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# Twinning in beef cows: preliminary results from embryo transfer studies

W.H. MCMILLAN, D.R.H. HALL, P.H. EVANS AND A.M. DAY[1]

AgResearch Whatawhata, Private Bag 3089, Hamilton, New Zealand.

## ABSTRACT

During the springs of 1990 and 1991 128 recipient cows were artificially inseminated (AI) and 7 days later one embryo was transferred (ET) to induce twinning. Twenty-six per cent (33/128) of recipients returned to oestrus within 25 days of AI. Pregnancy diagnosis at 35 days indicated 12%, 39% and 23% of recipients with 0, 1 or 2 foetuses. The expected calving rate was 63% of which 38% were expected to twin. Only 43% calved but 50% gave birth to twins. The results were similar in both years.

These results indicate that the combination of AI and ET result in normal pregnancy rates and higher than expected twin pregnancy rates at about five weeks after AI. However, losses from five weeks to calving are several times higher than expected, and are a major limitation to the achievement of 100% plus calving to one round of treatment. Early pregnancy diagnosis (day 35) may have precipitated such losses.

**Keywords:** beef cows, twinning, embryo transfer, embryo mortality.

## INTRODUCTION

Increasing the biological efficiency of farmed animals generally increases economic efficiency. Biological (ie beef) output in a beef breeding cow herd is made up of both the contribution of the calf and of the breeding female In most herds the contribution of the calf is much more important since several calves can be produced for sale over the lifetime of the cow. Twinning affords the opportunity to increase the number of calves weaned and thereby increase the total weight of calf weaned per cow. It is unlikely that other options exist to achieve such large potential gains in biological efficiency in beef cows.

The normal twinning rate in beef cow herds is 0-5% (Morris and Day, 1990). There are three main ways to achieve higher twinning rates in cattle. Firstly, some cows have a genetic propensity for natural twinning, and selection herds have been established in NZ (Morris and Day, 1990) and elsewhere (eg Gregory *et al.*, 1990). Secondly, induced twinning can be achieved in two ways. Induced twinning, through the artificial manipulation of ovulation rate, was first attempted around 1960 (Gordon *et al.*, 1962) using PMSG. More recently, FSH has been used but the latest developments have focused on immunisation against fragments of the protein hormone inhibin (Bindon and Hillard, 1992). The alternative method for inducing twinning is through embryo transfer (ET). Two embryos can be transferred, or as was the case in the present study, artificial insemination (AI) followed some days later by ET of a single embryo to supplement the recipients own embryo. Thirdly, the fostering of a single calf onto a recently calved cow is another means by which cows can rear two calves (McMillan *et al.*, 1993).

The primary aim of the present study is to describe the results from two years of study (1990 and 1991) where AI and ET were used in an attempt to induce twin-calving in cattle. A secondary aim was to test the hypothesis that the survival to term of the two embryos/calves was independent of each other. This was considered worth testing for two reasons. Firstly, with twinning in cows, as opposed to other species, calves share a common fused placenta and thus the loss of one calf could influence the fate of the second calf. Secondly, the direction of future research to enhance embryo survival in cattle could differ depending on the outcome of such an analysis.

## MATERIALS AND METHODS

### Recipient oestrus and AI

Oestrous was synchronised in 2-10 year-old suckling Hereford x Friesian cattle using a short term (9-12 day) CIDR™ treatment including oestradiol benzoate (McMillan and Macmillan, 1989). All 134 cows showing heat over the 96 h after CIDR™ withdrawal were potential recipients. All cows were inseminated once with Hereford semen at 48 h after CIDR™ withdrawal, irrespective of whether or not they had commenced oestrous activity. Thus, all calves born to these conceptions had the white head marking characteristic of the Hereford breed. Frozen-thawed semen was used in 1990 and fresh normally-processed or encapsulated semen in 1991 (Vishwanath *et al.*, 1992).

### Recipient ET and oestrous re-synchrony

Seven days after AI, all cows were palpated per rectum, to determine the site of ovulation. A frozen-thawed embryo from cows of several breeds (excluding Hereford) was then non-surgically transferred into cows with a palpable ovulation. Thus, all calves born to transferred embryos did not have the white head colour pattern characteristic of the Hereford breed. Embryos were recovered from superovulated donor

[1]AgResearch Ruakura, Private Bag 3123, Hamilton, New Zealand.

cows after slaughter in 1990 and after non-surgical recoveries in 1991. Half of the transfers were to either the uterine horn contralateral or ipsilateral to the ovary with the ovulation in 1990. All transfers were to the uterine horn contra-lateral to the ovary with the ovulation in 1991. Following ET, a CIDR™ was inserted into each recipient and withdrawn 21 d after AI so as to re-synchronise oestrus in non-pregnant recipients (and allow another fixed-time insemination). Recipients were then examined twice daily for 96 h for oestrous activity.

## Pregnancy and calving

Thirty five days after AI, all recipients which had failed to exhibit oestrous activity, and were thus presumed pregnant, were palpated per rectum by one of us (A.M.D.) and the number of foetuses determined. The number of calves present at birth was also recorded.

## Testing independence of survival of AI and ET embryos/calves

If the survival rate of one embryo (eg AI conceptus) is independent of the survival of a second embryo (eg ET conceptus), say each with a p chance of survival to term, then an independence (ie binomial) model would predict p2, 2pq and q2 of AI and ET recipients with 2, 1 and 0 calves at birth (where p+q=1). Thus, if p is assumed to be 60% then 36%, 48% and 16% of recipients would be expected to give birth to 2, 1 or 0 calves. Put another way, 84% of recipients would be expected to calve and 43% of these would be expected to calve twins. Data from this and other studies were used to test this hypothesis.

# RESULTS

## Oestrus and AI

A total of 75 and 59 cows were detected in oestrus in 1990 and 1991 respectively. A further 10% were in oestrus between 48 and 72 h. All were inseminated.

## Ovulation and ET

Overall, 128 (96%) cows ovulated with little difference between years (97% and 93%, NS). In only one instance was more than 1 ovulation present at palpation. Thus, 73 and 55 recipients received an embryo in 1990 and 1991 respectively. Embryos were transferred at the morula or blastocyst stage of development. Most recipients (85%) were within 24 h of synchrony of donors and no recipient was beyond 48 h.

## Return oestrus, pregnancy and calving

Site of deposition of embryo (ipsilateral vs contralateral to ovary with CL) did not influence embryo survival. Overall, 26% of recipients showed a return oestrus (27% vs 24% in 1990 and 1991). Pregnancy rate at 35 days after AI was 60% in 1990 and 67% in 1991. Of these, 36% and 38% respectively were expected to calve twins. However, actual calving rates were 44% and 42% respectively. Actual twin-calving rate in 1990 was similar to twin-pregnancy rate assessed at day 35 (38 vs 36%), but in 1991 more twin calves were born than expected at day 35 (65 vs 38%, $\chi^2 = 4.26$ P<0.05).

## Timing of loss of potential calves

A considerable wastage of potential calves occurred between AI, ET and calving. Based on a potential of 200 calves per 100 recipients at the time of ET, there was a maximum potential of 146 and 152 calves at day 25 in 1990 and 1991 respectively (assumes all non-return recipients were twin-pregnant). However, if we assumed that only recipients which finally calved twins were the only twin-pregnant recipients at day 25, the potential number of calves reduced to 101 and 125. At day 35 of pregnancy, the potential number of calves had reduced further to 82 and 93 in 1990 and 1991, assuming 100% accuracy of diagnosis. If adjustments are made for recipients diagnosed as single-pregnant but actually calved twins, and vice versa, a more accurate measure of potential number of calves at day 35 was 85 in 1990 and 102 in 1991. Proportionately more twins were diagnosed as single pregnancies in 1991, and hence the higher increase in potential calf numbers in that year. By calving time, the actual number of calves born was only 60 and 69 in each of 1990 and 1991. Clearly, about one third of the potential number of calves were present at birth compared with at ET.

## Independence of survival of AI and ET embryos/calves

In 1990, embryo survival was 47% following AI and 29% following ET. Assuming a model of independence of survival of the two embryos, the expected incidence of recipients with 0, 1 or 2 calves at term is 38, 48 and 14%. The actual incidences were 56, 27 and 17% ($\chi^2 = 13.91$, P<0.001). Since the expected and actual number of twin-calving recipients are similar ($\chi^2 = 0.42$, NS), it is clear that a disproportionate number of recipients are losing 2 rather than 1 embryo/foetus. In 1991 with embryo survival rates of 45% and 35% following AI and ET respectively, the expected ratios of recipients with 0, 1 or 2 calves is 36, 48 and 16% compared with 58, 15 and 27%. Again a major disparity is the low incidence of single-calving cows ($\chi^2 = 13.07$, P<0.001) and a higher incidence non-pregnant cows ($\chi^2 = 7.74$, P<0.01) compared with expectation. However, unlike the previous year's result, the incidence of twin-calving cows was higher than expected ($\chi^2 = 4.64$, P<0.05).

# DISCUSSION

The major finding in this study was that a combination of fixed-time AI followed 7 days later by ET could achieve a twin-calving rate of about 50%. This compares favourably with a 54% twin-calving rate in a larger study in Australia involving 654 calving recipients treated in a similar manner (Cummins *et al.*, 1992), but appears less than the 61% in 207 recipients reported in a Californian study (Guerra-Martinez *et al.*, 1990). In Japanese and Irish studies involving about 100 calving recipients each, twin-calving rates after transferring two embryos were 41 and 46% respectively (Suzuki *et al.*, 1989; Sreenan and Diskin, 1989). In the first reported studies on twin embryo transfer cattle, twinning rates were nil or very low (Rowson *et al.*, 1969 a, b), but advances in embryo handling and recipient management improved twinning rates to about 50% (Rowson *et al.*, 1971). Clearly, cows are capable of supporting twin pregnancies to term, but are limited by their low ovulation rate as demonstrated in the present study. In the future, it is likely that twin-ovulations will be induced in a high proportion of cows through the use of twinning vaccines based on the hormone inhibin (Bindon and Hillard, 1992). Until this vaccine technology is sufficiently developed, AI and ET will remain the most reliable technique for inducing twin-calving cows.

The second major, and unexpected, finding was the large loss in pregnant recipients between day 35 and calving: only 43% of recipients calved compared with an expected 63% at day 35. Roughly one third of recipients failed to calve after being diagnosed pregnant. Certainly, in the two other large studies referred to earlier actual calving rates have been above 60% (64% of 1021 and 325 recipients; Cummins *et al.*, 1992; Guerra-Martinez *et al.*, 1990 respectively), although only 43% of 208 recipients calved in the Japanese study (Suzuki *et al.*, 1989). The difference between early pregnancy rate and actual calving rate has been less than 10 percentage units in the two large studies involving 325 and 208 recipients (Guerra-Martinez *et al.*, 1990; Suzuki *et al.*, 1989, respectively).

It is difficult to explain the cause of such large losses in pregnancy rate. It is possible that attempted twin-pregnancy diagnosis at day 35 may have jeopardised the continuation of some pregnancies. Certainly, a subsequent pregnancy diagnosis at about day 60 indicated that most failed pregnancies had already failed by this time (McMillan, unpublished). Another possibility is that pasture toxins (eg zearelenone) could have prematurely terminated some pregnancies. Plasma levels of zearelenone have been reported to be high in dairy cows on nearby properties during the period when losses were occurring in our study (Towers, N.R., pers. comm). Clearly, other studies are required to determine the extent to which early palpation and pasture factors are a cause of this problem. It is unlikely that asynchrony between donors and recipients is a major cause of the loss since it would be reasonable to expect that pregnancy would not be initiated under such circumstances. Furthermore, most recipients were within a 24 h synchrony 'window' of donors in the present study. Other reports indicate that a 48 h synchrony 'window' is apparently well tolerated in cows (Rowson *et al.*, 1969 b). A further possibility is that pregnancy/non pregnancy diagnosis was in error at 35 days. This is unlikely given that the same operator has diagnosed pregnancy in several hundred yearling heifers for us at about the same early stage and pregnancy loss rate by calving has been low (under 5%).

The data from the present study do not support an hypothesis of independence of survival of embryos. This appears to be a consistent finding in other studies as well (Cummins *et al.*, 1992; Guerra-Martinez *et al.*, 1990; Suzuki *et al.*, 1989). It would thus seem that a disproportionate number of cows end up with 0 rather than 1 calf at term. Collectively, these data indicate that the chances of one embryo surviving, when two are initially present, is not independent of the chances of the other surviving. This raises the possibility that if one embryo/foetus fails, then so will the other, and vice versa. Certainly, such a possibility is more likely in cattle than other species as placental fusion is known to occur in the bovine. It would thus be tempting to speculate that, once a common placenta is functional in cattle, the loss of one foetus would jeopardise the loss of the other. Alternatively, the results may be explained by the fact that single contra-lateral pregnancies (ie pregnancy in the uterine horn *opposite* to the ovulating ovary) are do not occur naturally in cattle (Scanlon, 1972). Thus, if one embryo is initially present in each uterine horn, as in most recipients in the present study, the loss of the ipsilateral pregnancy (ie pregnancy in the uterine horn *adjacent* to the ovulating ovary) then the contralateral pregnancy would also be expected to fail. Under this scenario, the fate of the contralateral embryo depends absolutely on the fate of the ipsilateral embryo. In sheep at least, embryo loss in twin-ovulating ewes is 3-4 times higher if an embryo is the sole occupant of a contralateral rather than an ipsilateral uterine horn (Doney *et al.*, 1973).

In conclusion, AI and ET has the potential to achieve 100% plus calving to one round of treatment. In our study, twin-calving rates of about 50% were achieved, but a significant and unexplained loss of pregnancies after day 35 limited the achievement of this potential. The survival of embryos to term following AI and ET is not supported by an hypothesis of independence. The low survival rate of frozen-thawed embryos suggests that freeze-thawing procedures consistent with high embryo survival have yet to be developed.

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## REFERENCES

Bindon, B.M. and Hillard, M.A. 1992. Hormonal induction of twins in beef cattle. *Proceedings of the Australian Society of Animal Production* **19:** 439-440.

Boland, M.P.; Crosby, T.F. and Gordon, I. 1975. Twin pregnancy in cattle established by non-surgical egg transfer. *British Veterinary Journal* **131:** 738-740.

Cummins, L.J.; Clark, A.J.; McLeod, I.K.; Wilkins, J.F.; Williamson, P.J.; Hennessey, D.W.; Hillard, M.R.; Pashen, R.L. and Darrow, M.D. 1992. Induced twins by embryo transfer. *Proceedings of the Australian Society of Animal Production* **19:** 440-441.

Doney, J.M.; Gunn, R.G. and Smith, W.F. 1973. Transuterine migration and embryo survival in sheep. *Journal of Reproduction and Fertility* **34:** 363-367.

Gordon, I.; Williams, G.L. and Edwards, J. 1962. The use of serum gonadotropins (P.M.S.) in the induction of twin pregnancy in the cow. *Journal of Agricultural Science (Cambridge)* **59:** 143-198.

Gregory, K.E.; Echternkamp, S.E.; Dickerson, G.E.; Cundiff, L.V.; Koch, R.M. and Van Vleck, L.D. 1990. Twinning in cattle. Part I. Foundation animals and genetic and environmental effects on twinning rate. *Journal of Animal Science* **68:** 1867-1876.

Guerra-Martinez, P.; Dickerson, G.E.; Anderson, G.B. and Green, R.D. 1990. Embryo-transfer twinning and performance efficiency in beef cattle. *Journal of Animal Science* **68:** 4039-4050.

McMillan, W.H. and Macmillan, K.L. 1989. CIDR-B for managed reproduction in beef cows and heifers. *Proceedings of the New Zealand Society of Animal Production* **49:** 85-89.

McMillan, W.H.; P.H. Evans; Hall, D.R.H. and McLean, M.J. 1993. Twin-suckling in beef cows using foster calves: effects on calf and cow performance. *Proceedings of the New Zealand Society of Animal Production* **53:** In press.

Morris, C.A. and Day, A.M. 1990. Genetics and Physiology studies of cows in a twin breeding experiment. *Journal of Animal Breeding and Genetics* **107:** 2-6.

Rowson, L.E.A.; Lawson, R.A.S and Moor, R.M. 1969 a. Twinning in cattle. *Veterinary Record* **88:** 583-584.

Rowson, L.E.A.; Lawson, R.A.S and Moor, R.M. 1971. Production of twins by embryo transfer. *Journal of Reproduction and Fertility* **25:** 261-268.

Rowson, L.E.A.; Moor, R.M. and Lawson, R.A.S. 1969 b. Fertility following egg transfer in the cow; effect of method, medium and synchronisation of oestrus. *Journal of Reproduction and Fertility* **18:** 517-523.

Scanlon, P.F. 1972. Frequency of transuterine migration of embryos in ewes and cows. *Journal of Animal Science* **34:** 791-794.

Sreenan, J.M. and Diskin, M.G. 1989. Effect of unilateral or bilateral twin embryo distribution on twinning and embryo survival rate in the cow. *Journal of Reproduction and Fertility* **87:** 657-664.

Suzuki, T.; Sakai, Y.; Ishida, T.; Matsudu, S.; Muira, H. and Itoh, K. 1989. Induction of twinning in cross-bred heifers by ipsi-lateral frozen embryo transfer. *Theriogenology* **31:** 917-926.

Vishwanath, R.; McMillan, W.H. and Curson, B. 1992. Microencapsulation of bovine sperm: implications for semen preservation and artificial breeding. *Proceedings of the 44-th Ruakura Farmers' Conference,* pages 116-119.