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Genetic variation of metabolite and hormone concentration in UK Holstein-Friesian calves and the genetic relationship with economically important traits

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ABSTRACT

The decline of dairy cattle fertility worldwide remains a major concern, with conception rates to first service commonly below 40%. The length and severity of negative energy balance postpartum are unfavorably correlated with fertility, suggesting that the length and severity of negative energy balance and fertility are linked via several hormones or metabolites. These compounds therefore have the potential to predict fertility at a genetic level. The addition of a predictor trait for fertility into present fertility indices would accelerate genetic gain, particularly if it was expressed before adulthood. The objective of this work was to estimate the genetic variation in several metabolites and hormones in calves, and to determine their genetic relationships with fertility and production through sire predicted transmitting abilities (PTA; sires of calves sampled). Circulating concentrations of free fatty acids (FFA), glucose, growth hormone (GH), insulin, and insulin-like growth factor 1 (IGF-1) in male and female UK Holstein-Friesian dairy calves (average age \pm SD; 126 ± 12.7 d) were analyzed during 2 studies: data set 1 ($n = 496$ females; 1996–2001; 7 commercial dairy herds) and data set 2 ($n = 326$ females, $n = 256$ males; 2002–2006; multiple ovulation and embryo transfer breeding scheme). Univariate mixed models were fitted to the data using ASREML. Basal concentrations of FFA, glucose, GH, insulin and total IGF-1 were all moderately heritable in both sexes (heritability range \pm SE; 0.09 ± 0.05 to 0.66 ± 0.14). The sire PTA for protein percentage had significant regression coefficients and approximate genetic correlations with FFA and insulin, and the sire PTA for calving interval had significant regression coefficients and approximate genetic correlations with GH. Additive genetic variance

seems responsible for a moderate proportion of the phenotypic variation in important metabolites and regulatory hormones in male and female UK Holstein-Friesian dairy calves, therefore supporting further investigation into their use as juvenile predictors for fertility in the mature female.

Key words: metabolites, hormones, heritability, correlation

INTRODUCTION

The challenges faced by the UK dairy cattle industry are similar to those affecting dairying worldwide, and prominent among these is poor fertility (e.g., Royal et al., 2000). Although many countries now publish fertility indices, age and sex limitations on the existing measurements of fertility, combined with the large environmental variance associated with the trait, result in relatively low accuracies in these indices. For example, the UK fertility index is expected to slow the decline in fertility (Wall et al., 2003). The rate of genetic improvement in fertility might be improved by inclusion of a juvenile predictor trait (genetic indicator) in the fertility index. In particular, a trait measurable in the juvenile male but genetically correlated to female fertility would overcome both the delay and the sex limitations inherent in measuring fertility traits in the lactating female.

Potential genetic indicator traits arise from a consideration of the physiology of the lactating cow. During early lactation cows experience a period of negative energy balance (NEB), the extent of which is unfavorably correlated genetically with fertility (de Vries and Veerkamp, 2000; Dechow et al., 2002). During this period, changes occur in the circulating concentrations of glucose and regulatory hormones including FFA, growth hormone (GH), insulin, and IGF-1 (Butler, 2000; Roche et al., 2000; Jorritsma et al., 2003). Reist et al. (2002) have suggested that these changes reflect the duration and severity of NEB. However, these hor-

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Table 1. Number and age of calves (mean \pm SD¹) of each sex from each data set sampled

Data set	Sex	n	Age, d
Data set 1 (7 herds)	Female	496	129.2 \pm 8.3
Data set 2 (1 herd)	Male	256	127.1 \pm 11.0
	Female	326	120.4 \pm 16.8
	Total	1,078	126.0 \pm 12.7

¹Standard deviation of age at sampling.

mones and metabolites also affect reproduction such that during NEB, altered concentrations restrict follicle growth and steroidogenesis (Roche et al., 2000; Webb et al., 2004). This link highlights the possibility of a genetic relationship between energy balance and fertility and suggests several traits that, when measured in the calf, might serve as indirect genetic predictors for fertility in the adult.

The requirements for an indirect juvenile predictor include at least moderate heritability to allow genetically superior animals to be identified and for that superiority to be passed on to offspring. This was demonstrated by Hayhurst et al. (2007) for circulating concentrations of FFA, glucose, and insulin in 9-mo-old Danish Jersey, Red Dane, and Danish Holstein bull calves. To have predictive power, a juvenile predictor must also be genetically correlated to the trait in question; that is, fertility. Furthermore, it is beneficial in terms of data collection and structure if a trait to be used as a juvenile predictor is expressed in both sexes, genetically correlated in the 2 sexes, and genetically correlated with the trait in the mature animal. The aim of this study was to extend the previous analyses by Hayhurst et al. (2007) by using a different population of cattle (United Kingdom) at an earlier age and of a different breed (Holstein-Friesian), and by estimating the heritability of circulating concentrations of GH and IGF-1 in addition to FFA, glucose, and insulin in female as well as male calves. Additionally, to obtain an insight into the relationships between fertility and production traits in terms of hormone and metabolite concentrations, approximate genetic correlations between the traits were estimated using sire PTA.

MATERIALS AND METHODS

Animals and Experimental Design

Data collected during 2 UK studies (that followed similar protocols) were used: data set 1 (**D1-F**, $n = 496$ females; 1996–2001; 7 commercial dairy herds) and data set 2 [data set **D2-F**, $n = 326$ females and data set **D2-M**, $n = 256$ males; 2002–2006; multiple ovulation and embryo transfer (MOET) breeding scheme; Table 1]. Plasma samples were collected from a total of 1,078 Holstein-Friesian calves at an average age of

126 d (SD = 12.7 d). Ancestry was traced for at least 3 generations to construct a pedigree file ($n = 4,662$; pedigree data provided by Holstein UK, Scotsbridge House, Rickmansworth, UK).

In data set 1, female calves were raised commercially as replacement heifers, initially on milk powder, having been weaned by the time of blood sampling. The postweaning diet varied according to time of year and different management practices between individual farms. However, the majority of calves were fed dry cake rations, according to weight, and ad libitum hay. In data set 2, female and male calves were housed separately at 2 rearing units and calves were fed colostrum and milk replacer (Volac Heiferlac Instant, Volac International, Orwell, UK). In addition, barley straw was fed ad libitum, and calf pellets (HST Feeds, Crewe, UK) were fed in increasing amounts according to age and weight until weaning was complete between 9 and 12 wk of age. Postweaning calf pellets, hay, straw, grass, and maize silage were fed in varying quantities according to age, weight, and season. The differences in diet were accounted for in the batch effect (sampling day) as no 2 farms were sampled on the same day.

Experimental Procedure

On the day of sampling, calves were held in individual calf pens to be sampled and were left to become accustomed to their surroundings for between 30 and 60 min before the trial started. Animals were haltered and loosely tied except during blood sampling when they were restrained. Blood samples were taken directly into heparinized tubes by jugular venipuncture at fixed time intervals. Two blood samples (data set 1, 0 and 30 min; data set 2, 0 and 15 min) were used in these analyses. Further samples were taken for other purposes outside the current experiment later during the day. Blood samples were centrifuged ($1,500 \times g$, 15 min) and the plasma removed and frozen (-20°C) until laboratory analysis.

Hormone and Metabolite Assays

Growth hormone, insulin, and total IGF-1 concentrations were determined by validated noncompetitive

time-resolved immunofluorometric assays as described previously by Løvendahl et al. (2003), Løvendahl and Purup (2002), and Frystyk et al. (1995), respectively. Free fatty acid concentration was determined using a commercial assay kit (NEFA C kit, Wako Chemicals GmbH, Neuss, Germany) adapted to automated running on a Advia 1650 Bayer Opera system (Bayer, Raleigh, NC). Similarly, a commercial assay kit (Glucosehexokinase II; Bayer) adapted to the same automated system was used to determine glucose concentrations.

Sire PTA

Sire PTA for fertility and production traits (from 140 progeny-tested sires; MDC Breeding, Milk Development Council, Cirencester, UK; and calculated by Edinburgh Genetic Evaluation Service, Scottish Agricultural College, Penicuik, UK) were available for 1,035 of the 1,078 calves blood sampled. Ideally, genetic correlations between the hormone or metabolite with fertility and production of the calf sampled as a heifer would have been calculated. However, this was not possible (because of a lack of relevant data); therefore, as a compromise, the genetic regressions of the hormone or metabolite data on the sire PTA were calculated by fitting an additional term in the model for sire PTA. Approximate inferred genetic correlations estimated in this way are largely dependent on the genetic standard deviations of the separate traits, and therefore estimates should be interpreted with caution because of the possible bias this introduces. The sire PTA investigated were fertility index, 56-d nonreturn rate after first service, calving interval, days from calving to first service, and number of services per conception. As a secondary objective, the relationship with other sire PTA was investigated (305-d milk, fat, and protein yields in kilograms, fat and protein percentage, production index, profitable lifetime index, and BCS).

Statistical Analysis

The mean concentration of hormone or metabolite in the 2 plasma samples taken was used, and concentrations of FFA, GH, insulin, and IGF-1 were \log_{10} transformed to give approximately normally distributed residuals. Other types of transformation were investigated [square root(x), $1/(x)$, or $(x)^2$]; however, these did not improve (bring closer to zero) the coefficient of skewness to the same extent as the chosen transformation of \log_{10} . The distribution of glucose concentrations was approximately normal, thus no transformation was necessary.

Univariate mixed models were fitted to the data using ASREML software (version 2.00; Gilmour et al.,

2006). To investigate the possibility of combining the 3 subsets of data (D1-F, D2-F, and D2-M), they were first analyzed separately. The fixed effects in the initial model were age (d) and the experimental batch (sampling day; 131 batches with 2 to 48 calves each, mean \pm SD, 8.2 ± 9.9). The effect of batch accounts for many sources of variation including farm, day, month, and year of sampling. Although batch is often seen as a random effect [$\sim N(0, \sigma^2)$], it was fitted as fixed in this study. This was because although many batches fit the criteria of being a random variable, some did not because of transportation to the sampling site and a short time left to acclimate before sampling compared with the other batches.

These initial analyses highlighted that within each hormone or metabolite, the additive genetic variance for each of the 3 subsets was similar and, in addition to this, each estimate had a small standard error. However, the difference between the residual variance within each trait (for subsets D1-F, D2-F, and D2-M) was sizeable, the small standard errors being suggestive of significant differences. It appeared that in most cases, the residual variance in the D2-F subset was greatest and that in D1-F the smallest. A possible explanation for this was that D1-F was distributed over 7 privately owned commercial herds in which the experimental procedure was tightly controlled, whereas D2-M and D2-F were conducted at 2 young stock units for a large breeding company. In the case of D2-F, calves were transported from nearby rearing units to the testing station up to 60 min before sampling began, although this may not have been sufficient time to acclimatize. Many other models and methods were investigated to overcome the problems described, and although a tri-variate analysis (of the 3 data sets) would have been the preferred method because of the small size of the separate data sets, this was not possible. Therefore, to allow the analysis of the combined data (D1-F + D2-M + D2-F), extra random terms were fitted to account for the heterogeneous residual variance in subset D1-F, D2-M, and D2-F where necessary. The additional random terms were added for the 2 subsets with the highest residual variance within each trait.

The final model fitted the fixed effect of age, experimental batch, sex, the nested effect of batch within sex, and the random effect of the genetic relationships between animals by way of the pedigree file. Hence, the univariate model fitted to the combined data sets (D1-F + D2-M + D2-F) for each hormone and metabolite was

$$Y_{ijkm} = \alpha + \beta D_{ijkm} + B_i + S_j + SB_{ij} \\ + R1_{ijkm} + R2_{ijkm} + A_{ijkm} + \varepsilon_{ijkm},$$

where Y_{ijkm} = the hormone or metabolite concentration of animal m of batch i of sex j and data subset k . The fixed effects were α = intercept; βD_{ijkm} = regression on age of animal in days expressed as deviation from overall mean age with coefficient β ; B_i = the fixed effect of experimental batch ($i = 1$ to 131); S_j = sex ($j =$ female or male); and SB_{ij} = nested effect of batch within sex. Random effects were $R1_{ijkm}$ = first extra random term for extra residual variance for subset $k =$ D1-F, D2-M, or D2-F with the highest residual variance, distributed $N(0, \sigma_{E1}^2)$; $R2_{ijkm}$ = second extra random term for extra residual variance for subset $k =$ D1-F, D2-M, or D2-F with the second highest residual variance, distributed $N(0, \sigma_{E2}^2)$; A_{ijkm} = animal m $\left[N(0, \sigma_A^2 \mathbf{A}) \right]$, where \mathbf{A} is the numerator relationship matrix of animals available in the data; and ε_{ijkm} = error term $\left[N(0, \sigma_{E0}^2) \right]$.

Because of the database attributes as described above, 3 heritability estimates, one for each subset, were calculated for each hormone or metabolite. The variance components included in the phenotypic variance were dependent on which extra random terms were fitted for that subset.

For example, the univariate model fitted for glucose contained extra random terms for D2-M and D2-F because, out of the 3 subsets, these had the highest residual variance. Therefore, 4 components of variance were estimated: D2-M extra residual variance (σ_{E1}^2); D2-F extra residual variance (σ_{E2}^2); additive genetic variance (σ_A^2); and residual variance (σ_{E0}^2).

The 3 heritability (h^2) estimates for glucose were calculated as follows:

$$\text{D1-F } h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{E0}^2};$$

$$\text{D2-M } h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{E1}^2 + \sigma_{E0}^2};$$

$$\text{D2-F } h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{E2}^2 + \sigma_{E0}^2}.$$

Finally, sire PTA [for 305-d milk, fat and protein yield (kg), fat and protein percentage, production index, profitable lifetime index, fertility index, nonreturn rate 56 d after insemination, calving interval, DIM until the first AI, number of services per conception, and BCS) were added to the model one by one as a covariate (i.e.,

one PTA added and then removed before another PTA was added). The regression coefficient for the sire PTA gave an indication of the genetic relationship between the sire PTA and the hormone or metabolite. Because of the limitations of the data (as previously detailed), it was only possible to calculate approximate inferred genetic correlations (see the following equation; Royal et al., 2002) and because of this, these should only be used as a possible indication of a genetic relationship:

$$r_A = b \times \frac{\sigma_a \text{ sire PTA}}{\sigma_a \text{ hormone or metabolite}},$$

where r_A = the inferred genetic correlation; b = the regression coefficient for the sire PTA; σ_a = the genetic standard deviation of the sire PTA or the hormone or metabolite.

The genetic standard deviations for the sire PTA were obtained from several sources (Wall et al., 2003; R. Mrode, Scottish Agricultural College, Penicuik, UK; personal communication).

The significance of the fixed effects was assessed using Wald F statistics. The significance of variance components (including the animal; i.e., heritability) were tested using the likelihood ratio test by removing them from the model and comparing $-2 \times$ change in likelihood to the Chi-squared with 1 df. The log-likelihood values and Wald F statistics were obtained from ASREML.

RESULTS AND DISCUSSION

Hormone and Metabolite Concentrations

Concentrations of glucose, FFA, GH, insulin, and IGF-1 found in this study (Table 2) were comparable to those found in previous studies in calves of similar ages (Rowlands et al., 1983; Woolliams et al., 1992; Løvendahl et al., 1994; Taylor et al., 2004; Klotz and Heitman, 2006).

Heritability of the Hormones and Metabolites

Glucose, FFA, GH, and insulin all had moderate and similar heritabilities (heritability \pm SE, range 0.09 \pm 0.05 to 0.25 \pm 0.13; see Table 3), whereas that of IGF-1 was consistently high (heritability range 0.21 to 0.66). Furthermore, all heritability estimates were significantly different from zero ($P < 0.0005$ to $P < 0.025$). These findings are largely supportive of previous studies in 9-mo-old calves of different breeds and in both male and female calves (Løvendahl et al., 1994; Løvendahl and Jensen, 1997; Davis and Simmen, 2000; Grochowska et al., 2001).

Table 2. Number of calves sampled, mean (\pm SD) and residual variance ($\sigma_E^2 \pm$ SE) of glucose, FFA, growth hormone (GH), insulin, and IGF-1 concentrations in each subset (D1-F, D2-M, and D2-F)

Trait	Data set	n	Mean \pm SD ¹	$\sigma_E^2 \pm$ SE ²
Glucose	D1-F	493	4.26 \pm 0.83	0.17 \pm 0.05
	D2-M	256	5.10 \pm 0.75	0.24 \pm 0.05
	D2-F	320	4.73 \pm 0.87	0.49 \pm 0.06
FFA	D1-F	493	261.02 \pm 122.67	0.05 \pm 0.01
	D2-M	256	261.97 \pm 155.66	0.12 \pm 0.02
	D2-F	320	126.77 \pm 92.87	0.16 \pm 0.02
GH	D1-F	498	2.69 \pm 2.21	0.40 \pm 0.07
	D2-M	256	6.33 \pm 5.22	0.52 \pm 0.08
	D2-F	320	3.66 \pm 2.87	0.30 \pm 0.05
Insulin	D1-F	498	30.07 \pm 31.72	0.29 \pm 0.05
	D2-M	256	38.88 \pm 30.19	0.11 \pm 0.03
	D2-F	320	50.09 \pm 35.67	0.28 \pm 0.02
IGF-1	D1-F	489	159.98 \pm 75.52	0.05 \pm 0.03
	D2-M	254	263.51 \pm 110.34	0.04 \pm 0.02
	D2-F	317	126.13 \pm 78.65	0.29 \pm 0.05

¹Mean units: glucose: mmol/L; FFA: μ Eq/L, GH: ng/mL, insulin: pmol/L, IGF-1: ng/mL.

²Residual variance units: glucose: mmol/L, \log_{10} FFA, \log_{10} GH, \log_{10} insulin, \log_{10} IGF-1.

Fixed Effects Investigated

The fixed effects of batch, sex, and the nested effect of batch within sex were significant ($P < 0.001$) for each hormone or metabolite. Blood samples were collected on 131 occasions (131 batches) over a period of 9 yr. Therefore, batch includes variation due to time of sampling (e.g., technician, day, month, season, and year of sampling). Furthermore, batch also contained variation due to farm and diet in D1-F as none of the 7 farms in D1-F were sampled on the same day. The nested effect of batch within sex accounted for variation due to farm in D2-M and D2-F as these were sampled on the same day (i.e., same batch), but at separate units for each sex. The hormone or metabolite concentration was significantly affected ($P < 0.001$) by the sex of calf sampled, with males tending to have increased

concentrations (with the exception of insulin). Previous studies have also reported higher concentrations in male than in female calves ≤ 1 yr of age (e.g., Keller et al., 1979; Govoni et al., 2003).

The regression on age was only significant for insulin and IGF-1 ($P < 0.001$). Concentrations of IGF-1 and insulin increase with age (from birth until ≥ 15 mo of age; Plouzek and Trenkle, 1991; Skaar et al., 1994). In contrast, glucose (Rowlands et al., 1983) and FFA (Quigley et al., 1991) remain relatively stable over the range of ages at testing (range 72–167 d) and vary due to feeding, whereas GH concentrations decrease with age (from birth until ≥ 52 wk of age; Govoni et al., 2003). Growth hormone concentrations for animals in D1-F, D2-M, and D2-F were the average of 2 plasma samples taken 15 or 30 min apart; furthermore, the data were not edited to remove random pulses of GH.

Table 3. Estimated heritability (h^2) for plasma glucose, FFA, growth hormone (GH), insulin, and IGF-1 plus SE and significance in each subset (D1-F, D2-M, and D2-F)

Trait ¹	Data set	n	$h^2 \pm$ SE	P-value
Glucose	D1-F	1,029	0.23 \pm 0.11	0.009
	D2-F		0.13 \pm 0.06	0.009
	D2-M		0.20 \pm 0.10	0.009
FFA	D1-F	1,029	0.25 \pm 0.13	0.025
	D2-F		0.09 \pm 0.05	0.025
	D2-M		0.12 \pm 0.06	0.025
GH	D1-F	1,034	0.15 \pm 0.07	0.005
	D2-F		0.18 \pm 0.09	0.005
	D2-M		0.13 \pm 0.06	0.005
Insulin	D1-F	1,034	0.10 \pm 0.06	0.012
	D2-F		0.12 \pm 0.06	0.012
	D2-M		0.22 \pm 0.12	0.012
IGF-1	D1-F	1,020	0.55 \pm 0.13	0.000
	D2-F		0.21 \pm 0.05	0.000
	D2-M		0.66 \pm 0.14	0.000

¹Units for traits: glucose: mmol/L, \log_{10} FFA, \log_{10} GH, \log_{10} insulin, \log_{10} IGF-1.

Table 4. Genetic regression coefficients ($b \pm SE$) of the hormones and metabolites on the sire PTA, plus the significance of b and the approximate genetic correlation ($r_A \pm SE$)

Regression of ¹	$b \pm SE$	P -value	Approximate ² $r_A \pm SE$
FFA on protein percentage	-0.80 ± 0.28	0.004	-0.89 ± 0.31
Insulin on protein percentage	1.13 ± 0.46	0.015	0.84 ± 0.34
GH on calving interval	0.019 ± 0.0087	0.032	0.64 ± 0.30

¹Traits analyzed: \log_{10} FFA, \log_{10} insulin, \log_{10} growth hormone (GH).

²Genetic standard deviation (σ_a) used in calculation of approximate genetic correlation: FFA 0.1257, insulin 0.1879, GH 0.2606, protein percentage sire PTA 0.14, calving interval sire PTA 8.95.

The variance caused by the pulsatility of GH may in part have masked the effect of age, and the effect itself may be small over the narrow range of age at testing.

The Heterogeneous Nature of the Residual Variance

The heterogeneous nature of the residual variance in each data set can be seen in Table 2. This is probably because of inconsistencies in procedure, as described in the Materials and Methods. Another possible source of variation is feeding disturbance. Although food was available during sampling, it is probable that intake will be affected by the length of time left to acclimate, transport to the site, and human interaction. It is known that FFA concentration is particularly affected by the time since last feeding (Fox et al., 1991). Similarly, diurnal variation is also present in concentrations of glucose, insulin, and GH (Xing et al., 1991; Ndibualonji et al., 1997). Diurnal variation and variation due to time elapsed since last feeding cannot be accounted for in the genetic analysis of the present data, highlighting 2 sources of variation that could partially account for the unequal residual variation in the 3 data sets.

When determining the potential power of using indirect selection criteria within a breeding program, the certainty of a favorable genetic relationship must be carefully considered against the risk of incorrect selection decisions. This potential risk is higher when additional experimental variation cannot be tightly controlled. This study has highlighted the importance of controlled experimental protocols when working with physiological parameters for selection purposes. Although it provides confirmation that genetic variation is present in these parameters, caution should be used when considering specific magnitudes due to the heterogeneous nature of the residual variance.

Approximate Genetic Correlations of Hormones and Metabolites with Sire PTA

Of the many genetic regressions and correlations calculated, only those that were significant are presented (Table 4). The approximate genetic correlation between GH concentrations at 4 mo of age with the sire PTA for

calving interval was positive and significant (regression coefficient $\pm SE$, 0.019 ± 0.0087 , $P = 0.032$; genetic correlation $\pm SE$, 0.64 ± 0.30 , Table 4). Therefore, high GH at 4 mo of age is genetically associated with a longer calving interval in the cow. This might imply that high concentrations of GH at 4 mo of age might be associated with high concentrations of GH postpartum, which is often seen during NEB, and is associated with poor resumption of ovarian cyclicity and often subsequent longer calving intervals (Butler, 2000; de Vries and Veerkamp, 2000; Dechow et al., 2002). Previous experimental work has identified GH as a juvenile predictor of milk yield (Løvendahl et al., 1991; Woolliams et al., 1993), which was justified also by high GH of genetically high-yielding cows during lactation. Nonetheless, this dual nature of GH is consistent because there are well-established, moderately large negative genetic correlations between yield and fertility, and it may be that regulation of GH is a component of this association. Although for selection purposes accurate genetic parameters may be all that is needed for implementation, it may be necessary to delineate these systemic associations more accurately to fully understand the possible consequences of selection.

The approximate inferred genetic correlation between protein percentage sire PTA and insulin was positive, whereas that with FFA was negative, and both were significant (Table 4). Although insulin and FFA were associated with protein percentage PTA, there were no significant correlations with milk solids production, which is the trait of primary economic interest.

CONCLUSIONS

This research has indicated that additive genetic variance is responsible for a moderate proportion of the phenotypic variation in a selection of metabolites and regulatory hormones in male and female calves. Significant genetic relationships were present between FFA, insulin, and GH with protein percentage and calving interval sire PTA. Although the genetic correlation between GH and calving interval is promising, further work would be needed to confirm the relationship between the two. Nevertheless, GH may potentially be useful as

a juvenile selection criterion for fertility, provided this is following a strict experimental procedure allowing for several hours of acclimation before sampling to reduce unwanted experimental noise.

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