 to 60 are shown in Fig. 1. Hence, the fetal measurements for male and female fetuses were pooled for further statistical analysis. The comparative lengths of male and female buffalo fetus during early pregnancy are not available but at <100 days of gestation bovine male fetuses are reported to be heavier than female fetuses (Eley *et al.* 1978).

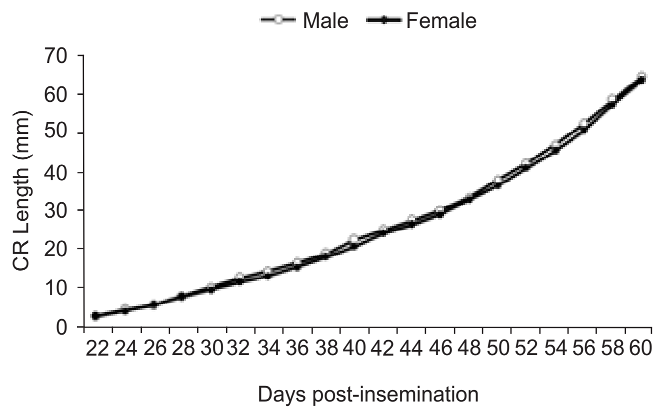

Fig. 1. Growth curve of male and female fetus.

In this study, 4 models i.e. exponential, Gompertz, logistic and monomolecular were attempted for best fit for calculation of fetal age and all models showed convergence. However, on the basis of mean absolute percentage error, mean absolute error and root mean square error, gompertz model was found the best fit (Fig. 2). Using Gompertz model the gestational age of buffalo fetus can be predicted from its crown-rump length with the following expression:

$$\text{Gestational age in days} = a.e^{\{-b.e(-c.fs)\}}$$

where, a, 64.675; b, 01.1915; c, 0.0431; e, exponential; fs, fetal size in mm.

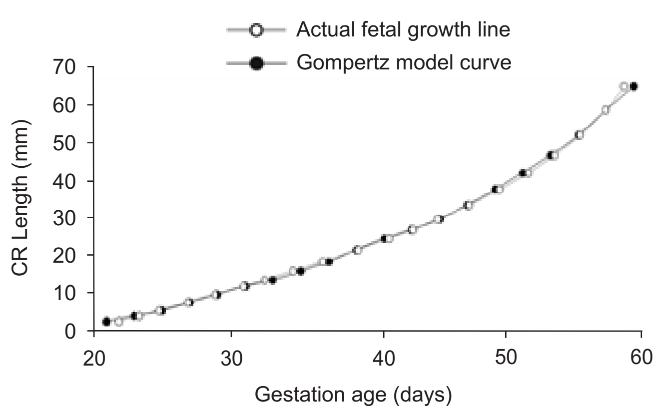

Fig. 2. Gompertz model of buffalo fetal growth for fetal age calculation.

Growth curves based on quadratic model (Curran *et al*. 1986), exponential model (Hughes and Davies 1989) and regression model (Kahn 1989) were also reported in bovines. Ali and Fahmy (2008) used regression models to evaluate these parameters in buffalo fetus. In our study, the relationship between CR length and gestational age best fitted in the gompertz model.

In the second phase of the experiment, crown rump length measurements of 74 pregnant buffaloes were fitted in the equation thus derived with Gompertz model and gestational age was calculated. The calculated gestational age was then compared to records of AI dates for these animals and accuracy was judged. The findings revealed that in majority of buffaloes (67.6%) estimated gestational age varied within ± 1 days from the recorded gestational age whereas in remaining buffaloes (32.4%), it deviated between 2 and 4 days either way (Fig. 3).

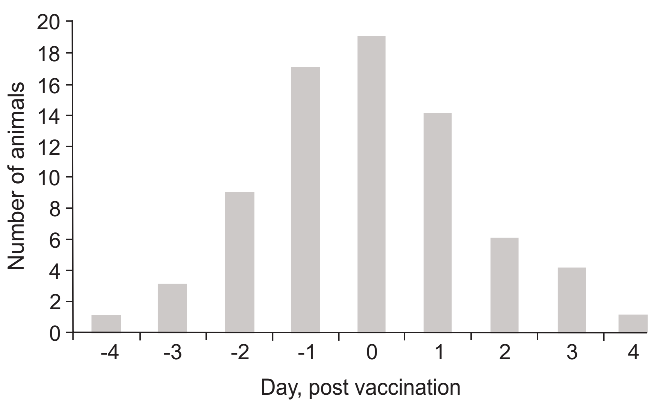

Fig. 3. Gestational age variation from actual recorded age (days) as calculated using Gompertz model.

Our model developed for estimation of gestational age in buffaloes proved quite accurate as majority of pregnant buffaloes (67.6%) in second part of the study were diagnosed within a variation of only ±1 days from actual recorded gestation age. Out of 74 estimates, a variation of $\geq\pm3$ days was predicted only in 9 cases. This variation widened to ±4 days in negligible (2.7%) cases. These minor variations between estimated and the actual gestational age could be due to individual differences in the growth rate of the fetus during pregnancy, errorneous measurement of crown rump length of the fetus due to inappropriate positioning of fetus during scanning or differences in parity and body weight of the dams, as the data were generated from pregnant heifers.

The mean gestational days at first detection of various fetal structures were as follows: heart beat, 27.1±0.3; amnion, 30.4±0.2; optic area, 32.4±0.2; forelimb buds, 34.8±0.2; tail bud, 36.7±0.2; optic lens, 40.8±0.3; umbilical cord, 40.9±0.2; split hooves, 42.7±0.3; fetal movements, 45.0±0.4 and genital tubercle, 49.8±0.4 days. Sonographically identifiable structures were detected 2-4 days earlier as compared to the previous study (Pawshe *et al*. 1994), probably due to higher frequency of the transducer used in the present study and thus better resolution.

From the above study it can be inferred that ultrasound examination can be carried out for fairly accurate assessment of early gestational age by measuring CR length of fetus. The technique also offers an added advantage of diagnosing pregnancy at an early age because non return rate is not a very good indicator of pregnancy especially in buffaloes due to less prominent signs of estrus.

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