Determining the number of calves in early pregnancy using real-time ultrasound imaging in beef cows induced to twin

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ABSTRACT

Details of the timing of pregnancy loss following induced twinning are unknown. The aim of this study was to evaluate real-time ultrasound as a method of determining early pregnancy status (0, 1 or 2 foetuses) in beef cows induced to twin using AI and ET. A 7.5 Mhz ultrasound probe was used intra-rectally to count foetuses in 2 experiments. In Exp. 1, 57 heifers were examined between 25 and 60 days of pregnancy and then slaughtered. In Exp. 2, up to 103 cows were examined between Day 25 & 60 of pregnancy, and final pregnancy status was confirmed using calving records. In Exp. 1, the accuracy of Day 25, Day 45 and Day 60 predictions of 2 foetuses was 100%, 20% and 100%, and 1/24, 5/33 and 1/33 twin pregnant heifers were predicted. The accuracy of correctly predicting twin outcomes was 33, 50 and 33% at the same 3 stages of pregnancy, but only 3, 2 and 3 twin pregnancies occurred, respectively. In Exp. 2, there was generally little difference between the accuracy of predicting twin pregnancies and the accuracy of the prediction. Thus, at Day 25, 35, 45 and 60, the accuracies were approximately 20, 47, 52 and 45%. Twenty recipients eventually calved twins. We conclude that real-time ultrasound imaging up to Day 60 is not an accurate technique for determining twin pregnancy status of cows. Loss of concepti between scanning and term did contribute substantially to this poor result in Exp. 2, but not in Exp. 1.

Keywords: ultrasound; pregnancy diagnosis; twinning; breeding cows; embryo transfer.

INTRODUCTION

Considerable research effort is currently focused on increasing the twin-calving rate in beef cattle in an attempt to increase both biological and economic efficiency (Herd *et al.*, 1993; Cummins, 1992; McMillan *et al.*, 1993). Complications during late pregnancy, calving and in the early post-natal period may be associated with twin pregnancies (McLeod *et al.*, 1992; Clark *et al.*, 1994). If additional management is required before and during calving in twinning cows, then reliable procedures are required to identify which cows are carrying twins. Information on the pregnancy status of cows (eg 0, 1 or 2 foetuses/calves present) may also be useful in research programmes aiming to study pregnancy loss following attempts to induce twinning (Wilkin *et al.*, 1994; McMillan *et al.*, 1993).

The aim of this study was to determine the relationship between twin-pregnancy diagnosis using ultrasound and twin-pregnancy outcome as determined at either slaughter (Experiment 1) or at calving (Experiment 2).

MATERIALS AND METHODS

Experiment 1

Fifty seven 2-year-old Hereford x Friesian heifers which each had two embryos transferred non-surgically at Day 7 (day of oestrus = Day 0), and had not returned to oestrus by Day 25, were used (Pugh *et al.*, 1993). On Day 25, 24 of the heifers were scanned per rectum using ultrasound to determine the number of foetuses present (ie 0, 1 or 2). The ultrasound probe was a 7.5 Mhz Aloka linear array device. The same day, all heifers were slaughtered and the uteri recovered and examined for foetuses. At Day 42 and Day 60, foetal number was determined following scanning in a group of 33 heifers which were slaughtered at Day 60 when the number of foetuses present were counted. Since no heifers were slaughtered following the Day 45 scans, the scan images were stored on video tape and these were re-examined if the number of foetuses present at slaughter at Day 60 did not match with the Day 45 prediction. The Day 60 slaughter data were then adjusted to fairly represent the Day 45 outcome only if total foetal loss was verified on the tapes.

Experiment 2

Up to 103 mixed age Hereford x Friesian cows which each had two embryos transferred non-surgically at Day 7 (day of oestrus = Day 0), and had not returned to oestrus by Day 25, were used on each scanning occasion. Foetal number was determined using ultrasound as in Exp. 1 on Day 25, 35, 45 and 60 of pregnancy. Images were stored on video tape. Final pregnancy status was determined at calving. No attempt was made to adjust records where foetal losses were known to occur, as we could not consistently determine whether 1 or 2 foetuses were present when tapes were re-examined. For the sake of simplicity, results related to only twinning are presented.

Defining Accuracy

In reporting data from studies of this kind, it is important to clearly define what is meant by accuracy. In this paper, we refer to accuracy of prediction and accuracy of predicting the outcome. In many clinical studies, the respective terms used are specificity and sensitivity. The accuracy of the prediction is defined as the ratio of correct predictions to total predictions expressed as a percentage. For example, if a total of 22 animals are predicted to have 2 foetuses present, but only 21 of the 22

are confirmed to have 2 foetuses, the accuracy of the prediction is 95%. The accuracy of predicting the outcome is defined as the ratio of predictions which matched outcomes, expressed as a percentage. Thus, using the previous example, if 25 animals were confirmed to be twin pregnant, only 21 of these were predicted, the accuracy of predicting the outcome is 84%. Overall accuracy is defined as the percentage of cases where predictions and outcomes coincided. In the remainder of the paper, all accuracy data will be defined as above.

RESULTS

Experiment 1

Overall accuracy at Day 25, 45 and 60 pooled over 0, 1 and 2 foetuses were 79, 85 and 97% respectively (Tables 1-3). However, the difference in overall accuracy at Day 45 and Day 60 (91%) was not significantly different to that at Day 25 ($c^2 = 1.30$, $P<0.1$). Only 2 of 7 twin predictions (29%) were correct compared with 51/57 (89%) single predictions ($c^2 = 12.30$, $P<0.001$). Conversely, only 3 of 7 (43%) twin outcomes were predicted compared with 51/56 (91%) single ($c^2 = 8.20$, $P<0.005$). The accuracy of prediction of heifers with no pregnancy (25/26, 96%) compared favourably with predictions of single pregnancies (89%, $c^2 = 0.33$, $P<0.1$). Furthermore, of 27 non-pregnant outcomes, 25 (93%) were correctly predicted. This result compares favourably with a 91% accuracy in predicting single outcome pregnancies. Collectively, these results indicate that accuracy associated with non-pregnant and single pregnancies contributed to a large extent to the high overall accuracy rate. Although twin accuracy were low, too few of the recipients were in fact twin pregnant.

TABLE 1: Number of heifers predicted to have 0, 1 and 2 calves present at Day 25 compared with number actually having 0, 1 and 2 calves, and accuracy of predictions and of predicting the outcome.

		Day 25 Predictions (# Heifers)				Accuracy of predicting the outcome
		0 Calves	1 Calf	2 Calves	All	
Day 25	0 Calves	9	2	0	11	82%
Outcome						
(# Heifers)	1 Calf	1	9	0	10	90%
	2 Calves	0	2	1	3	33%
	All	10	13	1	24	
Accuracy of Prediction		90%	69%	100%		79%

TABLE 2: Number of heifers predicted to have 0, 1 and 2 calves present at Day 45 compared with number actually having 0, 1 and 2 calves, and accuracy of predictions and of predicting the outcome.

		Day 45 Predictions (# Heifers)				Accuracy of predicting the outcome
		0 Calves	1 Calf	2 Calves	All	
Day 45	0 Calves	6	0	0	6	100%
Outcome						
(# Heifers)	1 Calf	0	21	4	25	84%
	2 Calves	0	1	1	2	50%
	All	6	22	5	33	
Accuracy of Prediction		100%	95%	20%		85%

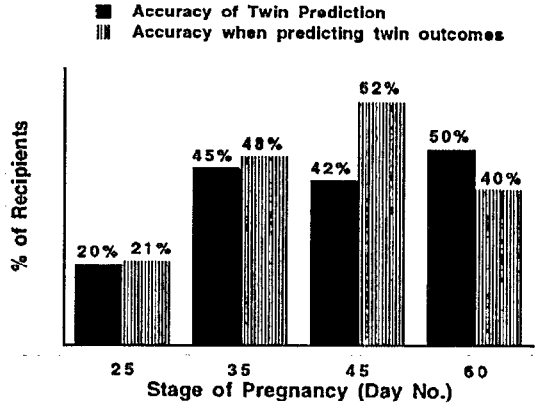
TABLE 3: Number of heifers predicted to have 0, 1 and 2 calves present at Day 60 compared with number actually having 0, 1 and 2 calves, and accuracy of predictions and of predicting the outcome.

		Day 60 Predictions (# Heifers)				Accuracy of predicting the outcome
		0 Calves	1 Calf	2 Calves	All	
Day 60	0 Calves	10	0	0	10	100%
Outcome						
(# Heifers)	1 Calf	0	21	0	21	100%
	2 Calves	0	1	1	2	50%
	All	10	22	1	33	
Accuracy of Prediction		100%	95%	20%		97%

Experiment 2

Approximately, 50% of cows which calved produced twins. The accuracy of twin prediction was lowest at Day 25 (20%) and varied from 42-50% between Day 35 and Day 60 (Figure 1). The accuracy of predicting twin outcomes was also lowest at Day 25 (21%) and varied from 40-62% subsequently (Figure 1).

FIGURE 1: Accuracy of twin predictions and predicting twin outcomes, based on calving (Exp. 2).



DISCUSSION

Overall accuracy in Exp. 1 was similar whether 0, 1 or 2 foeti were expected or achieved. However, it was more difficult to achieve a high accuracy with twins than singles, and single accuracies were comparable to non-pregnants. It is clear that it was difficult to achieve a high accuracy (>95%) of both prediction of twin pregnancy and predicting twin outcomes in both experiments when using real time ultrasound. The low incidence of twin pregnancies achieved in Exp. 1 means that the results should be treated with caution, although the results were similarly poor in Exp. 2 where twin pregnancies were about 33% of all calving outcomes. However, the timing of predictions and their immediate validation

at slaughter in Exp. 1 overcame the complication of loss of pregnancies between scanning and validation at calving that was apparent in Exp. 2.

It is difficult to explain why the accuracy of twin pregnancy diagnosis was so low, especially beyond Day 25. Total pregnancy loss after diagnosis and before calving was a source of error in Exp. 2, but accuracy rates were still generally below 80% if only calving cows were included in the analysis. Operator inexperience is an unlikely explanation for the poor results. Operators used in this study would have each completed at least 1000 scans each by the end of the study, and could not be described as inexperienced. It may be that the use of ultrasound in cattle for twin pregnancies is an inherently unreliable technique, although at least 19 out of 21 twin predictions between Day 50 and 80 were substantiated by calving in another study (Dobson *et al.*, 1993). We have other studies currently underway to assess our accuracy using the slaughter approach taken in Exp. 1, but with substantially more recipients and a higher expected twin pregnancy rate.

If our proposition is correct, that is, that ultrasound is an unreliable means for making twin predictions and for predicting twin pregnancies, then we are concerned about the publishing of papers with twin predictions using this technique. Furthermore, apart from the study by Dobson *et al.*, 1993, there do not appear to be other published reports that have reported validated accuracy either with (preferably) slaughter or with calving data. Instead, many reports of predicted twin pregnancy rates have appeared in the literature without validation (eg Agca *et al.*, 1994). In our experience, such validations are necessary to ensure meaningful interpretation of results.

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