

**ATHENRY RESEARCH REPORT**

**Effect of Holstein-Friesian cow genotype on hepatic expression of genes of the GH-IGF axis during early and mid-lactation**

It has been shown that the New Zealand Holstein Friesian (NZHF) has lower milk production but superior fertility compared to the North American Holstein Friesian (NAHF) strain in a pasture-based milk production system. The energy demands of early lactation exceed energy intake resulting in a 5-10 week period of negative energy balance (NEB). During positive energy balance, pulsatile release of growth hormone (GH) from the pituitary gland stimulates expression of insulin-like growth factor (IGF-I) in hepatocytes, resulting in increased hepatic IGF-I mRNA abundance and increased circulating concentrations of IGF-I. During NEB, however, the GH-IGF axis becomes uncoupled and circulating concentrations of IGF-I decline precipitously. It is hypothesised that differences in fertility between these two strains of Holstein Friesian may be due to greater negative NEB during early lactation, and a longer period of preferential partitioning of nutrients to milk production for the NAHF strain. These changes are likely to impact on components of the somatotrophic axis. The objective of this study was to examine the effect of cow genotype on the expression of genes in the GH-IGF axis during early and mid-lactation.

Ten mature NAHF cows and 10 mature NZHF cows were selected as representative of their respective strains from within the Teagasc, Moorepark Strain Comparison study. Liver tissue was collected from all cows at 35 and 150 days post-partum by puncture biopsy, snap frozen in liquid nitrogen, and stored at -80 °C. Cows were offered *ad libitum* grass silage plus 8 kg of concentrate per day at the time of the first biopsy and *ad libitum* zero grazed pasture plus 4 kg of concentrate per day at the time of the second biopsy. Total RNA was extracted using the TRIzol reagent and purified using the RNeasy minikit (Qiagen). Real-Time RT-PCR analysis was performed on genes involved in the IGF system using the ABI 7500 Fast Real-Time PCR System with the SYBR Green Master Mix (Applied Biosystems, Warrington UK). All samples were assayed in the same run, thereby, eliminating inter-assay variation, and the specificity of the reaction products was confirmed by melt curve analysis and gel electrophoresis.

**Table 71: Real Time RT-PCR analysis of genes involved in the synthesis and stability of IGF-1. Values are back-transformed least square means followed by the 95% confidence limits and are expressed as fg per µg of reversed transcribed RNA**

Gene	STRAIN		Fold change	P value	DAYS POSTPARTUM			
	NAHF	NZHF			35	150	Fold change	P value
IGF-1	0.32 (.23-.44)	0.51 (.37-.72)	1.6	< 0.05	0.32 (.22-.47)	0.51 (.34-.76)	1.6	0.15
GHR (tot)	0.51 (.37-.71)	0.47 (.33-.66)	1.1	0.71	0.6 (.42-.88)	0.4 (.27-.58)	1.5	0.15
GHR1A	0.03 (.02-.04)	0.03 (.02-.04)	-	0.65	0.04 (.03-.05)	0.02 (.02-.03)	2.0	< 0.05
IGFBP1	4.3 (2.8-6.8)	4.3 (2.7-6.8)	-	0.98	7.7 (4.9-12.3)	2.4 (1.5-3.8)	3.2	< 0.01
IGFBP2	3.4 (2.6-4.4)	3.1 (2.3-4.1)	1.1	0.62	5.5 (4.0-7.5)	1.9 (1.4-2.6)	2.9	< 0.001
IGFBPALS	0.06 (.04-.09)	0.1 (.07-.14)	1.7	0.06	0.06 (.04-.09)	0.1 (.07-.16)	1.7	0.11

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PCR products were sequenced to confirm their identity. Real-Time RT-PCR data were log transformed for normalisation of variances and were analysed using the MIXED procedure of SAS (SAS, 2003), with terms for day and strain and their interaction included in the model. Expression of the exogenous control, the kanamycin resistance gene was used as a covariate in the analysis.

Mean systemic concentrations of IGF-1 were higher in NZHF than NAHF during the post-transition period (97.5 vs 77.5 ng/ml;  $P < 0.05$ ). The results of the real-time RT-PCR analysis are summarised in Table 71. None of the genes analysed demonstrated a significant genotype by stage of lactation interaction (data not shown). IGF-1 was the only gene that was affected by cow genotype; mRNA abundance was 1.6 times greater in the NZHF breed ( $P < 0.05$ ). The abundance of acid labile subunit (ALS) mRNA tended ( $P = 0.06$ ) to be greater in NZ cows compared to NAHF cows. Across genotypes, mRNA abundance of IGFBP-1, IGFBP-2 and GHR1A decreased from day 35 to 150, whereas increased IGF-I and ALS mRNA were observed during this period.

It is concluded that the decrease in IGFBP-1 and IGFBP-2 mRNA abundance from days 35 to 150 is consistent with improving energy balance status. Consistent with greater IGF-I mRNA abundance, the NZHF strain also exhibited greater circulating IGF-I concentrations, and increased abundance of ALS (critical to IGF-1 stability), which may play a role in achieving superior reproductive performance. Holstein-Friesian cow genotype and stage of lactation have significant effects on the GH-IGF axis.

RMIS No. 5234

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### **The effects of negative energy balance on splenic gene expression in the *post-partum* dairy cow: consequences for immune function**

Negative energy balance (NEB) is a severe metabolic disease affecting high yielding dairy cows in the *post-partum* period. Increased mobilisation of body fats, to make up the energy deficit, cause by increased milk production results in an increased systemic concentrations of metabolites such as non-esterified fatty acids (NEFA), beta hydroxyl butyrate (BHB), the onset of oxidative stress and disruption of normal metabolism and physiology. The immune system is also depressed in early lactation and dairy cows are, therefore, more vulnerable to bacterial infections causing mastitis or endometritis at this time. The objective of this study was to determine the effects of NEB on immune function in the *post-partum* cow using a model of severe NEB (SNEB).

**Table 72: Gene classification according to canonical signalling pathways using IPA. (The %DEG is the proportion of DEG relative to the total no of genes in the specific canonical pathway. Gene names highlighted in bold are upregulated while those in normal font are down regulated)**

Pathway	p-value	%DEG	Genes
NRF2-mediated Oxidative Stress Response	0.0004	9.4	<b>TXN</b> , <b>AKR1A1</b> , <b>ATF4</b> , <b>HSPB8</b> , <b>SOD1</b> , <b>FTH1</b> , <b>DNAJC3</b> , <b>PRDX1</b> , <b>GSTP1</b> , <b>CBR1</b> , <b>AOX1</b> , <b>GSTK1</b> , <b>FTL</b> , <b>JUN</b> , <b>CAT</b> , <b>NQO1</b> , <b>CCT7</b>
Mitochondrial Dysfunction	0.0028	9.7	<b>NDUFS7</b> , <b>NDUFB7</b> , <b>PRDX5</b> , <b>APH1A</b> , <b>NDUFA6</b> , <b>UQCRC2</b> , <b>PARK7</b> , <b>SDHB</b> , <b>NDUFS4</b> , <b>NDUFB5</b> , <b>CAT</b> , <b>NDUFV1</b> , <b>UCP2</b> , <b>PRDX3</b> , <b>COX6A1</b> , <b>NDUFA2</b>
Endoplasmic Reticulum Stress Pathway	0.0251	16.7	<b>ATF4</b> , <b>DNAJC3</b> , <b>XBPI</b>
Natural Killer Cell Signalling	0.0263	6.4	<b>TYROBP</b> , <b>SH2D1A</b> , <b>LCP2</b> , <b>FYN</b> , <b>KIR3DL1</b> , <b>NCR3</b> , <b>FCGR3A</b>

Spleen tissue was removed *post-mortem* from five SNEB and five medium NEB (MNEB) cows 15 days *post-partum*. A bovine Affymetrix oligonucleotide array and bioinformatic analysis was used to determine differential gene expression, and Ingenuity Pathway Analysis (IPA) was used to explore significant gene networks. SNEB balance resulted in increased systemic concentrations of NEFA (P<0.001) and BHB (P<0.001) and in a reduction in circulating lymphocyte numbers (P<0.05). These effects were in turn associated with pleiotropic effects on splenic gene expression including signalling pathways and networks associated with oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress and natural killer cells (Table 72). SNEB was also associated with down-regulation of genes encoding IL-15, BCL-2 and IFN $\gamma$ ; up-regulation of gene encoding BAX and CHOP, favouring apoptosis with a potential negative impact on immune function.

RMIS No. 5234

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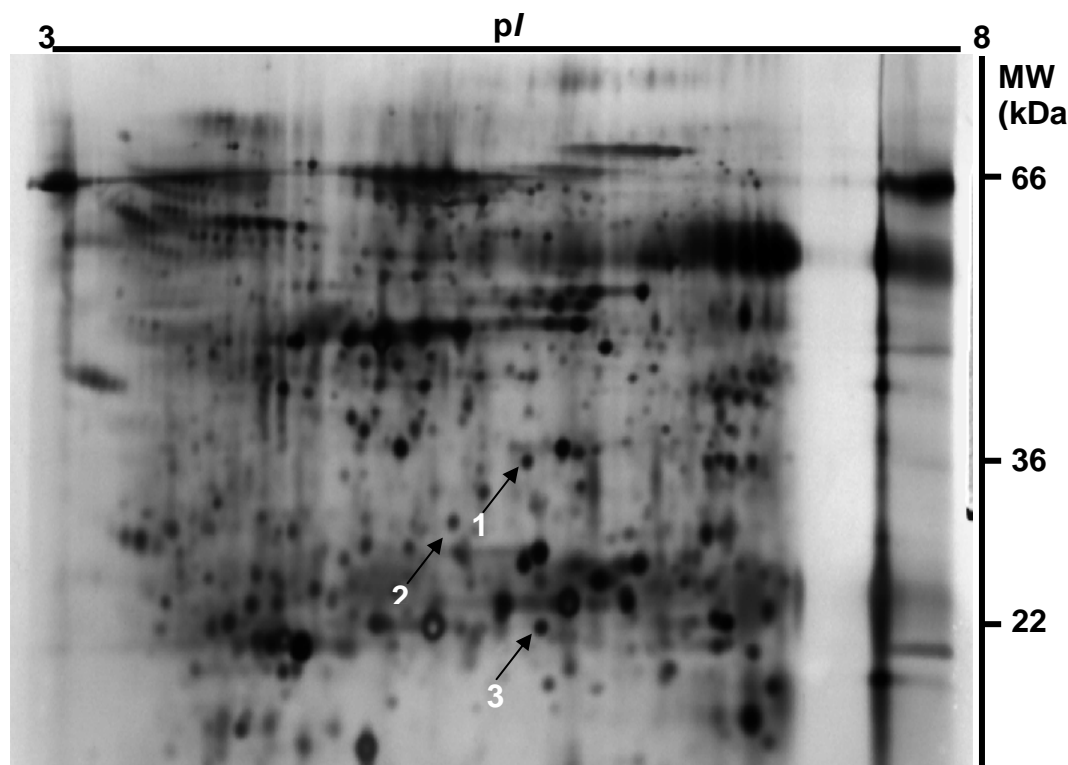
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### Effect of stage of cycle on the bovine uterine proteome

From the arrival of the bovine embryo into the uterus until implantation, the embryo is free floating surrounded by uterine fluid. The embryo is critically dependent on the uterine fluid for its normal growth and development, however, there is limited information on the protein composition of the fluid or how it is affected by stage of cycle. This study examined the intrauterine protein changes that occur between metestrus (Day 3) and late dioestrus (Day 15) in the bovine uterus using 2D electrophoresis. Spontaneously cycling, lactating Holstein-Friesian dairy cows at least 50 days *post-partum* were used. Uterine flushings (UF) were collected non-surgically from the uterine horns, ipsilateral and contralateral to the corpus luteum (CL) on Days 3 and 15 of the oestrous cycle. UF were rehydrated on a 24 cm 3-10 pH non-linear Immobiline DryStrip gel and isoelectric focused for 90kVh. The second dimension separation was carried out on a 12% SDS-PAGE gel (230x190x1.5mm). Separated proteins were detected using silver staining and imaged at 180nm using a CCD system. Image analysis and statistical analysis was carried out using Same Spots software (Nonlinear Dynamics, UK). The differentially expressed proteins were excised from a preparative gel stained with Colloidal Coomassie Blue. The excised proteins underwent LC/MS on LTQ (Thermo-Finnegan) and Sequest database search for identification. Three proteins were found to be upregulated on Day 15 compared to Day 3 and were identified as 1) aldose reductase ( $P<0.0001$ ), 2) plakoglobin ( $P<0.0001$ ) and 3) heat shock protein 27 ( $P<0.01$ ) (Figure 54 and Table 73).



**Figure 54. 2-DE map of proteins from uterine flushings on Day 15 of the oestrous cycle (n=12).**

Table 73: Proteins differentially expressed in uterine fluid between days 3 and 15 of the oestrous cycle						
Spot	Protein Identity <sup>a</sup>	Accession No. <sup>b</sup>	M <sub>r</sub> (kDa)	pI	Fold upregulated <sup>c</sup>	q-value <sup>d</sup>
1	Aldose reductase	P16116	35919.23	5.76	10	9.89E <sup>-11</sup>
2	Junction plakoglobin	Q8SPJ1	81820.84	5.75	2.3	1.19E <sup>-05</sup>
3	Heat shock protein beta 27	Q3T149	22393.06	5.98	1.5	0.0045

<sup>a</sup> Protein identity from Sequest search; <sup>b</sup> SWISS-PROT accession number; <sup>c</sup> Fold higher on Day 15 compared to Day 3; <sup>d</sup> Analysis by Same Spots (Non-Linear, UK).

Aldose reductase is an enzyme directly involved in the production of sorbitol and indirectly of fructose. Plakoglobin is a component of cellular junctions and its up-regulation may have a role in the function of the uterine glandular epithelium. Heat shock protein 27 (Hps27) may respond to potential stresses in the uterus or act as a molecular chaperone. Furthermore, Hsp27 was 1.4-fold higher (P<0.01) in the ipsilateral compared to the contralateral uterine horn on day 15 indicating a local modulating effect on the uterine proteome. Overall, while a number of proteins were found to be upregulated on Day 15 compared to Day 3, the exact nature of their function or regulation is not readily explained. However, it is likely that progesterone may play a role. This study indicates that the uterine environment of the cow is dynamic and that its composition is not only affected by stage of cycle but also by local environmental factors.

RMIS No. 5236

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**The association between metabolic hormones and metabolites during the early *post-partum* period and 1<sup>st</sup> service conception rate in dairy cows**

The poor reproductive performance experienced by high yielding dairy cows is, in part, due to their inability to meet the metabolic demands of lactation during the early *post-partum* period. This has contributed to a significant decline in fertility over the past 30 years. Recent studies have found some evidence of an association between the concentrations of metabolic hormones and metabolites during the *post-partum* period suggesting that they may have a concurrent and latent positive relationship with reproductive performance in dairy cows. The objective of the study was to determine the relationship between blood concentrations of insulin, IGF-I, β-hydroxybutyrate (BHB), glucose, non-esterified fatty acids (NEFA) and urea during the immediate *post-partum* period and subsequent conception rate in dairy cows.

For the study a total of 371 spring calving dairy cows in seven co-operating herds were used. Cows were blood sampled once weekly over a 4 week period (Week 1 to 4) with sampling commencing within 8 days of calving. Blood samples were collected *via* the coccygeal vein into lithium heparin vacutainers, initially placed in iced water, centrifuged at 2500 g for 15 minutes at 4°C, plasma extracted and stored at – 20°C until assay. Body condition score (on a scale of 1 (thin) to 5 (over fat) in increments of 0.25) was assessed on all cows during Week 1. Insulin was analysed using a solid phase time resolved fluoroimmunoassay. Glucose, NEFA, BHB and urea concentrations were analysed using appropriate kits and an ABX Mira auto analyser. Total plasma IGF-1 was measured by a non extracted two site

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immunodiometric assay. To avoid any confounding affects animals that experienced a difficult calving, severe lameness or other diseases were removed from the study. Cows were milked sampled once monthly (data obtained from the ICBF). Cows were inseminated according to individual farm policy and all cows were scanned for pregnancy at 30-50 days post AI. The relationships between the binary variable conception rate and continuous variables IGF1, insulin, glucose NEFA, BHB and urea, were evaluated using logistic regression SAS (2003) with terms for herd, parity, herd x variable included in each model; Herd x variable was not significant ( $P>0.50$ ) in all the analyses and consequently was removed from the final logistic model. The repeatability of metabolic hormone and metabolite variables across weeks was calculated as the ratio of the within group variance to the sum of the within and between group variances.

Repeatability estimates for metabolic hormones and metabolites are presented in Table 74. Repeatability is measured on a scale from 0 to 1 and indicates the usefulness of successive records in a particular trait, it also sets the uppermost limit for heritability.

**Table 74: Repeatability estimates for metabolic hormones and metabolites**

Variable	No. cows	No. Records	Repeatability	Variance
IGF-I	371	4	0.63	0.037
Insulin	371	4	0.34	0.034
Glucose	371	4	0.44	0.041
NEFA	371	4	0.38	0.037
$\beta$ HB	371	4	0.45	0.041
Urea	371	4	0.47	0.041

Conception rate to 1<sup>st</sup> service was 47% which contributed to a overall pregnancy rate of 87%. There was no association between body condition score at calving and conception rate to 1<sup>st</sup> service or overall pregnancy rate. The range of condition scores of cows at calving can be seen in Figure 55. There was no association between insulin, in each of the 4 weeks *post-partum*, mean insulin or changes in insulin from weeks 1 to 4 and conception rate to 1<sup>st</sup> service and similarly for NEFA, BHB and urea. Both concentrations of IGF-1 and glucose showed positive relationships with 1<sup>st</sup> service conception rate. The association between their mean concentration (XIGF-I; XGluc) and their changes ( $\Delta$ IGF-I;  $\Delta$ Gluc) in Weeks 1 to 4 and 1<sup>st</sup> service conception rate are summarised in Table 75. Neither 305-day milk yield nor composition were associated with these variables.

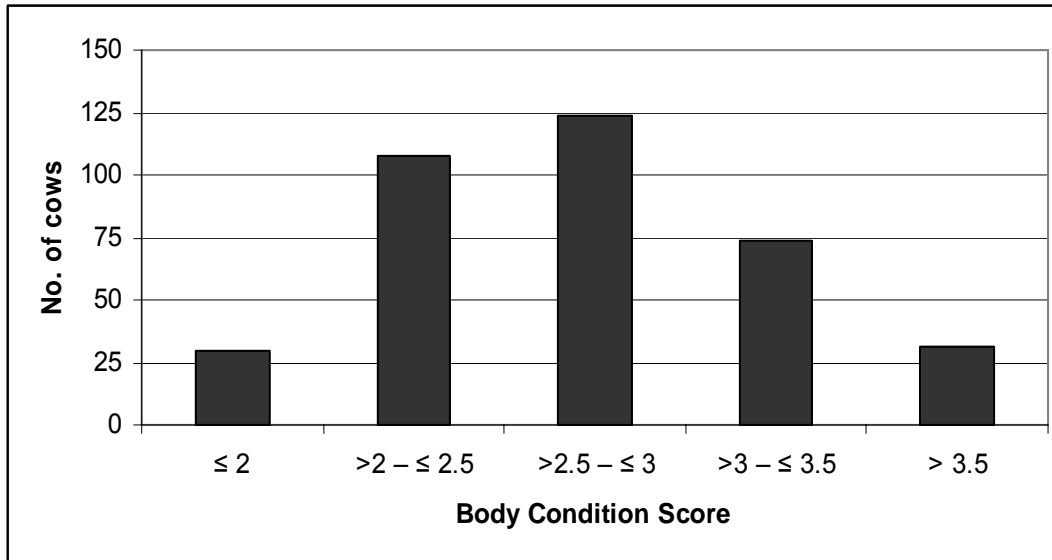


Figure 55. Range of condition scores of cows at calving.

**Table 75: The association between IGF-I and glucose during weeks 1 to 4 post partum. (Mean changes in concentration from Weeks 1 to 4 and conception to 1<sup>st</sup> service are presented as Odds Ratios with associated 95% Confidence Intervals (CI))**

Variable /Week	Odds ratio	CI	P-Value
<b>IGF-I</b>			
Week 1	1.01	0.998-1.009	P=0.22
Week 2	1.01	1.000-1.020	P=0.04
Week 3	1.07	1.001-1.003	P=0.03
Week 4	1.06	1.000-1.012	P=0.03
X IGF-I	1.008	1.001-1.010	P=0.02
Δ IGF-I	1.004	0.998-1.011	P=0.20
<b>Glucose</b>			
Week 1	1.63	0.921-2.883	P=0.09
Week 2	2.267	1.161-4.29	P=0.02
Week 3	2.435	1.145-5.182	P=0.02
Week 4	1.162	0.561-2.407	P=0.68
X Gluc	3.002	1.201-7.507	P=0.02
Δ Gluc	0.691	0.405-1.18	P=0.17

Blood metabolites indicative of energy balance had low repeatability during the first 4 weeks of the *post-partum* period, whereas IGF-I had a moderate repeatability and would indicate that it is well conserved with an individual animal and that fewer measurements are needed. There was no association with either body condition score at calving or total milk yield and composition and 1<sup>st</sup> service conception rate. Conception rate to 1<sup>st</sup> service was significantly associated with plasma concentrations of IGF-I and glucose over this 4 week period. This data would suggest that sampling for IGF-1 and glucose during the early *post-partum* period has the potential to provide us with markers of metabolic status and fertility.

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## Athenry Research Report

### **The effects of peri-ovulatory changes in endocrine and follicular activity on *corpus luteum* size, function and subsequent embryo survival**

The physiological mechanisms which contribute to observed differences in conception rate in heifers are not fully understood. Previous work from this laboratory has shown the critical period for embryo survival occurs during the first 16 days of gestation. The physiological changes which occur immediately before and during this period are paramount to the successful establishment of pregnancy. Previous studies have shown that embryo survival is positively associated with the concentration of progesterone (P4) during the early luteal phase. The relationship between the size of the ovulatory follicle and subsequent embryo survival rate is unequivocal. Furthermore, the relationship between follicle size and subsequent *corpus luteum* size and steroidogenic capacity also remains unclear. The objective of this study was to establish the relationships between these variables and embryo survival rate.

A total of 84 reproductively normal nuliparous beef heifers with a mean  $\pm$  S.D. live weight of  $459.6 \pm 38.3$  kg were oestrous synchronised using two injections of the prostaglandin F2 $\alpha$  (PG) analogue Cloprostenol administered 11 days apart (PG1 and PG2). Animals were fed grass silage *ad-lib* with a ME of 10.1 MJ/kg and a CP of 108 g/kg supplemented with 2 kg of concentrate having a ME value of 10.7 MJ/kg and CP of 154 g/kg indoors in a slatted floor arrangement through-out the experimental period, which lasted from day 15 pre- to d 35 post-oestrus and were subsequently turned out to pasture. Oestrus activity was monitored continuously using an electronic heat mount detection system (HeatWatch®) which were fitted at the time of PG2 administration. Heifers were visually observed for signs of oestrus activity every 3 h beginning 24 h after PG2. The time of initial standing event followed by successive mounting activity was taken as the onset of oestrus (d0). Heifers recorded in oestrus were inseminated by an experienced technician 5 to 21 h after onset using semen from a single ejaculate of a high fertility bull.

Ovarian structures were examined *per rectum* using an Aloka SSD-500V ultrasound scanner fitted with a 7.5-MHz transducer, starting 12 h after onset of oestrus and repeated every 6 h, thereafter, until ovulation (OV) occurred. Time of OV was determined from the time of the first scan on which the dominant follicle (DF) had disappeared minus 3 hours. The diameter of the DF was determined from the scan preceding its disappearance, measured as the average of the height and width of the follicle. Ovaries were re-examined on day 7 (d7) to confirm OV and to measure luteal structures.

Blood samples were collected twice daily at 0900 and 2100 h commencing 24 h after PG2 and continued until 48 h post-oestrus, with a further two samples taken 0900 h and 2100 h on day 7 post-oestrus. Retrospectively, all samples taken from 24 h post PG2 up until 36 h post-oestrus were used for estradiol analyses, a single sample was taken on days -1, 0 and 7 for IGF-1 analyses. Concentrations of progesterone were determined from mean of the two samples taken twice on day 7 post-oestrus. All blood samples were collected via jugular venipuncture into 10ml EDTA heparinised vacutainers.

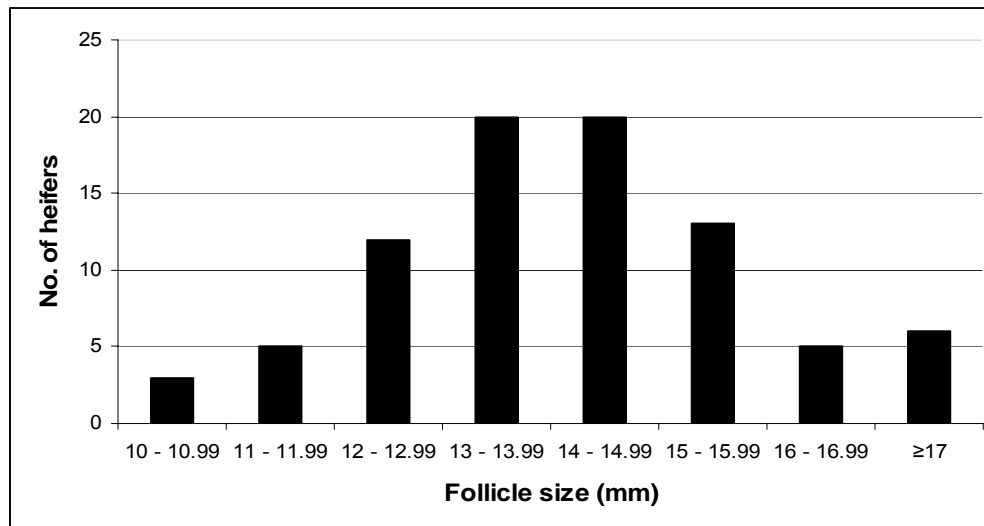
Blood samples were collected at 12 h intervals on d7 for prostaglandin (P4) and estradiol which were measured by RIA while IGF-1 was measured by IRMA. For P4 and IGF-1 a mean concentration of the twice daily samples was calculated and used for each animal. For estradiol its mean and maximum concentration and the change in concentration 12 h pre-oestrus to 36 h post-oestrus were used. Conception rate was confirmed by ultrasonography at day 30 (d30) and again on day 100 (d100) post AI. The relationships between the binary variable embryo survival by day 30 (pregnant/non-pregnant) and continuous variables was evaluated using logistic regression (PROC LOGISTIC, SAS, 2003).

Conception rate at d30 was 69% with only two of the heifers suffering foetal loss between d30 and d100. The mean ( $\pm$  S.D) interval to OV was  $27.4 \pm 5.90$  h after the onset of heat, associations between the chronological events surrounding AI and ovulation are summarised in Table 76. The interval from heat onset to time of ovulation did not affect embryo survival (OR = 1.042, P=0.38). Neither was there any relationship between intervals from heat onset to insemination any embryo survival. (OR 0.940, P=0.25)

**Table 76: Associations between timing of AI relative to either heat onset or ovulation and the timing of ovulation relative to heat onset with embryo survival by day 30 post AI**

Model	Odds ratio	95% CI	P-value
Interval from heat onset to ovulation and embryo survival at d30	1.042	0.956 – 1.136	P = 0.38
Interval from heat onset to AI and embryo survival at d30	0.940	0.846 – 1.044	P = 0.25
Interval from AI to ovulation and embryo survival at d30	1.059	0.983 – 1.140	P = 0.13

The mean  $\pm$  S.D. DF diameter was  $14.1 \pm 1.9$ mm and the range of ovulatory follicle sizes can be seen in Figure 56. The CL and lacuna size were measured as per follicles, CL volume was calculated using the equation  $\frac{4}{3}\pi r^3$  corrected by subtracting the lacuna volume where present and then denoted as the corrected CL volume.



**Figure 56. Range of Ovulatory follicle sizes.**

There was a tendency for a negative linear relationship between ovulatory follicle diameter and embryo survival (OR 0.799, P=0.09), with an increase in ovulatory follicle size leading to a reduced likelihood of embryo survival. Embryo survival was not related to CL diameter or corrected CL volume. There was a tendency for concentrations P4 to exhibit a positive linear relationship with embryo survival (Odds ratio 1.419, P>0.09). Neither maximum or mean estradiol concentrations, its change in concentration 12 h pre-oestrus to 36 h post-oestrus nor any of its individual measurements showed any relationship with embryo survival (P>0.05). Concentrations of IGF-1 either on individual days or their mean concentrations across the 3 days were not related to embryo survival (P>0.05).

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**Table 77: Relationship between ovarian and hormonal measurements and embryo survival on d30 post AI**

Variable	Odds ratio	95% CI	P-value
Ovulatory follicle size	0.799	0.615 – 1.039	P= 0.09
CL diameter	1.095	0.974 – 1.232	P> 0.13
Corrected CL volume	1.000	1.000 – 1.000	P< 0.24
Concentrations of IGF-1 d -1	1.003	0.997 – 1.009	P>0.39
Concentrations of IGF-1 d 0	1.003	0.998 – 1.008	P>0.29
Concentrations of IGF-1 d 7	1.003	0.998 – 0.998	P< 0.26
Maximum concentration of E2	1.098	0.748 – 1.612	P< 0.63
Mean concentration (-12h - +24h) of E2	1.003	0.624 – 1.613	P>0.99
Mean concentration of P4 on d 7	1.418	0.953 – 2.112	P>0.08

There was large variation around this time ovulation relative to heat onset. Neither the interval from heat onset to AI nor the interval from AI to ovulation affected embryo survival rate, not withstanding that some animals ovulated more than 30 h following insemination. This suggests that the viable lifespan of the sperm, at least for this bull, was greater than 30 h and would further question the practice of inseminating animals more than once as a means to improving fertility. Concentration of P4 on d7 was positively associated with embryo survival, again confirming the positive influence of progesterone during the early luteal phase on embryo survival. However the steroidogenic capacity of the CL could not be explained either by its size or by any stimulating effects of circulating estradiol and IGF-1 around heat or on day 7. The tendency for heifers ovulating larger follicles to have reduced fertility may be due to the production of incompetent oocytes produced from these large dominant follicles.

*RMIS No. 5546*

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### **Identification of the effects of short-term dietary restriction on gene expression in the anterior pituitary, hypothalamic, preoptic and the ventromedial hypothalamus tissues of cattle**

Negative energy balance (NEB) has an adverse effect on reproductive efficiency, especially in the dairy cow, with many cows remaining anoestrus for a number of weeks after calving. The hypothalamus and anterior pituitary are key reproductive tissues, mainly due to their roles in gonadotrophin releasing hormone (GnRH), luteinising hormone (LH), and follicle stimulating hormone (FSH) production and secretion. During NEB anoestrus cows fail to display GnRH, LH, or FSH concentration peaks due to the energy deficit. The hypothalamus also has roles to play in food intake. Some cows resume cyclicity earlier post calving than others. This indicates that there may be a significant genetic component to the bovine response to NEB.

The overall objective of this study is to use modern molecular biology techniques such as microarray technology and real time PCR to establish the genes that are uniquely expressed in heifers that are sensitive or tolerant to the NEB dietary restriction.

In order to complete a gene expression trial on the hypothalamus establishment of an experimental protocol for the dissection of particular nuclei within the hypothalamus is ongoing. This protocol establishment is a slow process due to the difficulty of hypothalamic nuclei dissection. There has been no published literature that performed a microarray study on the hypothalamus. However, some significant advances have been made and currently a custom made bovine brain matrix is under construction. This should allow for the repeatable and systematic dissection of the exact nuclei within the hypothalamus. In order to make this matrix, computed tomography (CT) scans of heifer skulls have been taken, and wax and silicone rubber moulds of the same animals' brains have been made. The brain matrix will make any results obtained more reliable and increase the scientific validity of the whole experiment. Also, the design of the brain matrix will be very useful for any future studies on hypothalamic function.

*RMIS No. 5756*

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### **Development of a DNA bank resource for Irish beef cattle**

The establishment of a DNA bank resource for Irish beef cattle with accompanying detailed phenotypic data is currently underway. This is an invaluable resource due to the extensive phenotypic data collected in Teagasc animals which accompanies these DNA samples. Traditional breeding programs involved mating sires and dams with high scores for the trait of interest. This process was repeated over many generations until the trait of interest was established and the entire process was extremely time-consuming; however DNA knowledge now allows us to fast track genetic improvement. The DNA bank resource can be used to determine the frequencies of single nucleotide polymorphisms (SNPs) associated with traits of economic importance such as fertility and feed efficiency in Irish beef breeds and also to investigate whether any deleterious consequences are expected in other important traits. The availability of thousands of SNPs spread across the genome for different livestock species opens up possibilities to include genome-wide marker information in prediction of total breeding values, to perform genomic selection. Furthermore, animals with varying genetic potential can be fed appropriately to improve their performance. The DNA bank is, therefore, invaluable in future development of DNA tests for economically important traits and in validating DNA tests which come into the market in an Irish context. This will improve the accuracy of the selection process and assist in increasing the rate of genetic progress, facilitating the identification of animals more suited to Irish production systems.

To date, protocols for the extraction and storage of DNA from blood and semen has been evaluated, optimised and standardised. Fully alarmed freezers and inventory systems have been purchased for optimal storage of DNA samples in a catalogued fashion. A database has been developed and is being employed to catalogue all sample information, such as DNA concentrations, quality, volumes and storage location. Phenotypic data is linked to those samples in a separate database. In total, DNA from 1167 cattle has been extracted and stored. These DNA samples have been included in a large 50k Illumina bovine SNP chip project and a number of fertility and feed efficiency candidate SNP studies are underway. Teagasc's establishment of the DNA bank has strengthened our collaboration with UCD and ICBF and will facilitate the successful integration and implementation of genomic selection into breeding programmes in Ireland in the near future.

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