INTERPRETIVE SUMMARY

Fertility genetic merit and the estrous cycle: By Cummins et al., Page 000. Reproductive efficiency in dairy cows has been declining internationally for the past five decades. The current study compared ovarian and circulating hormone measurements during one complete estrous cycle in cows with good or poor genetic merit for fertility traits but similar genetic merit for milk production traits. Superior genetic merit for fertility traits was associated with having a shorter estrous cycle, fewer follicular waves, a larger corpus luteum, greater circulating concentration of progesterone, a larger preovulatory follicle, and stronger behavioural estrus.

FERTILITY GENETIC MERIT AND THE ESTROUS CYCLE

Genetic merit for fertility traits in Holstein cows: II. Ovarian follicular and corpus luteum dynamics, reproductive hormones and estrus behaviour

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ABSTRACT

The objective of this study was to characterize the estrous cycle 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. A total of 37 lactating cows were enrolled on an 8-d CIDR-based protocol to synchronise estrus. 19 Fert+ and 12 Fert- cows that successfully ovulated a dominant follicle and established a corpus luteum underwent daily transrectal ultrasonography. Blood sampling was carried at 8 h intervals from d 0 to d 6 and from d 15 to ovulation, and once daily from d 7 to d 15. Blood samples were analysed for progesterone, estradiol, follicle stimulating hormone and luteinising hormone. Estrus behaviour was recorded using neck activity collars and mounting pads. Fert+ cows tended to have fewer (P = 0.07) follicular waves (2.2 vs. 2.7 waves) and had a shorter ($P < 0.05$) estrous cycle (21.0 vs. 25.1 d) than Fert- cows. There was no effect of genotype on day of first wave emergence or day of first wave dominant follicle peak diameter (all $P > 0.05$) but the peak diameter of the first wave dominant follicle tended to be larger ($P = 0.08$) in Fert- cows. During the first 13 d of the cycle, Fert+ cows developed a corpus luteum that was 16% larger (P = 0.08) than Fert- cows. Circulating progesterone concentrations were 34% greater ($P < 0.001$) in Fert+ than Fert- cows (5.15 vs. 3.84 ng/ml, respectively) from d 5 to d 13. During the final follicular wave, the interval from preovulatory follicle emergence to ovulation and the interval from preovulatory follicle dominance to ovulation were similar ($P >0.05$) in both genotypes. Maximum preovulatory follicle diameter was larger ($P < 0.05$) in Fert+ than Fert- cows (17.9 vs. 16.8 mm, respectively); however, circulating concentrations of oestradiol were not different (all $P > 0.05$) between genotypes. A greater proportion ($P < 0.05$) of Fert- cows ovulated to a silent heat than Fert+ cows (22% vs. 2%, respectively). Of cows

that showed behavioural estrus Fert+ cows had 41% greater ($P < 0.01$) mean activity count; however, no difference ($P > 0.05$) was seen in mounting behaviour between genotypes. These results demonstrate for the first time that genetic merit for fertility has pronounced effects on corpus luteum development, progesterone concentration, preovulatory follicle diameter and behavioural estrus.

INTRODUCTION

Reproductive efficiency in Holstein dairy cows has declined over the last 50 years in Ireland (Evans et al., 2006) and internationally (Royal et al., 2000, Washburn et al., 2002). Despite intensive research, the precise mechanisms contributing to this decline in dairy cow fertility remains poorly understood. Ovarian activity is under the control of the hypothalamic-pituitary-ovarian-uterine axis, and is central to reproductive success (Robinson et al., 2008). Increased incidence of ovarian abnormalities such as prolonged postpartum anestrus, cystic ovarian disease (Wiltbank et al., 2002), reduced estrus behaviour (Dransfield et al., 1998), and reduced circulating concentrations of progesterone (P4) and oestradiol (E2) (Wiltbank et al., 2006, Wiltbank et al., 2001) have all been cited as potential factors associated with declining reproductive performance in high-producing dairy cows.

Fertility performance of non-lactating Holstein heifers has remained relatively static during the last 50 years (Pursley et al., 1997, Sartori et al., 2002). The decline in fertility in lactating cows therefore appears to be a result of the energetic burden associated with initiation and maintenance of lactation (Butler and Smith, 1989). Previous studies have used this high fertility (non-lactating heifer or cow) vs. low fertility (lactating cow) animal model to determine characteristics of the estrous cycle

that contribute to poor fertility in lactating dairy cows (De La Sota et al., 1993, Sartori et al., 2004, Sartori, 2000, Wolfenson et al., 2004). A study comparing cows of high or low genetic merit for milk production demonstrated a negative association between circulating P4 concentration and genetic merit for milk production (Lucy, 2001). These studies have provided a valuable insight into the effects of lactation on characteristics of the estrous cycle, but they are confounded by effects of age, nutrition, energy status, body condition score (BCS), uterine environment, and other factors (Wolfenson et al., 2004).

We have recently shown that reproductive performance in pasture based production systems is more dependent on genetic merit for fertility traits than genotypic or phenotypic milk production (Cummins et al. 2012). The underlying physiological differences, however, between cows with good (Fert+) and poor (Fert-) genetic merit for fertility traits that are responsible for the observed differences in phenotypic fertility performance remain unknown. Therefore, the aim of this study was to characterize dynamics of ovarian follicle and corpus luteum (CL) development in Fert+ and Fert- cows. Specifically, we tested the hypothesis that genetic merit for fertility traits would affect ovarian follicular waves, preovulatory follicle development, CL volume, circulating concentrations of steroids and gonadotrophins, and estrus behaviour.

MATERIAL AND METHODS

Herd Establishment

The Economic Breeding Index (EBI) is a multi-trait profit index introduced in 2001, and has evolved to include 6 subindices (relative emphasis in parenthesis); milk

production (38.1%), fertility/survival (34.8%), calving performance (10.3%), beef carcase (7.2%), maintenance (6.1%) and health (3.6%) (http://www.icbf.com). The fertility subindex is comprised of 2 traits; calving interval (23.2%) and survival (11.5%). Good genetic merit for fertility traits requires negative EBV's for calving interval and positive EBV's for survival. The establishment of the herd was carried out during autumn 2007 using official dairy evaluations published by the Irish Cattle Breeding Federation (ICBF), and was outlined in detail by Cummins et al. (2012). The national dairy cattle database was screened for nulliparous spring calving heifers. Strict restrictions were placed on EBVs for milk production (between +200 kg and +900 kg) and proportion of Holstein genes (> 75%). Within this population, heifers with extreme positive (poor fertility) and negative (good fertility) EBV's for calving interval were identified. Poor fertility (Fert-) heifers were restricted to animals where sire and maternal grand-sire had positive EBV's for calving interval. Conversely, good fertility (Fert+) heifers were restricted to animals where the sire and maternal grand sire had negative EBV's for calving interval. Nulliparous Fert+ and Fert- cows were purchased in 2008 and 2009 (n = 28 of each genotype). Animals that calved between Jan 11 and April 10 2009 were enrolled in the current study (n = 37); 21 were Fert+ (6 first lactation and 15 second lactation) and 16 were Fert- (8 first lactation and 8 second lactation). The average (range) days in milk at the time of synchronization protocol initiation of the Fert+ and Fert- cows was 102 (52 to 134) and 108 (45 to 121), respectively. The EBVs of these cows 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.

Management System

For the duration of the study, cows were kept on a clean stand-off wood-chipped pad (Hickey et al., 2003) and given full time access to grass silage. Cows were individually fed 5 kg concentrate per day during the am and pm milkings. The concentrate composition on a fresh weight basis (relative proportion in parentheses) was citrus pulp (47%), corn gluten (47%), soya bean oil (3%) and minerals/vitamins (3%). The chemical composition of grass silage and concentrate is summarized in Table 2.

Milk yield was recorded at each milking using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). Milk composition (Fat, Protein and Lactose) was determined weekly from successive evening and morning samples by near-infrared reflectance spectroscopy (FT6000 Milkoscan instrument; DK-3400, Foss Electric, Hillerød, Denmark). Cow BCS was measured on d 21 of the cycle. Mean calving dates were February 11 (SD ± 21.2 d) and February 25 (SD ± 24.6 d) for the Fert+ and Fert- cows, respectively.

Estrous Synchronisation

Transrectal ultrasonography was carried out on all cows to determine utero-ovarian status (7.5-MHz transrectal transducer, Aloka SSD-900, Aloka Ltd., Tokyo, Japan). Cows that were diagnosed as anestrus or as having a uterine infection were removed from the study (two cows). The remaining 37 cows were enrolled on a standard 8-d controlled internal drug release device (CIDR, Pfizer Ireland, Dublin, Ireland) based protocol to synchronise estrus (Figure 1). On d -10 each cow was administered an i.m. GnRH agonist injection containing 10 µg buserelin (Receptal; Intervet Ireland, Dublin, Ireland), and a CIDR device containing 1.38 g P4 was inserted per vaginum.

On d -3 each cow was administered an i.m. $PGF_{2\alpha}$ injection containing 25 mg dinoprost tromethamine (Lutalyse; Pfizer Ireland, Dublin, Ireland). The CIDR devices were removed on d -2. Transrectal ultrasonography commenced on the day of expected estrus. Animals that failed to ovulate the dominant follicle (four Fert- cows) or that developed a cystic ovarian structure (two Fert+ cows) were removed from the study. The remaining 19 Fert+ and 12 Fert- cows that were successfully synchronized underwent frequent transrectal ultrasonography and blood sampling for one complete estrous cycle (Figure 1).

Transrectal Ultrasonography and Estrus Behaviour Measurements

Transrectal ultrasound was carried out daily. For all ovarian structures ≥ 5 mm, the largest image was frozen and cross-section measurements of height and width were recorded on ovarian charts. Ovulation was deemed to have occurred when a large dominant follicle could no longer be visualized and a luteal structure subsequently formed. This was set as d 0 of the estrous cycle. Starting 18 h after commencement of estrus behaviour at the spontaneous heat at the end of the estrous cycle, ultrasonography was carried out at 8 h intervals to determine the time of ovulation. Follicular data was combined for both ovaries. Follicle diameter was calculated as (Length + Width) / 2). The day of wave emergence was determined as the day when the retrospectively determined dominant follicle was ≤ 5 mm. The timing of follicle dominance was defined as the beginning of the greatest differences in growth rates between the 2 largest follicles (Ginther et al., 1997). The volume of a CL was calculated with the formula $V = 4/3 \times \pi \times \text{radius}^3$; if present, the volume of luteal cavities was removed from the final CL volume figure.

Cow activity and mounting data were recorded during the synchronized and spontaneous estrus using radiotelemetry transmitters (DDx HeatWatch, Denver, CO) and activity collars (Moomonitor, Dairymaster, Causeway, Co. Kerry, Ireland). HeatWatch pads were applied according to the manufacturers instructions 2 d prior to expected estrus. Activity collars were applied for the duration of the trial and raw activity data, averaged at 6 h intervals, were used as the index of cow activity during the spontaneous estrus. Cows required > 2 standing mounts to be deemed in estrus. The commencement of estrus was defined as the first standing mount where the second standing mount was followed within a 2 h period; similarly, the end of estrus was defined as the last standing mount where the previous mount was less than 2 h previous. The duration of estrus was determined for all cows that exhibited standing estrus, and was calculated as the interval from first mount to last mount.

Blood Sampling and Analysis

Blood samples were collected via coccygeal venipuncture into vacutainers (Becton Dickinson, Plymouth, UK) containing lithium heparin as an anticoagulant. Samples were centrifuged at 2,000 × g for 15 min at 5 ºC, plasma was decanted, and stored at -20 ºC until further analysis. Concentrations of P4 in plasma were determined in samples taken from d 0 to d 6 (at 8 h intervals), and from d 7 to ovulation (at 24 h intervals) using a commercially available solid-phase radioimmunoassay (Coat-A-Count Progesterone, Diagnostic Products Corporation, Los Angles, CA). The inter- and intra-assay coefficients of variation were 3.7% and 9.5 %, respectively. Circulating (E2 and FSH concentrations were measured in samples collected on d 0 to d 6 (at 8 h intervals), and from d 15 until ovulation (at 8 h intervals). FSH concentrations were quantified using a validated radioimmunoassay as described by

Crowe et al. (1997). The inter- and intra-assay coefficients of variation were 7.4% and 12%, respectively. Circulating concentrations of E2 were determined by radioimmunoassay following an extraction step (Prendiville et al., 1995) using Adaltis MAIA E2 Kit (Biostat, Stockport, UK). The inter- and intra-assay coefficients of variation were 16.5% and 19.3%, respectively.

Peak circulating P4 concentrations were calculated as the mean of the four greatest circulating P4 concentrations. The time when peak circulating P4 concentration occurred was determined as the mean of the 4 timepoints corresponding to the four greatest P4 concentration values. The day of luteolysis was determined as the day when circulating P4 concentration declined to 50% of peak and further declined to 25% of peak the following day. Circulating concentrations of E2 were graphed for each cow and peak concentrations, day of peak and the rate of increase from basal levels to peak were determined. 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.

Data Handling and Statistical Analysis

All statistical analysis and data handling was carried out using SAS (SAS Institute, 2006), with the exception that box plots were generated using R (R Development Core Team, 2011). Data from cows with atypical estrous cycles (depicted in Figure 5) were omitted if the variable in question was identified as an outlier for the particular genotype. Data were checked for normality. A Box-Cox transformation was used to normalise the distribution of P4, E2, and FSH data. Non-transformed P4, E2, and FSH data were used in Figures 2, 3, and 5 for illustrative purposes.

The effect of genotype on variables with repeated measures such as follicle and CL size measurements and blood hormone concentrations were determined using mixed models with cow nested within genotype as a random effect. A first-order autoregressive covariance structure with homogeneous variance provided the best fit for the data based on AIC values. Transformed data were used to calculate *P*-values, and the estimated group means and 95% confidence intervals reported are back-transformed values. The effects of genotype, parity, day of cycle, calving date, and their interactions were included in the final model statements where significant ($P < 0.1$).

The effect of genotype on continuous variables without repeated measures such as day of 1st wave emergence, 1st wave dominant follicle (DF) maximum diameter, the interval from emergence of final wave to ovulation, interval from dominance to ovulation and the ovulatory DF maximum diameter were determined using mixed models 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 was included as an adjustment variable, and significant effects ($P < 0.1$) were maintained in the final model.

Differences between genotypes for variables with a binomial distribution (the proportion of cow with 2 or ≥ 3 follicular waves, the proportion that ovulated or failed to ovulate, and the proportion that exhibited or failed to exhibit estrus were tested using Fishers exact test. The effect of genotype on the number of follicular wave was analysed using PROC NPAR1WAY and the Kruskal-Wallis test.

RESULTS

General Characteristics of the Estrous Cycle

The effect of genotype on ovarian measurements during the estrous cycle are summarized in Table 3. Genotype had a significant effect ($P = 0.01$) on cycle length; the interovulatory interval was 4.1 ± 1.08 d shorter in Fert+ cows compared with Fert- cows. Fert+ cows tended to have fewer ($P = 0.065$) follicular waves during the estrous cycle than Fert- cows (2.2 vs. 2.7 waves). There was no effect of genotype on the proportion of cows with 2 or ≥ 3 follicular waves ($P > 0.05$). Estrous cycles with two follicular waves were most common in both Fert+ (79%, 15/19 cows) and Fert- (54% 6/11 cows) cows. There was no effect of genotype on the mean daily number of follicles < 5 mm present on both ovaries during the first 13 days of the cycle (20.0 vs. 18.6 for Fert+ and Fert-, respectively). Of cows that successfully underwent luteolysis and developed a preovulatory follicle, a greater proportion ($P < 0.01$) of Fert- cows failed to ovulate the dominant follicle (0% vs. 18% for Fert+ and Fert-, respectively). Of cows that successfully ovulated the preovulatory dominant follicle, a greater proportion ($P = 0.02$) of Fert- cows failed to demonstrate behavioural estrus (2% vs. 22% for Fert+ and Fert-, respectively). Of cows that showed behavioural estrus a greater proportion ($P = 0.04$) of Fert- cows failed to ovulate a dominant follicle (0% vs. 14% for Fert+ and Fert-, respectively). There was no effect of genotype on the proportion of cows having multiple ovulations (2% vs. 7% for Fert+ and Fert-, respectively)

First Follicular Wave

The effect of genotype on follicular dynamics and circulating concentrations of FSH and E2 during the first follicle wave are summarized in Table 4. Cow genotype did not affect (all $P > 0.05$) day of emergence, number of follicles < 5mm, or day of dominant follicle peak diameter. There was no effect of genotype ($P = 0.11$) on the

mean size of the dominant follicle during the first wave (Figure 2). However, the maximum diameter of the first wave dominant follicle tended to be greater (1.31 ± 0.51 mm) in Fert- than Fert+ cows (P = 0.08). Circulating concentrations of FSH during the first follicular wave were similar for both genotypes (Figure 2). Cow genotype did not affect peak FSH concentrations, day of peak FSH concentrations or the increase in FSH from basal concentrations to peak concentrations (all $P > 0.05$). Fert+ cows took 18.1 ± 9.1 h less (P = 0.05) to reach peak FSH concentrations than Fert- cows. There was a significant genotype by day of cycle interaction ($P < 0.05$) for circulating E2 concentrations during the first follicular wave (Figure 2). There was no effect of genotype on peak E2 concentrations, day of peak E2 concentrations or the interval from peak FSH concentrations to peak E2 concentrations (all $P > 0.05$).

CL Volume and P4 Concentration

The effect of genotype on CL development and plasma concentrations of P4 during the first 13 d of the cycle are illustrated in Figure 3 and summarized in Table 5. During the first 13 d of the cycle, CL volume was 16% greater in Fert+ ($P = 0.08$) than Fert- cows. Circulating concentrations of P4 did not differ between Fert+ and Fert- cows during the first 5 d of the cycle (0.73 vs. 0.59 ng/ml, respectively). From d 5 to 13 of the cycle, circulating concentrations of P4 were approximately 34% greater ($P < 0.001$) in Fert+ cows than Fert- cows (5.15 vs. 3.84 ng/ml, respectively). Fert+ cows had greater peak plasma P4 concentrations (P = 0.02). The day of the cycle when peak plasma P4 concentrations occurred tended to be earlier (P = 0.09) in Fert+ than Fert- cows. Luteolysis occurred 2.9 d earlier (P = 0.03) in Fert+ cows than Fert- cows. The interval from luteolysis to ovulation was 1.0 ± 0.34 d shorter (P = 0.08) in the Fert+ compared with Fert- cows (Table 5).

Preovulatory Follicle Wave

There was no effect of genotype ($P > 0.05$) on the pattern of growth of the preovulatory dominant follicle from emergence to ovulation (Figure 6), with the exception that Fert+ cows ovulated a larger ($P = 0.03$) diameter follicle than Fert- cows (+1.14 ± 0.36 mm). Cow genotype had no effect ($P > 0.05$) on the number of follicles < 5 mm, the interval from preovulatory follicle emergence to ovulation or the interval form preovulatory follicle dominance to ovulation (Table 6). Circulating concentrations of E2 during the final follicular wave were similar ($P > 0.05$) for both genotypes (Figure 6). Cow genotype did not affect peak E2 concentrations, interval from peak E2 concentrations to ovulation or the increase in E2 from basal to peak concentrations (all $P > 0.05$).

Estrus Behaviour

The effect of genotype on estrus behaviour measurements is summarized in Table 7. Peak activity occurred 5.7 ± 2.2 h earlier ($P = 0.07$) and was 41% greater ($P < 0.01$) in Fert+ than Fert- cows. During the period from 30 h to 12 h before ovulation, Fert+ cows had significantly greater ($P < 0.05$) mean activity count than Fert- cows. There was no effect of genotype on the interval from first mount to ovulation or last mount to ovulation; however, the Fert+ group tended to have a longer ($P = 0.08$) overall duration of estrus than the Fert- group (7.5 ± 0.54 h and 5.9 ± 0.76 h, respectively). There was no difference in the number of mounts or the duration of each mount between genotypes.

Production Variables

Mean daily milk yield during the course of the estrous cycle did not differ ($P > 0.05$) between genotypes, (20.3 ± 0.54 kg/d and 19.5 ± 0.62 kg/d for the Fert+ and Fert- cows, respectively). Similarly there was no effect ($P > 0.05$) on genotype on milk solids yield (1.36 ± 0.039 kg/d vs. 1.31 ± 0.044 kg/d for Fert+ and Fert- cows, respectively). Fert+ cows tended to have greater BCS ($P = 0.09$) throughout the estrous cycle (2.96 ± 0.032 vs. 2.87 ± 0.036 units for Fert+ and Fert- cows, respectively).

DISCUSSION

This study provides compelling evidence that genetic merit for fertility traits has profound effects on the estrous cycle that are manifest in changes in ovarian follicle and CL measurements, circulating hormone concentrations and estrus behaviour. In the current study, factors that are known to affect ovarian function were similar in both experimental groups (age, nutrition, stage of lactation, phenotypic milk yield, genetic merit for milk production, proportion of Holstein genetics), but they were extremely divergent in genetic merit for fertility traits. Thus, this animal model can be used to help elucidate the physiological mechanisms that are responsible for subfertility in lactating Holstein cows in a pasture-based production system.

The present study indicated that genetic merit for fertility traits alters the length of the estrous cycle. Compared with Fert+ cows, Fert- cows took ~ 3 d longer to undergo spontaneous luteolysis and ~ 5 d longer to achieve a spontaneous ovulation in a natural cycle timed from a previous synchronized ovulation. There is a lack of consensus between studies comparing estrous cycles in heifers and lactating cows as to the association between cycle length and fertility; some found shorter cycle lengths

in heifers (Wolfenson et al., 2004) while others found no difference in cycle length (Sartori et al., 2004). In accordance with previous reports, cows in the current study predominantly had two waves of follicular development (Bleach et al., 2004, Townson et al., 2002). Townson et al. (2002) reported superior reproductive performance in cows with three follicular waves compared to cows with 2 follicular waves. The current study does not support that finding, as Fert+ cows tended to have fewer follicular waves than Fert- cows. A prolonged period of preovulatory follicle dominance in cows with two follicular waves was suggested by Townson et al. (2002) as a potential cause for decreased fertility performance. In the current study, no difference between genotypes was observed in the duration of dominance of the preovulatory follicle.

Following the preovulatory LH surge, theca and granulosa cells of the dominant follicle undergo luteinization. This involves a series of biochemical and morphological changes altering the steroidogenic pathway of both cell types to synthesize P4 and thus forming a CL (Niswender et al., 2000). The development of a functional CL and the timely increase in circulating concentrations of P4 play a crucial role in stimulating endometrial secretions necessary for embryonic growth and development (Garrett et al., 1988, Sreenan, 2001). It has been suggested that the reproductive failure of high producing dairy cows is partially caused by a delayed postovulatory rise in circulating P4 concentrations and inadequate circulating P4 concentrations during the luteal phase (Mann and Lamming, 2001). Elevated plasma P4 concentrations are necessary for processes essential for enhancing embryo development including maternal recognition of pregnancy (Mann and Lamming, 2001), functional changes in histotroph composition (Green et al., 2005), and structural changes in endometrial glandular duct density (Wang et al., 2007). In the

current study, genetic merit for fertility traits had a significant effect on circulating P4 concentrations. As early as d 5 of the cycle, Fert+ cows had greater plasma P4 concentrations and this difference was maintained until luteolysis (25% greater P4 plasma concentration). The essential role of circulating P4 concentrations for reproductive performance is well documented (Clemente et al., 2009, Lopes et al., 2007, Stronge et al., 2005). Studies comparing the estrous cycles of heifers and lactating cows found increased circulating P4 concentrations in heifers as early as d 3 (Wolfenson et al., 2004) and d 6 (Sartori et al., 2004) of the cycle.

Increased steroid metabolism has been suggested as a potential mechanism responsible for the reduced circulating P4 concentrations in lactating cows (Sangsritavong et al., 2002). In the current study, there was no difference in the size of the follicle that ovulated at the synchronized estrus. Following ovulation, however, Fert+ cows developed a CL that was 16% larger than Fert- cows. If it is assumed that the steroid production capacity per unit volume of CL is similar between genotypes, the observed differences in circulating P4 concentrations (+25%) can only partially be explained by the larger CL volume of the Fert+ cows. This suggests that other potential factors such as the abundance of steroidogenic substrates, intracellular steroidogenic pathways, luteal vascularisation, and metabolic clearance rate (Wiltbank et al., 2006) may also be involved in the observed differences in circulating P4 concentration. As no differences between genotype were observed in daily milk yield and DMI (Cummins et al. 2012), liver blood flow and steroid metabolic clearance rate would be expected to be similar. While the current study establishes that genetic merit for fertility traits affects circulating P4 concentrations, the etiology of increased circulating P4 concentrations and the mechanisms by which this is translated into superior reproductive performance warrant further investigation.

The ovarian dominant follicle is central to reproductive biology, playing a crucial role in most processes from primordial follicle development to successful maintenance of pregnancy (Lucy, 2007). In the current study, genotype had a significant effect on ovulatory follicle diameter, with Fert+ cows ovulating a larger dominant follicle. This finding is in agreement with studies that found a positive association between ovulatory follicle size and pregnancy rate in beef (Perry et al., 2005) and dairy cows (Lopes et al., 2007, Vasconcelos et al., 2001). The differences in ovulatory follicle diameter in the present study were not associated with differences in circulating E2 concentrations. This is probably because the dominant follicle in the final follicular wave was similar in size during the process of selection and dominance, and only deviated in size on the last day of the cycle. A notable feature of the current study was the large proportion (14%) of Fert- cows that underwent luteolysis, showed behavioural estrus but failed to ovulate the dominant follicle. This suggests that either the LH surge in these animals was inadequate to cause ovulation or the ovarian response to a normal LH surge was dysfunctional. Details of the duration and magnitude of the LH surge were not determined in the current study. Similar levels of ovulation failure (3.4% - 14.1%) have been reported in studies by López-Gatius et al. (2005) and Demetrio et al. (2007) in high yielding Holstein cows. Heat stress was cited as a potential cause for such high levels of ovulation failure (López-Gatius et al., 2005). In the current study, however, mean daily air temperatures ranged from 8.9 ºC to 18.8 ºC, indicating that heat stress was not a contributing factor to Fert- cows failing to ovulate. In addition, milk production was similar in both Fert+ and Fert- cows, and was low compared with TMR systems. Thus, level of milk production *per se* can be eliminated as a causative factor responsible for ovulation failure in the current study.

A cow's ability to show behavioural estrus and successfully ovulate a dominant follicle is a prerequisite for optimal timing of AI and subsequent oocyte fertilisation. The reduction in both the intensity and duration of estrus is a major contributing factor to the decline in reproductive efficiency in the modern dairy cow (Dobson et al., 2008, Law et al., 2009). Of cows that displayed standing heat in the current study, estrus behaviour differences between genotypes were minor. Of the cows that successfully ovulated, however, 22% of Fert- cows recorded no standing mounts compared to only 2% of Fert+ cows. Moreover, Fert+ cows recorded 41% greater peak activity at estrus than Fert- cows. These disparities highlight for the first time the role that genetic merit for fertility traits has on estrus behaviour. The performance of the Fert- cows is consistent with a previous study on high yielding Holstein cows (n = 463), that reported > 30% of ovulations occurred without any display of standing heat (Lopez et al., 2004). That study demonstrated that estrus behaviour was correlated with level of milk production (negative) and circulating E2 concentrations (positive). In the current study, however, both milk yield and circulating E2 concentrations were similar between genotypes. The exact underlying processes whereby genetic merit for fertility traits alters estrus behaviour remains unknown. Potential differences between genotypes could exist in the threshold concentrations of E2 required for estrus and the LH surge to occur, as has been documented between species (Fabre-Nys et al., 1993). Differences in luteal phase P4 priming of the neural mechanisms involved in GnRH release, as observed in sheep (Skinner et al., 2000), could also explain some of the variation.

CONCLUSION

Genetic merit for fertility traits had a significant effect on characteristics of the estrous cycle and were independent of management, plane of nutrition, stage of lactation, proportion of Holstein ancestry, and genotypic and phenotypic milk yield. The Fert+ group had shorter estrous cycles, greater circulating P4 concentrations, exhibited stronger estrus and ovulated larger follicles than the Fert- group. The differences in follicular dynamics and steroid concentrations may partially explain the superior fertility performance of the Fert+ cows. These results highlight the effect genetic merit for fertility traits has on measurable changes in ovarian function

ACKNOWLEDGEMENTS

The authors would like to thank Mr J.P. Murphy, Mr J. Kenneally, and the Moorepark farm staff for management and care of the animals. We also thank Ms. P. Furney (University College Dublin) for her technical assistance. The Teagasc Walsh Fellowship scheme, National Development Plan, and Dairy Levy funding is gratefully acknowledged.

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Table 1. The mean estimated breeding value[1] (and SD) for Fert+ and Fert- cows for milk production, survival, and calving interval

Genotype	Fert+	Fert-
Number of animals	21	16
NAHF (%)	91.2 (6.45)	93.2 (6.96)
Milk (kg)	+455.5 (133.9)	+433.2 (119.9)
Fat (kg)	+21.5 (7.37)	+17.5 (6.59)
Protein (kg)	+18.3 (5.43)	+17.1 (5.01)
Fat (g/kg)	+0.085 (0.117)	+0.025 (0.119)
Protein (g/kg)	+0.069 (0.049)	+0.06 (0.069)
Survival (%)	+3.12 (0.80)	-0.24 (1.22)
Calving interval (d)	-6.42 (1.44)	+5.49 (2.22)
Sire calving interval (d)	-9.14 (3.77)	+6.69 (2.17)
Maternal grandsire calving interval (d)	-5.14 (2.20)	+7.30 (4.59)

[1]All PTA's were obtained from the Autumn 2008 and autumn 2007 official dairy evaluations published by the Irish Cattle Breeding Federation and multiplied by two to convert to EBV's.

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Table 2. The mean (and SD) chemical composition of grass silage and concentrate supplement fed during the experimental period

Feed	Grass silage	Conc.
ADF (g/kg of DM)	359.1 (17.4)	
CP (g/kg of DM)	148.0 (20.0)	147.3 (18.3)
NDF (g/kg of DM)	521.8 (27.2)	316.3 (93.0)
Ash (g/kg of DM)	93.4 (12.3)	96.3 (4.8)
Crude Fibre (g/kg of DM)		102.5 (2.03)

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Table 3. The effect of genetic merit for fertility traits on estrous cycle characteristics

	Genotype			
Variable	Fert+	Fert-	SED[1]	*P*-Value
Number of animals	18	10		
Day of ovulation (d)	21.0	25.1	1.53	0.01
Number of follicular waves	2.2	2.7		0.07
Proportion with 2 follicular waves (n)	0.79 (15/19)	0.54 (6/11)		0.2
Proportion with $\geq$ 3 follicular waves (n)	0.21 (4/19)	0.45 (5/11)		0.2
Proportion that failed to ovulate[2]	0 (0/41)	0.18 (5/28)		0.009
Proportion that failed to exhibit estrus[2]	0.02 (1/41)	0.25 (7/28)		0.006
Proportion that ovulated but failed to exhibit estrus[2]	0.02 (1/41)	0.22 (5/23)		0.02
Proportion that exhibited estrus but failed to ovulate[2]	0 (0/40)	0.14 (3/21)		0.04
Proportion with double ovulations[2]	0.02 (1/41)	0.07 (2/28)		0.6

[1]SED =pooled standard error of the difference

[2] Combined data from synchronized and spontaneous heat

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Table 4. The effect of genetic merit for fertility traits on follicular measurements and circulating concentrations of E2 and FSH during the first follicular wave

	Genotype			
Variable	Fert+	Fert-	SED[1]	*P*-Value
Number of animals	19	11		
Day of 1st wave emergence (d)	0.97	1.70	0.62	0.2
No. of follicles > 5mm in 1st wave	2.71	2.96	0.34	0.5
Day of 1st wave DF peak diameter (Days)	7.73	8.55	0.67	0.2
1st wave DF max diameter (mm)	14.80	16.11	0.72	0.08
Day of peak FSH (d)	1.2	2.4	0.74	0.11
Peak FSH concentration (ng/ml)	0.34	0.33	0.03	0.8
Duration of increase of FSH to peak (hr)	37.6	55.7	13.0	0.05
Increase in FSH from basal levels to peak (ng/ml)	0.19	0.18	0.03	0.8
Interval from peak FSH to 1st wave DF max diameter (d)	6.5	6.8	0.52	0.5
Day of peak E2 (d)	4.6	4.7	0.63	0.8
Peak E2 concentration (pg/ml)	1.54	1.31	0.21	0.3
Duration of increasing E2 to peak (h)	55.5	59.1	13.23	0.07
Increase in E2 from basal levels to peak (pg/ml)	1.15	1.0	0.21	0.4
Interval from peak E2 to 1st wave DF max diameter (d)	6.3	7.5	0.76	0.14
Interval from peak FSH to peak E2 (d)	3.2	2.7	0.48	0.3

[1]SED =pooled standard error of the difference

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Table 5. The effect of genetic merit for fertility traits on circulating P4 concentrations, timing of luteolysis and interval from luteolysis to ovulation

	Genotype			
Variable	Fert+	Fert-	SED[1]	*P*-Value
Number of animals	19	11		
Day of cycle at peak P4 (d)	12.3	14.7	1.34	0.09
Peak P4 (CI in parenthesis) (ng/ml)	8.07 (7.25, 8.96)	6.5 (5.59, 7.55)		0.02
Interval form peak P4 to ovulation	8.2	10.8	0.76	0.002
Day of cycle at luteolysis	16.5	19.4	1.30	0.03
Interval from luteolysis to ovulation	4.3	5.3	0.49	0.05

[1]SED =pooled standard error of the difference

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Table 6. The effect of genetic merit for fertility traits on follicle measurements, circulating concentrations of E2 during the preovulatory follicle wave, and interval from peak E2 to peak estrus activity.

	Genotype			
Variable	Fert+	Fert-	SED[1]	*P*-Value
Number of animals	18	10		
No. of follicles > 5mm in ovulatory wave	2.72	2.73	0.48	0.9
Interval from emergence to ovulation (d)	9.62	9.06	0.60	0.3
Interval from dominance to ovulation (d)	6.29	5.75	0.6	0.4
Ovulatory DF max diameter (mm)	17.91	16.76	0.52	0.03
Synchronized estrous DF max diameter (mm)	16.7	16.0	0.71	0.3
Interval from peak E2 to ovulation (h)	32.6	36.4	4.7	0.4
Peak E2 concentration (pg/ml)	4.48	3.75	0.46	0.13
Duration of increasing E2 to peak (h)	42	45	7.1	0.7
Increase in E2 from basal levels to peak (pg/ml)	2.80	2.39	0.52	0.4
Interval from peak E2 to peak activity (h)	2.6	9.9	6.87	0.3

[1]SED =pooled standard error of the difference

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Table 7. The effect of genetic merit for fertility traits on estrus behaviour (SED).

	Genotype			
Variable	Fert+	Fert-	SED[1]	*P*-Value
Number of animals	15	8		
Interval from peak activity to ovulation (h)	32.8	27.1	3.16	0.065
Peak activity (counts)	168.5	119.7	15.98	0.005
Activity 12-30 hrs prior to ovulation	103.4	73.3	13.4	0.03
Interval from first mount to ovulation (h)	35.6	37.0	2.12	0.5
Interval from last mount to ovulation (h)	27.0	30.7	2.8	0.8
Duration of heat[2] (h)	7.53	5.86	0.93	0.08
Number of mounts[2]	12.1	12.8	2.50	0.6
Duration of all mounts[2] (sec)	20.7	20.8	4.90	0.9

[1]SED =pooled standard error of the difference

[2]Combined data from synchronized and spontaneous estrus (Fert+ n = 40; Fert- n = 21)

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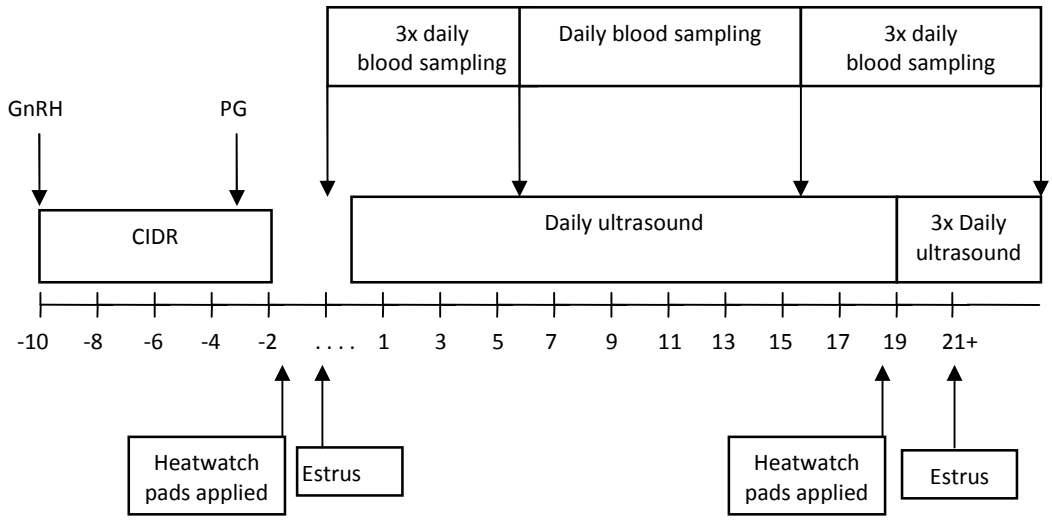

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638 **Figure 1.** Cummins
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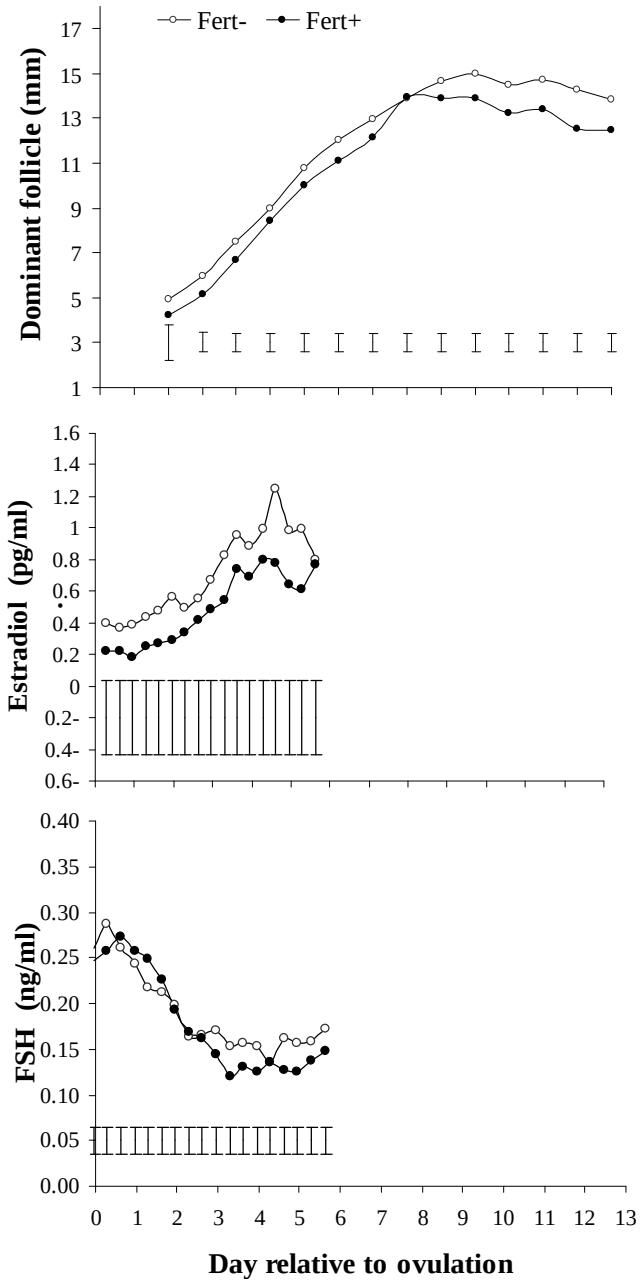

Figure 2. Cummins

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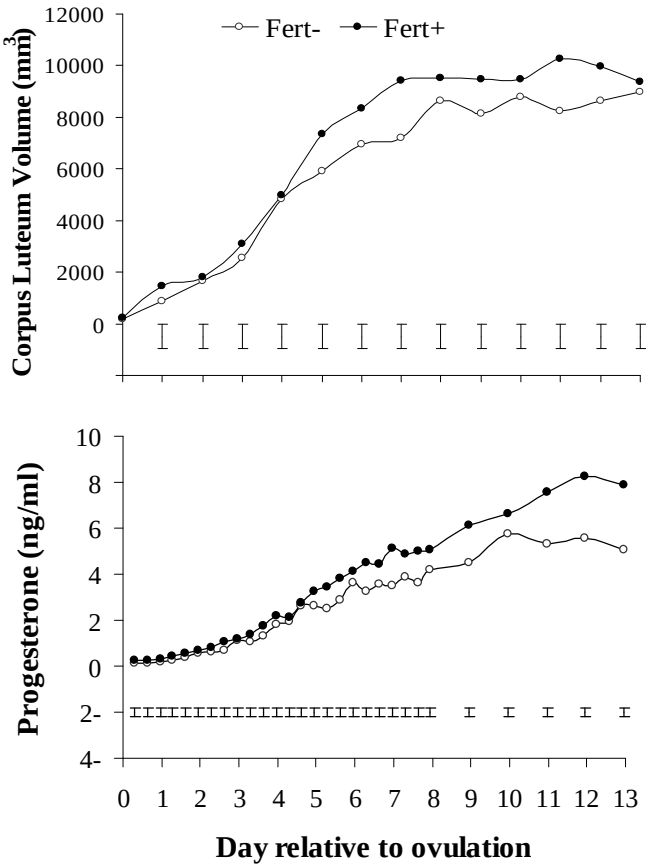

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660 **Figure 3.** Cummins
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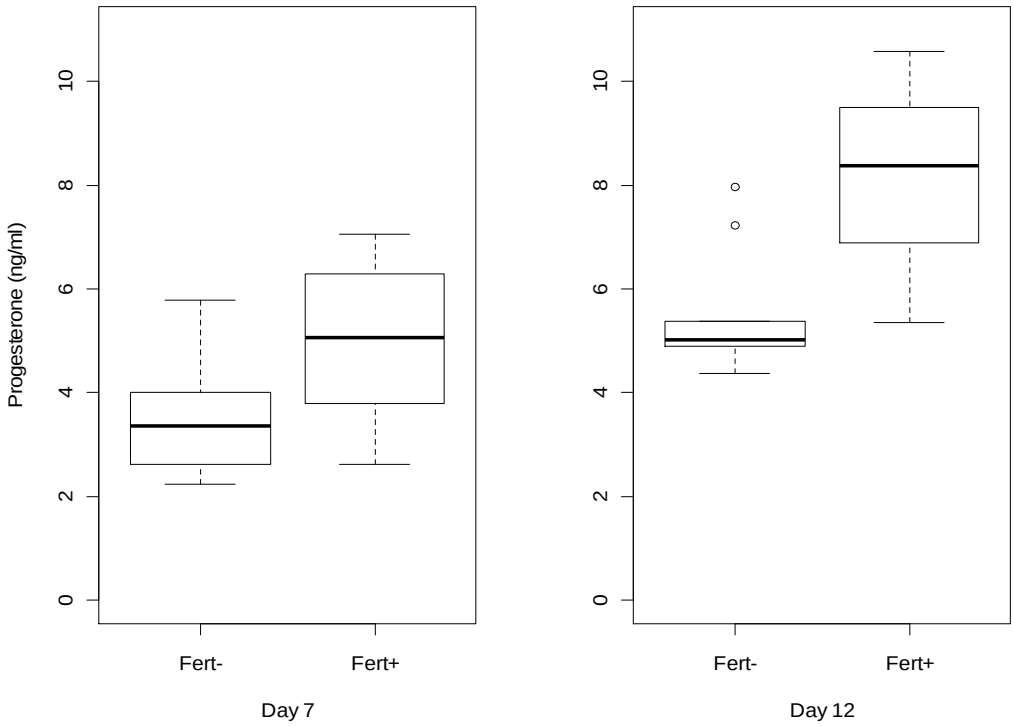

Figure 4. Cummins

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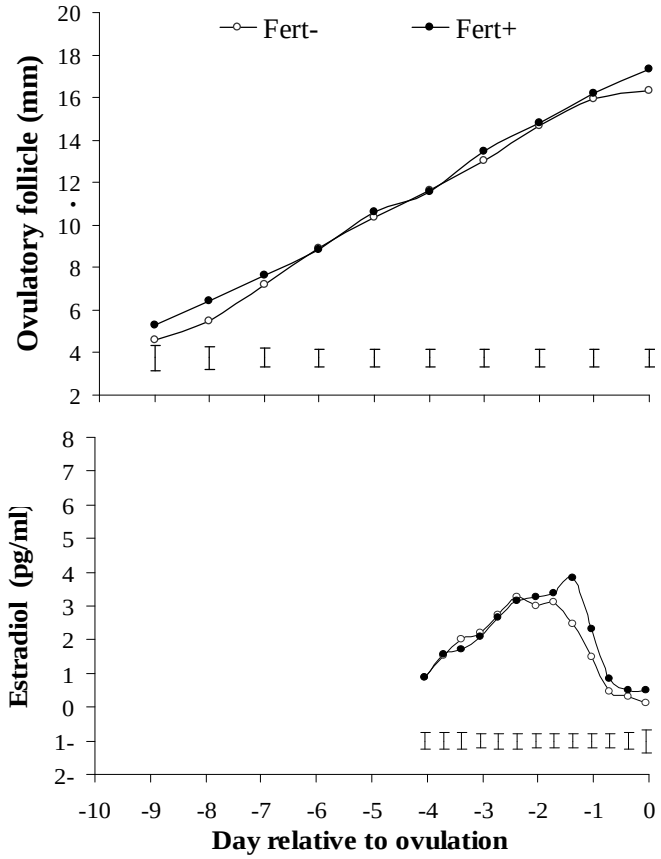

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680 **Figure 5.** Cummins
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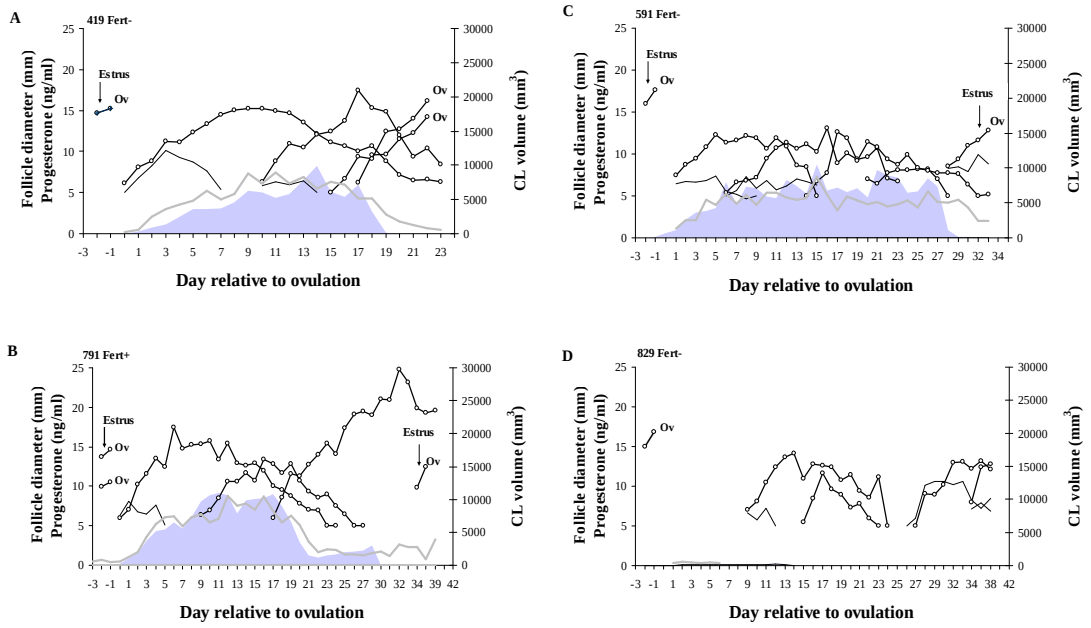

Figure 6. Cummins

Figure 1. Synchronisation protocol, blood sampling and ultrasound frequency for one complete estrous cycle relative to day of ovulation (d 0). CIDR = intravaginal P4-releasing device containing 1.38 g of P4; GnRH = GnRH agonist injections contained 10 µg buserelin; PG = prostaglandin $F_{2\alpha}$ analogue; OV = ovulation.

Figure 2. Dominant follicle size and circulating concentrations of E2 and FSH during the first follicular wave in 19 Fert+ and 11 Fert- cows. Vertical bars indicate the pooled standard error of the difference.
Top panel: No genotype ($P = 0.11$), genotype × day of cycle or genotype × parity effect was detected for first wave dominant follicle size (SED = 0.59 mm).
Middle panel: No effect of genotype or genotype × parity interaction was observed for plasma E2 concentrations ($P > 0.05$). There was a significant genotype × day of cycle interaction ($P < 0.05$) for E2 from 8 h prior to ovulation until d 6 of the cycle. The mean (95% confidence intervals in parentheses) circulating E2 concentrations were 0.40 (0.34, 0.46) and 0.48 (0.39, 0.59) for Fert+ and Fert-, respectively.
Bottom panel: No genotype, genotype × day of cycle or genotype × parity interaction effects were detected for plasma FSH concentrations ($P > 0.005$) from 1 day prior to ovulation until d 6 of the cycle. The mean (95% confidence intervals in parenthesis) circulating FSH concentration were 0.17 (0.15, 0.18) and 0.18 (0.16, 0.20) for Fert+ and Fert-, respectively.

Figure 3. Corpus luteum (CL) volume and circulating concentrations of P4 during the first 13 d of the cycle in 19 Fert+ and 11 Fert- cows. Vertical bars indicate the pooled standard error of the difference.
Top panel: CL volume tended to be larger in Fert- cows ($P = 0.08$) during the first 13 d of the cycle. No genotype × day of cycle or genotype × parity interactions were observed for CL volume ($P > 0.05$).
Bottom panel: Plasma P4 concentrations were significantly greater in Fert+ cows ($P < 0.01$) during the first 13 d of the cycle. No genotype × parity interaction was observed, but a significant genotype × day of cycle interaction existed ($P < 0.001$).

Figure 4. Box plots depicting circulating concentrations of P4 during d 7 and d 12 of the estrous cycle in 19 Fert+ and 11 Fert- cows. Plasma P4 concentrations were significantly greater ($P < 0.001$) in Fert+ cows on both d 7 and d 12 of the cycle. The mean (95% confidence intervals in parentheses) circulating P4 concentrations on d 7 were 5.00 ng/ml (4.33, 5.70) and 3.25 ng/ml (2.65, 3.96) for Fert+ and Fert-, respectively. The mean (95% confidence intervals in parentheses) circulating P4 concentrations on d 12 were 8.01 ng/ml (7.22, 8.86) and 5.40 ng/ml (4.65, 6.23) for Fert+ and Fert-, respectively.

Figure 5. Preovulatory follicle growth and circulating concentrations of E2 prior to ovulation in 18 Fert+ and 10 Fert- cows. Vertical bars indicate the pooled standard error of the difference.
Top panel: No effect of genotype, genotype × day of cycle or genotype × parity interaction was observed for preovulatory follicle diameter ($P > 0.05$).
Bottom panel: No effect of genotype, genotype × day of cycle or genotype × parity interaction was observed for plasma estradiol concentration ($P > 0.05$).

Figure 6. Patterns of follicular development (black lines: dominant follicle = thick lines with open circles; subordinate follicles = thin lines), luteal volume (dark grey line), and serum progesterone concentrations (light grey area) in cows with atypical estrous cycles.
A: a Fert- cow that ovulated a dominant follicle following behavioural estrus at the synchronized estrus, which resulting in the formation of a functional CL. Following luteolysis two co-dominant follicles from the third follicular wave ovulated at a silent estrus.
B: a Fert+ cow that ovulated two co-dominant follicles following behavioural estrus at the synchronized estrus, which resulted in the formation of two functional CL's. One CL underwent structural and functional luteolysis between d 19 and 20, but the second CL did not undergo luteolysis approximately 14 d later. Following luteolysis of the second CL, the growing dominant follicle of the third follicular wave failed to ovulate and continued to grow; forming a large persistent follicle. A fourth follicular wave emerged and the dominant follicle ovulated following behavioural estrus on d 39 of the cycle.

C: a Fert- cow that ovulated a dominant follicle following behavioural estrus at the synchronized estrus, and formed a functional CL. Luteolysis did not occur until d 28. The dominant follicle of the fifth follicular wave ovulated following behavioural estrus on 34 d of the cycle.
D: a Fert- cow that ovulated a dominant follicle at the synchronized estrus without behavioural estrus. The newly ovulated follicle failed to develop into a functional CL. The cow became anestrous and turnedover four anovulatory follicular waves. Ultrasound scanning ceased on d 38.