

Fluctuations in Energy Intake and Fertility in Cattle

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1. SUMMARY

Reproductive failure in dairy cows results in fewer calves born, lower milk sales, slower genetic progress and consequently, significant financial loss to the industry. Dairy cattle breed improvement programmes have, at least until very recently, focused primarily on increasing the yields of milk or milk solids. The resulting genetic improvement has led to significant increases in milk yield per cow but this increase is now associated with a significant decline in cow reproductive wastage. An important part of the Teagasc research programme in this area is to determine the time at which embryo loss occurs and also to determine whether the extent of the embryo loss is affected by the energy nutrition of the cow and to devise strategies to reduce its extent. This project has focused on the relationship between changes in dietary energy intake near the time of insemination and the extent and pattern of embryo survival. The main results are summarised in this report and detailed results of the several experiments involved have been published in the papers listed at the end of this report.

- The objectives of this project were to determine the effect of changes in energy intake near the time of insemination on embryo loss rate, on the timing of embryo loss and on the possible biological mechanisms involved.
- Cross bred heifers were provided with either high or low energy intakes that were based on pasture allowances calculated to provide either 0.8 or 2.0 times their maintenance requirements. These energy intakes were allocated for two weeks before and about five weeks after insemination. The effect of the changes in energy intake on embryo loss and on the time at which embryo loss occurred, relative to the time of insemination, was established. Possible associations between embryo loss and blood concentrations of progesterone, NEFAs, insulin and glucose were examined.

- A sudden reduction from a high to a low energy intake imposed for two weeks from the day of insemination reduced the subsequent embryo survival rate by 30 percentage points to a survival rate of 38%. When energy intake over this same period was either maintained or increased, embryo survival rate remained high (overall mean, 69%), within a range of 65-71%.
- The time at which embryo loss occurred was established. Embryo survival or pregnancy rates measured on days 14 and 30 after insemination and at full term were 68%, 76% and 72%, respectively. These results provide new information indicating that most embryo loss, at least in heifers, had occurred on or before day 14 after insemination.
- There was no evidence of any association between the short-term changes in energy intake either before or after AI and blood progesterone concentration. Neither was there any evidence that the detrimental effect of the sudden reduction in energy intake on embryo survival was mediated through changes in the systemic concentrations of non-esterified fatty acids (NEFAs) or insulin. There was a suggestion, however, that the detrimental effect of the reduced energy intake may operate through a reduction in systemic glucose concentrations.

2. INTRODUCTION

Reproductive failure in dairy cows results in fewer calves born, lower milk sales, slower genetic progress and consequently, significant financial loss to the industry. Dairy cattle breed improvement programmes have until very recently, focused primarily on increasing milk or milk solids yield. The resulting genetic improvement has led to significant increases in milk yield per cow but this is now associated with a significant increase in cow reproductive wastage. Following insemination the greatest increment of cow reproductive wastage occurs in the form of early embryo death with approximately 80% of all embryo deaths occurring in the first two to three weeks after insemination. High genetic merit, high yielding cows partition their feed energy mainly towards milk. In the early post-calving period, however, feed energy intake is unable to match that required to accommodate the increase in milk output with the result that cows are in negative energy balance for a period of up to 10 weeks. This negative energy balance together with the metabolic load resulting from a high milk output is associated with reduced cow fertility but the mechanisms involved are unclear.

Evidence from studies conducted with sheep and pigs shows that short term-changes in energy intake near the time of breeding can seriously affect the incidence of embryo loss, through an effect on systemic progesterone. There is no information on the effects of transient changes in energy intake around the time of insemination on embryo survival rate in the cow. Such information is essential in order to develop management and feeding strategies to reduce the level of embryo loss and enhance cow reproductive efficiency in a cost efficient manner.

Objectives

The objectives of this project were to investigate (i) the effect of short-term nutritional changes pre- and post-insemination on embryo survival rate, (ii) the timing of the embryo loss relative to the time of insemination and (iii) possible associations between energy intake changes and systemic progesterone and metabolite concentrations and embryo survival rate.

3. METHODOLOGY

Animals

Crossbred beef heifers (n= 296), 18 to 24 months old were used in a study conducted over two grazing seasons that extended from April to October in each year. Animals grazed the same pasture area in both years. The initial mean liveweight and body condition score (BCS) of the heifers was 401kg and 3.3 units, respectively. Liveweight and BCS were recorded at regular intervals throughout the project.

Pasture nutrition treatments

The pasture allowances were calculated on a dry matter basis according to the initial liveweight of the heifers and were designed to supply either 0.8 times (Low; L) or 2.0 times (High; H) their maintenance requirements for energy. The composition of the pasture in each of the two years is shown in Table 1.

	Year 1	Year 2
Dry Matter (g/Kg)	190 \pm 30	170 \pm 42
Digestibility (g/Kg)	770 \pm 65	780 \pm 95
Crude Protein	180 \pm 43	150 \pm 48
Water Soluble Carbohydrate (g/l)	22.0 \pm 0.99	15.1 \pm 0.69
Estimated Metabolisable Energy MJ/kg DM	11.0 \pm 0.05	11.0 \pm 0.10

Oestrus was synchronised in the heifers using two injections of prostaglandin at a 10-day interval. Checks for oestrous activity were carried out frequently after each prostaglandin administration. On the day of oestrus following the first prostaglandin administration heifers

were randomly allocated to either the low (L) or high (H) allowance for 10 days. At the oestrus following the second prostaglandin administration heifers were artificially inseminated (AI) with semen from one Limousin bull. On the day following AI the heifers were randomly re-allocated to the L or H allowance until either embryo recovery between days 14 and 16 or pregnancy diagnosis at day 30 (day of AI = day 0) by ultrasonic scanning. This resulted in four nutrition treatments L-L, L-H, H-H and H-L.

Embryo survival rate

A number of heifers from each nutrition treatment were randomly allocated to either undergo embryo recovery or to be left on the nutrition treatments until day 30 for pregnancy diagnosis by ultrasonography or to be held for record of calving.

Timing of embryo loss

The timing of embryo loss was measured as follows. One hundred and fifty eight of the total of 296 heifers that had been observed in oestrus and artificially inseminated were used in this study in the following manner. Following AI forty-seven heifers were randomly allocated to undergo embryo recovery at day 14 (AI = day 0) to measure early embryo survival rate and the remaining 111 heifers were ultrasonically scanned for the presence of a viable foetus at day 30. Seventy-two of the 84 heifers that had viable foetuses at the time of scanning were randomly selected to be held for record of calving.

Metabolite and progesterone measurements

In order to determine possible biological mechanisms involved in embryo survival or loss, blood concentrations of progesterone, non-esterified fatty acids (NEFAs), glucose and insulin were measured on selected days and the relationship between these concentrations and embryo loss was examined.

All nutritional, pasture, embryo and foetal survival, hormonal and metabolite data underwent appropriate statistical analysis.

4. RESULTS

Nutrition, liveweight and BCS

Liveweight and BCS response to the nutrition treatments imposed were predictable and are shown in Table 2.

Table 2. Effect of nutrition treatment on liveweight (LW) and body condition score (BCS) change (mean \pm s.e.) for two weeks before and after AI.

	Nutrition treatment				Max s.e.
	L-L	L-H	H-H	H-L	
LW change (Kg)	+3.1	+19.4	+33.7	-0.20	3.44
BCS unit change	-0.06	+0.23	+0.38	-0.12	0.09

Nutrition and oestrous response

The proportions of heifers observed in oestrus following the second prostaglandin administration were similar in both years and these data were therefore combined for analysis. A higher proportion of heifers exhibited oestrus in the low than the high pasture intake treatment (88% v 75%). Interval from prostaglandin treatment to onset of oestrus was similar for both treatment groups (L v H, 53 v 55 hours.)

Nutrition and embryo survival rate

Embryo survival rate, whether measured at embryo recovery, by ultrasonography or by record of calving was similar for both years of the study and therefore these data were combined for analysis. Embryo survival rate was not affected by liveweight or BCS or changes in these parameters. The effect of changes in energy intake on embryo survival rate is shown in table 3.

	Nutrition treatment			
	L-L	L-H	H-H	H-L
No. of heifers	66	65	60	56
No. pregnant	46	46	39	21
Embryo survival rate, %	70	71	65	38

When the energy intake was reduced from high-to-low embryo survival rate was reduced by about 30 percentage points compared to the other nutrition treatments. In the high-to-low group embryo survival was 38%. In the other groups embryo survival remained high at 70% for the low-to-low, 71% for the low-to-high and 65% high-to-high treatment groups. Measurements on embryos collected at day 14 after AI are shown in Table 4. There was no effect of nutrition treatment on embryo size in terms of either the length, diameter or estimated total protein content of the embryos collected.

	Nutrition treatment							
	L-L		L-H		H-H		H-L	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
Length (mm)	6.8 ± 1.74 (1.2 - 26.3)		2.0 ± 1.74 (0.72 - 3.0)		3.2 ± 1.97 (0.80 - 5.5)		2.6 ± 2.61 (0.60 - 5.2)	
Diameter (mm)	1.4 ± 0.14 (0.58 - 2.0)		1.0 ± 0.14 (0.67 - 1.6)		1.1 ± 0.16 (0.72 - 1.5)		1.4 ± 0.21 (0.44 - 2.0)	
Protein (µg)	85.1 ± 17.15 (27.3 - 285.1)		33.7 ± 17.15 (17.1 - 56.6)		48.0 ± 19.44 (17.9 - 82.6)		53.1 ± 25.72 (14.9 - 94.8)	

Timing of embryo loss

All heifers submitted for embryo recovery at day 14 had a single corpus luteum and the recovery rate of ova and, or, embryos was 79 % and the embryo survival rate was estimated to be 68% (Table 5).

No. heifers	47
No. heifers yielding ova/embryos (recovery rate)	37 (79 %)
No. unfertilised ova	4
No. degenerate ova / embryos	1
No. morphologically normal embryos	32
No. embryos surviving (%)	32/47 (68 %)

There was no difference in embryo survival rate measured on day 14, day 30 or at full term (see Fig 1). Of heifers confirmed pregnant with viable foetuses on day 30 and held for calving (n=72), three (4.2 %) failed to calve subsequently. All of the embryos recovered at day 14 were deemed to be grade 1 based on size and morphological examination.

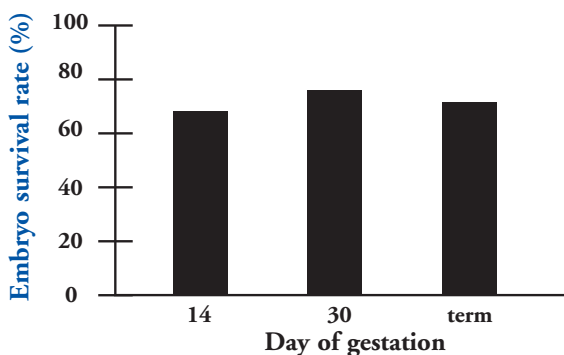


Fig. 1. Embryo survival rate in heifers after AI

Nutrition, progesterone and embryo survival rate

There was no effect of nutrition treatment on systemic progesterone concentrations measured during either the pre- or post-AI oestrous cycles (see Table 6).

		Nutrition treatment		
Oestrous cycle		L	H	Maximum s.e.
Pre-AI	Day 6	5.52	5.76	0.70
	Day 7	6.78	6.87	0.91
Post-AI	Day 6	5.69	5.00	0.40
	Day 7	7.07	6.57	0.52
	Day 13	11.20	11.69	0.92
	Day 14	12.55	12.49	1.00

Furthermore, progesterone concentration in turn was not related to either embryo length, diameter or protein content. In the second year of the trial progesterone concentration was higher on day 7 of the post-AI oestrous cycle in heifers that became pregnant than in those that failed to become pregnant.

Nutrition, metabolites and embryo survival rate

The effect of nutrition treatment on metabolite and insulin concentrations on day 7 of the pre-AI oestrous cycle and days 7 and 14 of the post-AI oestrous cycle is shown in Tables 7 and 8, respectively. As expected, NEFAs were higher in heifers on the low energy intake treatment and at the pre-feed sample time, and glucose concentrations were higher in heifers on the high energy intake treatment.

Table 7. The effect of pre-A.I. nutrition treatment and sample time relative to feeding on mean (\pm s.e.) metabolite concentrations measured on day 7 of the pre-A.I. oestrous cycle

Sample time	Pre-A.I. nutrition			
	Low		High	
	Pre-feed	Post-feed	Pre-feed	Post-feed
NEFA (mmol/l)	0.29 \pm 0.02	0.09 \pm 0.02	0.14 \pm 0.02	0.13 \pm 0.02
Glucose (mmol/l)	4.32 \pm 0.08	3.73 \pm 0.08	4.45 \pm 0.09	4.02 \pm 0.09
Insulin (μ iu/l)	7.16 \pm 0.65	7.3 \pm 0.65	9.42 \pm 0.73	10.93 \pm 0.73

Table 8. The effect of post-A.I. nutrition treatment and sample time relative to feeding on mean (\pm s.e.) metabolite concentrations measured on day 7 and day 14 of the post-A.I. oestrous cycle

Sample time	Pre-A.I. nutrition			
	Low		High	
	Pre-feed	Post-feed	Pre-feed	Post-feed
Nefa (mmol/l)				
Day 7	0.30 \pm 0.02	0.15 \pm 0.02	0.14 \pm 0.02	0.11 \pm 0.02
Day 14	0.32 \pm 0.03	0.26 \pm 0.03	0.21 \pm 0.03	0.16 \pm 0.03
Glucose (mmol/l)				
Day 7	4.31 \pm 0.07	3.68 \pm 0.07	4.38 \pm 0.08	4.09 \pm 0.08
Day 14	4.15 \pm 0.06	3.64 \pm 0.06	4.31 \pm 0.06	4.00 \pm 0.06
Insulin (μ iu/ml)				
Day 7	7.22 \pm 0.75	8.71 \pm 0.75	9.52 \pm 0.82	12.13 \pm 0.82
Day 14	6.14 \pm 0.59	7.20 \pm 0.59	8.06 \pm 0.65	7.56 \pm 0.65

5. DISCUSSION

The results of this project demonstrate for the first time that short-term fluctuations in energy intake can have a significant deleterious effect on embryo survival rate in cattle. A reduction in energy intake from twice to 0.8 times maintenance requirements for a period of two weeks immediately after insemination resulted in a reduction in embryo survival rate of about 30 percentage points.

Despite the documented relationship between energy intake and systemic progesterone in sheep and pigs, there is no evidence from the results of the study presented here that changes in energy intake affect systemic progesterone concentrations in heifers. Previous published reports on the effect of nutrition on progesterone concentrations in cattle are equivocal. Some authors have reported that restricting energy intake results in lower systemic progesterone concentration, others have reported a higher progesterone in heifers fed a restricted plane of nutrition, while others have failed to find any effect of level of nutrition on progesterone concentrations. In the present study there was no effect of nutrition treatment on progesterone measured in year 1, however, in year 2 progesterone was lower on days 5, 6 and 7 post-AI in the L-H treatment group. Progesterone has been positively correlated with embryo size in sheep and cattle and it is interesting to note that embryos from the Low-High pasture intake group tended to be smaller. This result, however, was not significant probably due to the large variation within a small set of morphological data. There was no evidence that embryos from the L-H treatment group were less viable, based on the recorded embryo survival rate of 71 %. It is the timing of the progesterone rise after ovulation that is of critical importance to the development of the early embryo and it has been reported that a delayed rise in progesterone soon after ovulation is associated with smaller and potentially less viable embryos at day 16. In the present study there was no association between progesterone concentration at day 7 of the oestrous cycle and embryo size. Reducing energy intake immediately after insemination (H-L treatment) resulted in a reduction in embryo survival rate of about 30 percentage points but

there is no evidence to suggest that this effect was mediated through changes in systemic progesterone concentrations.

Attempts have been made in several cattle studies to link the concentration of metabolites in the blood with reproductive performance, though these have been largely unsuccessful. High circulating concentrations of NEFA have been associated with reproductive failure in some studies. However, in this project, despite the elevated NEFA concentrations in the low fed heifers, indicative of a higher degree of adipose mobilisation, there was no indication of any association with reproductive failure. Heifers on the low pasture allowance pre- and post-AI (L-L) consistently had higher concentrations of NEFAs and yet the embryo survival rate in this group was high.

Carbohydrate insufficiency can also result in elevated blood urea nitrogen as protein is catabolised. However, systemic urea and ammonia were largely unaffected by nutrition treatment in this study, suggesting that the energy restriction imposed was not severe enough to result in significant mobilisation of body protein reserves. Clearly the nutrition treatments altered the energy status of heifers without having a significant effect on protein metabolism. The effects on embryo survival may therefore be assumed to be as a result of short-term changes in energy status and independent of protein metabolism. The animals on the high to low (H-L) pasture allowance had the lowest plasma glucose concentrations on day 7 post-AI. This group also had the lowest embryo survival rate. Cattle embryos have an increased requirement for glucose from around the 8 - 16 cell stage of development and particularly at about day 7, at the start of blastocyst formation. Furthermore, glucose uptake by cattle embryos at this time is positively correlated with their capacity to develop further. Low glucose concentrations in a critical period post-A.I., as a result of a sudden reduction in energy intake may, at least in part, be responsible for the reduction in embryo survival observed in this study.

Insulin is known to affect follicle cell function in several species including cattle. However, there was no apparent association between systemic insulin concentrations and embryo survival in the present study.

This study provides further clarification of the timing of embryo loss in heifers and shows, for the first time, that most embryo loss occurs before day 14 after insemination. Assuming a fertilisation rate of 90 % the incidence of embryo loss measured in the present study at day 14 was 22 percentage points with no further loss by day 30. Between day 30 and full term, three pregnancies (4.2%) were spontaneously terminated. This level of foetal loss is consistent with the published literature. There are, however, few similar studies to date which allow direct comparison with the pattern of embryo loss presented here. The results presented here are the first to demonstrate that most embryo loss has, in fact, occurred by day 14 or earlier after AI. Further definition of the pattern of embryo survival or loss rate is essential.

5. CONCLUSIONS

The detrimental effects of long-term negative energy balance on cow fertility have been documented but there is no information on the effects of short-term changes in energy intake around the time of insemination. Such information is essential in order to develop management and feeding strategies to reduce the level of embryo loss and enhance cow reproductive efficiency in a cost efficient manner. This project focused on the relationship between changes in dietary energy intake near the time of insemination and the extent and pattern of embryo survival. The main conclusions are outlined below and detailed results of the several experiments involved have been published in the papers listed at the end of this report.

- A sudden reduction from a high to a low energy intake imposed for two weeks from the day of insemination reduced the subsequent embryo survival rate by 30 percentage points to a survival rate of 38%. When energy intake was either maintained or increased, embryo survival rate remained high overall (69%), within a range of 65-71%.
- The results provide new information indicating that most embryo loss, at least in heifers, occurs in the first two weeks after insemination. Embryo survival or pregnancy rates were similar whether measured on days 14 or 30 after insemination or at full term.
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The results provide no evidence of any association between the short-term changes in energy intake either before or after AI and blood progesterone concentration. Neither is there any evidence that the detrimental effect of the sudden reduction in energy intake on embryo survival was mediated through changes in the systemic concentrations of non-esterified fatty acids (NEFAs) or insulin. There is a suggestion, however, that the detrimental effect of the reduced energy intake may operate through a reduction in systemic glucose concentrations.

9. PUBLICATIONS ARISING FROM THIS PROJECT

1. Dunne, L.D., Diskin, M.G. and Sreenan, J.M. (2000). Embryo and foetal loss in beef heifers between day 14 of gestation and full term. *Animal Reproduction Science*, 58, 39-44.
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