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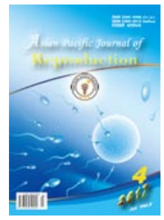

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Establishment of fetal age equations based on ultrasound measurements in cross−bred Holstein cows

Kanoknan Somnuk, Pim Wannapakorn, Waraporn Raksapol, Bunlue Kornmatitsuk, Sudsaijai Kornmatitsuk✉

Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand

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ABSTRACT

Objective: To establish fetal age equations based on ultrasound measurements in cross-bred Holstein cows in Thailand. **Methods:** The animals were bred for 30-120 d before pregnancy diagnosis using transrectal ultrasonography with real-time B-mode and specify 5 or 7.5 MHz linear array transducer. Parameters examined included crown rump length (CRL), trunk diameter (TrD) and eye diameter (ED). **Results:** One hundred and two cows had been examined, 80 of them were pregnant and contained at least one measurable characteristic as defined in the methods. Regression analysis and curve estimation were implied. The most frequently visualized parameters were TrD (42/80; 52.50%) and CRL (41/80; 51.25%) whereas ED (13/80; 16.25%) was least frequently detected. In our study, the equations to estimate fetal age (indicated as Y) from CRL and TrD were established with very high correlation coefficients as follows, Y = 22.679+12.005 (CRL)-1.042 $(CRL)^2$, R^2 = 0.950, P<0.001 and Y = 14.583+29.878 (TrD)-3.759 $(TrD)^2$-0.225 $(TrD)^3$, R^2 = 0.950, P<0.001. The equation from ED was Y=107.582-98.928 (ED)+61.116 $(ED)^2$-9.221 $(ED)^3$ but with very low correlation coefficient (R^2 = 0.673, P=0.021). Comparing between our equations (CRL and TrD) and the ones embedded in the commercially available ultrasounds, the higher fetal age was estimated based on the same value of parameters (P<0.05). In conclusion, the most reliable equations created in our study were from CRL and TrD. **Conclusions:** Comparing with others, Thai cross-bred fetus clearly showed slower growth rate and thus, to use our CRL's and TrD's equations for estimating fetal age seems more reliable than to use the ones embedded in the commercial ultrasounds.

1. Introduction

Pregnancy diagnosis plays an important role economically in cattle. The diagnosis includes detection of abnormal fetus, twins, gender and estimation of gestation age. Rectal palpation has been used for pregnancy diagnosis in cattle for many years[1]. However, this method is reliable earliest after 45 d post-breeding and criticized to be invasive[2,3]. Nowadays, ultrasound has been widely used for cattle reproduction and provided the practitioners a way to gather more information than via rectal palpation[4-7]. One of the main advantages of using ultrasound is early detection of pregnancy, usually after 27 d post-breeding[6,8,9]. Other advantages include monitoring fetal development and viability as well as estimating fetal age and sex[10-12].

Gestation age is significant for predicting parturition date, mating management plan and recording reproductive data of unknown cattle, so as to reduce production loss and over operating costs. Fortunately, aging pregnancy using fetal measurements has proven to be accurate[12-15] *i.e.*, by measuring of crown-rump-length (CRL), of which determined along the direct line between the crown and the ischium. CRL, however, may only be determined over a relatively short period due to the limited size of the screen of most ultrasound scanners. It is hardly possible to correctly measure CRL once the fetuses have reached a length of more than 10 cm[12,16,17]. Trunk diameter (TrD), a suitable measurement can be made whether the images are in cross section or longitudinal section, but a cross section may be preferred. TrD measurements may be used throughout the fetal stage as far as the lower portion of the trunk is still in the scanning field[8,18-20]. Eye diameter (ED), by optimally positioning the ultrasound probe, a section which shows the largest diameter of the eye can also be obtained but becomes less precise after day 130 post-breeding [16,21].

Most modern ultrasound scanners are embedded with fetal age equation of pure-bred Holstein cow based on measurements of various structures[22,23]. However, genetic make-up of the conceptus and its interactions with environmental factors play an important role on fetal development and gestation in the cows. Not only the sire's and dam's contribution to difference in genes but certain limited factors including number of fetuses, sex, parity or age of cattle, heat or cold stress, nutrition[24-26] possibly affect on potential for growth and on inaccuracy of fetal age estimation of cross-

✉Corresponding author: Sudsaijai Kornmatitsuk, Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand.
Tel:66−2−441−5242
Fax: 66−2−441−0937
E−mail: sudsaijai.kor@mahidol.ac.th

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bred Holstein cows in Thailand. Therefore, the study was aimed to establish the precise fetal-age equation based on ultrasound measurements in Thai cross-bred Holstein cows.

2. Materials and methods

2.1. Animals

One hundred and two healthy cross-bred Holstein-Friesian cows (over 75% HF) were included in the study. The animals were housed at the commercial farms. Seventy were from Saraburi province and the others were from Kanchanaburi province with a free-stall system and fed 4 times a day with total mixed ration (protein at 12%, crude fiber at 15% and total digestible nutrients at 64%), at least 14 kg DMI. The experimental protocol was reviewed and approved by the local animal ethics committee at Mahidol University, following the procedure of the National Research Council, Bangkok, Thailand. On the days of ultrasound scanning, the animals were already bred by means of artificial insemination between 30-120 d.

2.2. Ultrasound examination

The ultrasound examinations were performed using real-time, B-mode, diagnostic scanners. 1) Falco Vet®, Esoata-Pie Medical, Italy, and 2) Aloka®, Hitachi Aloka, Japan. Both were equipped with a 5/7.5-MHz rectal linear-array transducer. At each examination, an attempt was made to firstly record crown-rump length, a straight line between the fetal crown and the origin of the tail, trunk diameter, the widest diameter or at level of umbilical cord attachment and lastly, eye socket diameter, the two furthest removed points on the border between the anechoic eyeball and the hyperechoic surrounding orbit in all cases[21].

2.3. Images interpretation

All parameters were measured in centimeters. The First parameter was CRL, which is the measurement of the length of bovine fetus from the top of the head (crown) to the bottom of the buttocks (rump) (Figure 1).

The second parameter measured was Trunk diameter. The measurement of the trunk should be taken at its widest point, in the region of the last ribs, at the level of the liver and stomach. In general, for this purpose a transverse section through the abdomen should be obtained (Figure 2).

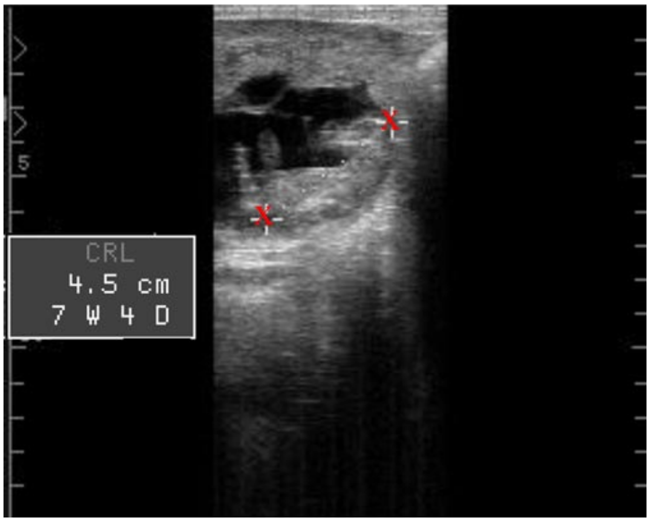

Figure 1. CRL (the length of bovine fetus) measurements from the top of the head (crown) to the bottom of the buttocks (rump).

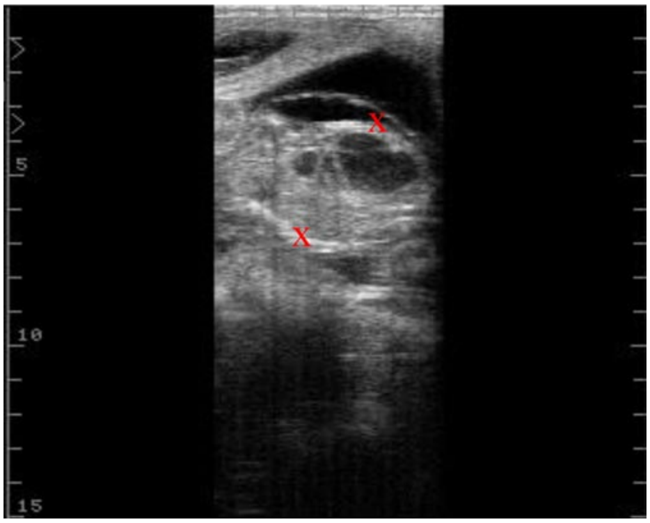

Figure 2. TrD measurements.

A transverse section through the abdomen at its widest point, in the region of the last ribs, at the level of the liver and stomach.

The third parameter measured was ED. The eye is the organ that is most frequently available for fetometry by transrectal ultrasonography during mid to late stages of pregnancy. For the highest possible degree of accuracy, the largest diameter should be measured between the two furthest removed points on the border between the anechoic eyeball and the hyperechoic surrounding orbit in all cases (Figure 3).

Figure 3. ED measurements.

The largest diameter between the two furthest removed points on the border between the anechoic eyeball and the hyperechoic surrounding orbit.

2.4. Statistical analysis

Analysis of the dataset was performed by regression analysis and curve estimation with SPSS 17. Age was added as dependent variable and fetal parameters (CRL, TrD and ED) as independent variables. Model used to analyze were as follows: linear regression, logarithemic regression, quadratic regression, and cubic regression.

The linear regression line had an equation as Y = a + bX, where X was the explanatory variable and Y was the dependent variable. The slope of the line was b, and a was the intercept.

The logarithmic equation has the general form as Y = m*ln(x) + b. Logarithmic data will exhibit a straight-line relationship when graphed with the x values on a log scale and the y values on a linear scale. The y-intercept was b and the slope was m.

The form of a quadratic equation was given by $Y = a + b_1x + b_2x^2$, where the constant term was a, the linear term was b_1, and b_2 was quadratic term.

Lastly, the cubic equation has the form as $Y = a + b_1x + b_2x^2 + b_3x^3$. The constant was a. The constant plus a linear term was b_1 and the constant plus a linear term plus a quadratic term was b_2. A cubic term was represented as b_3.

For each parameter, the selection of equation was based on the highest correlation coefficient (R^2). The analytical method of repeated measurement ANOVA was applied for determine whether significant differences exist between dependent-variable scores. The level of significant was considered when $P<0.05$. Unless otherwise stated, paired *t*-test was applied.

3. Results

One hundred and two cows had been examined, 80 of them were pregnant and contained at least one measurable characteristic as defined in the methods. TrD was visualized most frequently (42/80; 52.50%) as well as CRL measurement (41/80; 51.25%) but ED (13/80; 16.25%) least frequently. The relationship between gestation age and each of three parameters was represented as regression models, coefficients of determination (R^2) and the *P*-value were showed in Table 1.

Table 1

The relationship between ultrasound measurements of CRL, TrD, ED and gestation age.

Measurement	Regression model	R^2	*P*-value
CRL	Linear	0.887	0.000
	Logarithmic	0.943	0.000
	Quadratic*	0.950	0.000
	Cubic	0.949	0.000
TrD	Linear	0.938	0.000
	Logarithmic	0.931	0.000
	Quadratic	0.951	0.000
	Cubic*	0.956	0.000
ED	Linear	0.595	0.005
	Logarithmic	0.656	0.003
	Quadratic	0.663	0.009
	Cubic*	0.672	0.021

The symbol * indicates the selected regression model.

The results of the current study showed that 2 of 3 parameters (TrD and CRL) were highly significant correlated with gestational age ($P<0.001$). CRL showed the highest correlation coefficient (R^2 = 0.950) in quadratic regression model of which the equation was $Y=22.679+12.005x-1.042x^2$ (Figure 4).

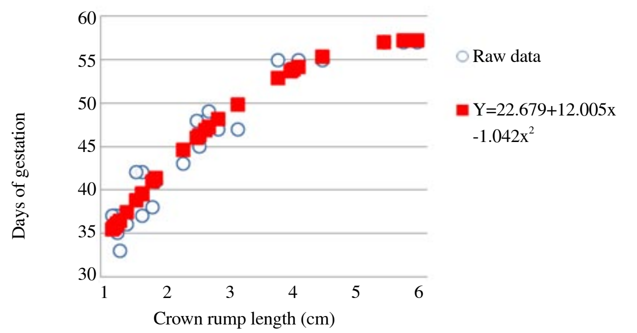

Figure 4. Quadratic regression curve showing the relationship between the CRL (cm) and gestation age (days) in cross-bred Holstein cows.

The regression model for correlation between TrD and gestation age that presented highest correlation coefficient was cubic curve (R^2=0.956). The equation for calculating gestation age was $Y=14.583+29.878x-3.759x^2-0.225x^3$ (Figure 5).

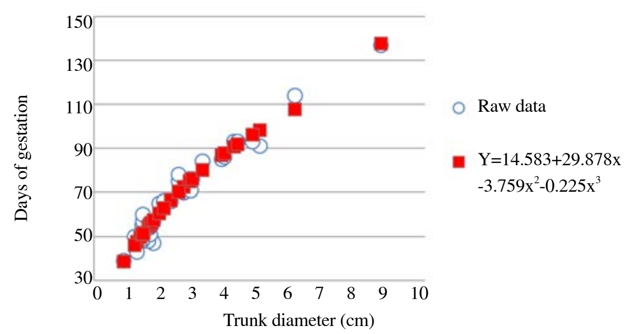

Figure 5. Cubic regression curve showing the relationship between the TrD (cm) and gestation age (days) in cross-bred Holstein cows.

Figure 6 represented the regression curve showing the relationship between ED and gestation age. The correlation coefficient was highest in cubic model (R^2=0.672) but lowest when compared with curves of the other two parameters. The equation for estimating related from ED was $Y=107.582-98.928x+61.116x^2-9.221x^3$ (Figure 6).

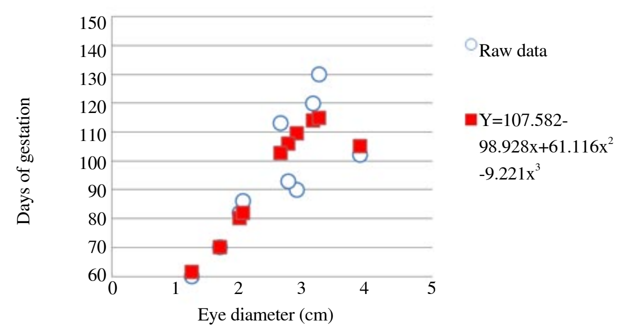

Figure 6. Cubic regression curve showing the relationship between the ED (cm) and gestation age (days) in cross-bred Holstein cows.

Figures 7 and 8 showed the comparison between our equation and the ones created by White *et al.*[22] and Kahn[23] to estimate the fetal age. The regression line of CRL and TrD parameters and fetal age tended to go in the same direction comparing between 3 equations. However, when statistically compared, with the same values of parameters, the days of gestation calculated by our equation were higher than the calculations by White's and Kahn's equations (P<0.05).

Similarly, when comparing with Kahn's equation, the fetal ages estimated by our equation using ED as parameter were higher (P<0.05) (Figure 9).

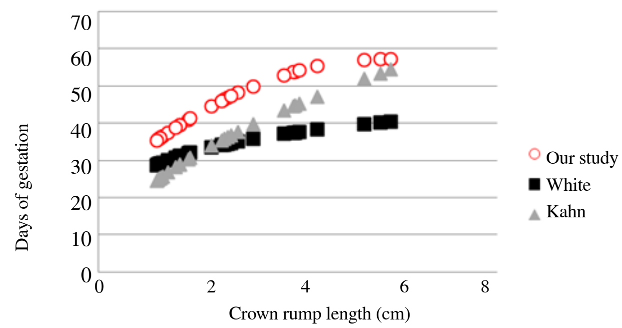

Figure 7. Regression curve for our equation of CRL (cm) comparing with White's and Kahn's equation.

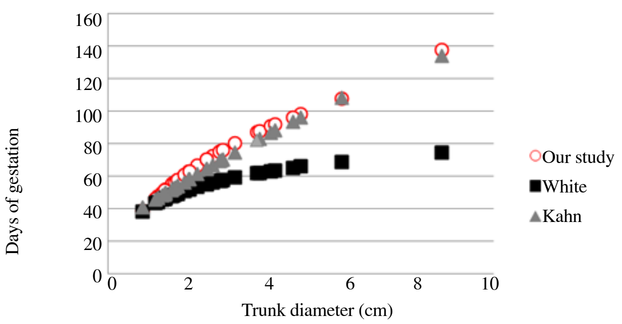

Figure 8. Regression curve for our equation of TrD (cm) comparing with White's and Kahn's equation.

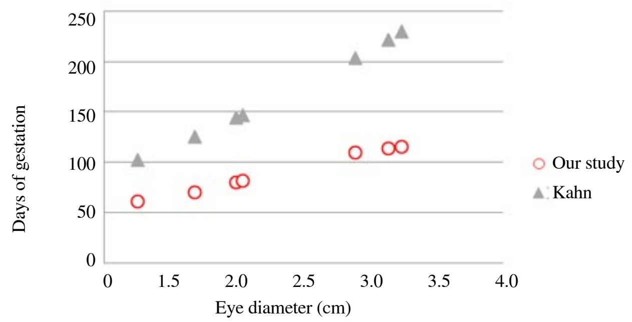

Figure 9. Regression curve for our equation of ED (cm) comparing with White's and Kahn's equation.

4. Discussion

The estimation of the day of pregnancy is a frequently requested application of ultrasonic imaging in cattle after aging pregnancy using fetal measurements has proven to be accurate[12,27]. Most of modern ultrasound scanners are embedded with fetal age equation of pure-bred Holstein cows based on measurements of various structures, most popularly created by White *et al.*[22] and Kahn[23]. However, the precision of age estimation is influenced by many factors including number of fetuses, sex, parity or age of cattle, heat or cold stress, nutrition, and genetic as well as breed of sire or dam[12,15].

The CRL is the measurement of the length of embryo from the top of the head (crown) to the bottom or tail head (rump)[21]. In our study, the relationship between fetal age and CRL showed strong positive correlation coefficient (R^2 = 0.950) in quadratic regression. The selected equation of CRL was Y=22.679+12.005x-1.042x^2. In general, CRL can be used between 31-83 d of gestation. After day 60, data was useless because by that time it exceeded the screen width for most scanners when the fetus was parallel with the top of screen. In addition, it is sometimes difficult to obtain a full, longitudinal section. Though CRL was the least variable parameter, but it was most chosen for gestation age prediction only in 30-60 d of pregnancy[12,16,17,23,28].

Measurement of trunk should be taken at its widest point, in the region of the last ribs, at the liver and stomach. In our study, the strongest positive correlation coefficient (R^2 = 0.956) was showed in cubic regression. The equation of TrD selected is Y=14.583+29.878x-3.759x^2-0.225x^3. From day 60 to 70 of pregnancy, the trunk diameters of bovine fetuses were 20-30 mm. It was increase by 0.9 mm per day to reach 100 mm around day 150 of pregnancy. Trunk measurements can be used throughout the fetal stage until the lower portion of the trunk is beyond the depth of ultrasound field (*e.g.* using a 3.5 MHz probe at day 140)[18-20,23].

Eye measurement seems more suitable for estimate late fetal age[21]. Our ED's equation is Y=107.582-98.928x+61.116x^2-9.221x^3. However, the correlation coefficient was rather low (R^2=0.670). The low correlation coefficient as such was possibly according to the inadequate amounts of data collected. White *et al.*[22] stated that ultrasound probe was not able to access to fetal head in posterior presentation position before calving 3-5 mo. After cranial presentation was established (1 and 2 mo before pregnancy), failures to detect an eye were primarily problem because the fetus was too deep; often only the front limbs and part of head were reachable. Thus, this parameter may not be as practical for estimating fetal age as CRL and TrD.

When we compared the fetal age calculating from CRL and TrD using our equations with the ones created by White *et al.*[22] and Kahn[23] which are popularly embedded in the commercial ultrasounds, curtain conflicts were found. Based on the same value of measured parameter, the fetal age estimated by our equation was higher than the ones estimated by White *et al.*[22] and Kahn[23] equations. This may indicate that the fetus of Thai cross-bred cattle had slower growth rates compared to pure-bred ones according to many factors discussed elsewhere.

To conclude, the most reliable equations created in our study were from CRL and TrD. Comparing with others, Thai cross-bred fetus clearly showed slower growth rate and thus, to use our CRL's and TrD's equations for estimating fetal age seems more appropriate than to use the ones already embedded in the ultrasound machine.

Conflict of interest statement

We declare that we have no conflict of interest.

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