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## Calf and heifer development and the onset of puberty in dairy cows with divergent genetic merit for fertility

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### Abstract

A research herd with divergent genetic merit for fertility was established to better understand the underlying drivers of fertility in dairy cattle. This paper describes the establishment of this herd, heifer growth and development, and the effect of divergence in fertility breeding value (FBV) on the timing of puberty. The average FBV of the high and low fertility groups of heifers was +5.6 and -4.6, respectively. The heifers were weighed fortnightly, with body condition score (BCS) and stature recorded at six, nine, 12 and 15 months of age. Weight and age at puberty were defined as the first day when plasma progesterone was >1 ng/ml in two of three consecutive once weekly samples. There was no effect of FBV on live weight (LWT), BCS, nor stature by age as the heifers matured. High-fertility heifers attained puberty at a lighter LWT (271 vs. 296 kg; SED 4.3 kg), an earlier age (358 vs. 379 days; SED 6.0 days) and a lower proportion of estimated mature LWT (51 vs. 55%; SED 0.7%), compared with Low-fertility heifers. Therefore, the premise that high genetic merit for fertility is positively associated with reaching puberty earlier is supported. Further work is required to support and assess the value of incorporating a puberty trait into the FBV.

**Keywords:** growth; puberty; genetic merit; fertility

### Introduction

High reproductive performance contributes to the productivity and sustainability of seasonal dairy production systems (Verkerk 2003). In New Zealand, genetic merit for fertility is expressed as the estimated fertility breeding value (FBV) and measured using eight predictor traits. The predictor traits are whether cows are mated within 21 days of the planned start of mating (PM21) in first, second and third parity cows (expressed as a binomial trait); calved in the first 42 days after the planned start of calving (CR42) in second, third and fourth parity cows (as a binomial trait); milk volume in a cows' first lactation and body condition score (BCS) in a cows' first lactation at 60 days in milk (DairyNZ, 2016a).

Improvements in estimating the genetic component of fertility are required, as gains in the accuracy of the evaluations would advance the rate of genetic gain and provide on-farm benefits in animal production and farm profitability. Moderate correlations between heifer and cow fertility traits are reported for cows both in New Zealand and internationally (0.38 to 0.66; Pryce et al. 2007; Liu et al. 2008; Tiezzi et al. 2012; Berry et al. 2014; Bowley et al. 2015; Amer et al. 2016). Therefore, heifer fertility traits potentially provide an avenue to achieving greater gains in cow fertility and allows selection on fertility traits within heifers, where a surplus exists. To explore such opportunities, a unique herd of Holstein-Friesian (HF) female dairy cattle with divergent FBV (high and low) has been established, while keeping other traits such as milk production and LWT equivalent. The objectives here are: firstly, to describe the establishment of the herd, secondly, to describe the growth of the heifers, and thirdly, to evaluate

the effect of selection for high and low FBV on the timing of puberty.

### Materials and methods

#### *Contract mating and calf collection*

This work was undertaken with animal ethics approval (Ruakura AE approval #13574 and modification approval #2096). A targeted breeding programme was used to select high and low FBV cows with at least 14/16<sup>th</sup> HF breeding among commercial dairy herds. Dams were contracted by June 2014 (high FBV n=1299, low FBV n=1483), from which 919 high and 855 low FBV dams were expected to calve to contracted sire inseminations.

A bull-allocation plan was generated for all cows, which limited the inbreeding co-efficient to <12%, produced a calf of  $\geq 15/16$ <sup>th</sup> HF breeding, and allocated semen for three inseminations per dam, and aimed to achieve a separation of at least 10 units in FBV between the high and low FBV progeny. In addition, the plan aimed to ensure overlap among the groups and constrain the group average breeding values (BV) within the larger of the two SDs for milk volume, fat, protein, liveweight and ancestry (% North American HF). The mating events occurred between Oct and Dec 2014.

#### *Calf collection and parentage verification*

A total of 640 heifer calves were collected between June and Sept 2015 following farmer-reported birth of a heifer calf to a contract-mated cow. The mean date of birth was 3 Aug (range 6 July to 10 Sept) for the high and 7 Aug (range 25 June to 24 Sept) for the low FBV group. The average age at collection was 9 days (SD 5.4 days) for the

high and 8 days (SD 4.4 days) for the low FBV calves. The calves were collected from 379 herds, with 52% from the Waikato, 20% from Taranaki, 13% from the Bay of Plenty, and 15% from the Wellington and Hawkes Bay regions of the North Island.

A tissue sample (ear notch) was collected from every calf and their dam at the time of collection to verify parentage of both the calf and dam via DNA testing (Genemark, LIC, Hamilton, New Zealand). Calves were only retained for heifer rearing where the sire and maternal grandsire were of consistent genetic merit for fertility (low being negative, and high being positive FBV).

#### *Calf and heifer rearing*

Calves were reared for a 13-week period within a common environment at a single calf rearing facility. On arrival, calves were placed in indoor pens with eight others, and received milk once daily (allocated 5 L per day) and calf meal *ad libitum* for seven weeks. Calves were then transferred to larger mobs of 30 to 40 calves outdoors where they were fed pasture, pasture silage and meal (*ad libitum*) until to weaning. The high and low FBV heifers were relocated to a grazing property at around 95 days of age. There, they were managed in four age-based mobs

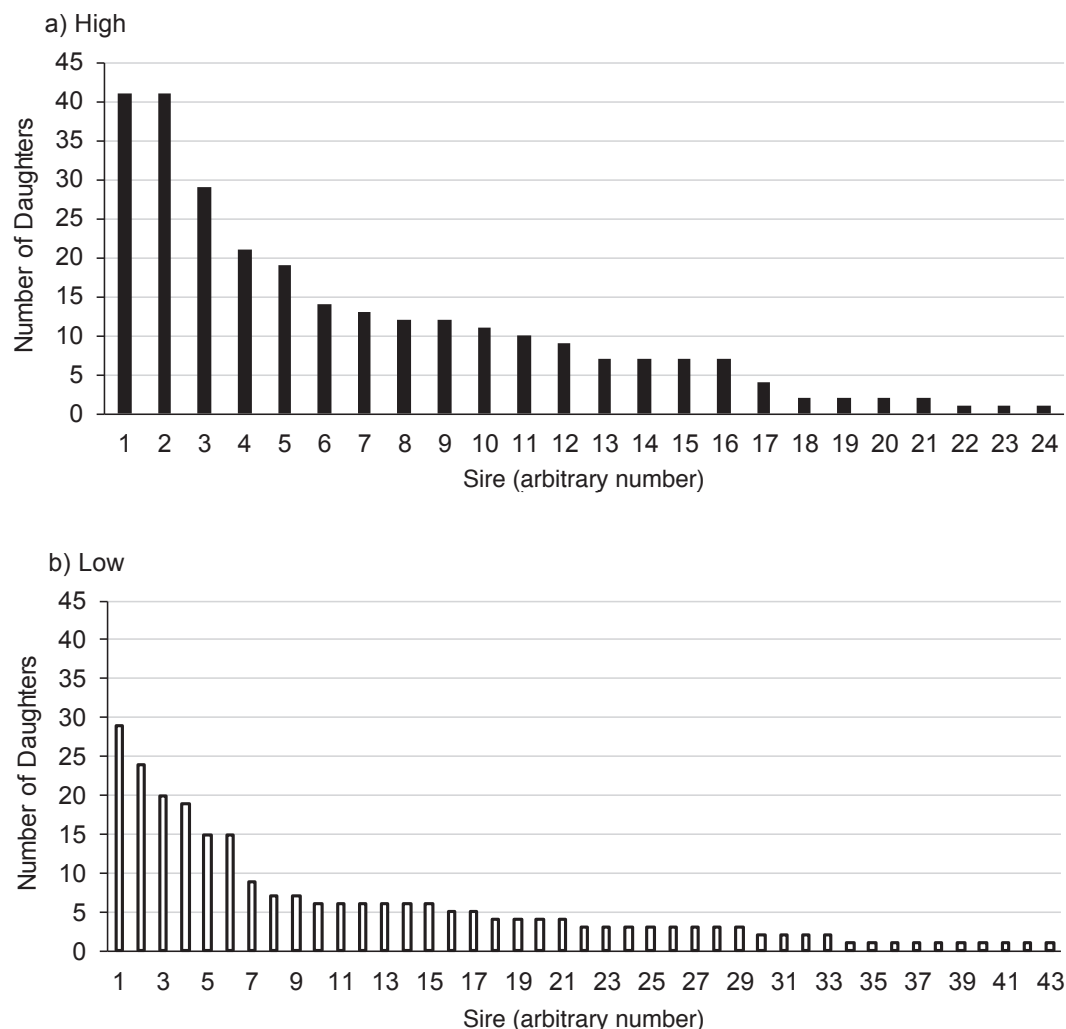
of approximately 130 to 150 heifers per mob to achieve industry LWT targets (DairyNZ, 2016b). Heifers were fed predominantly on ryegrass pasture, with the sward including kikuyu and chicory. Supplementary feed (palm kernel extract and pasture bailing/silage) was used by the grazier as required to achieve targeted weight gains.

As of January 2017 a total of 524 heifers were in the research herd (high FBV n=275, low FBV n=249). A total of 116 (high FBV n=49, low FBV n=67) heifers were removed. The reasons for removal were; failed parentage (high FBV n=35; low FBV n=40); poor conformation and freemartin (high FBV n=2, low FBV n=6); poor health (high FBV n=6, low FBV n=7) and deaths (high FBV n=6, low n=14). The remaining heifers were sired by 24 high ( $11 \pm 11.5$  daughter per sire [mean  $\pm$  SD]; range of between 1 to 41 daughters per sire) and 43 low FBV sires ( $6 \pm 6.5$  daughters per sire; range of between 1 to 29 daughters per sire; see Figure 1). A summary of the Breeding Worth, Breeding Values (BV) for key traits and ancestry of the heifers present in January 2017 are presented in Table 1.

#### *Weight, body condition and stature*

Calves were first weighed at 9 days of age, or the day after arrival for those calves >9 days of age at collection

**Figure 1** Daughters per sire for heifers bred to have a) high and b) low genetic merit for fertility



**Table 1** Description of the genetic merit of the high and low fertility heifers including the estimated Fertility Breeding Value, Breeding Worth, the remaining estimated Breeding Values (BV), Production Worth and Ancestry (estimates are from the Feb 2016 Animal evaluation run). Estimates are parent averages and are presented as the mean and standard deviation (SD).

Item	Genetic merit for fertility <sup>1</sup>			
	High	SD	Low	SD
Heifers (n) <sup>2</sup>	275	-	249	-
Fertility BV	5.6	0.72	-4.6	1.34
Breeding Worth	156	21.4	89	29.7
Production Worth	108	25.5	131	30.6
Volume BV	746	164.5	805	158.7
Fat BV	18.2	5.06	24.1	6.63
Protein BV	25.6	3.75	28.5	4.75
Liveweight BV	34.7	11.78	37.7	10.45
Body condition score BV	0.05	0.058	-0.08	0.067
Gestation length BV	-3.2	2.07	-1.4	2.23
Residual survival BV	37	55.7	33	72.8
Total longevity BV	361	39.0	156	79.8
Somatic cell count BV	-0.06	0.133	0.13	0.169
Ancestry (North American %)	56	6.2	62	8.4

<sup>1</sup> The aim was to generate two groups of calves with at least a 10-unit separation in Fertility BV, and to ensure overlap among the groups and constrain the group average breeding values (BV) within the larger of the two SDs for; milk volume, fat, protein, live weight, and ancestry (% North American HF). <sup>2</sup> Current heifers as of January 2017.

(14 ± 6.8 days of age; [mean ± SD]), with the mobs weighed fortnightly subsequently. Body condition (1 to 10 scale (Roche et al. 2007)) and stature were measured at approximately six, nine, 12 and 15 months of age. Stature comprised measures including height at the withers, girth at the shoulder, and length from the shoulder to tail-head.

#### *Plasma sampling, progesterone analyses and puberty variables*

Once-weekly blood sampling for determination of plasma progesterone concentrations was initiated when heifers reached 190 to 200 kg and continued until puberty was confirmed or until the 27th October 2016. Blood was sampled via a coccygeal vessel into vacutainers containing lithium heparin, and stored in iced water. The samples were transported to the laboratory at the end of the sampling day and centrifuged for plasma harvest. Plasma was stored in duplicate aliquots at -20°C.

A commercial, double antibody radioimmunoassay kit was used to determine plasma progesterone concentrations in accordance with the manufacturer's instructions (ImmuChem Progesterone Double Antibody RAI, MP Diagnostics, USA) but with the reagents and samples/standards were halved in volume. The inter- and intra-assay coefficients of variation for a high standard were both 8%, and for the low standard they were 14% and

10% respectively (n=25 assays). The minimal detectable concentration was 0.18 ng/ml.

We defined achievement of puberty when progesterone was >1 ng/ml in at least two of three consecutive once-per-week blood samples, with timing of onset of puberty defined as the date when the first of these samples was >1 ng/ml (McNaughton et al. 2005).

The puberty variables estimated were; age at puberty, LWT at puberty (based on the daily weight gain during that fortnightly period that was multiplied by the days since the last weight measure and added to the weight at the beginning of the fortnightly period), and the percentage of expected mature LWT at puberty (calculated by dividing the LWT at puberty by the estimated mature cow LWT using the industry standard estimate of 500 kg plus the liveweight BV for that individual (DairyNZ, 2016c).

## Statistical analysis

### *Stature, body condition and live weight*

Variables measured at six, nine, 12 and 15 months were analysed as repeated measurements in GenStat 17.1 (VSN International 2014) using random coefficient regression (quadratic) and including the fixed effects of fertility group, age expressed as linear and quadratic and the interaction of fertility group with the linear and quadratic age effects and the random effects of sire, herd (of origin), mob, animal, and linear and quadratic age effects nested within animal.

Each variable was then analysed at each measurement time (>14 days, relocation to grazier (95 days) and six, nine, 12 and 15 months) using mixed models that included the fixed effects of age as a covariate along with fertility group, herd (of origin), sire, mob (for measurements at the grazier) and animal were fitted as random effects. LWT data used at six, nine, 12 and 15 months were restricted to measures no more than seven days away from the actual age of the individual.

### *Puberty*

Analyses of time to puberty were restricted to the current population (high n=275, low n=249), as identified in Table 2. The CENSOR procedure in GenStat 17.1 (VSN International 2014), including fertility group as the treatment factor and date of birth fitted as a covariate, was used to obtain estimates for censored data for age, LWT, and proportion of mature LWT at puberty. These actual and censor-estimated data were then analysed using mixed models that fitted sire and herd as random effects and fertility group as a fixed effect.

Proportions analyses using generalised linear models in GenStat 17.1 were used to evaluate the differences in the proportions that reached puberty by the time of mating and at the end of progesterone sampling.

## Results

### *Stature, body condition and weight*

The high FBV calves were 1.4 kg lighter at 4 to 14 days of age (P=0.03), and 2 kg lighter (P=0.05) at the time

**Table 2** Weight, girth, length, height, and body condition as calves and as six, nine, 12 and 15 month old heifers<sup>1</sup>, and the puberty measures for the animals with high and low genetic merit for fertility.

Item	Age / Timing	Genetic merit for fertility				
		High	Low	SED	P value <sup>a</sup>	P value <sup>b</sup>
Weight (kg)	< 14 days <sup>2</sup>	44.5	45.9	0.64		0.03
	Prior to transport to grazier (95 days) <sup>3</sup>	96.3	98.4	1.07		0.05
	6 months	149	151	1.8	<0.01	0.36
	9 months	220	221	2.4		0.57
	12 months	273	277	2.8		0.26
Girth (cm)	15 months	352	357	3.1		0.09
	6 months	124	124	0.4	0.35	0.49
	9 months	139	139	0.5		0.74
	12 months	150	151	0.5		0.32
Length (cm)	15 months	165	166	0.5		0.21
	6 months	91	91	0.5	0.17	0.25
	9 months	101	101	0.4		0.22
	12 months	107	106	0.5		0.25
Height (cm)	15 months	117	117	0.5		0.76
	6 months	97	97	0.4	0.91	0.92
	9 months	106	106	0.4		0.84
	12 months	113	113	0.4		0.87
Body condition	15 months	119	120	0.42		0.35
	6 months	4.8	4.8	0.04	0.76	0.71
	9 months	5.0	5.0	0.03		0.92
	12 months	5.2	5.2	0.04		0.86
	15 months	5.4	5.4	0.03		0.62

<sup>a</sup> repeated measures analyses including 6, 9, 12, 15 P value for FBV interaction with Linear age (interactions of FBV Group with time); <sup>b</sup> P value for FBV group (high vs low). <sup>1</sup> Data is limited to those animals where the measure was within seven days of six, nine, 12 and 15 months of age (minimum numbers of animals included at each time point being 274 for the high and 247 for the low FBV groups). Those outside the seven days were excluded. <sup>2</sup> Data from 239 high and 236 low FBV heifers had a live weight measure prior to 14 days of age and included in the analysis. Those excluded were collected at >14 days of age, a reflection of the variation in when farmers reported calving events and the collection of calves. Heifers collected after 14 days of age were excluded. <sup>3</sup> Mean age of transport to grazier; high FBV 95 days (SD 2.5 days; n=275); low FBV 95 days (SD 3.5 days; n=249).

of relocation to the grazier (approximately 95 days of age; Table 2). An interaction ( $P < 0.01$ ) between FBV group and time, was detected, reflecting the 5 kg lower ( $P = 0.09$ ) LWT in the high compared with the low FBV heifers at 15 months of age (Table 2). There was no fertility group effects nor interaction of fertility group with age evident for heifer girth, length, height or body condition (Table 2).

### Puberty

High FBV heifers attained puberty at a lighter (271 vs. 296 kg; SED 4.3 kg), and an earlier age (358 vs. 379 days; SED 6.0 days), compared with low FBV heifers ( $P < 0.01$ ). The high FBV heifers also attained puberty at a lower percentage of estimated mature LWT compared with the low FBV heifers (51% vs. 55% of estimated mature LWT; SED 0.7%;  $P < 0.01$ ).

More ( $P < 0.01$ ) of the high FBV group had reached puberty by the planned start of mating date (4 Oct 2016); 93% (255/275) vs. 77% (192/249; SED 3.1%) of the low FBV heifers. After three weeks of mating, 99% (271/275) of high and 88% (220/249; SED 2.2%;  $P < 0.01$ ) of low FBV heifers had reached puberty.

### Discussion

This is the first report that we are aware of that determined whether genetic merit for fertility of dairy cows, which is primarily based on reproductive phenotypes collected during the first to third lactation, is associated with the timing of puberty. The most notable finding was that puberty occurred at a lighter LWT, and earlier age, in the high compared with the low FBV group. Additionally, the high FBV group achieved sexual maturity at a lower percent of mature LWT, and not unexpectedly, more high FBV heifers had reached puberty by the start of mating. Considering that there were no differences in stature development, BCS nor LWT at six, nine, 12 nor 15 months of age, between high and low FBV heifers, we conclude that the link between puberty and genetic fertility is based on differential responses to cues for sexual maturity driven by body development.

Time to puberty in the current study is consistent with other reports for the HF breed managed under pasture-based, seasonal systems. Macdonald et al. (2007) reported that HF heifers with a 1990's-type genotype and  $\geq 85\%$  NZ ancestry achieved puberty, on average, at a LWT of 253 kg at 356 days of age and those with  $< 15\%$  NZ ancestry ( $> 85\%$  North American ancestry achieved puberty at a

LWT of 274 kg at 373 days. The pattern of puberty onset in the previous reported study is similar to that in the current study. Macdonald et al. (2007) also reported differences in the LWT and age at puberty among three divergent strains of HF, subsequently, to be linked with divergence in fertility (Macdonald et al. 2008). In fact, the comparative differences between the high and low FBV heifers in the age and LWT at puberty of the current study to those of the different HF Strains, are remarkably similar.

Heifer growth targets aim for 60% of their mature LWT by first mating, at 15 months of age (DairyNZ, 2016b). Heifers that reach this target are more likely to have attained puberty (McNaughton, 2003; Macdonald et al. 2005). Based on the progesterone definition of puberty used we would expect fewer low FBV heifers to have reached these target LWT as fewer had reached puberty by the start of mating (98% vs. 76% for the high and low FBV heifers). However, as a group the estimated percent of mature LWT at 15 months of age is 65-66% (calculate as the liveweight BV + 500 kg / mean LWT at 15 month of age; high FBV 352 kg / 535 kg = 65%; low FBV 357 kg / 538 kg = 66%). These data support the industry targets, as targets that are conservative and allow for a margin of error, which if reached by individual animals would have most heifers reach puberty prior to mating. Whether the current data is used to review and revise the industry targets remains to be considered.

As more High FBV heifers reached puberty by the start of mating, it may be expected that these heifers will conceive and calve earlier, which would lead to better fertility subsequently (Pryce et al. 2007). Earlier calving provides a greater opportunity to recover from calving, ovulate and then become pregnant early. Additionally, heifers with an earlier onset of puberty may also have a shorter postpartum anoestrous period, as the reproductive endocrine events that occur at puberty are similar to the events that control postpartum anoestrus. Further work is planned to test the hypotheses including that heifers achieving an early onset of puberty will also have shorter postpartum anoestrous intervals, and that this association will confer an advantage for subsequent reproductive outcomes.

As improvements in estimating the genetic component of fertility are sought, puberty traits are strong candidates to improve the accuracy and, potentially, advance the rate of genetic gain for fertility. In particular, puberty information is obtained approximately one year earlier than first calving date, and two years earlier than second calving date in the daughters of young bulls being assessed based on the performance of their daughters for the first time. A puberty trait for the future that could be easily and efficiently collected on large numbers of heifers should improve the rate of genetic progress for fertility in the New Zealand dairy herd. One choice may be the use of oestrus activity (via KAMAR or tailpaint recording) if blood sampling is deemed inappropriate. Limitation of this work include the use of single bred and use of two rearing environments (a single calf rearer and grazier). Future work

should investigate breed and environmental effects on the genetics of puberty. Which would in turn support, further work assessing the value of incorporating a puberty trait into the FBV.

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