The use of fetal femur length for estimation of gestational age in cattle

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SUMMARY

The aim of the present research was to study the relationship between the femur and gestational age and thus generate an equation which would be able to estimate fetal age in cows. 23 dairy cows were examined every after one week transrectally using B−mode real−time ultrasound scanner with a 7.5MHz linear probe (Mindray DP 6600, Mindray Szechuan, China). The dairy cows were examined at Large Animal Research Unit of Massey University New Zealand between day 60 and day 120 of pregnancy. Ultrasonography examination commenced the 20th of July 2015 and was finished in September 2015. The predicted gestation age in dairy cows had a chance of being 21.7 days greater and 21.6 days less than actual gestational age. The findings from this study show there is a moderate positive linear correlation between femur length and gestational age in the dairy cows (R^2 =0.64). However, this relation did not have good agreement to justify the use of equation developed to be used in estimation of gestational age.

Key words: dairy cows, gestational age, agreement, femur length

INTRODUCTION

Gestation is the period between conception and birth, and gestational age is therefore the time since conception. In cattle, gestational age is most commonly estimated using rectal palpation or transrectal ultrasonography. In the present study, the focus is onthe use of transrectal ultrasonography. This is a quick, safe and non-invasive technique that can be used to diagnose pregnancyas early as 28 days after conception (Racewicz and Jaskowski, 2013). Transrectal ultrasound can also be used to determine gestational age, based on the relationship between time since conception and size of the conceptus (Varol *et al*., 2001) and fetal viability based on the presence of a beating heart (Lamb*et al*.,2015).Importantly transrectal ultrasound has not been found to affect embryonic or fetal viability(Kahn, 1992; Ball and Logue, 1994).

The key rationale for estimating (or confirming) gestational age is that doingsowill allow prediction of expected calvingdate; in dairy cattle this prediction can be used toidentify drying-off date and to plan for calving (e.g. identifying labour requirements) (Doize*et al*., 1997). Inextensivesystems, drafting cows based on fetal age at particular time points during the year may be easierthan trying to locate and remove all bulls from a paddock (Jephcott, 2009).

A huge range of parameters are potentially measureable using transrectal ultrasound including biparietal diameter, femur length, crown-rump length, thoracic diameter and abdominal diameter and non-fetally-based measures such as placentome diameter or corpus luteum size.

Femur length measurements

The femur is is the most proximal bone in the hind limb of the cow, articulating with the acetabulum in the pelvic bone to form the hip joint whereas distally there is a knee joint. As for BPD, studies in multiple species have shown a very strong correlation between gestational age and femur length including goats (Rihab *et al*., 2012), sheep (Noia *et al*., 2002), buffalo (Terzano *et al*., 2012) and hyena (Place *et al*., 2002) as well as cattle (Kahn, 1989). In cattle the association is seen in all breeds though there can be significant differences between them (Table 1)

A wide range of parameters that can also be measured by ultrasonography have been shown to be highly correlated with gestational age, including: crown rump length (r=0.91), head (biparietal) diameter (r=0.95), head length (r=0.94), trunk diameter (r=0.95), nose diameter (r=0.95), uterine diameter (r=0.93) (all results from White *et al*., 1985) and femur length (r=0.99; Kahn 1989). Femur measurements are illustrated in Figure 1. Femur measurement is considered to be accurate only when the image shows two blunted ends, i.e. the extension to the greater trochanter and the head of the femur are not included.

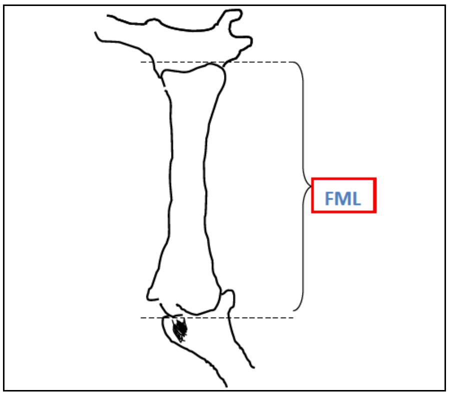

Figure 1. Representation of reference points for femur length (FML) used when measuring using ultrasound

Table 1. Fetal legs traits during various stages of gestation in cattle

	Gestational age		
Breed	3 months	6 months	9 months
German Angus	4.3	19.7	32.7
Galloway	3.2	17.1	33.3
Holstein Friesian	5.6	21.3	38.5
Belgian Blue	4.6	19.2	36.4

Table 1: Effect of breed on change in leg length (cm) with gestational age (Source: Mao *et al*., 2008).

Postmortem data has shown that significant cartilaginous development of the long bones has begun by ~7 weeks of gestation with ossification beginning a few weeks later being detectable from around 74 days of gestation (the first identification of calcium phosphate deposits), with ossification centres being seen in all bones by day 81 (Trujillo *etal*., 2011). So although femur measurement is possible from 49 days, measurement using bobly landmarks is only feasible only from ~80 days onwards Femur measurement is much less practicable in older fetuses. Kahn (1989) reported that by month 4 of gestation only 60% of fetuses could have measurements made of their hind limb area. By month 7 this figure had decreased to 25%, while in months 9 and 10 no measurement of the hind limbs was possible. This means that femur measurement as an estimate of gestational age needs to be restricted to fetuses <160 days.

MATERIALS AND METHODS

Study animals

The animals used in this study belonged to the large animal teaching unit (LATU) of Massey University. Sixtynon-lactating 2.5-year-old dairy cows (Friesian and Friesian cross Jersey) were used.

In order to ensure that the exact date of conception was known, allthe cows used in this study had been synchronised using an intravaginal progesterone plus GnRH-PGF$_{2\alpha}$-GnRH program (Adeyinka *et al*., 2014), with pregnancy diagnosis undertaken 6 weeks after synchronisation. The cows were all inseminated on 14th May 2015.

Ultrasound equipment

The uteri of the selected cows were examined transrectally, using a B-Mode real-time ultrasound scanner with a 7.5 MHz linear probe (Mindray DP6600, Mindray Szechuan, China). Fetal femur length measurements were made in the dairy cattle from 20th July to 1st October 2015. This meant they were scanned between days 60 to 130. Measurements were made in weekly basis.

Measurement of femur length

Femur length was measured and defined as the length of the diaphysis of the femur (see Figure 1) diaphysis at both ends (line x to x) as shown in figure 1. Once a suitable image was obtained it was recorded digitally before transfer to a desktop computer for image analysis using the image processing and analysis programme Image J (Figure 2).

Figure 2. Example of a digital image showing measurement of femur length

Statistical analysis

All analysis were undertaken using SPSS 24 (IBM, USA)

Regression analysis

A regression analysis of gestational age against femur length was undertaken to establish the strength of the association between the measure and the best equation for predicting gestational age from the measurements.

Limits-of-agreement analysis

Predicted age (based on the regression equation) was calculated from the femur length, and a mean/difference plot (Bland *et al*., 1990) created. Regression analysis was then undertaken to identify whether there was a significant association between mean and difference (and the variance of that difference), and the limits-of-agreement then calculated (Bland and Altman 1999). Femur length for each cow at each given time point was used to create the predicted gestational age. The agreement between the predicted and the actual gestational age in terms of femur length for dairy cows was identified by the limits of agreement. The two versions of limits of agreement were used. One by (Bland and Altman, 1999) where the limits of agreement were calculated using the standard deviation of the difference between the predicted and the actual gestational age. The other one as suggested by (Bland and Altman, 2007) for repeated measurement where the true value varies, taking into consideration the association between method difference and gestational age.

RESULTS

Regression analysis

The association of fetal femur length with gestational age is illustrated in Figure 3

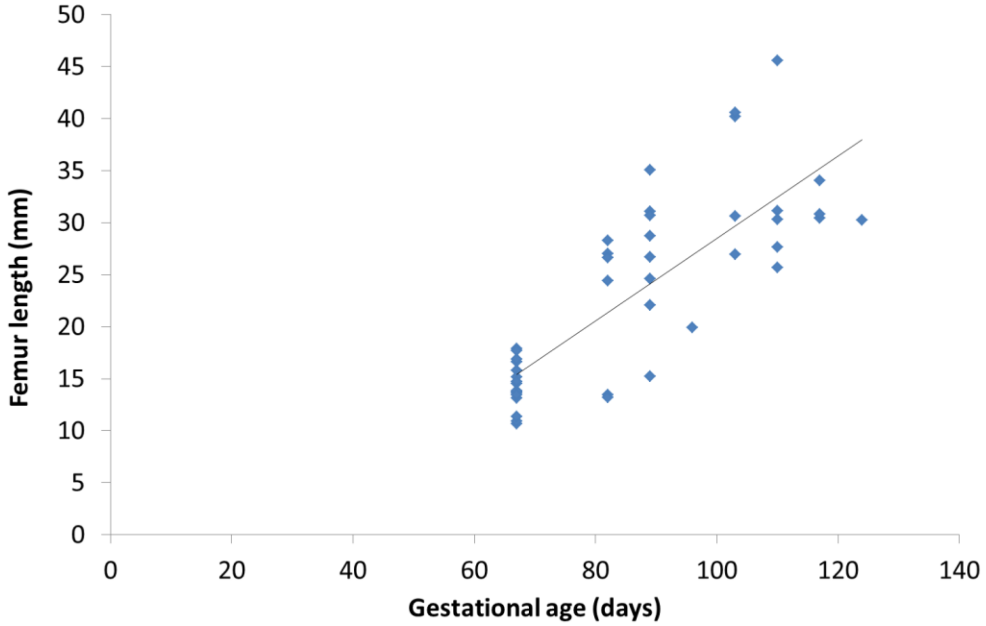

Figure 3. Relationship between femur length (mm) and gestational age for dairy cows measured using transrectal ultrasonography. Solid line: line of best fit.

The results of the regression analyses are summarised in Table 2

Table 2. Association between gestationalage andfemur length measured using transrectal ultrasound

Measure	Type	Prediction equation from gestational age (days)	R^2
Femur (mm)	Dairy	0.40*age - 11.1	0.64

Limits-of-agreement analysis

The limits-of-agreement plot based on the femur size is shown in Figure 4. Overall there was no evidence of bias (mean difference [SEM] was -0.025 [2.05]). There was a moderate association between mean and difference (R^2=0.124; p=0.018) and a strong association between mean and variance of the difference (R^2=0.447; p<0.001). The limits-of-agreement analysis thus took account of these associations and suggests that at 80 days, 95% of the differences between predicted and actual gestational age will be between -19 and +16 days whereas at 120 days the equivalent figures will be -29 and +46 days. So at 80 days ~74% of differences will be ≤10 days from the line of best fit, whereas at 120 days ~21% of differences will be ≤10 days.

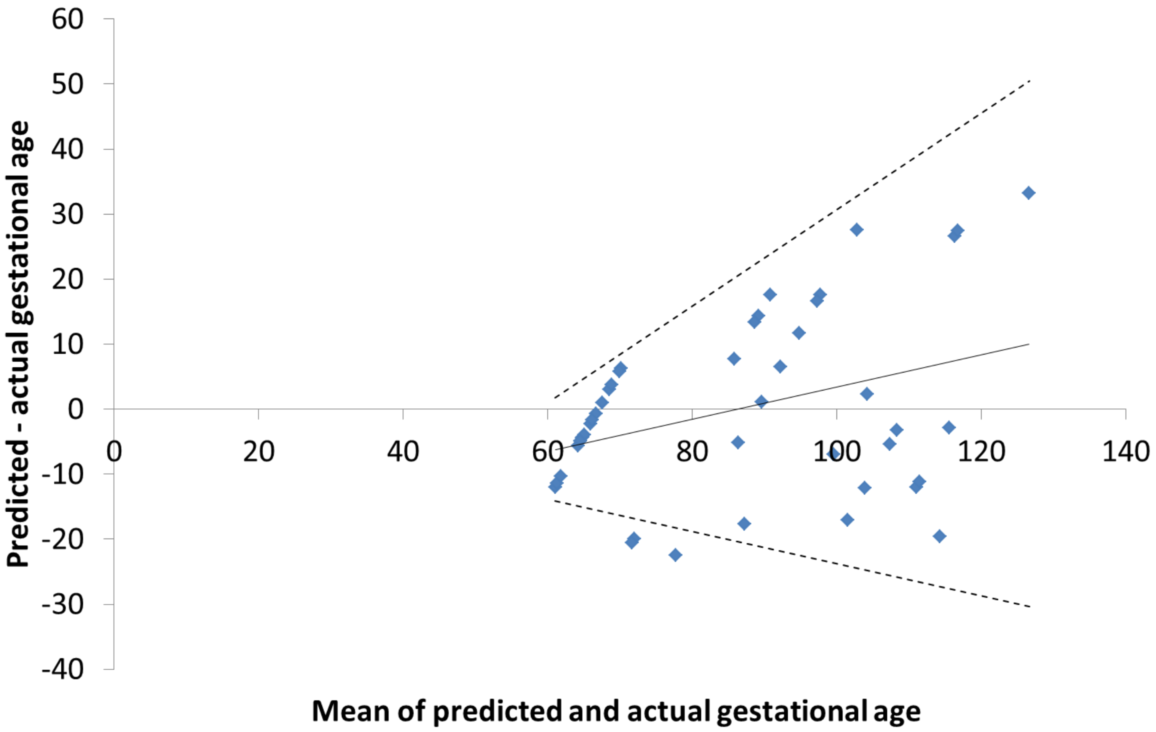

Figure 4. Showing scatter plot of the difference between predicted and actual gestational age and the mean of predicted actual gestational age (days). The dark blue line is the best-fit line; the doted line is for unadjusted limits of agreement.

DISCUSSION

In cattle, measurement of femur length is one of the commonly used methods of estimating gestational age; however very few studies have properly assessed the agreement between femur length and gestational age. Many studies have focussed on correlation and relationship between means which are not appropriate for assessing agreement as they ignore the variation between individual results (variance of the difference) and the change in that variance as measurements increase.

In this study there was a significant association between femur lengthand gestational age. The good association in this study (R^2=0.64) was poorer than some previous results (e.g. Kahn 1989, White *et al*., 1985), but other studies reported similar good values (Terzano2012). It is not clear why correlations between studies. In this study, in an attempt to mimic what was feasible under New Zealand conditions, measurement was undertaken as soon as the measure could be identified, so it is likely that measurement time was short relative to previous studies. In addition, the beef cattle used in this study were unused to handling, so were difficult to scan safely, again increasing the likelihood of errors. Another possibility which could have decreased correlations in this study is the relatively high gestational age of many of the fetuses when they were first scanned which would again reduce association.

This is the first study that has specifically focussed on the agreement between estimates of gestational age from femur and actual gestational age. Previous papers (e.g. White *et al*., 1985 and Kahn 1989), have principally focussed on correlation and when they have included measures of agreement have not taken account of change in agreement with time; e.g. White *et al*. (1985) reported residual standard deviations ranging from 4.5 days for crown-rump length to 12.6 days for uterine diameter, but did not take into account the increase in standard deviation with time shown on their graphs. The impact of time on agreement is particularly important under New Zealand conditions as, although in intensive systems most cows are scanned before 42 days (Fitzgerald *et al*., 2015), in New Zealand the majority of pregnant cows are >80 days when scanned with many cows scanned at a later stage (Brownlie *et al*., 2015).

The limits-of-agreement analysis showed that for femur length (in dairy cows) there was a large increase in the predicted differences with increasing gestational age; i.e. estimation of gestational age became less precise as pregnancy progressed. This is consistent with the results shown by previous studies of fetal size such as Khan (1989) and White *et al*., (1985) where graphical representation of the data from the fetus showed that variance increased with gestational age, and Adeyinka*et al*., (2014) who found no effect of gestational age on the precision of its estimation using placentome length. This lack of association was observed irrespective of whether the regression equation from this dataset or that from Adeyinka*et al*., (2014) was used. Femur size became increasingly difficult to access as gestational age increased.

It is concluded that measurement of gestational age is a crucial part of the pregnancy diagnosis process. This study is the first to compare the agreement between predicted and actual gestational age for fetal femur length. However, this study has confirmed that the precision of these measurements decreases significantly as gestation progresses and by 120 days of gestation. Measuring femur length was by far the most difficult measure and by 120 days was much less precise than other fetal measurements studied before like placentome size and biparietal diameter. Femur measurement should be restricted to use only in early gestation and then it should be used alongside with other fetal measurements.

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