



End of Project Report

ARMIS 4009

NUTRITION AND OESTROUS AND OVARIAN CYCLES IN CATTLE

Authors

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CONTENTS

	Page
Summary	1
Introduction	3
Overall Objectives	4
Study 1.	
Temporal endocrine, metabolic and ovarian follicle changes during dietary restriction, anoestrus and during resumption of oestrous cycles in beef heifers.	4
Study 2.	
The effect of either acute dietary restriction or dietary over-supply on ovarian follicle growth and on endocrine and metabolic hormone concentrations in beef heifers.	14
Study 3.	
The effect of acute dietary restriction on the incidence of anovulation and on periovulatory oestradiol and gonadotrophin concentrations in beef heifers	22
Conclusions and Implications	30
Publications arising from this project	31

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Summary

- Moderate dietary restriction of beef heifers over a long period resulted in failure of ovulation and onset of anoestrus after an average of 174 days. Re-feeding, even at a level of 1.8 times maintenance requirements, did not result in an immediate resumption of ovulation and oestrous cycles. A strong inverse relationship was recorded between the time period required on the restricted diet to cause cessation of ovulation and the time period required to cause resumption of ovulation following the re-introduction of high plane feeding.
- Moderate dietary restriction did not affect the number of ovarian follicle waves per oestrous cycle but reduced the growth rate and maximum diameter of the dominant follicle prior to onset of anoestrus. After the onset of anoestrus, dominant follicles continued to grow and regress but follicle growth rate and maximum diameter were reduced compared with cyclic heifers.
- During anoestrus, LH pulse frequency and systemic concentrations of IGF-I and oestradiol were significantly reduced. However, the systemic concentration of FSH was unaffected by dietary restriction, suggesting that FSH is not a limiting factor to ovulation in dietary-induced anoestrous heifers. Following an increase in dietary intake, LH pulse frequency, systemic concentrations of IGF-I and the diameter of the dominant follicle increased as heifers approached the resumption of ovulation and normal oestrous cycles.
- Close similarities between follicle wave dynamics and reproductive and metabolic hormone secretion were observed in the dietary-restricted anoestrous heifer and in the postpartum cow. The dietary-restricted anoestrous heifer is therefore a good model for determining specific nutritional effects on different aspects of reproduction and is a relevant model for the beef cow.

- Acute dietary restriction of beef heifers, to 0.4 M, induced anovulation in a high proportion (60%) of heifers within 2 weeks. Failure of ovulation was always preceded by the absence of a gonadotrophin surge and in some cases preceded by the absence of a pro-oestrus increase in the concentration of oestradiol.
- Acute dietary restriction caused an immediate suppression in the growth rate and maximum diameter of the dominant follicle. This suppression occurred in the absence of any observed effect on the frequency of LH pulses.
- Acute dietary restriction caused an increase in the magnitude of the FSH rise associated with the emergence of a new follicular wave in cyclic heifers but did not affect any LH parameter measured. In ovariectomised heifers acute dietary restriction caused a linear increase in the concentration of FSH and an increase in the amplitude of LH pulses.

Introduction

In spring calving beef and dairy herds, good reproductive performance, resulting in an early and compact calving pattern just prior to the onset of grazing is central to production and economic efficiency. However, a major problem limiting efficient production, especially in beef herds, is that the interval from calving to the resumption of regular oestrous cycles is prolonged, resulting in late calving and in the failure of many cows to become pregnant. One of the management factors that influences the duration of this interval is cow nutrition. However, the effects of nutrition, both pre- and post-partum, are confounded with the effects of suckling, maternal-offspring bonding, and the energy demands of lactation in the beef cow. In the dairy herd while genetic improvement and better management practices have led to the production of large quantities of milk per cow, this is associated with a decline in reproductive efficiency. This decline in reproductive efficiency is due, at least in part, to a prolonged interval from calving to the resumption of regular oestrous cycles. In high yielding dairy cows negative energy balance during the first 3-4 weeks postpartum is highly correlated with the interval to resumption of regular oestrous cycles. However, in the dairy cow the effects of nutrition on reproduction are also confounded by the effects of lactation.

Until the biological mechanisms by which nutrition influences oestrous and ovarian cycles are defined it will not be possible to develop management and feeding strategies to optimise reproductive performance in either beef or dairy cows. It is imperative to establish how changes in nutrition, whether of short- or long-term duration, specifically affect the cow's endocrine, metabolic and physiological systems. Such information is necessary in order to devise management and feeding strategies that will enhance reproductive efficiency in a cost-effective manner.

Overall Objective

The overall objective of this project was to establish the effects of both long- and short-term changes in nutrition on ovarian follicle dynamics and on the systemic concentrations of metabolic and reproductive hormones. In order to avoid the confounding effects of lactation, suckling and maternal–calf bonding, beef heifers were used in a series of three studies.

Experimental Studies

Study 1. Temporal endocrine, metabolic and ovarian follicle changes during dietary restriction, anoestrus and during resumption of oestrous cycles in beef heifers.

Background: Cyclical patterns of hormone secretion and ovarian follicle growth and development are prerequisites for the establishment and maintenance of regular oestrous cycles in cattle. An increase in the frequency of luteinising hormone (LH) pulses and an increase in oestradiol production by the dominant follicle (DF) on the ovary are essential for the first postpartum ovulation to occur. Extended postpartum anoestrous intervals in both beef and dairy cows result from a failure of successive DFs to ovulate because of inadequate LH pulses. Nutrition plays a major role in determining the duration of the anoestrous interval but the specific effects of nutrition on the reproductive axis are impossible to delineate because of the confounding factors that apply to lactating animals. There is little published information on reproductive and metabolic hormone secretion or on follicle wave dynamics during periods of negative energy balance, when animals (a) remain cyclic, (b) become anoestrus or (c) recommence regular oestrous cycles.

Specific objective

The specific objectives of this study were to determine the temporal endocrine, metabolic and ovarian follicular changes during these three phases.

Materials and Methods

Twenty three Hereford x Friesian cyclic beef heifers with an average liveweight of 374 kg and a body condition score (BCS) of 2.8, fed a grass silage and concentrate diet supplying the nutritional requirements for their maintenance (1.0 M), were used. Using ultrasonography, ovarian follicle dynamics were monitored over one oestrous cycle at the start of the study, and again during the fourth and seventh oestrous cycles following feed restriction to 0.66 M. Ovarian follicle dynamics were then monitored during the anoestrous phase and following recommencement of high plane feeding until the second ovulation had occurred. During each of these periods, blood concentrations of oestradiol, luteinising hormone (LH), follicle stimulating hormone (FSH) and insulin-like growth factor-I (IGF-I) were determined. Following the onset of anoestrus, all heifers were immediately returned to a diet of 1.0 M for the duration of 6 anovulatory follicle waves (about 60 days; Recovery 1), after which they were randomly allocated to diets supplying either 1.0 M (Recovery 2) or 1.8 M (Recovery 3) until oestrous cycles had resumed (See Figure 1). Heifer liveweight and BCS score were monitored weekly.

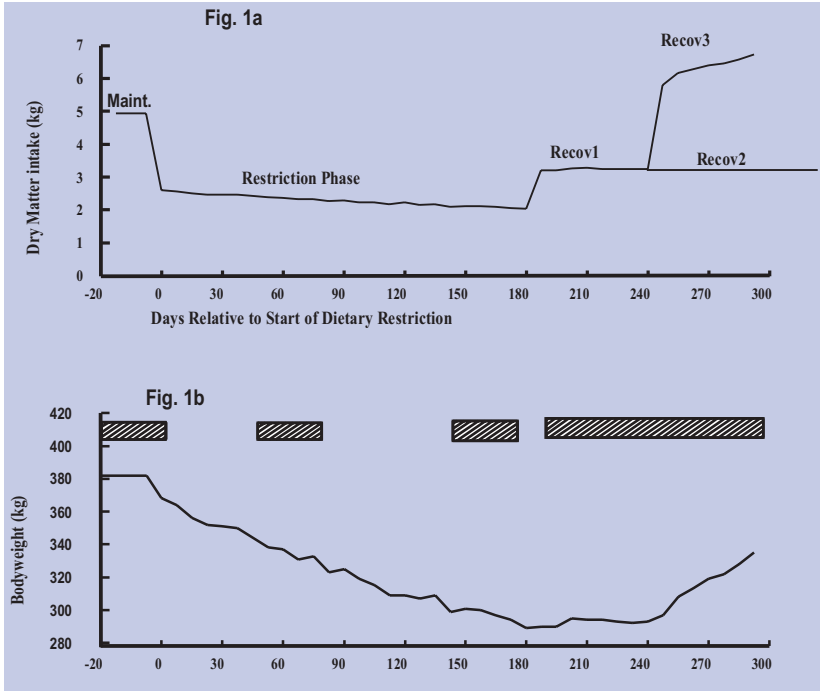


Fig 1a. Schematic of dietary intake (kg DM heifer⁻¹ day⁻¹) during maintenance, restriction and recovery phases. Fig 1b. Associated changes in body weight throughout the study. Periods of daily ultrasound scanning blood sampling for FSH and LH at known stages of dominant follicle growth are shown as

Results

Production changes: During the first 24 weeks of dietary restriction, there was a linear decline in liveweight averaging 3.5 kg/heifer/week. Body condition score (BCS) declined in a linear and quadratic fashion [BCS=2.8-0.04 (week after start of dietary restriction) + 0.000124 (week after start of dietary restriction)²; R² = 0.86; P<0.01]. Heifers became anoestrus at an average of 174 days after start of dietary restriction at which time their average weight was 286 kg, or 76% of their original liveweight, and their average BCS was 2.1 units.

Follicle dynamics: The reduction in dietary intake did not influence oestrous cycle length or the number of follicle waves during the oestrous cycle. However, the measurements recorded after the fourth and seventh cycle (following 60-80 days and 120-140 days of dietary restriction, respectively) indicated a reduction in the growth rate and maximum diameter of the DF (Table 1).

Table 1: Effect of dietary restriction on dominant follicle characteristics

	DIETARY TREATMENT		
	Control Diet	Restricted Diet	
		Cycle 4	Cycle 7
Growth rate (mm/day)	1.13 ^a	1.0 ^b	1.0 ^b
Max. diameter (mm)	11.3 ^a	10.7 ^b	10.4 ^b
Persistence (days)	8.8 ^a	13.3 ^b	13.3 ^b
Cycle length (days)	20.3 ^a	20.0 ^a	20.7 ^a

Within row, treatments with different superscripts are significantly different (P < 0.05).

During the “Recovery Phase” when heifers were still anoestrus, an increase in nutritional supply from 1.0 to 1.8 M resulted in an increase in the growth rate and maximum diameter of successive DFs, until the resumption of ovulation. A typical follicle wave profile is shown in Figure 2.

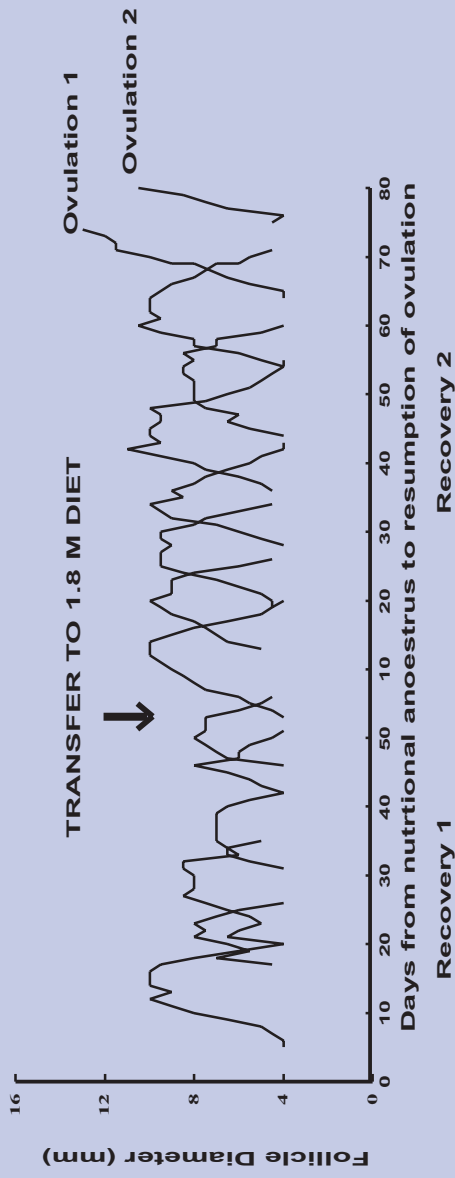


Figure 2: Pattern of dominant follicle growth from day of dietary induced anoestrus (Day = 0) until the second ovulation during the recovery phase.

Reproductive hormone changes: Measurements during the fourth and seventh cycles showed that there was no effect of chronic dietary restriction on LH pulse characteristics or on oestradiol concentrations. However, during anoestrus, the mean systemic concentrations of oestradiol and LH pulse frequency were both reduced compared with concentrations during the time that heifers were cyclic, and increased as the time of recommencement of oestrous cycles approached. There was no effect of chronic nutritional restriction or of re-feeding on the systemic concentration of FSH at any stage of the follicle wave, demonstrating that FSH did not limit ovulation in nutritionally induced anoestrous heifers.

Relationship between days to anoestrus and days to resumption of oestrous cycles: Heifers resumed cyclicity when, on average, they had attained a liveweight of 342 kg (91% of their original liveweight) and a BCS of 2.4 units. There was an inverse relationship ($R^2 = 0.78$) between days to onset of anoestrus (when heifers were restricted to 0.67 M) and days to the resumption of ovulation, when heifers were fed 1.0 M or 1.8 M, (Figure 3). Animals, which took the longer time to become anoestrus, were the first to resume ovulation on recovery, while animals, which took the shorter time to become anoestrus, were slowest to resume ovulation on re-feeding.

Metabolic hormones: There was a linear decrease in the concentration of IGF-I towards the onset of anoestrus (Figure 4a), and a linear increase in the concentration of IGF-I towards the resumption of cyclicity (Figure 4b).

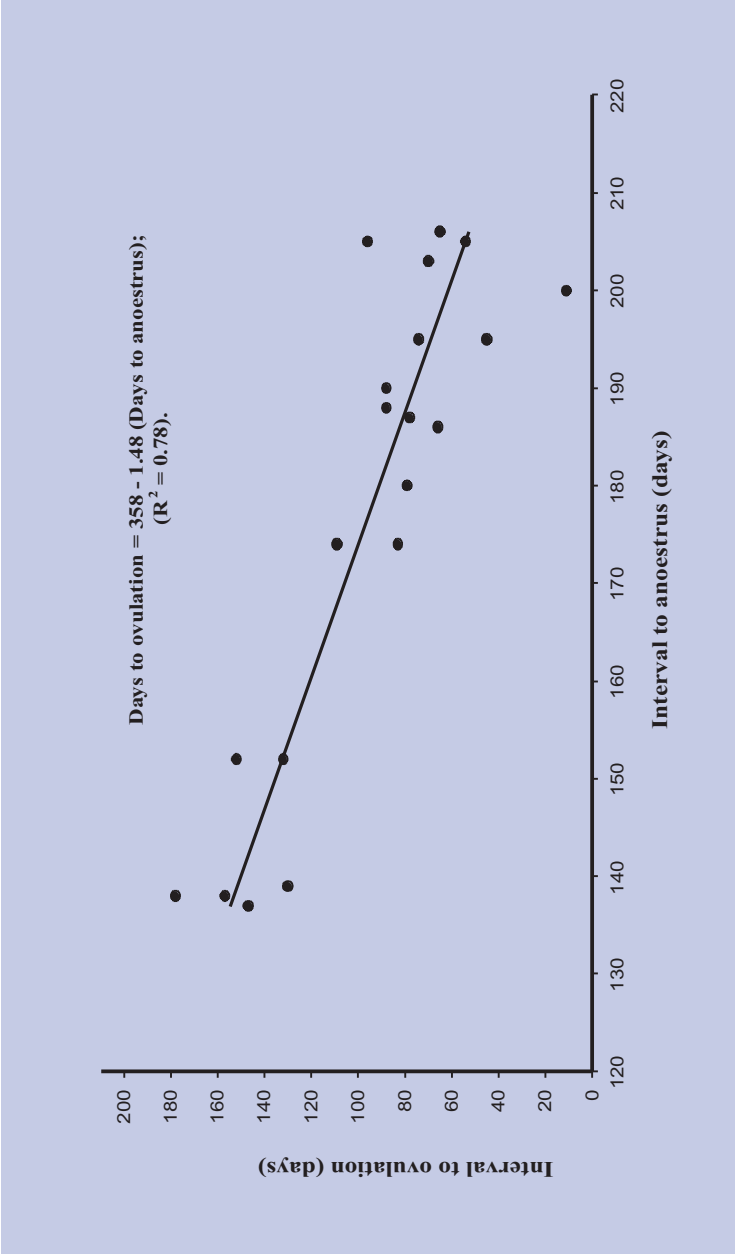


Figure 3: Fitted regression line for beef heifers showing relationship between days on restricted diet to anoestrus and days on recovery of ovulation. Values for individual animals are presented.

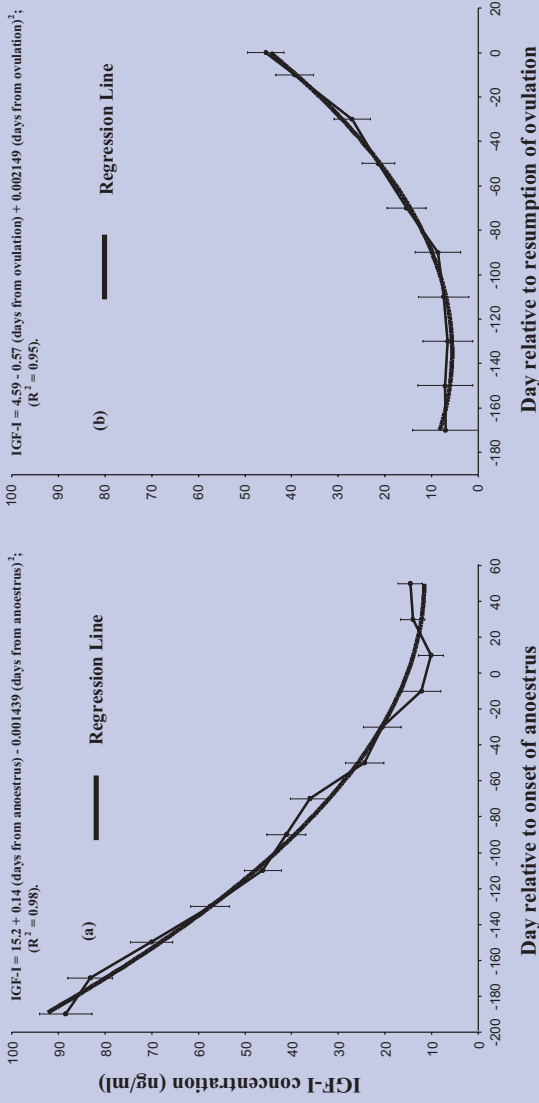


Fig 4a: Mean plasma concentration of IGF-I in beef heifers fed a restricted diet of 0.66 M until they became anoestrus (Day 0), and for a further 60 days when fed a diet of 1.0 M. Fig 4b. Mean plasma IGF-I concentration in nutritionally induced anoestrus beef heifers prior to resumption of oestrus cycles Day 0.

Discussion

Dietary restriction caused a decrease in growth rate and diameter of the DF, but did not change the number of follicle waves during the cycle, or the pattern of FSH secretion relative to follicle wave emergence. There was a linear decrease in peripheral IGF-I concentration as anoestrus approached. Increasing dietary intake, to initiate resumption of ovulation, was associated with an increase in the growth rate and diameter of the DF, but with no change in the pattern of FSH release. The frequency of LH pulses and the systemic concentrations of IGF-I and oestradiol increased as animals approached resumption of oestrous cycles.

At the level of dietary restriction applied here (fed at 67% of maintenance) heifers did not become anoestrus until an average of 25 weeks after commencement of the dietary restriction. Heifers had lost 24% of their initial body weight by this time. Heifers recommenced oestrous cycles after an average 8.4 weeks on the high diet and when they weighed 9% less than their starting weight. The BCS at anoestrus was very consistent, with 82% (19/23) of heifers having a BCS of between 2.0 and 2.25. This indicates a minimum BCS threshold below which nutritional anoestrus is induced. It has been shown in other studies, that dairy and suckler cows in poor BCS at calving have a much longer postpartum anoestrous interval than cows in good BCS. The results of this study would strongly suggest that for cows in poor condition at calving, an increase in weight and/or BCS must occur before they can resume cyclicity. The results of this study might also explain the inconsistency of results following the use of current pharmacological methods to induce oestrus in cattle that are in a low BCS. Cows in a low BCS may be more susceptible to prolonged postpartum anoestrus in association with suckling or lactational demands from milk production.

There was significant variation in the length of time heifers spent on the high diet (1.8 M) until ovulation and recommencement of oestrous cycles began. A strong inverse relationship was recorded between the time period required on the restricted diet to cause cessation of ovulation and the time

period required to cause resumption of ovulation following the re-introduction of high plane feeding. This indicates considerable between-animal variation in response to under-nutrition. This variation between animals may be due to differences in metabolic rate, differences in how individual animals prioritise energy allocation, differences in the sensitivity of the hypothalamic-pituitary-ovarian axis to dietary restriction or to genetic differences.

While dietary-induced anoestrus was characterised by a decrease in systemic concentrations of IGF-I and a decrease in diameter and the growth rate of the DF, nevertheless, dominant follicles continued to grow and regress during anoestrus. Increasing dietary intake caused an increase in diameter of the DF, increased concentrations of IGF-I and increased LH pulse frequency as heifers approached resumption of ovulation. This sequence is very similar to the sequence of endocrine and physiological events prior to resumption of oestrous cycles in postpartum cows and indicates that the anoestrous heifer is an appropriate model to study nutrition-reproduction interactions in cows. Furthermore, this model has the advantage of removing lactation, suckling, maternal-offspring bonding and postpartum interval as confounding factors.

Study 2. The effect of either acute dietary restriction or of dietary over-supply on follicle growth and on endocrine and metabolic hormone concentrations in beef heifers.

Background: Long-term effects of relatively mild negative energy balance (Study 1), in the absence of confounding effects of lactation, show that systemic concentrations of LH are not affected until animals become anoestrus by which time they have lost about a quarter of their initial body weight. This suggests that gonadotrophin secretion in cattle is relatively insensitive to nutritional restriction. While growth of the dominant follicle was clearly suppressed by long-term chronic dietary restriction, it is not clear whether this effect is mediated through ovarian hormones or other local ovarian factors or through direct effects of nutrition on the hypothalamic–pituitary axis. During their productive life, cattle can experience acute changes in nutritional supply both over and more importantly, under-supply such as after the onset of lactation. It is important, therefore, to establish the effects of acute changes in nutritional supply on reproductive function.

Specific objectives

The specific objectives of this study were to (i) establish the effects of acute dietary under- and over-supply on ovarian follicle dynamics and on the systemic concentration of gonadotrophins and steroids and (ii) determine whether the effects of nutrition are steroid or non-steroid mediated.

Materials and Methods

The study was conducted using 20 intact and 18 long-term ovariectomised (ovx) beef heifers with average liveweights of 441 and 446 kg, and body condition scores of 3.2 and 3.3, respectively. Heifers were initially fed a grass silage and concentrate diet (fed 1:1 on a DM basis) supplying the equivalent of 1.2 times maintenance (1.2 M). The use of ovariectomised heifers, which were subjected to the same dietary treatments as the intact heifers, allowed

determination of whether the effects of nutrition were steroid independent or steroid dependent. The experimental design used is summarised in Figure 5. Basically, the oestrous cycles of all the intact heifers were synchronised using an intravaginal Controlled Internal Drug Releasing (CIDR) device and prostaglandin (PG).

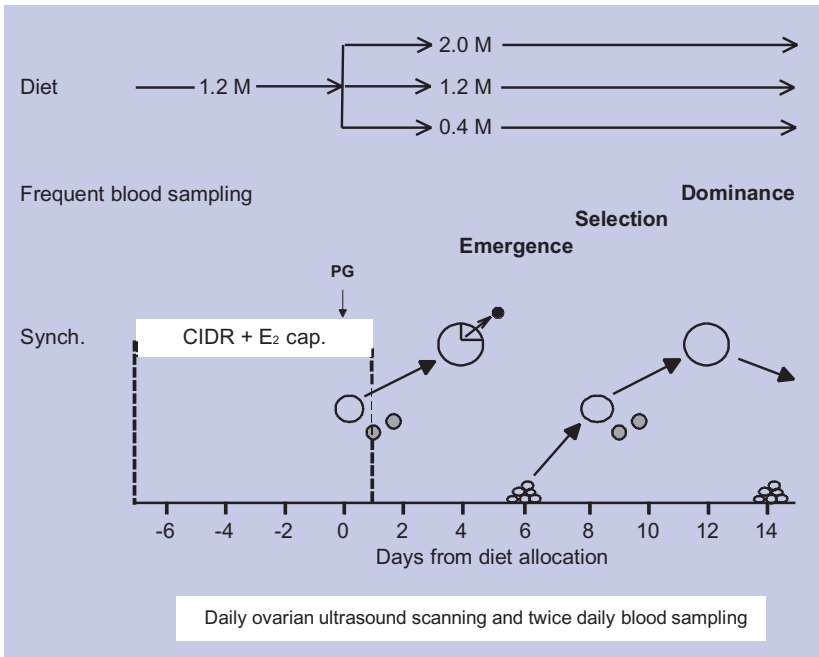


Figure 5: Diagrammatic representation of the oestrous synchronisation and dietary treatments imposed on the intact heifers and their relationship to the time of ovulation and follicle growth. Ovx heifers underwent the same synchronisation and dietary treatments and blood sampling was conducted at similar times to the intact heifers.

One day before CIDR withdrawal, all heifers were allocated randomly, within ovarian status, to diets supplying the requirements for 0.4 , 1.2 or 2.0 M. Prior to diet allocation, ovx heifers were given the same pre-experimental 'synchronisation' treatment as that given to the intact heifers, but without the PG injection.

Daily ovarian ultrasonography was performed on all intact heifers and follicles of ≥ 3 mm diameter were measured and recorded from the time of CIDR insertion until emergence of the second follicle wave after the synchronised ovulation. Blood samples were taken as appropriate and assayed for determination of FSH, oestradiol, progesterone, IGF-I, insulin and non-esterified fatty acids (NEFA). At emergence of the first follicle wave, frequent blood samples were collected for determination of LH pulse characteristics. Heifers were blood-sampled again upon selection of the DF (selection phase), and again three days later during the dominance phase, when the DF approached its maximum diameter. The same blood-sampling regime was simultaneously conducted on ovariectomised heifers, approximately 6, 9 and 12 days after diet allocation for 'emergence', 'selection' and 'dominance' phases, respectively.

Results

Production changes: From diet allocation to emergence of the second post-ovulation follicle wave, just over two weeks later, heifers fed 0.4 M lost an average of 39 kg of liveweight while heifers fed 1.2 M lost 14 kg and heifers fed 2.0 M gained 8 kg. Whether heifers were intact or ovx did not affect liveweight change. Neither diet nor ovarian status affected BCS.

Follicle dynamics: After CIDR withdrawal there was no effect of diet on the maximum diameter of the pre-ovulatory DF. Restriction to 0.4 M caused a decrease in the growth rate, maximum diameter and estimated volume of the first DF of the subsequent oestrous cycle (Table 4).

Table 4: Effect of diets supplying the requirements for 0.4, 1.2, or 2.0 M on follicle wave dynamics in beef heifers.

	Diets		
	0.4 M	1.2 M	2.0 M
a) Synchronised ovulatory follicle			
No. of heifers ovulating	6 / 8	7 / 7	5 / 5
Maximum diameter (mm)	13.9 ^a	14.5 ^a	14.1 ^a
b) First follicle wave post-synchronisation			
Maximum diameter of DF (mm)	10.1 ^a	12.9 ^b	12.7 ^b
Day of maximum diameter	10.1 ^a	10.5 ^a	10.2 ^a
Number of days growing	5.3 ^a	5.5 ^a	5.2 ^a
Growth rate (mm/d)	1.11 ^a	1.60 ^b	1.58 ^b

Within row, treatments with different superscripts are significantly different ($P < 0.05$)

Reproductive hormones: The LH pulse characteristics (mean concentration, pulse frequency and pulse amplitude) of intact heifers varied from the emergence phase through to the dominance phase of the first follicle wave, but did not vary in the ovx heifers sampled simultaneously (Figure 6). However, LH pulse frequency was much greater in ovx than in intact heifers because of the absence of ovarian negative feedback effects.

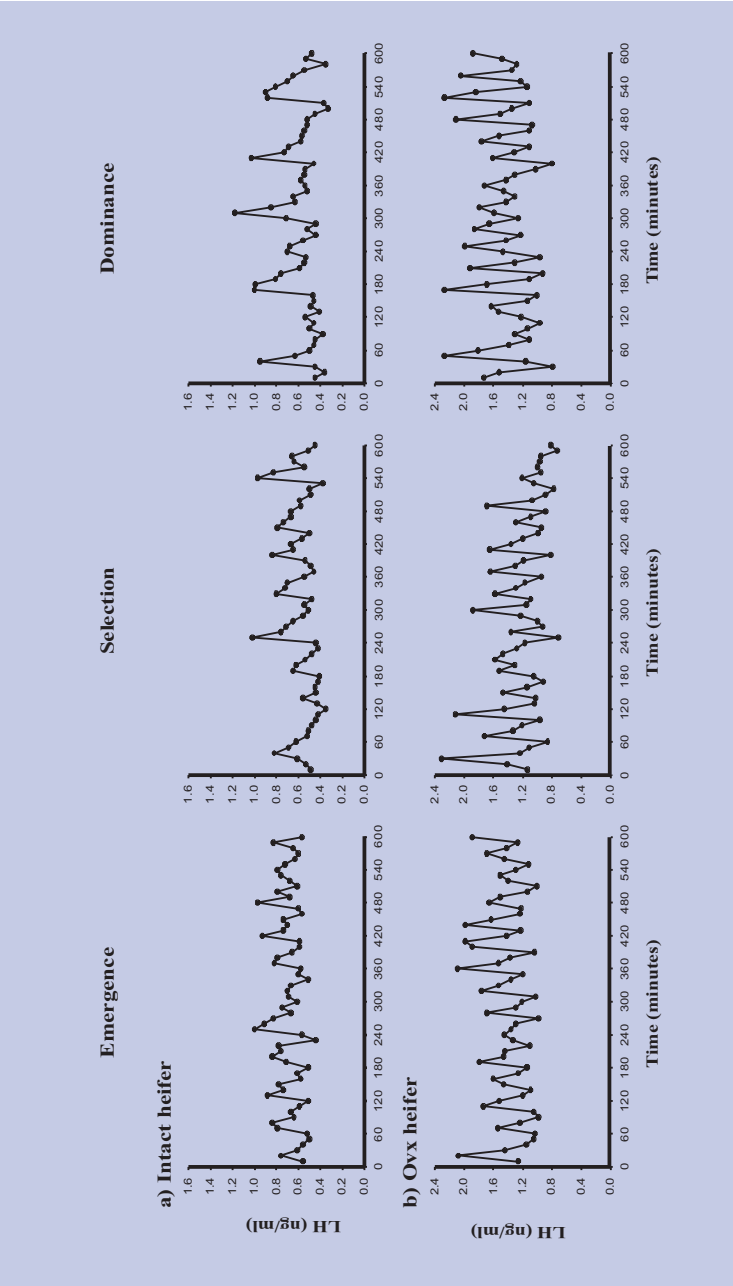


Figure 6: Typical LH profiles of a) an intact heifer at emergence, selection and dominance phases of development of the first follicle wave of the oestrous cycle, and b) an ovx heifer blood sampled at similar times relative to the day of diet allocation.

The LH pulse frequency decreased and the pulse amplitude increased as intact heifers moved into the luteal phase of the oestrous cycle, but there was no effect of diet. The pattern of LH secretion was similar in ovx heifers throughout, but the amplitude of LH pulses was higher in heifers restricted to 0.4 M. In intact heifers, restriction to 0.4 M caused an increase in the magnitude of the FSH increase preceding new follicle wave emergence, compared with heifers fed 1.2 or 2.0 M. The systemic concentration of FSH between successive pre-emergence increases was not affected by diet. In ovx heifers there was a linear increase in the plasma concentration of FSH following restriction to 0.4 M, unlike heifers fed 1.2 or 2.0 M diets 2.0 M where the concentration tended to decline over time (Figure 7).

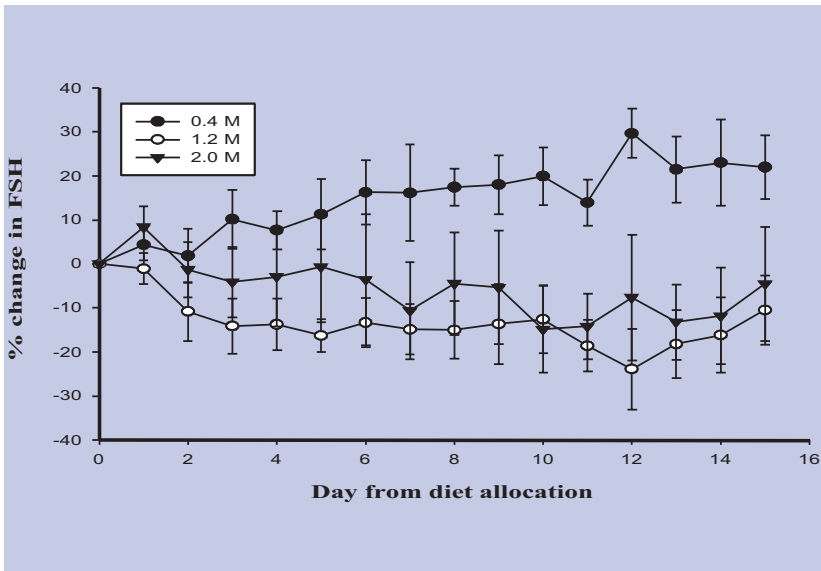


Figure 7: Percentage change in plasma FSH concentration of ovariectomised heifers when randomly reallocated to diets supplying 0.4, 1.2, or 2.0 M from day 0, after previously being fed a diet of 1.2 M.

While the concentration of both oestradiol and progesterone varied according to the day of the oestrous cycle, there was no effect of diet on either hormone.

Metabolic hormones: There was evidence of an interaction between ovarian status (intact vs. ovx) and diet on the plasma concentration of IGF-I. In intact heifers, concentrations of IGF-I increased over the first 4-6 days following diet allocation in association with development of the pre-ovulatory DF but concentrations decreased on ovulation. After 14 days of dietary treatment, concentrations of IGF-I were significantly lower in heifers fed 0.4 M than in those fed 1.2 M. At this time, the concentration of IGF-I in heifers fed 2.0 M were intermediate and were not significantly different from those of heifers fed either 0.4 or 1.2 M. In ovx heifers fed either 0.4 or 2.0 M, concentrations of IGF-I decreased over time while heifers fed 1.2 M had relatively stable IGF-I concentrations.

Neither insulin nor NEFAs were good indicators of metabolic status as the plasma concentrations of both were more closely related to the time of sampling than to the particular diet fed. Concentrations of insulin were significantly greater in post-feed than in pre-feed blood samples but the magnitude of the difference increased with the level of feeding. Concentrations of NEFA in pre-feed blood samples were significantly greater than in post-feed samples but, within a particular sample time, the concentration of NEFA in serum decreased in proportion to the level of energy feeding.

Discussion

Acute dietary restriction of cyclic heifers from 1.2 to 0.4 M caused a decrease in the growth rate and maximum diameter of the DF in the first wave. This effect occurred without any related dietary effect on any LH characteristic measured. The lack of a dietary effect on LH pulse frequency suggests that GnRH pulse frequency was unaffected. Plasma concentrations

of oestradiol were also unaffected and this is consistent with the lack of a dietary effect on LH, the key hormone involved in the regulation of oestradiol production in the follicle. Dietary restriction was associated with an increase in the magnitude of the FSH rise that precedes new follicle wave emergence but, it is unlikely that such an increase would have affected the subsequent growth rate of the DF during a period of low systemic FSH. Thus, the effects of acute nutritional restriction on follicle development are suggestive of local growth factor effects on DF growth, possibly mediated through IGF-I and its binding proteins within the follicle. It is also possible, however, that the effect of acute nutritional restriction on follicle growth may be related to the decline in the systemic concentration of IGF-I, which began approximately four days after diet allocation. The failure of a number of heifers, restricted to 0.4 M, to ovulate suggests that acute nutritional deprivation might affect ovulation of the pre-ovulatory follicle formed from the DF present at the start of dietary restriction. Thus while heifers are sensitive to acute nutritional restriction, the mechanism appears to operate at the ovarian rather than at the hypothalamic-pituitary level in contrast to other species.

The feeding of different amounts of the same diet to provide a broad range of nutritional supply had been anticipated to cause changes in plasma insulin and NEFA concentrations. However, there were significant diet x day x sample time interactions in respect of these parameters, so that neither insulin nor NEFA proved to be useful indicators of metabolic status due to effect of sampling time rather than diet on their systemic concentrations. A declining concentration of IGF-I was a more useful indicator of low nutritional status than insulin, but the effects of diet on the concentration of IGF-I were confounded by complex interactions involving both ovarian status and day relative to diet allocation. This seriously limit the effectiveness of either IGF-I or indeed NEFAs as good indicators of acute nutritional deficiency in cattle.

Study 3. The effects of acute dietary restriction on the incidence of anovulation and on periovulatory oestradiol and gonadotrophin concentrations in beef heifers.

Background: Chronic negative energy balance (NEB) causes a linear decrease in the maximum diameter of successive DFs, eventually resulting in anoestrus due to suppressed LH pulse frequency in the final oestrous cycle before anovulation (Study 1). Acute periods of more severe dietary restriction also cause a decrease in the growth rate and maximum diameter of DFs, but the decrease in growth rate is not related to changes in LH (Study 2). In the latter study, a number of heifers failed to ovulate following acute nutritional restriction but, due to limited numbers, it was not established whether failure of ovulation was related to the nutritional restriction. The effects of acute nutritional restriction on periovulatory oestradiol and gonadotrophin concentrations or, on the ability of the DF to ovulate, are not well established. Detrimental effects of dietary restriction on reproduction may not only induce anovulation in some animals, but it may also compromise fertility in animals that do express oestrus and ovulate. Pregnancy rate to first service in Holstein cows has declined over the past 30 years in both the U.S. and in Europe and this decline has been partly attributed to severe negative energy balance (NEB) in the early postpartum period, especially in high genetic merit cows where the feed requirements for milk production are greater than the dry matter intake capacity of the cow. Severe NEB in the early postpartum period may affect follicular development, oocyte competence and ultimately, pregnancy rate.

Specific objective

The specific objectives of this study were to determine whether acute dietary restriction, from 1.2 times maintenance to 0.4 M, in beef heifers would affect (i) the ability of dominant follicles to ovulate, (ii) the endocrine mechanism(s) responsible for anovulation and (iii) pregnancy rate following ovulation of growth restricted follicles.

Materials and Methods

The study was conducted using 41 cyclic beef heifers with an average liveweight of 406 kg and body condition score of 2.9. Heifers were initially fed a grass silage and concentrate diet (fed 1:1 on a dry matter basis) supplying the equivalent of 1.2 times maintenance (1.2 M). The experimental design, based largely on that of Study 2, is summarised in Figure 8.

The oestrous cycles of all heifers were synchronised using a CIDR device and prostaglandin. One day before CIDR withdrawal, heifers were allocated randomly to diets supplying the requirements for 0.4 (n = 20) or 1.2 M (n = 21). Six days after ovulation of the DF present at CIDR removal (synchronised DF), luteolysis was induced using PG to allow ovulation of the first DF of the subsequent oestrous cycle (first DF). All heifers observed in oestrus were inseminated. Pregnancy diagnosis was carried out by ultrasonography at 30 days post-insemination and confirmed 20 days later. Ovarian ultrasonography and blood sampling was conducted at appropriate intervals throughout. The time of ovulation and new follicle wave emergence was determined. During each periovulatory period, 19 heifers (0.4 M, n = 9; 1.2 M, n = 10) were blood-sampled at 4-hourly intervals until ovulation or regression of the DF to detect the pro-oestrous oestradiol increase, gonadotrophin surge and periovulatory pre-emergence FSH increase.

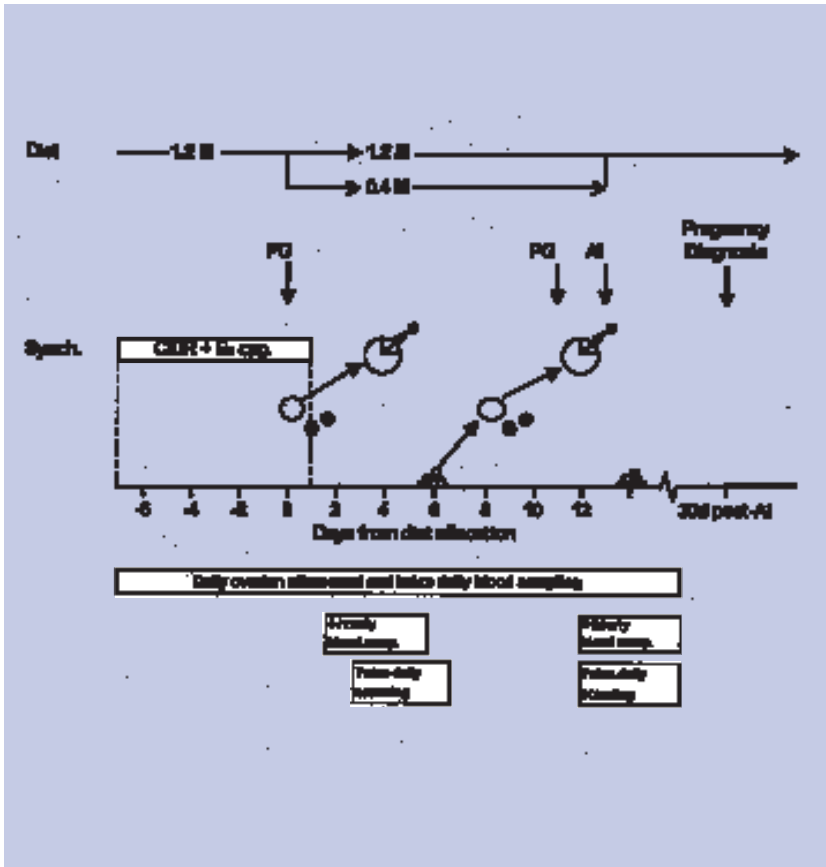


Figure 8. Diagrammatic representation of the oestrous synchronisation and dietary treatments imposed on heifers and their relation to ovulation of the DF present at progesterone withdrawal (synchronised DF) and ovulation of the first DF of the subsequent oestrous cycle (first DF). Ovarian scanning and blood sampling was performed during each periovulatory period as shown in boxes above

Results

Production changes: Heifers fed the restricted diet (0.4 M diet) had lost more liveweight (-25.6 kg) than heifers on the 1.2 M diet (-2.6 kg) after two weeks but, there was no change in BCS in either group.

Incidence of anovulation: The average size of the synchronised DF was similar in all heifers at the start but the maximum diameter attained was reduced in heifers fed 0.4 M (Table 6). Two of the heifers fed 0.4 M failed to ovulate this smaller DF which regressed allowing new follicle wave emergence to occur. The growth rate and maximum diameter of the first DF to develop after the synchronised DF were significantly reduced in heifers fed 0.4 M compared with those fed 1.2 M (Table 5). Following administration of PG, the first DF failed to ovulate in 10 of 18 heifers fed 0.4 M, while all 20 heifers fed 1.2 M ovulated the first DF.

Reproductive hormones: All heifers blood-sampled had a pro-oestrus increase in plasma oestradiol concentration associated with development of the synchronised DF following CIDR withdrawal, the magnitude of which was unaffected by diet. Subsequently, all heifers fed 1.2 M had a coincident LH and FSH surge and the DF ovulated, while only 7 / 9 heifers fed 0.4 M had coincident LH and FSH surges and ovulated. The heifers that failed to ovulate had no further increase in oestradiol concentrations or concomitant pre-ovulatory type LH and FSH surges. However, they had an increase in the plasma concentration of FSH preceding emergence of the subsequent wave emergence (Figure 9c). Following first ovulation, administration of PG seven days later caused luteolysis in all heifers on both dietary treatments. However, 45% of the heifers fed a 0.4 M diet had no pro-oestrus increase in oestradiol concentration nor a gonadotrophin surge (Figure 9b). Where ovulation did occur, diet had no effect on the magnitude of the pro-oestrous increase in concentration of oestradiol. Plasma concentrations of FSH, during the pre-emergence increase, tended to be higher in heifers fed 0.4 M than those fed 1.2 M but this was not significant. Oestrus was detected in 7/18 (39%) of heifers fed 0.4 M and in 20/21 (95%) of heifers fed 1.2 M following administration of PG on the sixth day after ovulation of the synchronised DF.

Pregnancy rates: All heifers detected in oestrus were inseminated. Ten heifers fed 0.4 M diet did not exhibit oestrus activity and were not inseminated. Subsequently all 10 heifer were confirmed anoestrus. The pregnancy rate at 30 days post-insemination, defined as the number of heifers pregnant/number of heifers inseminated, was 57% and 55% in heifers fed 0.4 M and 1.2 M, respectively.

Table 5. The effect of diets supplying 0.4 M or 1.2 M on follicle wave dynamics in beef heifers.

	Nutrition Level		
	0.4 M	1.2 M	P
No. of heifers	20	21	
<u>Synchronised DF</u>			
Maximum diameter attained (mm)	11.7	12.6	**
No. anovulatory heifers	2 / 20	0 / 21	NS
<u>First new follicular wave:</u>			
Growth rate of DF (mm/d)	0.96	1.28	**
Maximum diameter of DF (mm)	10.6	13.8	***
No. anovulatory heifers	10 / 18	0 / 21	***
Total no. anoestrous heifers	20	12 / 20	**

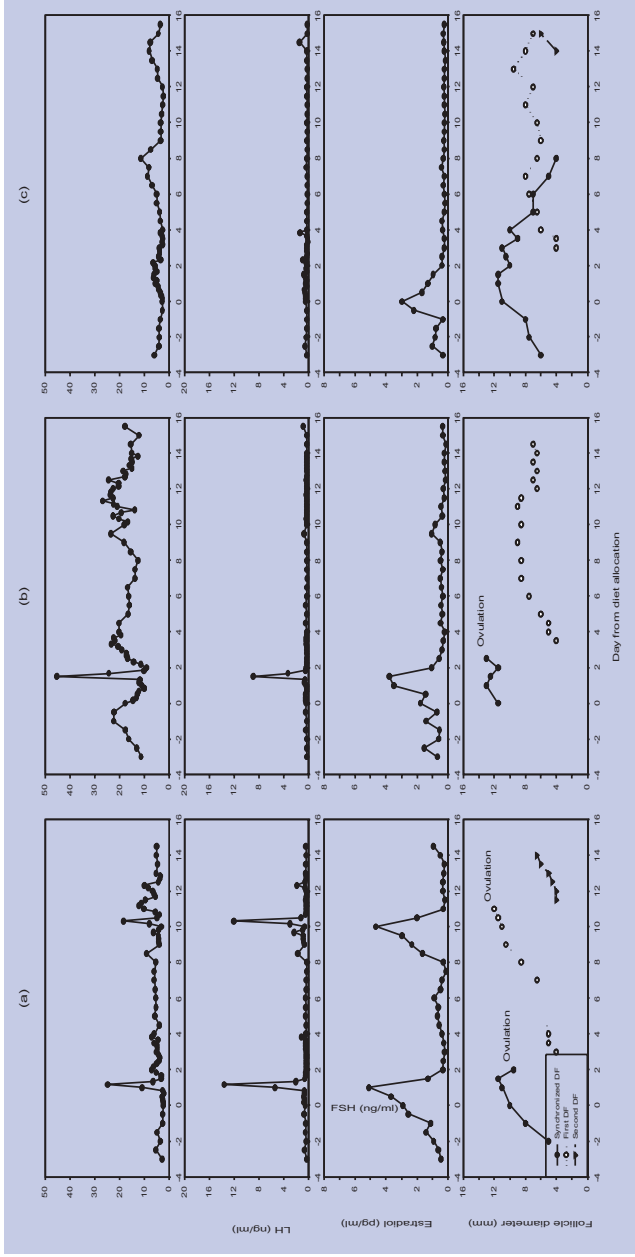


Figure 9. Typical hormone and follicle profiles from heifers fed the 0.4 diet, but which (a) remained cyclic throughout, (b), became anoestrus at the first DF (c), or became anoestrus at the synchronised DF.

Discussion

Acute dietary restriction from 1.2 to 0.4 M for a period of two weeks resulted in failure of the DF to ovulate in 60% of heifers. Failure of ovulation was always associated with the absence of a preovulatory LH surge. However, the absence of an LH surge was not always associated with the absence of a pro-oestrus increase in the plasma concentration of oestradiol. Where ovulation did occur following approximately 14 days of acute restriction, pregnancy rate was similar to that of heifers fed 1.2 M throughout.

In the heifers, which failed to ovulate the DF present at CIDR withdrawal while there was a pro-oestrus increase in the plasma concentration of oestradiol, there was no LH surge. Failure of ovulation of the DF present at CIDR withdrawal in a proportion of the heifers is consistent with the previous study (Study 2) where the DF present at CIDR withdrawal failed to ovulate in 25% of heifers. The presence of a pro-oestrus increase in the concentration of oestradiol appears to depend on whether anovulation occurs after 4 to 5 days or after a longer period (13 to 15 days) of restriction to 0.4 M. This suggests that the mechanism(s) leading to anovulation may depend on the duration of acute nutritional restriction.

In the current study, where the first DF of the next oestrous cycle failed to ovulate, the absence of a pro-oestrus increase in the concentration of oestradiol suggests that there were insufficient LH pulses to stimulate oestradiol synthesis and secretion. Low systemic concentrations of oestradiol would be incapable of inducing a preovulatory LH surge, which in turn would prevent ovulation occurring. While the absence of an LH surge resulted in ovulation failure, dietary effects on follicle growth rate were evident much earlier. Dietary restriction of energy decreased the growth rate and maximum diameter of DFs in both chronically (Study 1) and acutely restricted heifers (Study 2). In the present study, acute restriction from 1.2 to 0.4 M caused an immediate suppression of the maximum diameter attained by the DF present on withdrawal of the CIDR device.

Due to the high incidence (60%) of ovulation failure among heifers fed 0.4 M, the effect of acute nutritional restriction on pregnancy rate could not be adequately established. However, the initial indications are that neither oocyte competence nor ability to establish a pregnancy were affected by acute nutritional restriction in those heifers that exhibited oestrous activity and ovulated.

In summary, this experiment confirmed that acute dietary restriction suppresses the growth rate and maximum diameter of dominant follicles, resulting in the failure of dominant follicles to ovulate in 60% of heifers. We suggest that failure of ovulation is due to the absence of an LH/FSH surge, which in some cases is preceded by the absence of a pro-oestrus increase in the concentration of oestradiol. Pregnancy rate in heifers that did ovulate was not compromised by short-term acute dietary restriction.

Conclusions and Implications

The studies carried out in this project show that while moderate dietary restriction, equivalent to about 0.7 M, does not immediately affect oestrous cyclicity, animals do become anoestrous after about 6 months of restriction, when they reach a body condition score (BCS) of 2.1. All animals became anoestrous at this BCS suggesting that this may be the threshold below which cattle become nutritionally anoestrous. Furthermore, once nutritional anoestrus becomes established, resumption of normal oestrous cycles does not immediately occur for some time after increasing dietary intake. Heifers in nutritional anoestrus required almost 60 days on a high plane of nutrition and a gain of about of a half a unit of BCS before oestrous cycles resumed. This may explain the inconsistent results arising from application of current pharmacological methods to induce oestrus in cattle in low BCS. Clearly, cattle should not be allowed to decline in body condition score below 2.25 in order to avoid the possibility of nutritional anoestrus becoming established, which clearly is extremely difficult to reverse.

A strong inverse relationship was recorded between the time period required on the restricted diet to cause cessation of ovulation and the time period required to cause resumption of ovulation following the re-introduction of high plane feeding. This indicates considerable between animal variation in response to under-nutrition. The variation may be due to different metabolic rates and lower maintenance requirements, differences between animals in priorities for energy allocation or differences in the sensitivity of hypothalamic-pituitary-ovarian axis to dietary restriction or to genetic differences. This warrants further study, particularly in relation to genetic effects which may be exploitable. This study also shows that LH and the IGF-I system are the key reproductive hormone and metabolic regulators, respectively, involved in nutritional regulation of oestrous cycles in cattle.

It is clear from Studies 2 and 3 that more acute nutritional restriction results in immediate detrimental effects on follicle growth and on gonadotrophin and oestradiol secretion, resulting in 60% of the heifers becoming anoestrous after two weeks of dietary restriction. This is the first study to demonstrate and quantify such an acute nutritional effect in cattle. This again emphasizes the importance of maintaining high dry matter intakes in animals, particularly high yielding dairy cows in early lactation in order to maintain good subsequent reproductive performance. Because the effects were not consistent in all animals it indicates possible genetic differences between animals in their response to dietary restriction.

Publications arising from this Project

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3. **Stagg, K., Diskin, M.G., Roche, J.F. and Sreenan, J.M.** 1995. Association between FSH concentrations and follicle growth during normal oestrous cycles and nutritional anoestrous in heifers. Journal of Reproduction and Fertility Abstract Series No. 15: 66 (Abstr.)
4. **Stagg, K., Diskin, M.G., Roche, J.F. and Sreenan, J.M.** 2000. Endocrine and follicular dynamics during energy induced cessation and resumption of oestrous cycles in beef heifers. (Prepared for submission to Journal of Animal Science)
5. **Mackey, D.R.** (1999) The effect of acute nutritional restriction on follicular growth and gonadotrophin secretion in cattle. Ph.D. Thesis, National University of Ireland, Dublin.
6. **Mackey, D.R., Sreenan, J.M., Roche, J.F. and Diskin, M.G.** 1997. The effect of acute changes in energy intake on follicle wave turnover in beef heifers. Irish Journal of Agricultural and Food Research 36: 95-96 (Abstr.).

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