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Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits

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ABSTRACT

This study investigated the hypothesis that dairy heifers divergent in genetic merit for fertility traits differ in the age of puberty and reproductive performance. New Zealand's fertility breeding value (FertBV) is the proportion of a sire's daughters expected to calve in the first 42 d of the seasonal calving period. We used the New Zealand national dairy database to identify and select Holstein-Friesian dams with either positive (POS, +5 FertBV, n = 1,334) or negative FertBV (NEG, −5% FertBV, n = 1,662) for insemination with semen from POS or NEG FertBV sires, respectively. The resulting POS and NEG heifers were predicted to have a difference in average FertBV of 10 percentage points. We enrolled 640 heifer calves (POS, n = 324; NEG, n = 316) at 9 d ± 5.4 d (± standard deviation; SD) for the POS calves and 8 d ± 4.4 d old for the NEG calves. Of these, 275 POS and 248 NEG heifers were DNA parent verified and retained for further study. The average FertBV was +5.0% (SD = 0.74) and −5.1% (SD = 1.36) for POS and NEG groups, respectively. Heifers were reared at 2 successive facilities as follows: (1) calf rearing (enrollment to ~13 wk of age) and (2) grazier, after 13 wk until 22 mo of age. All heifers wore a collar with an activity sensor to monitor estrus events starting at 8 mo of age, and we collected weekly blood samples when individual heifers reached 190 kg of body weight (BW) to measure plasma progesterone concentrations. Puberty was characterized by plasma progesterone concentrations >1 ng/mL in at least 2 of 3 successive weeks. Date of puberty was defined when the first of these samples was >1 ng/mL. Heifers were seasonally bred for 98 d starting at ~14 mo of age. Transrectal ultrasound was used to confirm pregnancy and combined with activity data to estimate breeding and pregnancy dates. We measured BW every 2 wk, and body condition and stature at 6, 9, 12, and 15 mo of age. The significant FertBV by day interaction for BW was such that the NEG heifers had increasingly greater BW with age. This difference was mirrored with the significant FertBV by month interaction for average daily gain, with the NEG heifers having a greater average daily gain between 9 and 18 mo of age. There was no difference in heifer stature between the POS and NEG heifers. The POS heifers were younger and lighter at puberty, and were at a lesser mature BW, compared with the NEG heifers. As a result, 94 ± 1.6% of the POS and 82 ± 3.2% of the NEG heifers had reached puberty at the start of breeding. The POS heifers were 20% and 11% more likely to be pregnant after 21 d and 42 d of breeding than NEG heifers (relative risk = 1.20, 95% confidence interval of 1.03–1.34; relative risk = 1.11, 95% confidence interval of 1.01–1.16). Results from this experiment support an association between extremes in genetic merit for fertility base on cow traits and heifer reproduction. Our results indicate that heifer puberty and pregnancy rates are affected by genetic merit for fertility traits, and these may be useful phenotypes for genetic selection.

Key words: puberty, pregnancy, heifer, genetic, fertility

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INTRODUCTION

Before 2000, both the phenotypic reproductive performance and the genetic merit for fertility traits of lactating dairy cows were declining (Berry et al., 2014; Pryce et al., 2014). This decline led many dairy genetics organizations to extend breeding objectives to include fertility traits (Miglior, 2002; Miglior et al., 2005; Harris et al., 2006; Egger-Danner et al., 2015). As a result, genetic merit for fertility traits has been improving, with albeit modest yearly gains estimated at <0.2 percentage points (Pryce et al., 2014).

Under traditional, pedigree-based genetic evaluation approaches, the rate of genetic gain in fertility can be accelerated by increasing the accuracy and volume of phenotypic data, as well as finding novel and earlier genetically correlated traits from the progeny of sires. Opportunities such as increasing the accuracy of existing industry data may be implemented quickly, but promise only modest gains. Whereas, incorporation of new fertility traits that can be evaluated earlier or that have a greater heritability are expected to produce greater gains (Berry et al., 2014; Carthy et al., 2014; Bowley et al., 2015; Jenkins et al., 2016). For these reasons, interest in the evaluation of novel fertility traits such as resumption of cycling postpartum, estrus behaviors, and pregnancy loss is increasing (Petersson et al., 2007; Bamber et al., 2009; Berry et al., 2014; Fleming et al., 2015; Lucy, 2019). These novel measures may be the next generation of traits that increase genetic gain in fertility, leading to further improvements in cow reproductive performance.

A strong candidate trait that increases the rate of genetic gain in fertility should be determined earlier than those in current use, have a heritability greater than current traits, and be positively correlated with the key outcomes being selected for, such as pregnancy rate. Traits of interest could include the age at puberty and heifer pregnancy rate. These candidate traits are measured before calving-related trait and have a greater heritability than traditional reproductive traits captured after calving (Morris et al., 2000, 2011). Additionally, phenotypic and genetic correlations between heifer traits and subsequent fertility suggest that heifers that calve early have better fertility as cows (Pryce et al., 2007; Tiezzi et al., 2012), indicating that selection for heifer fertility traits could result in better cow fertility. Additionally, Wathes et al. (2014) identified a positive relationship between heifer reproductive performance (e.g., age at first calving) and subsequent calving interval. Yet, other studies have reported weak or no genetic or phenotypic association between heifers and cow fertility (Raheja et al., 1989; Mion et al., 2019).

To identify candidate traits that accelerate the rate of genetic gain for fertility, we wanted to understand the underlying biological differences between heifers with high and low values for New Zealand's fertility breeding value (**FertBV**). We generated a unique population of genetically divergent animals that represented a research resource to support the evaluation of traditional and novel measures related to cow fertility and reproduction. Previous research investigated phenotypic differences of cows that were divergent in genetic merit for fertility traits, identifying differences in the timing of conception, conception, luteal and follicular function, uterine health, and the somatotropic axis in cows with positive or negative genetic merit for fertility traits (Cummins et al., 2012a,b,c; Moore et al., 2014). Another study identified heifers based on genomic selection for heifer conception rate and daughter pregnancy rates in the United States, with these authors reporting a range of heifer traits (Veronese et al., 2019a,b). In the current study, we report evaluations on heifer calves through to their first successful breeding period. Specifically, we hypothesized that the onset of puberty and reproductive performance would differ for heifers of high and low FertBV. A secondary hypothesis was that no difference in the growth and development of the heifers with high and low FertBV would be observed. To test these hypotheses, we measured the age at puberty, the pregnancy outcomes during their first breeding season, and the heifers' growth and development.

MATERIALS AND METHODS

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved this study and all manipulations (AE application #13574).

Establishing the Research Herd

The process for dam selection, contract breeding, and calf collection is depicted in Supplemental Figure S1 (http://dx.doi.org/10.17632/343t97cpdr.2)and described herein.

Breeding Strategy. We used a customized, seasonal breeding strategy between October and November 2014 to produce heifer calves with a predicted high (**POS**, +5%) and low (**NEG**, −5%) genetic merit for fertility traits via assortative breeding between parents with POS and NEG estimated FertBV as defined by the New Zealand national genetic evaluation scheme (evaluation run Feb 2014). The FertBV is expressed as the percentage of a sire's daughters that are predicted to calve in the first 42 d of the calving season. Therefore, a FertBV of +5 equates to 5% more daughters calving in the first

42 d of the calving season, and −5 equates to 5% fewer daughters calving in the first 42 d compared with a 0 FertBV. At the time, the FertBV was estimated from 8 predictor traits as follows: presented for breeding within 21 d of the planned start of seasonal breeding in lactations 1, 2 and 3; recalving in the first 42 d after the planned start of seasonal calving in lactations 2, 3, and 4; and milk volume and BCS at 60 DIM in the cow's first lactation (DairyNZ, 2018).

We generated a breeding plan that was targeted to produce the desired difference in the FertBV of the offspring, and for the criteria set using the MateSel (Kinghorn, 2011). This approach modeled the mating outcomes and produced a customized breeding strategy that could achieve the 10-percentage point difference in FertBV in the offspring. Additionally, the customized breeding strategy was designed to limit the inbreeding coefficient of the offspring with the average inbreeding coefficient of 2.8 ± 1.44% (mean ± SD, target <6.3%). It also aimed to limit the expected parent averages for milk volume breeding value (**BV**), fat BV, protein BV, BW BV (DairyNZ, 2018), and ancestry (% North American Holstein-Friesian; **HF**) to be within 1 standard deviation (**SD**) of each other, and to produce calves of >15/16th HF. The predicted mean BV, the SD, and commentary on achievement of predicted traits for the heifer offspring are summarized in Supplemental Table S1 (http://dx.doi.org/10.17632/343t97cpdr.2).

Dam Selection. Suitable dams were selected from the New Zealand Dairy Industry Good Animal Database (https://www.dairynz.co.nz/animal/animal-evaluation/animal-database/data-access/). These cows had >3 herd tests in the 2012 to 2013 lactational season, and more than 89% complete pedigree information that confirmed the sire and maternal grandsire were POS or NEG FertBV. The cows were ≥14/16th HF, had calved in the first 42 d of the seasonal calving period, with the expectation that this would optimize reproductive outcomes to the contracted breeding, and were less than 8 yr old and had a high likelihood of remaining in the herd. In addition, candidate dams had no recorded markers for genetic-based diseases. Candidate dams were eligible if they came from herds that were free of tuberculosis, Johne's disease, and enzootic bovine leukosis. Herd owners with suitable dams were enrolled for contracted inseminations. The contracted inseminations consisted of 1,334 POS and 1,533 NEG dams from 669 commercial herds and were inseminated with their respective allocated semen (Supplemental Figure S1). Due to the relatively low number of confirmed breedings and expected calvings of NEG compared with POS dams (55% vs. 69%; Supplemental Figure S1), we used the New Zealand Dairy Industry Good Animal Database to identify additional mating between NEG sires and NEG dams. Only dams that had fulfilled the criteria as outlined above were considered. We identified 129 pregnant NEG dams that were recorded breeding with a NEG FertBV sire. The NEG FertBV progeny had an expected birth date in the same calving season as the other calves. The dams were identified before calving and enrolled so that they underwent the same processes precalving. We collected 24 heifer calves from these 129 dams identified. These were undistinguishable throughout calf collection, calf rearing, and heifer rearing.

Sire Selection. Sires with POS and NEG FertBV were selected based on semen availability. Semen from sires with sufficient stock for 3 inseminations of each dam was distributed for repeated rounds of inseminations, if required. In total, 24 POS (FertBV 5.1% ± 1.67; mean ± SD) and 43 NEG sires (FertBV −6.1% ± 2.33) were used.

Calf Collection and Parentage Verification. We obtained 640 female calves from 379 herds during the 2015 seasonal calving period (Supplemental Figure S1). The mean date (± SD) of birth was August 3 ± 14 d (n = 324) for the POS group, and August 7 ± 15 d (n = 316) for the NEG group. The average age at collection (±SD) was 9 ± 5.4 d for the POS calves and 8 ± 4.4 d for the NEG calves. We verified the parentage of the calf and paternity of their dam via DNA testing from an ear-notch tissue sample using the commercial parentage panel available through Genemark (LIC, Hamilton, New Zealand). Retained calves (POS, n = 289; NEG, n = 276) had a known sire and maternal grandsire of corresponding genetic merit for fertility (Supplemental Figure S1). The numbers of calves collected per sire are summarized in Supplemental Figure S2.

Calf and Heifer Rearing

All calves were reared for 13 wk at a single facility (Parklands Road, Te Awamutu, New Zealand; latitude −38.018759, longitude 175.440412). On arrival, calves were placed in indoor pens in groups of 9 calves and fed 3.5 L of milk replacer once daily (Ancalf, 26% protein, NZAgbiz, 2020) with commercial calf muesli (20% protein, SealesWinslow Ltd., Morrinsville, New Zealand) ad libitum for 7 wk. From wk 8 to 13, calves were grazed outdoors in groups of 30 to 40 where they were grazed a predominantly ryegrass (*Lolium perenne*) pasture and grass silage, and had access to ad libitum calf muesli (20% protein, SealesWinslow Ltd.). Heifers

were moved to a grazing property at an average age of 95 d (SD = 2.9 d; State Highway 16, Waimauku, New Zealand; latitude −36.757141, longitude 174.458980). At the grazing property, the heifers were grouped into 4 age-based herds of 130 to 150 heifers on arrival from the rearer, with the POS and NEG heifers represented across grazing herds. Heifers grazed on ryegrass pasture, with the sward including kikuyu (*Pennisetum clandestinum*) and chicory (*Cichorium intybus*). Supplementary feeds (palm kernel expeller and pasture baleage and silage) were fed to the heifers when insufficient pasture was available to ensure heifer growth rates were consistent with industry BW targets (DairyNZ, 2016b). By February 2017, the research herd consisted of 524 heifers (275 POS and 249 NEG). A summary of the breeding worth, BV for key traits (animal evaluation run Jan 2017), ancestry, and their respective dams and sires are presented in Table 1.

Table 1. Mean (and SD) of breeding worth and component traits of heifers with positive or negative genetic merit for fertility traits that were available for the reproductive phenotypes including numbers (n), the date of birth, fertility breeding value (BV), breeding worth, and the components traits, as well as ancestry of the heifers, and the fertility BV and breeding worth of their dams and sires

Variable per estimated genetic merit[1]	Genetic merit for fertility trait			
	Positive		Negative	
	Mean	SD	Mean	SD
Heifer (n)	275	—	249	—
Date of birth (d/mo; d)	3 Aug	14	7 Aug	15
Estimated genetic merit				
Fertility BV[2] (%)	5.0	0.74	−5.1	1.36
Breeding worth[3] (NZ$/yr)	109	21.4	40	30.7
Volume BV[4] (kg)	654	165.1	732	157.6
Fat BV[4] (kg)	11.3	5.46	17.8	6.57
Protein BV[4] (kg)	17.8	6.57	23.2	4.57
BW BV[4] (kg)	37	12.5	40	10.1
BCS BV[5]	0.07	0.068	−0.08	0.071
Gestation length BV (d)	−3.2	2.07	−1.4	2.23
Residual survival BV	54	58.1	30	72.7
Total longevity BV (d of life)	300	47.6	74	82.4
SCS BV[6]	−0.11	0.140	0.10	0.175
Ancestry[7] (North American %)	56	6.3	62	8.4
Inbreeding coefficient[7] (%)	2.6	1.23	3.1	1.62
Dam[8] (n)	273	—	246	—
Fertility BV[2] (%)	4.6	1.00	−3.6	1.62
Breeding worth[3] (NZ$/y)	89	28.7	39	29.9
Sire[9] (n)	24	—	43	—
Fertility BV[2] (%)	5.1	1.67	−6.1	2.33
Breeding worth[3] (NZ$/yr)	132	34.8	35	46.3

[1]New Zealand Animal Evaluation (NZAEL) animal evaluation run date Jan. 2017.

[2]Fertility BV is a percentage value consisting of the lactating cow's ability to start cycling (a binary trait called PM21, representing success vs. failure at being presented for breeding in the first 21 d of the herd's breeding period, from first, second, and third parity cows) and a lactating cow's ability to conceive (a binary trait called CR42, representing success vs. failure for recalving in the first 42 d of the herd's calving period, from second-, third-, and fourth-parity cows; DairyNZ, 2017).

[3]Breeding worth (NZ$/yr) is the NZ$ net farm income/5 t of DM, which is assumed to be fed per cow per year. (At time of writing, US$ equivalent POS BW is US$77 and NEG BW is US$28.)

[4]The breeding plan aimed to reduce the variation in the BV for milk volume, fat, protein, BW, and ancestry (% North American Holstein-Friesian) to be within 1 SD and produce calves of >15/16th Holstein-Friesian breeding.

[5]Body condition unit is a measure of subcutaneous fat deposits (Roche et al., 2004), calculated using records collected on primiparous 2-yr-old heifers. These records are collected in early lactation. Raw scores are converted into a d 60 lactation equivalent, and then enter the animal evaluation model. A breed neutral adjustment has been applied to this BV, such that the breed average for this trait is 0 across all breeds.

[6]SCS is the log-transformed SCC, which is derived from milk testing (DairyNZ, 2017).

[7]Ancestry and inbreeding coefficient data were received from animal evaluation following parentage checks (Feb. 2016).

[8]Twin heifer calves were collected from 5 dams.

[9]Parentage verified sires of the calves. More negative fertility BV were used due to the reduced availability of semen and to achieve the inbreeding criteria set for the expected offspring.

Body Weight, ADG, BCS, and Stature

Average age at first BW measurement was 9 d (SD = 5.0 d; weighed using static scales, Gallagher, Hamilton, New Zealand). Thereafter, BW was measured once every 2 wk. Average daily gain was calculated from the following periods: 9 d to 3 mo, 3 to 6 mo, 6 to 9 mo, 9 to 12 mo, 12 to 15 mo, 15 to18 mo, and 18 to 21 mo of age. Heifer stature and BCS (1–10 scale; Roche et al., 2004, 2007) were measured at 6, 9, 12, and 15 mo of age. Stature measures were height (vertical distance from the ground to the top of the withers), girth (circumference of the animal measured directly behind the front legs), and length (horizontal distance between the bottom of the pin bones to the top of the withers; Macdonald et al., 2007).

Plasma Sampling, Progesterone Analyses, and Puberty Variables

Weekly blood sampling for determination of plasma progesterone concentrations started when heifers were approximately 190 kg of BW (Macdonald et al., 2007) and continued either until puberty or until 3 wk after the start of the breeding season for those that had not reached puberty by this time. Blood was collected from the coccygeal vessel into evacuated blood tubes containing lithium heparin (BD Vacutainers, BD New Zealand, Auckland, New Zealand). Samples were placed in iced water and transported to the laboratory at the end of the sampling day and centrifuged (at 4°C, 1,900 × *g* for 12 min) for plasma harvest. Plasma was stored in duplicate aliquots at −20°C until analysis for progesterone. A commercial double antibody radioimmunoassay kit was used to determine plasma progesterone concentrations in accordance with the manufacturer's instructions (ImmuChem Progesterone Double Antibody RIA, MP Biomedicals LLC, Irvine, CA). The inter- and intra-assay coefficients of variation for a high standard were 8% and 8%, respectively, and for the low standard they were 14% and 10%, respectively (n = 25 assays). The minimal detectable concentration was 0.18 ng/mL.

Puberty was defined to have occurred when progesterone concentrations were >1 ng/mL in at least 2 of 3 consecutive weekly plasma samples (Macdonald et al., 2007). Date of puberty was the day when the first of these samples was >1 ng/mL. Age at puberty (d) and estimated BW at puberty were calculated. Estimated BW at puberty was calculated using the BW, the ADG, and the age at puberty. Percentage of expected mature BW at puberty was calculated using the estimated mature cow BW using the industry standard estimate for HF cows plus the genetic merit for BW (BW BV) for that individual (DairyNZ, 2016a).

Heifers (n = 15; 14 NEG, 1 POS) that had not reached puberty based on the plasma progesterone concentrations by 21 d after the start of seasonal breeding underwent transrectal ultrasonography with a 5 to 15 MHz probe (SonoScape S6V, Euromed Medical Systems, Auckland, New Zealand). Heifers that had a corpus luteum were returned to the herd without treatment, but heifers that were corpus luteum (CL)-negative (n = 4 NEG heifers) had a reproductive treatment to stimulate ovulation. Animals received an intravaginal P4-releasing device (CIDR, Zoetis New Zealand Limited, Auckland, New Zealand) from 0 to 7 d (insertion = d 0), gonadorelin (Ovurelin 100 mg i.m.; Bomac Laboratories Ltd., Auckland, New Zealand) on 0 d, and 500 mg of cloprostenol i.m. on 7 d (Ovuprost, Bayer Animal Health NZ, Auckland, New Zealand).

Heifer Breeding

We maintained the heifers in their 4 grazing herds throughout the 98-d breeding season (starting October 4, 2016). Thirty-five 15-mo-old Jersey bulls were commingled with each of the grazing herds at a ratio of 1 bull per 20 heifers (6–8 bulls per group) with the remaining bulls held in reserve to be rotated on a regular basis. The Jersey bulls were sourced from a single supplier, were health and fertility tested (before the breeding season), vaccinated for leptospirosis and bovine viral diarrhea, and had BCS of ≥4.5 (scale of 1–10; Roche et al., 2004) 42 d before the start of the breeding season.

Estrus Events and Estrus Rate

Estrus events were monitored using the SCR Heatime HR system (SCR Engineers Ltd., Netanya, Israel), which included the collar-mounted Heatime sensor attached to the upper left side of a collar worn at the cranial part of each heifer's neck at approximately 213 d before the start of the breeding season (200 d, SD = 11.2 d, range 154–235 d). The Heatime sensors collected both activity and rumination data (via microphone) and sent data wirelessly every 2 h to a receiving unit connected to a base computer (Burfeind et al., 2011; Silper et al., 2015a,b). As the heifers did not visit a central yarding point on a daily basis, data collection occurred via 9 receiver stations (routers; including WIFI nodes), and 2 repeater units (with solar panels) were deployed at high points close to water troughs around the grazing property to allow for continuous data transmission to the base computer. Each heifer's activity and rumination data were translated into an index value (0–100) that represented weighted SD from its own basal activity. A system heat was logged when

the threshold was reached for an episode of high activity, using the manufacturer's setting (SCR Engineers Ltd.).

We calculated the proportion of heifers with a SCR system heat (**SH**) alert during the first 21 and 42 d of the seasonal breeding period (**SH21**, **SH42**), as well as the interval from the start of breeding to the first SH alert.

Pregnancy Diagnoses, Pregnancy Rates, and Losses

Fetal aging was undertaken at 3 time points to enable accurate pregnancy diagnosis and identify early embryo losses. All heifers were examined 49 to 51 d after the start of the breeding season. Nonpregnant heifers, or those detected with a pregnancy less than 30 d old, were enrolled for a second pregnancy diagnosis at 79 d after the start of the breeding season. Confirmation of pregnancy included identification of heartbeat to indicate the presence of a viable fetus. The final pregnancy diagnosis included all heifers and was undertaken 44 d after bulls were removed. The method involved transrectal ultrasonography using a 5- to 15-MHz probe (SonoScape S6V, Euromed Medical Systems, Auckland, New Zealand) or Easi-Scan using a 3- to 7-MHz probe (BCF Technologies, Auckland, New Zealand).

Pregnancy rates were defined as the proportion of heifers diagnosed pregnant by 21 (**PR21**), 42 (**PR42**), 63 (**PR63**), and 98 d (**PR98**) relative to the start of the breeding season that were viable at the pregnancy test (i.e., pregnancy losses are not included in the pregnancy rate estimates). Pregnancy loss was defined as a heifer that was pregnant at the first or second pregnancy test, but was not pregnant or pregnant with a younger fetus, at the final pregnancy diagnosis.

Statistical Analysis

We undertook the analyses using SAS/STAT 15.1 (SAS Institute Inc., 2018). Body weight was analyzed as repeated measurements using random coefficient model with fertility group (POS, NEG), age in days up to third-degree polynomial, and their interactions included as fixed effects, and cow, intercept, and day included as random effects. The random coefficients were specified to have bivariate normal distribution (type = un), whereas ADG, stature, and BCS were subjected to repeated measures ANOVA using mixed models approach (Proc Mixed). Fertility group (POS, NEG), month, and their interaction were included as fixed effects, and cow, sire, grazing herds, and original herd were included as random effects. The covariance patterns model was autoregressive heterogeneous [type = arh(1)] to account for increasing variances within and decreasing correlations between measures with increasing age. Results from the repeated measures are presented as adjusted means with standard errors of the difference.

Cox proportional hazard models (Proc PHReg) with censoring variables were used to analyze age, BW, and percentage of mature BW at puberty, as well as time from the start of breeding to first SCR SH alert and time to conception. The models included fertility group (POS, NEG) as fixed effect and sire as random effect. Date of birth (number of days after June 1, 2015) was included as covariate for the analyses of puberty. For time to first breeding and conception, covariates were age at puberty or day of puberty relative to start of breeding and BW at puberty (due to autocorrelation, only 1 age variable was included at any single time). Puberty observations were censored for those cows that had not reached the puberty threshold by the end of progesterone sampling and were allocated a puberty date of +30 d relative to the start of seasonal breeding. Observations for time from start of breeding to first SCR SH and time to conception were censored if the animal had not been bred or had not conceived by the end of seasonal breeding, and were allocated a time of +104 d relative to the start of breeding. Time to events are presented as survival curves with 95% confidence interval (**CI**), median, and hazard ratio (**HR**) with 95% CI. Hazard ratios for covariates were assessed as offsets from the mean and expressed in units of 10 (d).

Probability of heifers reaching puberty by breeding start date and reproductive variables were analyzed using binary logistic regression (Proc GLIMMIX). Reproductive parameters included SH21 and SH42, being pregnant after 21, 42, 63, 84, and 98 d relative to the start of breeding (PR21, PR42, PR63, PR84, PR98), losing a pregnancy, and being pregnant after pregnancy loss. The models included fertility group (POS, NEG) as fixed effect, and sire as random effect. Date of birth (number of days after June 1, 2015) were included as covariate for the analysis of puberty. For reproductive parameters, covariates used were age at puberty or day of puberty relative to start of breeding and BW at puberty (due to autocorrelation, only 1 age variable was included at any single time). Results of event ratios are presented as adjusted mean percentages and absolute counts for POS and NEG fertility group, and relative risk (**RR**) with 95% CI for POS versus NEG fertility. Hazard ratios for continuous covariates with 95% CI were assessed as offsets from the mean and expressed in units of 10 (d).

Analysis of animal losses was undertaken with Fisher's exact 2 × 2 test. Three analyses were undertaken: (1) losses associated with parentage errors as a proportion of the total calves collected, (2) losses due

Table 2. The ADG, girth, length, height, and BCS for the heifers with positive or negative genetic merit for fertility traits; data are presented as adjusted means and the standard error of the difference (SED)

		Genetic merit for fertility traits				Model *P*-value[1]		
Variable	Age	Positive	Negative	SED	*P*-values[2]	Fertility	Mo	Fert × Mo
ADG (kg/d)	Mean	0.74	0.75	0.008	0.147	0.147	<0.001	0.003
	4 d–3 mo	0.63	0.64	0.010	0.555	—	—	—
	3–6 mo	0.63	0.62	0.011	0.466	—	—	—
	6–9 mo	0.80	0.79	0.013	0.658	—	—	—
	9–12 mo	0.58	0.62	0.013	0.002	—	—	—
	12–15 mo	0.88	0.92	0.013	0.003	—	—	—
	15–18 mo	0.88	0.91	0.019	0.056	—	—	—
	18–21 mo	0.76	0.73	0.029	0.458	—	—	—
Girth (cm)	Mean	144	145	0.4	0.377	0.377	<0.001	0.260
	6 mo	124	124	0.5	0.908	—	—	—
	9 mo	139	139	0.5	0.875	—	—	—
	12 mo	150	151	0.5	0.323	—	—	—
	15 mo	165	166	0.4	0.053	—	—	—
Length (cm)	Mean	104	103	0.4	0.230	0.230	<0.001	0.401
	6 mo	91	90	0.5	0.095	—	—	—
	9 mo	101	100	0.4	0.114	—	—	—
	12 mo	107	106	0.4	0.533	—	—	—
	15 mo	117	117	0.5	0.933	—	—	—
Height (cm)	Mean	109	109	0.4	0.893	0.893	<0.001	0.561
	6 mo	97	97	0.5	0.921	—	—	—
	9 mo	106	106	0.5	0.963	—	—	—
	12 mo	113	113	0.5	0.834	—	—	—
	15 mo	119	120	0.5	0.514	—	—	—
BCS[3]	Mean	5.1	5.1	0.02	0.926	0.926	<0.001	0.658
	6 mo	4.7	4.8	0.03	0.629	—	—	—
	9 mo	5.0	4.9	0.03	0.667	—	—	—
	12 mo	5.2	5.2	0.04	0.562	—	—	—
	15 mo	5.4	5.4	0.03	0.742	—	—	—

[1]Repeated measures analyses, *P*-values for genetic merit for fertility traits (fertility), linear age in months (mo), and their interaction (Fert × Mo).

[2]*P*-values for genetic merit for fertility traits (positive vs. negative) at each age (mo).

[3]BCS scored on a 1–10 scale (Roche et al., 2004, 2007)

to health (unsound + deaths + euthanized or culled) as a proportion of the total calves collected, and (3) total losses (failed parentage + unsound + death + euthanized or culled + not pregnant) as a proportion of the total calves collected. Descriptive data of the categories (failed parentage, unsound, deaths and euthanized or culled) and the subcategories within each category are presented in Supplemental Table S2 (http://dx.doi.org/10.17632/343t97cpdr.2).

RESULTS

The ADG, BW, Stature, and BCS

A significant fertility by month interaction for ADG ($P = 0.003$; Table 2) was evident. The interaction between FertBV and time was such that NEG FertBV heifers had a greater ADG between 9 to 12 mo and 12 to 15 mo of age ($P < 0.01$; Table 2), with an ADG advantage of 0.02 kg/d between 15 and 18 mo ($P = 0.056$). Average daily gain was least from 4 d to 6 mo of age and 9 to 12 mo of age (0.58 and 0.64 kg/d), periods that align with late winter to early spring, and the following autumn to winter, respectively. There was an increase in ADG between 12 and 15 mo of age (0.88 and 0.92 kg/d), corresponding to the next spring to early summer.

Significant fertility by day interaction for BW was evident ($P < 0.001$; Figure 1). This interaction was such that the heifers with NEG genetic merit for fertility traits were increasingly heavier as the heifers aged, such that the NEG heifers were 8 kg heavier on average by 21 mo of age (NEG = 470 kg, POS = 462 kg, standard error of the difference = 2.9 kg; Figure 1). There was no effect of FertBV nor interactions with age (mo) on heifer girth, length, height, nor BCS (Table 2).

Puberty and Reproductive Parameters

Heifers with POS genetic merit for fertility traits reached puberty earlier and at a lighter BW and lesser percentage of mature BW. The median age, BW, and percentage of mature BW for the POS heifers was 358 d, 274 kg, and 51%, respectively, and the NEG heifers

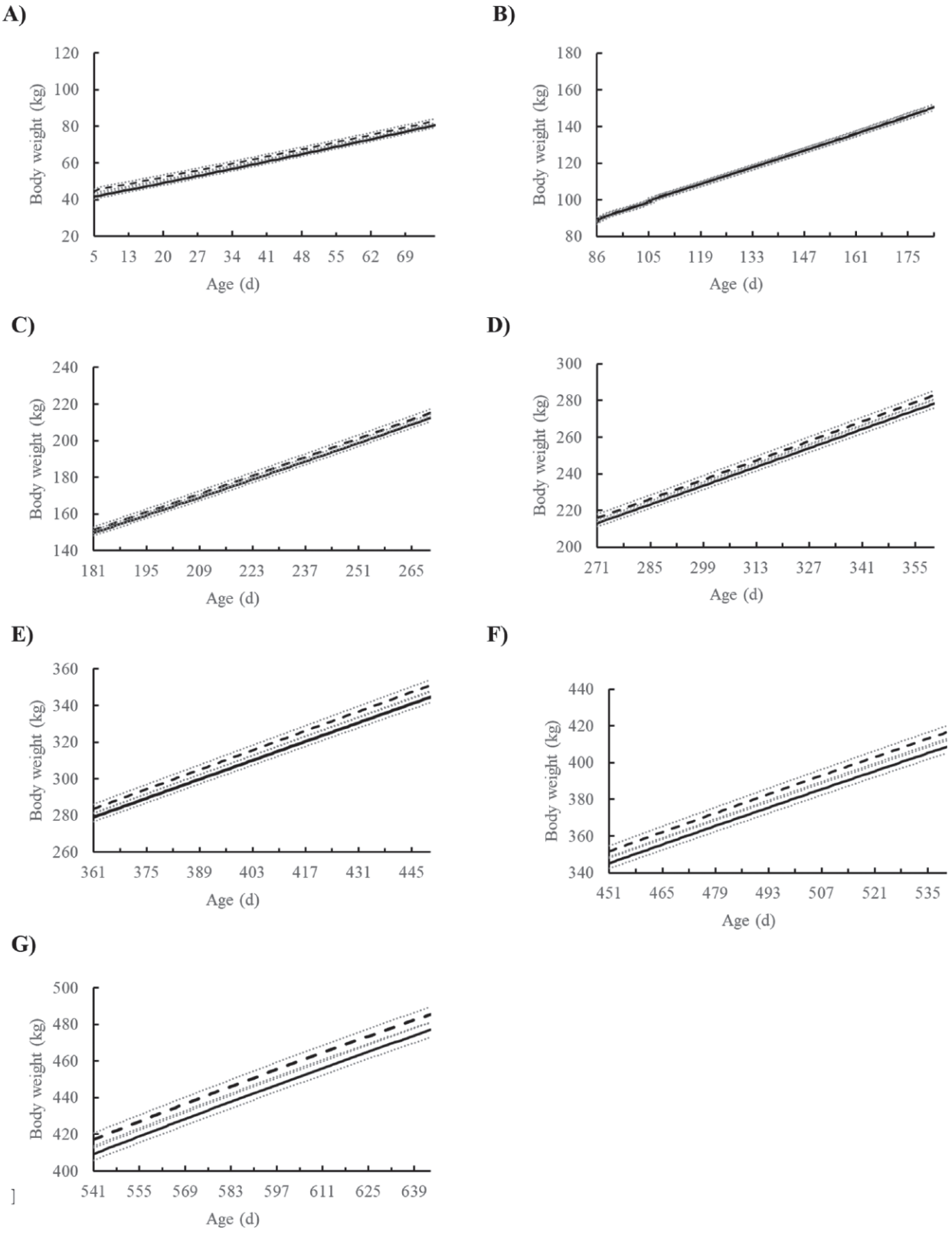

Figure 1. Average BW of heifers with positive (solid line) or negative (dashed line) genetic merit for fertility traits from 8 to 644 d of age. Data represent the estimated means and 95% CI (dotted lines) for each group. The standard error of the differences (SED) are not included due to scale (SED range: positive, 0.89–2.86 kg; negative, 0.93–3.02 kg). Fertility, $P < 0.001$; quadratic fertility × day, $P < 0.001$. Data are arbitrarily grouped as follows: (A) d 5–75, (B) 76–180 d, (C) 181–270 d, (D) 271–360 d, (E) 361–450 d, (F) 451–540 d, and (G) 541–644 d, respectively.

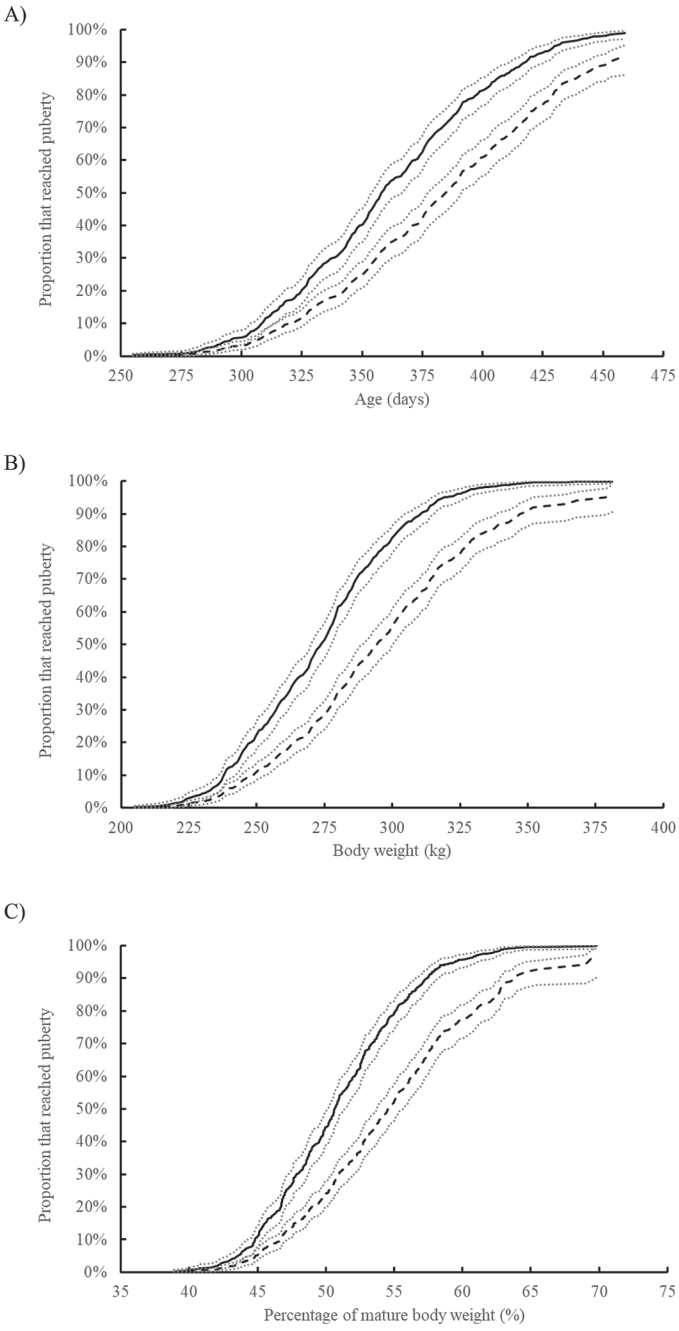

Figure 2. Survival estimations from the Cox proportional hazard model of (A) age (d, $P < 0.001$), (B) BW (kg, $P < 0.001$), and (C) percentage of estimated mature BW (%) at puberty ($P < 0.001$) of heifers with positive (solid line) and negative (dashed line) genetic merit for fertility traits. Dotted lines represent the 95% CI.

were 385 d, 294 kg, and 55%, respectively (Figure 2). The HR for reaching puberty was greater in POS than NEG heifers for age at puberty (HR = 1.98, 95% CI = 1.45–2.70, $P < 0.001$), BW at puberty (HR = 2.37, 95% CI = 1.71–3.29, $P < 0.001$), and percentage of mature BW at puberty (HR = 2.25, 95% CI = 1.68–3.01, $P <$ 0.001). Figure 2 depicts the proportion of heifers reaching puberty with increasing age, BW, or percentage mature BW.

At the start of the seasonal breeding period, 94 ± 1.6% of the POS and 82 ± 3.2% of the NEG heifers had reached puberty ($P < 0.001$). Indeed, POS genetic merit for fertility traits, relative to NEG, had a 14% greater chance of reaching puberty before the start of the seasonal breeding period (RR = 1.14, 95% = CI 1.0 –1.18). The chance of reaching puberty at the start of breeding was dependent on date of birth, such that for every 10 d born later, the chance of reaching puberty decreased by 9% (HR = 0.90, 95% CI = 0.86–0.94, $P < 0.001$).

There was no difference in the proportion of heifers that had a recorded SH during the first 21 or 42 d of breeding season (SH21 or SH42; Table 3). The median time from planned start of breeding to first SH was 11.3 d for the POS and 12.1 d for the NEG FertBV heifers. There were no effects of age at puberty, BW at puberty, nor reaching puberty before start of breeding on whether the heifer had a recorded SH alert (Table 3).

The difference in heifer pregnancy rate at PR21 and PR42 between the POS and NEG group was 12.6 and 8.6 percentage points in favor of the POS heifers, respectively ($P = 0.025$, and $P = 0.032$; Table 3). As breeding progressed, the difference between the POS and NEG heifers reduced to 5 percentage points, with a difference of 4.2 percentage points at the end of breeding ($P = 0.039$; Table 3). POS heifers were 20% and 11% more likely to be pregnant after 21 d and 42 d of breeding than NEG heifers (RR = 1.20, 95% CI = 1.03–1.34, $P = 0.025$; RR = 1.11, 95% CI = 1.01–1.16, $P = 0.032$). With few pregnancy losses, there was no difference between the POS and NEG heifers. There were no effects of age and BW at puberty or puberty relative to breeding on the PR parameters.

The POS FertBV heifers conceived earlier than the NEG heifers (Figure 3). The heifers with POS genetic merit for fertility traits conceived 3.6 d earlier (median 13.0 vs. 16.6 d, $P = 0.001$). At any given time during the breeding period, 40% more POS heifers conceived ($P = 0.001$; Table 4) compared with the NEG fertility heifers. Neither age, BW at puberty, nor puberty expressed as days relative to the start of breeding were associated with time conception (Table 4).

Animal Losses

The sources of animal losses between the time calves were collected (~9 d of age) and final pregnancy diagnosis are described in Table 5. There were no differences in the proportion of heifers with POS or NEG genetic merit for fertility traits that failed parentage

Table 3. Effect of genetic merit for fertility traits (positive or negative) and age at puberty (Age Pub), BW at puberty (BW Pub), and time of puberty relative to the start of breeding (Day rel BS) on heifer reproductive parameters; data are presented as adjusted group mean proportions (counts), and relative risk (RR) with 95% CI for the effect fertility, and group mean estimates with SEM for potential confounders

	Genetic merit for fertility traits				Confounder		
Variable	Positive	Negative	*P*-value	RR	Confounder	RR (95% CI)	*P*-value
Total heifers[1] (n)	275	248					
PR21[2]	74.9% (205)	62.3% (163)	0.025	1.20 (1.03–1.34)	Age Pub	1.01 (0.99–1.03)	0.571
					BW Pub	1.02 (0.99–1.05)	0.246
					Days rel BS	1.00 (0.99–1.02)	0.898
PR42[2]	90.1% (247)	81.5% (204)	0.032	1.11 (1.01–1.16)	Age Pub	1.00 (0.98–1.01)	0.686
					BW Pub	1.01 (0.99–1.02)	0.359
					Days rel BS	0.99 (0.98–1.00)	0.204
PR63[2]	93.4% (256)	87.9% (219)	0.073	1.06 (0.99–1.10)	Age Pub	1.00 (0.99–1.01)	0.739
					BW Pub	1.01 (0.99–1.02)	0.445
					Days rel BS	0.99 (0.98–1.00)	0.162
PR98[2]	96.5% (264)	91.2% (227)	0.033	1.06 (1.01–1.08)	Age Pub	1.01 (1.00–1.01)	0.124
					BW Pub	1.00 (0.98–1.01)	0.508
					Days rel BS	1.00 (1.00–1.01)	0.473
FinPR[2]	98.0% (269)	93.8% (232)	0.039	1.04 (1.00–1.06)	Age Pub	1.00 (1.00–1.01)	0.158
					BW Pub	0.99 (0.98–1.00)	0.210
					Days rel BS	1.00 (1.00–1.01)	0.716
Pregnancy loss[3]	1.9% (5)	3.3% (9)	0.523	0.57 (0.00–29.9)	Age Pub	0.94 (0.29–2.91)	0.615
					BW Pub	1.13 (0.24–4.96)	0.497
					Days rel BS	1.01 (0.38–2.65)	0.909
Pregnant after loss	72.2% (3)	16.9% (2)	0.326	4.28 (0.00–64.9)	Age Pub	0.00 (0.00–1.43)	0.872
					BW Pub	0.34 (0.00–4.8)	0.641
					Days rel BS	0.00 (0.00–1.25)	0.329
SH21[4]	78.0% (215)	81.2% (201)	0.552	0.96 (0.18–1.21)	Age Pub	1.00 (0.89–1.08)	0.921
					BW Pub	1.00 (0.84–1.11)	0.823
					Days rel BS	1.00 (0.90–1.07)	0.729
SH42[4]	82.5% (228)	85.6% (211)	0.541	0.96 (0.17–1.16)	Age Pub	1.00 (0.91–1.07)	0.634
					BW Pub	0.99 (0.84–1.08)	0.505
					Days rel BS	1.00 (0.92–1.06)	0.893

[1]One heifer (negative) was excluded from analysis, as she was euthanized before final pregnancy diagnosis.

[2]Pregnancy rates are defined as the proportion of heifers identified as pregnant by d 21 (PR21), 42 (PR42), 63 (PR63), 98 (PR98, end of the seasonal breeding) of the seasonal breeding period, where the pregnancy was still viable at the final pregnancy test 44–45 d after bulls were removed.

[3]Losses between confirmed pregnant and the pregnancy diagnoses on February 16–17 2017 (30–120 d of gestation, approximately).

[4]SCR system heat (the automated monitoring of estrus events using the SCR Heatime HR system; SCR Engineers Ltd., Netanya, Israel) alert during the 21 (SH21) and 42 (SH42) d of the breeding season.

testing ($P = 0.54$). Significantly more heifers with NEG genetic merit for fertility traits were removed due to ill health compared with the POS group (POS, 14/324; NEG, 27/316; $P = 0.034$). For total removals, fewer heifers with POS genetic merit for fertility traits were removed (55/324) compared with the NEG fertility group (83/316; $P < 0.01$).

DISCUSSION

The earlier onset of puberty (younger and lighter) and greater pregnancy rate in heifers with a POS compared with NEG FertBV support our hypothesis that heifers divergent in genetic merit for fertility traits differ in their reproductive performance. To our knowledge, this is the first reported example in which direct selection for genetic merit for fertility traits, estimated using reproductive traits from lactating cows, has resulted in an earlier onset of heifer puberty. The effects of the FertBV on heifer reproductive phenotypes reported here align with recent findings of the effect of genetic selection on detailed reproductive phenotypes (Cummins et al., 2012a,b; Veronese et al., 2019a,b).

This indirect selection for earlier puberty occurred even though the FertBV consisted of 6 binomial reproductive traits related to recalving rates during lactations 2, 3, and 4, and breeding rates during the first 3 wk of seasonal breeding collected during lactations 1, 2, and 3 (DairyNZ, 2018). Previous studies have reported an indirect effect on puberty when selecting for productivity traits. In a study evaluating the effects of 20 yr of genetic improvement in New Zealand dairy cows, greater overall genetic merit led to heifers reaching puberty later. It was identified that New Zealand heifers representing the genetic potential from the 1970s reached puberty earlier compared with New Zealand heifers with genetics from the 1990s (Macdonald et al., 2007). In the same study, both groups of New Zea-

Table 4. Effect of genetic merit for fertility traits (positive or negative) and potential confounders on heifer reproductive parameters; data are presented as median time from start of breeding to SCR system heat[1] and to conception, and hazard ratio (HR) with 95% CI for the effect fertility and per 10 d for potential confounders

Variable	Positive (median)	Negative (median)	*P*-value	HR (95% CI)	Effect[2]	HR per 10 d (95% CI)	*P*-value
Time to system heat (d)	11.3	12.1	0.305	1.11 (0.91–1.36)	Age Pub	1.00 (0.99–1.00)	0.798
					BW Pub	0.99 (0.99–1.01)	0.526
					Days rel BS	0.99 (0.99–1.00)	0.979
Time to conception (d)	13.0	16.6	<0.001	1.40 (1.15–1.72)	Age Pub	1.00 (0.99–1.00)	0.663
					BW Pub	1.00 (0.99–1.01)	0.376
					Days rel BS	0.99 (0.99–1.00)	0.246

[1]SCR system heat refer to the automated monitoring of estrus events using the SCR Heatime HR system (SCR Engineers Ltd., Netanya, Israel), which included the collar-mounted Heatime sensor. A system heat was logged when the threshold was reached for an episode of high activity, using the manufacturer's setting.

[2]Age Pub = age at puberty; BW Pub = body weight at puberty; Days rel BS = days relative to the start of the breeding season (BS).

land heifers (1970s and 1990s) reached puberty earlier than heifers with 1990s North American genetics and at a lighter percentage of mature BW (McNaughton et al., 2002; Macdonald et al., 2007). In other studies (Garcia-Muniz, 1998) identified that HF heifers from lines with greater mature BW (with high proportion of North American ancestry) and larger stature were older and heavier when they reached puberty when compared with heifers with low genetic merit for mature BW (with predominantly New Zealand ancestry) or smaller in stature. Hence, the onset of puberty can be influenced by numerous factors, such as ancestry, mature BW, and fertility traits from lactating cows. To better understand these factors, data not biased by our study design (selection for POS and NEG FertBV) is needed. Therefore, focus should be on generating an unbiased data set to robustly determine correlations among ancestry, BW, fertility traits, and management factors on the onset of puberty.

The extent of the differences in the age and BW at puberty between the heifers with POS and NEG FertBV were unexpected. The difference in the onset of puberty reported here is comparable with changes in the onset of puberty reported previously when undertaking single trait section. For example, in a long-term study focused on direct genetic selection for earlier puberty, over 7-yr of single selection puberty was shifted by 62 d (Morris and Amyes, 2005; Morris et al., 2011). This large shift

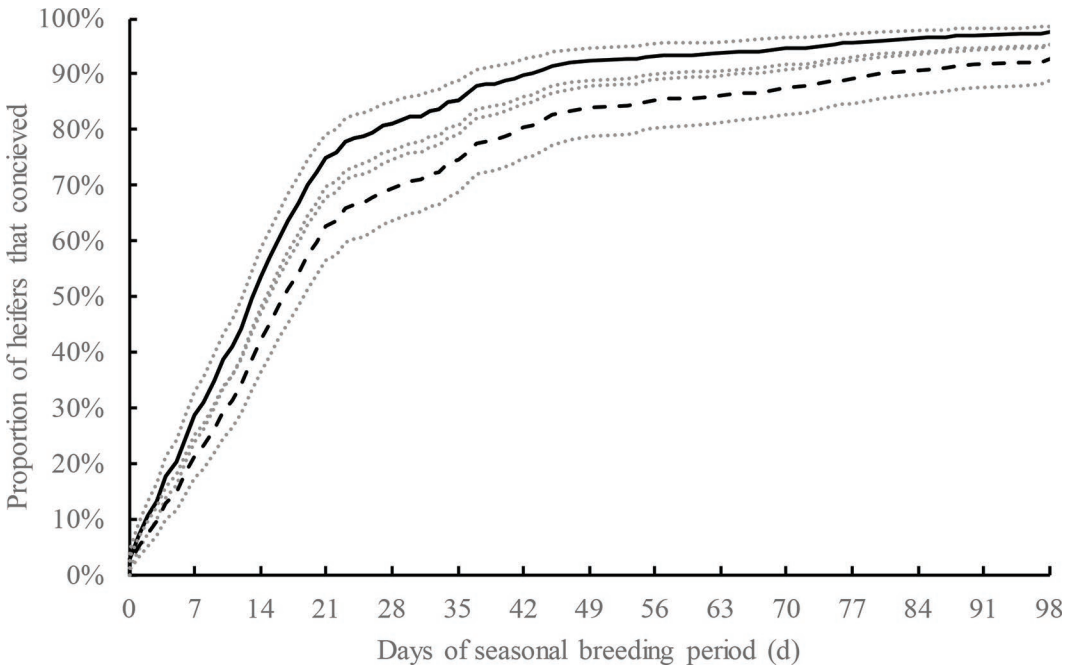

Figure 3. Survival estimations from the Cox proportional hazard model of time to conception (d, $P = 0.001$) of heifers with positive (solid line) and negative (dashed line) genetic merit for fertility traits. Dotted lines represent the 95% CI.

in the onset of puberty was possible because of the single trait selection approach as well as the heritability of puberty traits. The heritability of puberty has a large reported range from 0.10 to 0.67 in beef and dairy heifers. This range in heritability reflects both the measures used and the study size (Martin et al., 1992; Morris and Hickey, 2004). From our results, the difference in the age at puberty between the POS and NEG FertBV heifers suggests early onset of puberty is correlated with the FertBV. The mechanisms altered to result in such large differences remain to be elucidated.

In the current study, there was no difference in stature nor BCS, even though NEG FertBV heifers had a small numeric advantage with BW and ADG between 9 to 21 mo of age. As previously discussed, mature BW and genetic ancestry can affect the onset of puberty, and the breeding approach resulted in the NEG FertBV heifers having 4 kg greater BW BV and 6% greater North American ancestry (Table 1). This small effect is suggestive of the NEG FertBV having greater size, although there was no difference in stature of the heifers when evaluated at 6, 9, 12, and 15 mo of age. What proportion of the difference in puberty is explained by the difference in BW BV and ancestry remains to be determined.

Management of heifers has a significant effect on when heifers reach puberty. Previous studies have identified that the age at puberty is inversely related to ADG or nutrition levels, such that heifers with low ADG are older at puberty (Patterson et al., 1992; Schillo et al., 1992; Macdonald et al., 2005). Yet, we observed that the NEG FertBV heifers were heavier and had a greater ADG after they reached 9 mo of age, and the NEG FertBV had a greater ADG up to 15 mo of age (approximately 0.04 kg/d, which is equivalent to 1.2 kg BW over 30 d). Based on the information available, we propose that in this study, ADG and nutrition were not the main contributors to the difference in the onset of puberty reported. The role of body composition and stature at maturity is to be determined.

Industry recommendations identify that the average heifer reaches puberty between 43 and 47% of mature BW (DairyNZ, 2016b). These recommendations align with the range reported by McNaughton et al. (2002) of the 2 New Zealand strains (1990s and 1970s) at 43% of mature BW, and the North American strain reaching puberty at 47% of mature BW. Our results identified that the heifers reached puberty at 51% and 55% of mature BW. It remains to be seen whether the industry expectations that the average heifer on commercial farms reaches puberty at 43 to 47% of mature BW continues to be appropriate. To ensure industry recommendations are robust, estimates of BW at puberty from commercial herds should be evaluated.

If ADG and nutrition are not the key factors controlling the onset of puberty as previously reported (Macdonald et al., 2005; Patterson et al., 1992; Schillo et al., 1992), the underlying mechanisms controlling the difference in puberty in this study remain to be determined. As the current study selected for extremes in genetic merit for fertility traits, it is plausible that inherent difference in the hypothalamus and pituitary signals

Table 5. Heifer losses between 9 d of age (at collection) and the final pregnancy diagnosis (>18 mo of age) for the 2 lines of positive (POS) or negative (NEG) genetic merit for fertility traits

Variable	Genetic merit for fertility traits		Total	*P*-value
	POS	NEG		
Total collected (n)	324	316	640	
Failed parentage verification to sire or maternal grandsire (n)	35	40	75	
(%)[1,2,3]	(10.8)	(12.7)	(11.7)	
Unsound[3,4] (conformation/freemartin; n)	2	6	8	
(%)[1]	(0.6)	(1.9)	(1.3)	
Deaths[3,4] (n)	6	14	20	
(%)[1]	(1.9)	(4.4)	(3.1)	
Euthanized or culled[3,4] (n)	6	7	13	
(%)[1]	(1.9)	(2.2)	(2.0)	
Not pregnant[3] (n)	6	16	22	
(%)[1]	(2.2)	(6.4)	(3.4)	
Heifers remaining (n; May 2017)	269	233	502	<0.01
(%)[1]	(83.0)	(73.7)	(78.4)	

[1]Percentages of those heifers collected.

[2]Failed parentage Fishers exact 2 × 2 test: POS, 35 from 324; NEG, 40 from 316; $P = 0.54$.

[34]All losses due to parentage failure, health (unsound, deaths, euthanized or culled) and not pregnant. Fishers exact 2 × 2 test: POS, 55 from 324; NEG, 83 from 316; $P < 0.01$.

[4]Losses due to health (unsound, deaths, euthanized or culled) after calves with failed parentage are removed. Fishers exact 2 × 2 test: POS, 14 from 289; NEG, 27 from 270; $P = 0.035$.

determine the timing of puberty. A deeper knowledge of whether the biological mechanisms that control puberty differ between the POS and NEG FertBV lines may support the discovery of new candidate traits that benefit cow reproductive performance.

The earlier puberty in the heifers with POS genetic merit for fertility traits meant that these heifers had 1 more estrus event, on average, before the start of breeding. This can provide significant effects on pregnancy outcomes, as heifers bred on the second or third estrus have 36% greater conception and 20% greater pregnancy rates compared with those bred on the first estrus (Byerley et al., 1987; Perry et al., 1991). Our finding aligns with that of Funston et al. (2012), who reported that overall pregnancy rates in heifers was directly influenced by the proportion of heifers showing estrus before the beginning of the breeding season. Future solutions that aim to optimize reproductive outcomes for seasonally bred heifers should be cognizant of the gains that could be achieved if heifers are postpubertal (second or third estrus) early in the breeding season.

In the current study, the focus was on the benefits within the current breeding season. However, long-term benefits have also been reported. Heifers that are well grown, and heifers that get pregnant early in the breeding season, calve earlier and have improved lifetime production and reproduction (Pryce et al., 2007; Wathes et al., 2014; Dennis et al., 2018; Handcock et al., 2020). The extent and consistency of benefits under commercial conditions requires a larger data set to quantify the benefits under commercial environments. Additional value may be generated by understanding these relationships across different farm systems (seasonal twice a day milking, once a day milking, split calving in spring and autumn, year-round calving).

The breeding priorities of many countries are focused on breeding cows most suited for that specific dairying industry (dairy system), with increasing importance on a balance between productivity, profitability, and robustness. This focus has put more emphasis on breeding for traits associated with cow reproductive performance and health (Miglior et al., 2005; Cole and VanRaden, 2018). Yet, few breeding approaches include heifer traits, and none include heifer puberty. Three points that make age at puberty an attractive candidate trait to consider in selection indices are as follows: (1) the heritability is better than that reported for traditional traits in use currently for estimating genetic merit for fertility, (2) puberty occurs earlier in life than the current (lactational) traits used, and (3) there are benefits in heifers reaching puberty and conceiving early with respect to their longevity in the herd. We believe that generating data sets that support robust evaluation of genetic and phenotypic correlations between puberty, heifer conception and pregnancy rate, and traits currently in the animal evaluation models is the next step to progressing this area. The difficulties will be associated with achieving appropriate recording (widespread or targeted approach), and acceptance that these phenotypes may be from a limited number of heifers (reference population). Puberty data on a large scale could be estimated using plasma progesterone or automated systems to capture puberty (pedometer or activity collars). There are, however, trade-offs that will need to be accepted including frequency of data, bias data sets, accuracy, and volume of data that can be collected.

CONCLUSIONS

In the current study, we demonstrated that selecting for extreme positive POS (+5) genetic for fertility based on the New Zealand FertBV estimated from predictor traits collected during lactations 1 to 4 will produce heifers that reach puberty earlier, with greater pregnancy rates during their first breeding period. This effect is independent of heifer growth rates. Our results indicated that heifer puberty and pregnancy rate are potential earlier predictor traits than the cow fertility traits used currently. Furthermore, understanding how selection for genetic merit for fertility traits has altered the physiological and genetic mechanisms controlling puberty may provide additional early indicators for subsequent cow fertility.

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