Genetic merit for fertility traits in Holstein cows: I. Production characteristics and reproductive efficiency in a pasture-based system

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ABSTRACT

The objective of the present study was to characterize the phenotypic performance of cows with similar proportions of Holstein genetics, similar genetic merit for milk production traits, but with good (Fert+) or poor (Fert–) genetic merit for fertility traits. Specifically, we tested the hypothesis that cows with a negative estimated breeding value for calving interval would have superior fertility performance and would have detectable differences in body reserve mobilization and circulating concentrations of metabolic hormones and metabolites compared with cows that had a positive estimated breeding value for calving interval. For the duration of the study, cows were managed identically as a single herd in a typical grass-based, spring-calving production system. A total of 80 lactation records were available from 26 Fert+ and 26 Fert– cows over 2 consecutive years (2008 and 2009). During yr 1, cows were monitored during a 20-wk breeding season to evaluate reproductive performance. Milk production, body condition score (scale 1 to 5), body weight, grass dry matter intake, energy balance, and metabolic hormone and metabolite data were collected during both years. The Fert+ cows had greater daily milk yield (19.5 vs. 18.7 kg/d), shorter interval from calving to conception (85.6 vs. 113.8 d), and fewer services per cow (1.78 vs. 2.83). No difference between groups in grass dry matter intake, energy balance, or body weight was observed. The Fert+ cows maintained greater BCS during mid (2.84 vs. 2.74 units) and late lactation (2.82 vs. 2.73 units). Circulating concentrations of insulin-like growth factor-I were greater throughout the gestation-lactation cycle in Fert+ cows (148.3 vs. 128.2 ng/mL). The Fert+ cows also had greater circulating concentrations of insulin during the first 4 wk of lactation (1.71 vs. 1.24 μ IU/mL). Analysis of records from national herd data verified the association between genetic merit for fertility traits and phenotypic reproductive performance;

Fert+ cows ($n = 2,436$) required 11.1 d less to recalve than did Fert– cows ($n = 1,388$), and the percentage of cows that successfully calved for the second time within 365 and 400 d of the first calving was 8 and 13% greater for Fert+ compared with Fert– cows, respectively. These results demonstrate that genetic merit for fertility traits had a pronounced effect on reproductive efficiency, BCS profiles, and circulating concentrations of insulin-like growth factor-I.

Key words: genetic selection, fertility trait, reproduction, insulin-like growth factor-I

INTRODUCTION

The efficiency of pasture-based milk production systems is based on maximizing milk production from low-cost grazed grass (McCarthy et al., 2007). The success of these systems is largely dependent on achieving a compact calving pattern to coincide with the start of the grass growing season. The maintenance of this compact calving pattern is reliant on the cow's ability to successfully calve, complete uterine involution, return to cyclicity, exhibit estrus, conceive, and maintain a pregnancy in a window of 85 d, thus maintaining a 365-d calving interval. The relative importance of female fertility is markedly greater in seasonal calving systems than in year-round calving systems (Veerkamp et al., 2002). Failure to maintain the target calving interval of 365 d in a seasonal calving system results in financial losses at farm level (Evans et al., 2006b). This arises through decreased volumes of saleable milk and increased voluntary and involuntary culling rates and breeding costs (Plaizier et al., 1997).

Despite the crucial role fertility plays in maximizing economic output, reproductive efficiency in dairy cows has decreased during the past half century both in Ireland (Evans et al., 2006a) and internationally (Royal et al., 2000; Washburn et al., 2002). In Ireland, some of this decrease has been attributed to the intense selection for milk production traits and the associated introgression of North American Holstein genes (Horan et al., 2004). An extensive study of performance indicators on 14 spring-calving dairy farms between 1990 and

Received July 19, 2011.

Accepted November 2, 2011.

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2001 in Ireland confirmed these trends (Evans et al., 2006a). During this 11-yr period, the pedigree index for milk volume increased by 25 kg per year, the proportion of Holstein genes increased from 8 to 63%, and the calving rate to first service decreased from 55% in 1990 to 44% in 2001.

To address the problem of decreasing fertility, the Irish national breeding program shifted from being predominantly focused on milk production traits to a more holistic multi-trait index called the Economic Breeding Index (**EBI**; Veerkamp et al., 2002). Introduced in 2001, the EBI included production and nonproduction traits, thus identifying animals of superior genetic merit for delivering on-farm profit (Berry et al., 2007). Since its introduction, the EBI has evolved to include 6 subindexes (relative emphasis in parenthesis); milk production (38.1%), fertility and survival (34.8%), calving performance (10.3%), beef carcass (7.2%), maintenance (6.1%), and health (3.6%; <http://www.icbf.com>). The fertility subindex comprises 2 traits: calving interval (23.2%) and survival (11.5%). Good genetic merit for fertility traits requires negative EBV for calving interval and positive EBV for survival.

Previous studies evaluating the effects of genetic influences on reproductive efficiency have compared animals of varying genetic merit for milk production (Buckley et al., 2000) or varying Holstein ancestry (Horan et al., 2004). In both cases, large differences in reproductive performance were reported. The precise mechanisms responsible for fertility differences remain poorly understood. In previous studies comparing models of good and poor fertility, observed differences in phenotypic fertility performance were generally confounded with genetic merit for milk yield and phenotypic milk production. Therefore, the aim of this study was to characterize the phenotypic performance of cows with similar genetic merit for milk production traits and similar proportions of Holstein genes, but divergent genetic merit for fertility traits (genotypes Fert+ and Fert-). Specifically, we tested the hypothesis that cows with negative EBV for calving interval would have superior fertility performance, would be less reliant on body reserve mobilization, and would have detectable differences in circulating concentrations of metabolic hormones and metabolites compared with cows that had positive EBV for calving interval.

MATERIALS AND METHODS

Herd Establishment

Using the autumn 2007 official dairy evaluation published by the Irish Cattle Breeding Federation (ICBF, Bandon, Co. Cork, Ireland), the national dairy cattle database was screened for heifers due to calve for the

first time in Spring 2008. Restrictions were placed on the EBV for milk production (between +200 and +900 kg) and proportion of Holstein genetics (>75%). Within this population, heifers with extreme positive (i.e., poor fertility) and negative (i.e., good fertility) EBV for calving interval were identified. Poor-fertility (**Fert-**) heifers were restricted to animals where both the sire and maternal grand-sire had positive EBV for calving interval. Conversely, good-fertility (**Fert+**) heifers were restricted to animals where both the sire and maternal grand sire had negative EBV for calving interval. Heifers identified as being available for purchase were screened for infectious diseases. A total of 18 nulliparous Fert- and 18 nulliparous Fert+ cows passed the Moorepark Biosecurity Protocol and were purchased and moved in January 2008 to the Moorepark Animal & Grassland Research and Innovation Centre in Fermoy, Co. Cork, Ireland (55°10'N 8°16'W).

The process of selection described above was repeated with the same criteria (as explained above) during autumn 2008 and an additional 8 nulliparous Fert- and 8 nulliparous Fert+ heifers were purchased. The EBV of the 2 genotypes are summarized in Table 1. Within the Irish national herd, these animals were representative of the top quartile in genetic merit for milk production, whereas the Fert+ and Fert- groups represented the top 20% and bottom 5% for calving interval, respectively.

Feed and Management System

Animals were managed identically as 1 herd in a typical grass-based, spring-calving production system. Following parturition, cows were turned out to grass in early February until mid-November and grazed under a rotational grazing system, as described by Dillon et al. (1995), in a predominantly perennial ryegrass (*Lolium perenne* L.) sward. Fresh pasture was allocated daily to the herd following morning milking using temporary fencing. During yr 1, the mean daily herbage allowance of 13 ± 1.45 kg of DM/cow per day was supplemented with 3.9 ± 1.21 kg of DM/cow per day of concentrate. Similarly during yr 2, the mean daily herbage allowance of 14.3 ± 1.29 kg of DM/cow per day was supplemented with 4.0 ± 1.69 kg of DM/cow per day. The mean pre-grazing herbage yield was $1,337 \pm 494$ kg of DM/ha in yr 1 and $1,146 \pm 396$ kg of DM/ha in yr 2. Cows were dried off 80 d before expected calving date, housed in a freestall barn and given full time access to a TMR of grass silage (90%) and concentrate (10%).

Animal Measurements

Cows were milked twice daily at 0730 and 1630 h. Milk yield was recorded at each milking using electronic

Table 1. The mean estimated breeding value¹ (and SD) for the 2 groups of Holstein cows studied based on their milk production, individual calving interval, sire calving interval, and maternal grandsire calving interval

Item	Yr 1 ²		Yr 2 ³	
	Fert+	Fert–	Fert+	Fert–
No. of animals	18	18	23	21
Holstein (%)	92 (5.6)	93 (6.3)	91 (6.0)	93 (6.2)
Milk (kg)	+468 (161.8)	+526 (153.8)	+462 (133.4)	+478 (145.2)
Fat (kg)	+18.8 (7.96)	+19.2 (7.52)	+22 (7.34)	+17 (6.86)
Protein (kg)	+18.4 (6.36)	+20.8 (6.42)	+18.8 (5.52)	+17.8 (6.1)
Fat (g/kg)	+0.032 (0.1112)	–0.014 (0.1464)	+0.088 (0.112)	–0.016 (0.133)
Protein (g/kg)	+0.062 (0.0542)	+0.068 (0.0922)	+0.072 (0.046)	+0.042 (0.0855)
Survival (%)	+3.2 (0.88)	+0.2 (1.08)	+3.2 (0.78)	–0.2 (1.16)
Calving interval (d)	–6.4 (1.584)	+5.86 (3.434)	–6.36 (1.41)	+5.86 (2.88)
Sire calving interval (d)	–8.94 (4.336)	+8.12 (3.368)	–9.06 (3.6)	+7.3 (3.044)
Maternal grandsire calving interval (d)	–5.22 (2.54)	+6.48 (3.414)	–5.44 (2.496)	+7.92 (4.992)

¹All PTA were obtained from autumn 2008 and autumn 2007 official dairy evaluations published by the Irish Cattle Breeding Federation and multiplied by 2 to convert to EBV.

²Fert+ = good-fertility cows; Fert– = poor-fertility cows.

³In yr 2, the Fert+ group comprised 15 second- and 8 first-parity cows, and the Fert– group comprised 13 second- and 8 first-parity cows.

milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (fat, protein, and lactose) was determined weekly from successive evening and morning samples by mid-infrared reflectance spectroscopy using a FT6000 MilkoScan instrument (DK-3400; Foss Electric, Hillerød, Denmark). Cow BW was measured weekly and BCS (Edmonson et al., 1989) was assessed every 2 to 3 wk throughout the study. During lactation, BW and BCS measurements were taken immediately after morning milking by a single operator. Individual animal grass DMI (**GDMI**) measurements were carried out on 3 occasions during yr 1 at wk 13, 28, and 35 postpartum (SD $\pm$ 3.2 wk for all time points) and on 1 occasion in yr 2, at wk 13 postpartum (SD $\pm$ 3.9 wk) using the n-alkane technique (Mayes et al., 1986) as modified by Dillon and Stakelum (1989). Supplemental concentrate fed at the time of GDMI measurements was added to the GDMI figure to calculate total DMI (**TDMI**). Energy balance (**EB**) was estimated as the difference between energy intake and the sum of the energy required for maintenance and milk production. The French net energy (NE) system was used, where 1 unité fourragère lait (UFL) is the NE content of 1 kg of air-dried barley for milk production (Vermorel, 1989).

Blood Sampling and Laboratory Analysis

In yr 1, blood samples were collected once every 2 wk throughout lactation. In yr 2, blood samples were collected weekly for 3 wk before parturition, twice weekly during the first 4 wk of lactation, once weekly from wk 5 to 9, and once every 2 wk thereafter until the end of lactation. Sampling took place after morning milking and before returning to fresh pasture. Blood samples were collected via coccygeal venipuncture into

Vacutainers containing lithium heparin as an anticoagulant (Becton Dickinson, Plymouth, UK). Samples were placed in a centrifuge for 15 min at $2,000 \times g$, plasma was decanted, and stored at -20°C until further analysis. Blood plasma was analyzed for the metabolites NEFA, BHBA, and glucose concentrations by enzymatic colorimetry (NEFA kit supplied by Wako Chemicals GmbH, Neuss, Germany; BHBA and glucose kits supplied by ABX Mira, Montpellier, France). Plasma insulin concentrations were determined using a solid-phase fluoro-immunoassay (AutoDELFIA; PerkinElmer Life and Analytical Sciences, Turku, Finland), with appropriate kits (Unitech BD Ltd., Dublin, Ireland). Inter- and intra-assay coefficients of variation were 17.5 and 8.5%, respectively. Circulating IGF-I concentrations were quantified using a validated double antibody RIA, following ethanol:acetone:acetic acid extraction as described by Enright et al. (1989). Inter- and intra-assay coefficients of variation were 15.6 and 15.8% respectively. For all hormone assays, each genotype was equally represented in each assay and all samples for a cow of a given genotype were completed in a single assay.

Reproductive Management

The reproductive performance of the herd was monitored in yr 1 during a 20-wk breeding season with a mating start date of April 14. Before the mating start date, all cows greater than 30 DIM were examined using transrectal ultrasonography (7.5-MHz transrectal transducer, Aloka SSD-900; Aloka Ltd., Tokyo, Japan) to determine utero-ovarian status. One cow from each genotype was diagnosed with endometritis, and were treated with an i.m. PGF_{2 α} injection containing 25

mg dinoprost tromethamine (Lutalyse; Pfizer Ireland, Dublin, Ireland), followed by intrauterine cephapirin (Metricure; Intervet International BV, Boxmeer, the Netherlands) 2 to 4 d later after observation of estrus. During the breeding season, heat detection was carried out a minimum of 3 times daily with the aid of tail paint. Cows detected in heat were inseminated with frozen-thawed semen from sires of their own genetic group to generate replacement heifers. For each sire used, semen was from a single ejaculate, and sperm viability and quality was verified before use in the experiment. All inseminations were carried out by a single AI technician during the breeding season. Pregnancy diagnosis was carried out by transrectal ultrasound at 30 to 36 d and again at 60 to 66 d post-insemination to measure the rate of late embryo loss. Final pregnancy status was determined by transrectal ultrasound 80 d after the completion of the breeding season. In yr 2, breeding was delayed to allow collection of biological samples of interest and, hence, no fertility performance indicators were available.

Data Handling

All data handling was carried out using SAS (SAS Institute Inc., Cary, NC). Data were checked for normality. A Box-Cox transformation was used to normalize the distribution of IGF-I, insulin, NEFA, and BHB data. Linear interpolation was used to calculate values for every day of the study for blood metabolites, hormones, and BCS, and the estimated daily values were collapsed into weekly means. Circulating concentrations of blood metabolites, metabolic hormones, and BCS data for each cow were analyzed over the full gestation-lactation cycle [from wk -3 (-6 for BCS) to wk 42]. These data were also divided into 4 time periods [dry period: from wk -3 (-6 for BCS) to wk 0 prepartum; early lactation: from wk 1 to 12 postpartum; midlactation: from wk 13 to 28 postpartum; and late lactation: from wk 29 to 42 postpartum]. Additionally, separate analysis was carried out on blood metabolite and hormone concentration data during wk 1 to 4 of lactation. The week of BCS nadir was determined by identifying the earliest postpartum occurrence of the lowest BCS value recorded during the first 15 wk of lactation.

Study Replicated Using National Herd Data

Data from the Irish national herd were also used to determine whether results from the Fert+/Fert- herd were consistent with on-farm performance records from cows of similar genetic merit. Data on first-parity Holstein dairy cows calving between the years 2006 and 2010 inclusive ($n = 501,922$) were extracted from the

Irish Cattle Breeding Federation database. Herds that were predominantly spring calving, where 80% of cows calved between January 1 and July 31, were retained. Herds with less than 5 cows in any year were removed for that year. Only cows from a known AI sire and AI maternal grand sire ($n = 185,354$) were retained. The pedigree index for each cow was calculated as $0.5 \times \text{sire EBV} + 0.25 \times \text{maternal grandsire EBV}$. To avoid any environmental covariance between cow phenotypic performance and cow pedigree index, the preceding year's official dairy evaluations published by the Irish Cattle Breeding Federation were used to estimate cow pedigree index. Restrictions were placed on cow EBV for milk production (between +256 and +514 kg), calving interval (Fert+: sire < -3.5 , maternal grandsire < -3.5 ; Fert-: sire > 3.5 , maternal grandsire > 2) and proportion of Holstein genes ($> 71\%$) in line with the selection criteria set out for the Fert+ and Fert- cows used in the controlled study. A total of 2,371 Fert- and 4,859 Fert+ first-lactation cows in 2,560 herds were available for inclusion in the analysis. The number of cow records available for each of the 5 yr (2006–2010) were 460, 978, 1,198, 1,801, and 2,793, respectively. An additional 79,408 first-parity herdmates of these animals were included in the analysis for improved estimation of fixed effects, especially contemporary group effects.

Standardized 305-d milk, fat, and protein yields were estimated by area under the curve from d 0 to 305 (Olori and Galesloot, 1999). Milk fat and protein percent was calculated by dividing fat and protein yields by milk yield. The fat-to-protein ratio was calculated by dividing fat yield by protein yield. Calving interval was defined as the number of days from the date of first calving to the date of second calving. Animals that failed to recalve or had calving intervals outside the range of 300 to 800 d were removed from the analysis of fertility variables. Two separate traits were used to describe the survival of an animal to its second lactation: recalving within 365 d of first calving (the target for a seasonal calving system) and recalving within 400 d of first calving (a minimum target for a seasonal calving system).

An algorithm was used to generate contemporary groups for herd-year-season at first calving for milk production and calving interval data separately. The algorithm initially grouped first-parity animals within herd that had a calving date within 10 d of each other. If the number of records within any contemporary group was less than 7, they were combined with the next contemporary group, if the start date of one contemporary group and the end date of the other contemporary group was < 182 d. Only contemporary groups with a minimum of 5 animals were retained. A total of 86,638 animals in 4,290 contemporary groups

remained for inclusion in analysis of milk production data, whereas 49,661 animals in 3,572 contemporary groups remained for inclusion in the analysis of calving interval and survival.

Statistical Analyses

Experimental Herd Data. All statistical analyses were carried out using SAS (SAS Institute Inc.). The effect of genotype on variables with repeated measures, such as weekly production variables (milk yield, milk fat, protein and lactose concentrations, BCS, and BW), GDMI, TDMI, EB variables (yr 1), and blood metabolite and hormone concentrations were determined using mixed models with cow nested within genotype as a random effect. A first-order autoregressive covariance structure with homogeneous variances among weekly records within cow-parity provided the best fit to the data. Transformed data (IGF-I, insulin, NEFA, and BHBA) were used to calculate *P*-values, and the estimated group means and 95% confidence intervals were derived from back-transformed values. The effect of genotype, parity, calving date, year, lactation week, and their interactions were included in the final model where significant ($P < 0.1$). Where an interaction existed between genotype and parity, orthogonal contrasts were used to compare differences between genotypes within parity.

The effect of genotype on continuous variables without repeated measures such as number of services, calving to first service interval (all cows were served at least once), calving-to-conception interval, GDMI, TDMI, and EB variables (yr 2), and selected BCS variables (i.e., BCS at calving, postpartum BCS nadir, week of BCS nadir, and changes in BCS from parturition to nadir and from nadir to end of lactation) were determined using the mixed model with cow nested within genotype as a random effect. The effect of genotype, parity, and the interaction between genotype and parity were tested, calving date and year were included as adjustment variables, and significant effects ($P < 0.1$) were maintained in the final model.

Differences between genotypes for variables with a binomial distribution (conception rate to first service, pregnancy rate to first and second service, submission rate, 42-d pregnancy rate, embryo mortality, and overall pregnancy rates) were tested using the Fisher exact test. The interval from mating start date to conception was used to calculate a Kaplan-Meier estimate of survival function. Log-rank tests were used to compare the resulting survival analysis curves. For illustrative purposes, the proportion of cows pregnant was extrapolated from the survival distribution function for each genotype.

A Wilmink (Wilmink, 1987) exponential model curve was fitted to daily milk yield to generate lactation profiles for each lactation. The Wilmink function is described by the equation $y_t = a + be^{-0.05t} + ct$. In this model, y_t represents daily milk yield at day t of lactation. The a parameter represents the height of the curve and a negative b value and c value represent the rate of increase of daily milk yield during the initial lactation stage and the rate of decline from peak, respectively. Regression parameters were estimated separately for each cow-parity. Residuals of predicted daily milk yield were then calculated as the difference between the predicted and the actual at each day of lactation. The mean square prediction error was calculated as the standard deviation of the calculated residuals.

National Herd Data. Fixed effects linear models were used to quantify the association between genotype and milk production and fertility variables. Herd-year-season contemporary group, proportion of Holstein genes, and age at first calving were included as fixed effects in the model where significant ($P < 0.1$). The association between genetic merit and recalving within 365 d of first calving and recalving within 400 d of first calving was determined using generalized linear mixed models; herd-year-season, age at first calving, and proportion of Holstein genes were included as adjustment variables, where significant ($P < 0.1$).

RESULTS

Milk Production

Mean daily milk production and the parameters for the Wilmink exponential function are summarized in Table 2. During the full lactation, genotype had a significant effect ($P < 0.05$) on milk yield, with Fert+ cows having greater milk yield (19.5 ± 0.02 kg/d) than Fert– cows (18.7 ± 0.02 kg/d). Milk solids yield tended to be greater ($P = 0.09$) in Fert+ cows (Figure 1). Mean milk fat and lactose concentrations did not differ (both $P > 0.05$) between the Fert+ and Fert– cows, but Fert– cows tended to have greater milk protein concentration ($P = 0.07$).

The Wilmink function fit the daily milk yield data well, with a median coefficient of determination value of 0.72. The Fert+ cows tended to have a higher ($P = 0.08$) lactation curve (a parameter) compared with Fert– cows (Figure 1). No effect of genotype or genotype by parity interaction was observed for either the rate of increase to peak milk yield (b parameter), or the rate of decline from peak milk yield (c parameter). Days postcalving when peak daily milk yield occurred did not differ between genotypes (31.9 and 29.0 d for the Fert+ and Fert– cows, respectively).

Table 2. The effect of genetic merit for fertility traits on mean daily milk production performance over the complete lactation

Variable	Genotype ¹			P-value		
	Fert+	Fert–	SED ²	Genotype	Genotype × week	Genotype × parity
Number of animal records	41	39				
Milk yield (kg/d)	19.54	18.69	0.39	0.02	0.2	0.2
Protein (g/kg of milk)	33.2	33.6	0.02	0.07	0.06	0.09
Fat (g/kg of milk)	40.7	41.3	0.05	0.3	0.2	<0.001
Lactose (g/kg of milk)	46.7	46.6	0.01	0.4	0.9	0.3
Milk solids ³ (kg)	1.43	1.39	0.02	0.09	0.7	0.3
Wilmink parameter						
<i>a</i> (peak milk yield)	28.79	27.34	0.91	0.08	—	0.4
<i>b</i> (rate of increase to peak milk yield)	–7.13	–5.83	1.66	0.4	—	0.9
<i>c</i> (rate of decline from peak milk yield)	–0.061	–0.060	0.0038	0.7	—	0.9
DIM to peak ⁴ (d)	31.9	29.0	5.14	0.5	—	0.8

¹Fert+ = good-fertility cows; Fert– = poor-fertility cows.

²SED = pooled standard error of the difference.

³Combined yield of fat and protein.

⁴The day when the Wilmink parameter *a* was estimated.

Reproduction and Fertility

The reproductive performance of the herd was monitored during yr 1 of the study on 36 first-parity cows. The calving period ranged from January 8 to April 14, with the median calving date being February 2 and 9, for the Fert– and Fert+ groups, respectively. The effect of genotype on reproductive performance is summarized in Table 3. No effect of genotype on interval from calving to first service or 21 d submission rate was observed, but the interval from calving to conception was significantly shorter ($P < 0.05$) for Fert+ (85.6 ± 7.0 d) compared with Fert– (113.8 ± 7.8 d) cows. Pregnancy rate to first and second service and the 6 wk in-calf rate tended to be greater ($P = 0.08$ and $P = 0.09$, respectively) for the Fert+ cows. The number of services per cow was fewer ($P < 0.05$) for the Fert+ compared with the Fert– cows over the course of the breeding season. The proportion of cows establishing pregnancy during the course of the breeding season is illustrated in Figure 2, and indicates the difference between genotypes in the interval from mating start date to successful pregnancy establishment (effect of genotype, $P < 0.05$).

Energy Balance, DMI, BCS, and BW

No difference between Fert+ and Fert– cows was observed for GDMI (12.4 and 12.1 kg/d, respectively) or TDMI (15.4 and 15.1 kg/d, respectively) across the 3 measurement time points in yr 1. In addition, no effect of genotype was observed on mean daily EB at the DMI measurement time points (2.87 ± 0.23 vs. 3.09 ± 0.23 UFL/d, Fert+ and Fert–, respectively). Parity had a significant effect ($P < 0.001$) on GDMI in yr

2, being greater in parity 2 (17.5 ± 0.39 kg/d) than parity 1 (12.5 ± 0.24 kg/d) cows. No effect of genotype or genotype by parity interaction was observed for EB or GDMI at the single measurement time point in yr 2. The BCS and BW profiles of both genotypes are illustrated in Figure 3. No differences between genotypes were observed for BW, but Fert+ cows had greater BCS during early ($+0.13$ units, $P = 0.06$), mid ($+0.1$ units, $P < 0.05$), and late ($+0.09$ units, $P < 0.05$) lactation compared with Fert– cows (Table 4). A significant genotype by week interaction for BCS occurred during the gestation-lactation cycle and early lactation time periods. The difference between genotypes was greatest in first-parity cows at calving and during early lactation (both $P < 0.05$). The Fert+ cows tended to have greater ($P = 0.07$) BCS at nadir, but no differences were observed for week of lactation at nadir, or changes in BCS from parturition to nadir and from nadir to end of lactation.

Circulating Concentrations of Blood Metabolites and Metabolic Hormones

No differences between genotypes were observed during the dry period (all $P > 0.05$) for plasma concentrations of metabolites (Table 5). A significant genotype by parity interaction was observed for plasma NEFA concentration ($P < 0.05$) during early lactation; first-parity Fert+ cows had greater circulating concentrations compared with first-parity Fert– cows, but no differences were observed in second-parity cows. Genotype had no effect ($P > 0.05$) on circulating concentrations of NEFA or glucose during the gestation-lactation cycle. During early lactation, Fert+ cows had a greater ($P < 0.01$) mean circulating concentration of BHBA

Figure 1. The mean daily milk yield and milk solids yield profiles of the good-fertility (Fert+) and poor-fertility (Fert-) cows during the full lactation. Vertical bars indicate the pooled standard error of the difference (SED). Top panel: mean daily milk yield was greater ($P < 0.05$) in Fert+ cows (SED = 0.39 kg/d) than in Fert- cows. No genotype $\times$ week or genotype $\times$ parity effects were detected. Bottom panel: Mean milk solids yield tended to be greater ($P = 0.094$) in Fert+ cows (SED = 0.02), whereas no effect of genotype $\times$ week or genotype $\times$ parity interaction was observed.

compared with Fert- cows (0.340 vs. 0.276 mmol/L, respectively), but no differences were detected during mid or late lactation. The effect of genotype on mean plasma concentrations of IGF-I and insulin during the gestation-lactation cycle are illustrated in Figure 4. The Fert+ cows had greater ($P < 0.01$) mean circulating concentrations of IGF-I throughout the study period compared with Fert- cows. No overall effect of genotype was observed on mean circulating concentrations of plasma insulin during the gestation-lactation cycle ($P > 0.05$); however, mean plasma insulin concentrations were greater ($P < 0.05$) in Fert+ (1.71 μ IU/mL) than in Fert- cows (1.24 μ IU/mL) during the first 4 wk of lactation.

Analysis of National Herd Data

Performance of first-parity cows from the Irish national herd ($n = 7,230$) that fit the selection criteria of the Fert+/Fert- herd are summarized in Table 6. A significant ($P < 0.01$) association was observed between genotype and 305-d milk yield, with Fert+ cows having greater milk yield ($5,556 \pm 11.0$ kg) than did Fert- cows ($5,503 \pm 15.9$ kg). No differences ($P > 0.05$) were observed between genotypes for 305-d protein or fat yield, but Fert- cows had greater mean milk fat ($P < 0.05$) and protein ($P < 0.001$) concentration than did Fert+ cows. Fert+ cows took 11.1 d less ($P < 0.001$) to recalve than did Fert- cows. The proportion of cows that re-calved within 365 d (+8%) and within 400 d (+13%) were greater for Fert+ compared with Fert- cows ($P < 0.001$).

DISCUSSION

This study clearly indicates that genetic merit for fertility traits has a profound effect on phenotypic fertility performance. Other factors that are known to affect fertility performance were similar for both groups (herd management, plane of nutrition, proportion of Holstein genes, and genetic merit for milk production traits). Furthermore, production and fertility data for Fert+ and Fert- cows obtained from the Irish national herd database substantiated the findings from the experimental herd. Hence, this unique herd represents a powerful animal model for future fertility research to elucidate the biological mechanisms underpinning differences in fertility without being confounded by milk production.

The superior reproductive performance observed in the Fert+ cows confirms the strong association between genetic merit for fertility traits and the subsequent reproductive performance. In agreement with our findings, Horan et al. (2004) suggested that cows of Holstein or New Zealand Friesian origin that had been selected for superior fertility and survival exhibit superior reproductive performance. Similarly, Coleman et al. (2009) reported differences in pregnancy rate to first service and 6-wk in-calf rate between Holstein cows with a modest 2.46 d difference in EBV for calving interval (12.24 d difference in the current study) but with similar genetic merit for milk yield. These results confirm the contribution of poor genetic merit for fertility traits to suboptimal fertility performance. Incorporation of fertility traits in national breeding programs can reverse these trends (Norman et al., 2009).

An antagonistic relationship between genetic merit for milk production and fertility has been reported in lactating dairy cows (Pryce et al., 2004; Evans et al.,

Table 3. The effect of genetic merit for fertility traits on reproductive performance during a 20-wk breeding season

Variable	Genotype ¹		SED ²	P-value
	Fert+	Fert–		
Number of animal records	18	18		
Mean calving date at first parturition (d)	Feb 15	Feb 09	7.71	0.5
Calving-to-first service interval (d)	74.2	80.1	7.00	0.4
Calving-to-conception interval (d)	85.6	113.8	10.69	0.01
No. of services per cow	1.78	2.83	0.51	0.05
No. of services per pregnancy	1.44	2.23	0.41	0.06
21-d submission rate (%)	83.3	72.2	—	0.7
Conception rate to first service (%)	55.6	33.3	—	0.3
Pregnancy rate to first and second service (%)	83.3	50	—	0.08
42-d pregnancy rate (%)	72.2	41.2	—	0.09
Late embryo mortality (%)	0	11.1	—	0.5
Overall pregnancy rate (%)	88.9	72.2	—	0.4

¹Fert+ = good-fertility cows; Fert– = poor-fertility cows.

²SED = pooled standard error of the difference.

2006a). Cows with high genetic merit for milk yield prioritize the partitioning of nutrients toward milk production, rendering them susceptible to prolonged periods of NEB and BCS loss, resulting in impaired reproductive function (Butler and Smith, 1989). In the current study, genetic merit for milk yield was similar for both groups, but Fert+ cows maintained higher daily milk yield throughout lactation compared with Fert– cow. When daily milk output was expressed as milk solids yield, however, the difference between genotypes was

modest. These results suggest that on a grass-based system, Fert+ cows were more capable of expressing their genetic potential for milk yield. This concurs with Buckley et al. (2003), who reported a positive association between phenotypic milk yield and reproductive performance when genetic merit for milk yield and proportion of Holstein genes were included as adjustment variables in the statistical model. However, a lack of consensus exists within the literature on the association between phenotypic milk yield and dairy cow fertility. No association (Patton et al., 2007; LeBlanc, 2010), a negative association (Nebel and McGilliard, 1993; Sakaguchi, 2011), and a positive association (Buckley et al., 2003; Mackey et al., 2007) between phenotypic milk production and measures of reproductive performance have been reported. Despite these seemingly conflicting results, early lactation EB and associated BCS changes have been consistently demonstrated to affect fertility performance (Roche et al., 2009).

Mobilization of body reserves during early lactation is part of an orchestrated series of adaptations in high-yielding dairy cows to support mammary milk synthesis (Bauman and Currie, 1980). The severity and duration of NEB during early lactation has been reported to influence fertility (Butler and Smith, 1989). As a subjective visual assessment of adipose tissue reserves, BCS has a strong association with NEB and fertility (Butler, 2003). In the present study, early lactation trends in BCS suggested that Fert+ cows maintained a higher threshold BCS level. This occurred despite maintaining a higher milk yield throughout lactation. The difference in BCS levels seen in early lactation were more pronounced during mid and late lactation, when the Fert+ cows maintained a higher BCS level, indicating superior energy status. These findings concur with Berry et al. (2003), who reported that BCS during mid to late

Figure 2. The interval in days from mating start date (MSD) to conception for good-fertility (Fert+) and poor-fertility (Fert–) cows. The y-axis represents the proportion of animals pregnant. The Fert+ cows conceived at a significantly greater rate than did the Fert– cows ($P < 0.05$). The median number of days (95% CI in parentheses) for 50% of the Fert+ cows to conceive was 19 d (13, 38 d), compared with 68.5 d (36, 98 d) for 50% of the Fert– cows to conceive.

Figure 3. The mean BCS and BW profiles of the good-fertility (Fert+) and poor-fertility (Fert-) cows. Vertical bars indicate the pooled standard error of the difference (SED). Top panel: no genotype effect was observed for mean BCS ($P = 0.207$, $SED = 0.065$ units) during the gestation-lactation cycle. A significant genotype $\times$ week interaction ($P < 0.05$) for BCS was observed, with the interaction of genotype $\times$ parity tending toward significance ($P = 0.072$). Bottom panel: no genotype, genotype $\times$ week, or genotype $\times$ parity effect was detected for BW ($SED = 8.96$ kg).

lactation had the strongest genetic relationship with fertility. Roche et al. (2006) concluded that cows with a strong genetic drive to produce milk maintained lower BCS throughout lactation. The ability of the Fert+ cows to produce more milk while also maintaining BCS at a higher threshold would suggest that differences in DMI existed. Neither DMI nor calculated EB were different, however, during the 5 measurement periods throughout lactation. The earliest DMI measurement was recorded, on average, 13 wk postpartum (for yr 1 and 2), and perhaps differences in DMI existed before

Figure 4. Circulating concentrations of IGF-I and insulin during the gestation-lactation cycle of good-fertility (Fert+) and poor-fertility (Fert-) cows. Top panel: plasma IGF-I concentrations were significantly higher in Fert+ cows ($P < 0.01$) throughout the study period. The mean (95% CI in parentheses) circulating IGF-I concentrations were 148.3 (139.2, 157.6) and 128.2 (119.1, 137.7) for Fert+ and Fert-, respectively. No genotype $\times$ week or genotype $\times$ parity interactions were observed for IGF-I. Bottom panel: no effect of genotype or genotype $\times$ parity interaction was observed for plasma insulin concentrations ($P > 0.05$). A significant genotype $\times$ week interaction ($P < 0.05$) for insulin was observed during the study period. The mean (95% CI in parentheses) circulating insulin concentration were 2.89 (2.71, 3.07) and 2.83 (2.64, 3.03) for Fert+ and Fert-, respectively.

this measurement. Further work to measure daily intakes is required to more closely examine DMI and EB relationships in this animal model.

The link between metabolic status and reproductive efficiency involves the complex integration and regulation of endocrine and metabolic signals (Chagas et al., 2007). Alterations in circulating concentrations of blood

Table 4. The effect of genetic merit for fertility traits on mean BCS and BW variables

Variable	Genotype ¹		SED ²	P-value		
	Fert+	Fert–		Genotype	Genotype × week	Genotype × parity
No. of animals records	41	39				
Mean BW (kg)	509.7	515.8	8.96	0.5	0.1	0.4
Mean BCS (units)	2.96	2.88	0.065	0.2	0.04	0.07
BCS early lactation ³ (units)	3.01	2.88	0.076	0.06	0.03	0.02
BCS midlactation ³ (units)	2.84	2.74	0.049	0.03	0.9	0.2
BCS late lactation ³ (units)	2.82	2.73	0.043	0.02	0.7	0.2
BCS at calving ⁴ (units)	3.65	3.55	0.097	0.3		0.003
BCS at nadir (units)	2.63	2.53	0.06	0.07		0.2
Week of nadir (wk)	10.7	10.2	0.87	0.6		0.5
BCS change (units)						
Calving to nadir	1.02	0.98	0.09	0.8		0.08
Nadir to drying off	0.25	0.37	0.12	0.4		0.9

¹Fert+ = good-fertility cows; Fert– = poor-fertility cows.²SED = pooled standard error of the difference.³Early lactation = wk 1 to 12 postpartum, midlactation = wk 13 to 28 postpartum, late lactation = wk 29 to 42.⁴BCS at calving was the BCS measured on the week of calving or less than 2 wk before calving.

metabolites and metabolic hormones, such as IGF-I, insulin, glucose, NEFA, and BHBA have been demonstrated to play important roles in the dialog between nutritional status and the reproductive axis (Butler, 2000, 2003; Leroy et al., 2008). In the current study, genetic merit for fertility traits had no effect on circulating concentrations of glucose or NEFA, but plasma concentrations of BHBA were higher during early lactation in Fert+ cows. In support of these findings, Patton et al. (2007) found concentrations of plasma NEFA and glucose measured during the first 4 wk of lactation to have no association with calving to ovulation interval or conception rate. A possible reason for the lack of as-

sociation between blood metabolite concentrations and measures of reproductive performance in the current study is that levels of NEFA, BHBA, and glucose were within critical thresholds for healthy cows. Outside these thresholds, the likely development of postpartum metabolic disorders has been shown to have detrimental effects on fertility (Nydham et al., 2009).

The uncoupling of the somatotrophic axis in early lactation is a characteristic of high-producing cows and occurs when the liver becomes unresponsive to growth hormone and hepatic IGF-I output is decreased (Lucy, 2001). The observed differences in circulating IGF-I concentrations in the current study indicate a genetic

Table 5. The effect of genetic merit for fertility traits on mean circulating metabolite concentrations¹

Variable	Genotype ²		P-value		
	Fert+	Fert–	Genotype	Genotype × week	Genotype × parity
Dry period (wk –3 to 0)					
NEFA (mmol/L)	0.157 (0.119, 0.206)	0.155 (0.119, 0.204)	0.9	0.8	0.07
BHBA (mmol/L)	0.231 (0.188, 0.281)	0.190 (0.154, 0.232)	0.2	0.9	0.06
Glucose (mmol/L)	2.84 (2.51, 3.16)	2.77 (2.44, 3.10)	0.8	0.4	0.3
Early lactation (wk 1–12)					
NEFA (mmol/L)	0.341 (0.290, 0.389)	0.320 (0.269, 0.379)	0.9	0.9	0.06
BHBA (mmol/L)	0.340 (0.304, 0.379)	0.276 (0.243, 0.312)	0.009	0.2	0.3
Glucose (mmol/L)	2.68 (2.49, 2.88)	2.65 (2.44, 2.86)	0.8	0.5	0.06
Midlactation (wk 13–28)					
NEFA (mmol/L)	0.108 (0.094, 0.124)	0.109 (0.094, 0.127)	0.9	0.9	0.08
BHBA (mmol/L)	0.275 (0.243, 0.31)	0.247 (0.216, 0.282)	0.2	0.7	0.4
Glucose (mmol/L)	2.57 (2.37, 2.77)	2.59 (2.37, 2.8)	0.9	0.7	0.1
Late lactation (wk 29–42)					
NEFA (mmol/L)	0.077 (0.067, 0.088)	0.068 (0.058, 0.080)	0.2	0.06	0.3
BHBA (mmol/L)	0.304 (0.264, 0.349)	0.264 (0.223, 0.312)	0.2	0.4	0.3
Glucose (mmol/L)	2.58 (2.36, 2.80)	2.49 (2.24, 2.74)	0.6	0.5	0.2

¹Geometric mean (95% CI).²Fert+ = good-fertility cows; Fert– = poor-fertility cows.

Table 6. The effect of genetic merit for fertility traits on milk production and reproductive performance within the Irish national herd during the years 2006 to 2010

Variable	Genotype ¹		SED ²	P-value
	Fert+	Fert–		
Milk production ³				
No. of animal records	4,859	2,371		
305-d milk yield (kg)	5,556	5,503	19.3	0.007
305-d protein yield (kg)	188.8	189.2	0.61	0.5
305-d fat yield (kg)	219.4	218.3	0.75	0.1
Protein (g/kg of milk)	34.1	34.5	0.01	<0.001
Fat (g/kg of milk)	39.9	40.1	0.01	0.04
Fat-to-protein ratio	1.17	1.16	0.003	0.003
Reproductive performance ⁴				
No. of animal records	2,436	1,388		
EBV for calving interval ⁵ (d)	–5.6 (1.21)	5.2 (1.44)		
Calving interval (d)	391.6	402.6	2.65	<0.001
Recalve within 365 d (%)	41	33	1.6	<0.001
Recalve within 400 d (%)	77	64	1.6	<0.001

¹Fert+ = good-fertility cows; Fert– = poor-fertility cows.

²SED = pooled standard error of the difference.

³Milk production data available for 2006 to 2010 inclusive.

⁴Reproductive performance data available for 2006 to 2009 inclusive.

⁵Standard deviation in parentheses.

effect in the regulation of the somatotrophic axis. The ability of the Fert+ cows to maintain greater circulating concentrations of IGF-I is consistent with a large body of evidence linking increased IGF-I with improved reproductive outcomes (Taylor et al., 2004). Contrary to previous findings, the observed differences in circulating plasma IGF-I concentrations were independent of alterations in nutrition, Holstein ancestry, milk production, or genetic merit for milk production (Lucy et al., 2009), highlighting for the first time the effect of genetic merit for fertility traits on key components of the somatotrophic axis. The precise mechanisms by which Fert+ cows maintained greater concentrations of IGF-I warrant further examination.

Insulin plays a central role in the metabolism of body tissues, acting as an indicator of energy status. In the present study, plasma insulin concentrations were greater for the Fert+ cows during the first 4 wk of lactation. In studies where early postpartum dairy cows were subjected to a hyperinsulinemic-euglycemic clamp infusion, it was shown that elevated circulating insulin concentrations directly affected dominant follicle estradiol output (Butler et al., 2004), and the recoupling of the somatotrophic axis through increasing hepatic GHR 1A and IGF-I mRNA expression (Butler et al., 2003). Elevated plasma insulin concentrations as a result of dietary manipulation during the first 50 to 100 d postpartum resulted in shorter intervals from calving to ovulation and from calving to conception, and increased conception rate (Gong et al., 2002). In vitro studies have demonstrated that increased insulin concentrations stimulate granulosa cell proliferation and

progesterone steroidogenesis (Spicer and Echternkamp, 1995). The early rise in plasma insulin concentrations observed in Fert+ cows during the immediate postpartum period could be important in the regulation of the somatotrophic axis and reproductive events in the postpartum period.

CONCLUSIONS

Genetic merit for fertility traits had a significant effect on reproductive efficiency in the current study. The Fert+ group had an improved calving-to-conception interval, fewer services, and conceived more quickly after breeding began than did the Fert– group. The observed differences in reproductive performance were independent of management, plane of nutrition, proportion Holstein ancestry, genotypic and phenotypic milk yield, and blood metabolite concentrations of NEFA and glucose. The Fert+ cows maintained greater BCS and circulating concentrations of IGF-I throughout lactation and had greater circulating concentrations of insulin during the first 4 wk of lactation. This supports the premise that animals with superior fertility are less reliant on body reserves for milk synthesis, thus reducing the degree to which the somatotrophic axis is uncoupled. These results highlight the effect of genetic merit for fertility on phenotypic reproductive performance, which may not necessarily be to the detriment of milk production.

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