REVISED

Running Head: EFFECT OF STRAIN OF DAIRY COW ON FERTILITY

The effect of strain of Holstein-Friesian cow on size of ovarian structures, periovulatory circulating steroid concentrations, and embryo quality following superovulation.

M. A. de Feu[1, 2], J. Patton[1], A. C. O. Evans[2], P. Lonergan[2], S. T. Butler[1*]

[1]Teagasc, Moorepark Dairy Production Research Centre, Fermoy, Co. Cork, Ireland;

[2] School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

* Corresponding author: stephen.butler@teagasc.ie,

Telephone/Fax: +353- (0)25-42252/ +353-(0)25-42340

Keywords: cow, oestradiol, progesterone, embryo quality, superovulation

Abstract

When managed under grass-based systems of production, the NZ strain of Holstein-Friesian cow has superior reproductive performance compared to the NA strain despite having similar SCM yields. This study compared the ontogeny of early pregnancy events in NZ and NA cows. Ten NZ and 10 NA cows were submitted to a superovulation protocol on three occasions. Blood samples were collected daily from every cow from day -3 to +7 relative to a synchronised oestrus during each superovulation protocol. Pre-ovulatory oestradiol concentrations, follicle diameter, post-ovulatory progesterone concentrations, CL diameter, and circulating insulin-like growth factor-I concentrations did not differ between the two strains. Uteri were non-surgically flushed 7 d post AI, embryos were isolated and graded. The proportion of transferable embryos recovered was higher ($P<0.01$) in the NZ cows compared with the NA cows. A greater ($P=0.01$) proportion of the recovered structures were at the blastocyst stage in the NZ cows. Peak SCM yield and BCS at the time of peak SCM yield were not different between strains. However during the experimental period the NA cows maintained significantly higher daily SCM yields, whereas the NZ cows replenished significantly greater levels of BCS. The results indicate that differences in periovulatory steroid concentrations and size of ovarian structures do not explain the differences in embryo quality between the two strains. However, strain differences in nutrient partitioning from the time of peak SCM yield through late lactation may provide the key signals responsible for superior embryo quality in NZ cows.

Introduction

The British Friesian was the predominant breed of dairy cow in Ireland until the mid 1980's. In the last 20 years, however, the use of North American (NA) Holstein-Friesian (HF) genetics has dominated. The proportion of NAHF genes increased from 8% in 1990 to 63% in 2001 (1), the impetus for this being primarily to improve the rate of genetic gain for milk production. This policy of selecting primarily for increased milk yield resulted in cows capable of high milk production, but also resulted in reproductive performance that was suboptimal for efficient seasonal-calving pasture-based systems of production (2,3). In the period between 1990 and 2001, fertility, measured in terms of calving rate to a single insemination, declined at a rate of almost 1% per annum in Irish dairy cows (1). However, despite intense research efforts in the area, the underlying mechanisms responsible for the compromised reproductive performance of the modern dairy cow remain poorly understood.

It has been demonstrated that embryo quality on day 5 after insemination (based on stage of development, blastomere compactness, and cellular debris) is inferior in high yielding lactating cows compared to non-lactating cows (4), suggesting that the energetic demands of lactation in high producing cows may have an adverse effect on early embryo development. Similarly, embryonic loss in subfertile (repeat breeder) dairy cows is evident from day 6-7 post AI, as the morula develops into a blastocyst (5). By comparison, in beef heifers, where energy balance is generally positive and the metabolic burden of lactation does not exist, little embryo loss occurs before day 8 post-insemination (6).

Secretion of progesterone (P4) by the corpus luteum (CL) is essential for the successful establishment and maintenance of pregnancy. Progesterone concentrations

during both the cycle preceding and following insemination affect embryo survival, with evidence that insufficient or indeed excessive concentrations of P4 are detrimental (7). Previous reports have found that a more rapid rate of rise in post-ovulatory P4 concentrations improved embryo survival (8). In agreement with this, previous studies have observed that low P4 concentrations on day 5 to 7 of pregnancy were associated with lower fertility (9), and it has also been reported that supplementary P4 enhances the development of conceptuses in beef cows(10). It has been speculated that the relationship between successful embryonic development and P4 is probably mediated through beneficial effects of P4 on the uterine environment and also concluded that successful recognition of pregnancy requires an adequate degree of embryonic development and an appropriate pattern of P4 secretion following ovulation (11).

The Teagasc Research Centre located at Moorepark, County Cork, Ireland has established herds of New Zealand (NZ) and NA strains of cattle with diverse genetic backgrounds. On a grass-based system of milk production, these strains produce approximately similar levels of milk solids over the course of a lactation, but have marked differences in reproductive performance (2,3). Hence, these different strains represent a unique and powerful tool to elucidate the biological mechanisms leading to compromised fertility. The aim of this study was to determine whether or not differences in size of ovarian structures, steroid concentrations, and early embryo development could be detected between the NZ and NA strains, which could explain, at least partly, the observed differences in conception rate.

Materials and Methods

Experimental design and Animals

The pedigree index for each cow was calculated as 0.50 × sire predicted difference + 0.25 maternal grandsire predicted difference + 0.125 × maternal great-grandsire predicted difference. The predicted difference of the sires and maternal grandsires were from the February 2004 international evaluations of the INTERBULL Animal Center (Uppsala, Sweden) using the technique known as MACE (multiple-trait across-country evaluation).

Ten NZ Holstein-Friesian cows, genetically selected on the basis of milk solids production, feed efficiency and survivability in a grass-based seasonal system of production (12), and 10 NA Holstein-Friesian cows, genetically selected on the basis of high milk production, were used in this study. The top 50% of HF cows in the Moorepark herd based on pedigree index for milk production were inseminated with semen from five North American HF sires to generate the NA strain. The five sires were chosen on the basis of their superior pedigree index for milk production. The average proportion of HF genes in the NA strain was 90%. The NZ animals were imported as embryos from New Zealand and implanted into 13-month-old HF heifers at Moorepark. The embryos were generated by mating the highest available genetic merit New Zealand HF cows (based on the New Zealand genetic evaluation system, Breeding Worth) to five New Zealand HF sires. On average, 87·5% of the NZ strain ancestry were New Zealand HF. Jersey contributed up to a maximum of 12.5% ancestry, with the remaining ancestry composed of North American HF. The co-ancestry co-efficient between the NA and NZ strains based on 6 generations of pedigree depth was 0.72%, and therefore the overall level of genetic similarity among the two strains is very low. The mean pedigree indices (based on Irish proofs with

Interbull conversions) of the two strains for milk production, calving interval and survival are reported in Table 1. Detailed descriptions of the two strains have been previously described (2), and further information on the milk production and bioenergetic status of the particular animals used in the current study is also available (13).

Mean calving dates and lactation number were 25th February (s.d. 18 days) and 3.8 (s.d. 1.1) for the NA strain, and 2nd March (s.d. 17 days) and 4.4 (s.d. 0.5) for the NZ strain. Cows in both groups were allocated 4 kg of concentrate per day and *ad libitum* grazed grass (primarily *Lolium perenne*) throughout the experimental period. All animals had exhibited normal oestrous cycles and were clinically healthy before the treatment started and throughout the study period. Cows were milked twice daily, and milk yield was recorded at each milking. Milk composition (fat, protein and lactose concentrations) were determined once per week on an AM and PM sample by near-infrared reflectance spectroscopy (Milkoscan 605; Foss Electric, Hillerød, Denmark). Solids-corrected milk (SCM) yield was calculated using the equation of Tyrrell and Reid (14).

Oestrus synchronization and superovulation protocol

All cows were submitted to a superovulation protocol on three occasions between July and November 2005. The superovulation protocol and blood sampling regime is illustrated in Fig. 1. Each superovulation protocol took place over a 33-day period. Oestrus was synchronized in all cows using an intravaginal P4-releasing device (Eazi-breed CIDR, containing 1.94g P4 Ph. Eur., InterAg, New Zealand) inserted for 9 days. On the day prior to CIDR removal, $PGF_2\alpha$ (500 µg cloprostenol sodium, BP (Vet) Coopers, Berkhamsted, England) was administered intramuscularly

(i.m.) at 8AM. Tail paint was applied on the day of CIDR removal and cows were observed for oestrus behaviour for the following four days. Commencing on day 10 following oestrus, follicle stimulating hormone (FSH; Folltropin, Bioniche Animal Health Europe Ltd, Clonee, Co. Meath, Ireland) was administered i.m. twice daily at 12-hr intervals in decreasing doses over a four day period (Table 2).

Prostaglandin $F_2\alpha$ analogue was administered i.m. concomitant with the sixth injection of FSH. All cows were inseminated with frozen-thawed semen collected from a single ejaculate of a Holstein-Friesian bull (Dairygold A.I., Mallow, Co. Cork, Ireland.) at 36 and 48 hrs after the final injection of FSH. Kinship between the bull used for insemination and the cows on the study was examined by looking at the co-ancestry over the 3 preceding generations. The bull used for insemination had a co-ancestry co-efficient of 2.8% with the NA cows and 0.3% with the NZ cows based on 6 generations of pedigree analysis. The co-ancestry co-efficient for the NA cows is similar to the national inbreeding coefficient for Holstein-Friesian females born in Ireland in 2004 (15). At each of the three superovulation treatments, cows were removed from the protocol if one of the following occurred: (i) no ovulation at reference heat; (ii) no super-stimulatory response to FSH treatment; (iii) no ovulation following FSH treatment.

(Insert Table 2 here)

Transrectal ultrasonography

Ovarian structures were examined by linear array ultrasonography using a 7.5-MHz transrectal transducer (Aloka SSD-900; Aloka Ltd., Tokyo, Japan). Ultrasound scans were carried out at four time-points for each superovulation protocol: two days

after CIDR removal to determine follicle diameter on day of oestrus; the final day of blood sampling (day 7, relative to the reference heat) to verify the presence and diameter of the CL; on the day before AI to determine how many large follicles were present in response to FSH treatment; and on the day prior to flushing to determine the number of CL's on each ovary.

Blood sampling and hormone analysis

Blood samples were collected daily during the reference heat period of each superovulation protocol at 8 AM, commencing on Day 3 prior to oestrus and continuing until day 7 after oestrus (see Fig. 1). Blood samples were collected from the coccygeal vessels into lithium heparin vacutainers (Becton Dickinson, Plymouth, United Kingdom). Blood samples were collected during the reference heat period rather than during the heat associated with the superovulation treatment to avoid confounding effects of exogenous gonadotropin administration and variable follicle and CL numbers on circulating steroid concentrations. Samples were centrifuged at 2000 × *g* for 15 minutes at 5 °C. The plasma was harvested and decanted into 1.5 ml tubes, sealed with an air-tight cap and stored at -20 °C until further analysis. Oestradiol (E2) concentrations were analysed from day -3 until day 0 (oestrus). Progesterone concentrations were measured in plasma samples taken from day of oestrus (0) until day 7 following oestrus. Circulating insulin-like growth factor–I (IGF-I) was determined in plasma samples taken on day 6 (relative to oestrus).

The concentration of E2 in plasma was determined by radioimmunoassay following extraction (16) using E2 MAIA kits (Biostat, UK). Inter- and intra-assay coefficients of variation were 21.9 and 3.8% (n = 3).

The P4 assays were carried out using a time-resolved fluoroimmunoassay (Autodelfia; PerkinElmer Life and Analytical Science, Ballymount, Dublin 12, Ireland) using P4 kits (Unitech BD Ltd., Dublin, Ireland). Inter- and intra-assay coefficients of variation were 27.5 and 4.4% (n = 2).

Circulating IGF-I concentrations were quantified using a validated double-antibody radioimmunoassay following ethanol-acetone-acetic acid extraction (17). Recombinant human IGF-I (R&D Systems Europe, UK) was used as a standard and as the iodinated tracer. The assay was carried out as described by Echternkamp et al. (18). Inter- and intra-assay coefficients of variation were 11.7 and 14.3% (n = 2).

Embryo recovery and evaluation of embryo quality

Uteri were non-surgically flushed on day 7 post AI by an experienced technician using standard techniques. Each uterine horn was flushed with 500 ml of phosphate buffer saline (PBS). Following flushing, the recovered lavage was filtered through an embryo filter (Miniflush Embryo Recovery System, mesh size 44µm, Minitub, Germany). The fluid was examined for oocytes/embryos under a stereomicroscope and the recovered structures were isolated and graded according to the criteria of the International Embryo Transfer Society (IETS) (19). Morphological assessment and grading of the embryos was carried out by an embryologist blind to the strain of the dams. $PGF_2\alpha$ was administered to all cows immediately after the flushing procedure.

Data handling and statistical analyses

All statistical analyses were carried out using SAS (SAS Inst. Inc., Cary, NC). Pedigree data was obtained from Holstein UK (www.holstein-uki.org), and co-ancestry co-efficients were calculated using PROC INBREED. The number of cows

that had a true reference heat (ovulation followed by an increase in circulating P4 concentrations) once, twice or three times was 0 and 1, 5 and 2, and 5 and 7 for the NZ and NA cows, respectively. The number of cows successfully flushed once, twice or three times was 5 and 6, 2 and 1, and 2 and 2 for the NZ and NA cows, respectively, resulting in 15 successful flushes for the NZ cows and 14 successful flushes for the NA cows. For each flush the proportion of recovered structures that were transferable (morulae and blastocysts), the proportion of recovered structures that were morulae and the proportion of recovered structures that were blastocysts were calculated. For all flushes yielding transferable embryos the proportions at the morula and blastocyst stages were also calculated. This data was then analysed using the Mann-Whitney non-parametric test with Wilcoxon scores, and Fishers exact test was used to compare differences between strains. Each superovulation and embryo flushing event was considered independent.

The progesterone area under the curve (P4AUC) was calculated from day of oestrus until day 7 post-oestrus for each cow during each reference heat. Peak E2, diameter of the dominant follicle on day of oestrus, diameter of the CL on Day 6 post-oestrus, P4AUC, and the P4 concentration on days 5 to 7 post-oestrus (P4D5-7) were analysed using repeated measures with the MIXED procedure of SAS. An unstructured covariance structure was used for the P4AUC and P4D5-7 analysis, and a first order autoregressive covariance structure was used for other variables based on best fit according to Akaike's Information criterion and Schwarz's Bayesian criterion (20). Strain, flush number and the interaction between strain and flush number were fixed effects, and cow within strain was included as a random effect. Lactation number and calving day of the year were included as adjustment variables for the IGF-I analysis but were removed because they were not significant. The coefficient of

variation was examined as an indicator of the repeatability of the progesterone measurements (P4AUC and P4D5-7) across flushes. The coefficient of variation is known to have a non-normal distribution and the numbers of animals with responses for each reference heat was small (5 for NZ and 7 for NA) so the data were analysed non-parametrically. A signed rank test was used for the complete group of responses (PROC UNIVARIATE) and an exact Wilcoxon two-sample test (PROC NPAR1WAY) was used to compare the two groups.

Results

Milk yield and body condition score

Peak solids corrected milk yield was not different between the NA and NZ strains (39.4 vs. 38.0 kg/day; P = 0.3), but over the course of the experimental period, the NA strain had significantly higher SCM yield compared to the NZ strain (24.3 vs. 20.9 kg/day, P = 0.005; Figure 2). There was no difference in BCS between NA and NZ strains at the time of peak milk production (2.70 vs. 2.85; P = 0.25). During the remainder of the lactation the NA strain both mobilised a greater amount of body reserves and failed to replenish body condition whereas the NZ strain commenced partitioning nutrients to body reserves from mid-lactation onwards resulting in significant differences in BCS during the experimental period (2.47 vs. 2.74, P = 0.03; Fig. 2).

(Insert Figure 2 here)

Ovarian structures and circulating steroid concentrations

The diameter of the preovulatory dominant follicle on the day of oestrus (17.1 vs. 17.5 mm; P = 0.7) and the diameter of the CL on day 7 post-oestrus (24.8 vs. 24.6 mm; P = 0.8) were not different between the NZ and NA strains, respectively. There were no differences between strains in peak pre-ovulatory E2 concentration (Table 3), P4D5-7 concentration, or P4AUC (Table 4 and Fig. 3). The repeatability of P4AUC and P4D5-7 were examined by comparing the coefficient of variation at each reference heat for all cows with 3 successful reference heats (n = 12). For both variables, the coefficient of variation was different from zero (P < 0.001), indicating low repeatability. There was no difference is the Wilcoxon scores for P4AUC CV (mean score 6.20 vs. 6.71; P = 0.8) or P4D5-7 CV (6.6 vs. 6.43; P = 0.9) between the NZ and NA strains, respectively, indicating no difference in the behaviour of the coefficient of variation between the two groups. There was no difference between strains in circulating IGF-I concentrations (flush 1: 82.0 *vs* 68.8 ng/ml; flush 2: 98.7 *vs* 93.7 ng/ml and flush 3: 108.3 *vs* 99.1 ng/ml for NZ and NA, respectively, pooled error = 10.8 ng/ml; P = 0.45).

(Insert figure 3 here.)

(Insert Table 3 here.)

(Insert Table 4 here.)

Embryo recovery and quality

The total number of CL and structures recovered are summarized in Table 5. The proportion of transferable embryos (morula and blastocyst) and the proportion of blastocysts recovered were higher for the NZ cows compared to the NA cows (P < 0.01 and P = 0.01, respectively). Of the transferable embryos recovered, the

proportion at the blastocyst stage tended (P = 0.099) to be higher in the NZ cows and consequently the proportion of embryos at the morula stage tended to higher in the NA cows (Table 5).

Insert Table 5 here

Discussion

Previous studies have indicated that events around the time of ovulation are associated with likelihood of successfully establishing a pregnancy. These include, but are not limited to, the diameter of the ovulatory follicle prior to ovulation (21,22), circulating oestradiol on the day of oestrus (22), rate of progesterone rise following ovulation (23), and circulating concentrations of metabolic hormones, e.g., IGF-I and insulin (24,25). This study was carried out to elucidate potential physiological mechanisms responsible for the differences in conception rates between the NZ and NA Holstein Friesian dairy cows that have been previously reported in studies using large animal numbers (2,3).

In the current study, a greater proportion of the embryos recovered from the NZ cows were transferable compared to the NA cows. Furthermore, of the transferable embryos recovered the proportion at the blastocyst stage was higher in the NZ cows. This indicates that the factors responsible for the previously reported differences in conception rate between these strains are manifest as early as 7 days after insemination. It is generally accepted that morulae and blastocysts are equally likely to establish a pregnancy in multiple ovulation and embryo transfer programmes. However, it was previously reported that the transition from morula to blastocyst represents a major area of embryo loss in sub-fertile repeat breeder dairy cows (5). The results of the current study indicate that the NZ strain makes the transition from morula to blastocyst earlier than their NA counterparts. Previous studies have examined early embryo mortality in lactating dairy cows, and concluded that embryo mortality could be detected as early as day 5 after oestrus (26). Our results indicate that marked differences between NZ and NA cows in the proportion of transferable embryos could be detected by day 7 after oestrus. The greater proportion of

transferable embryos yielded by the NZ cows is consistent with reports of superior pregnancy rates and reduced numbers of non-pregnant cows at the end of the breeding period (2,3). In a recent preliminary report examining genetic variation in the quality of embryos recovered from heifers undergoing superovulation, it has been concluded that there was significant genetic variation in embryo quality, that the trait was moderately heritable (h^2 = 0.13), and that embryo quality could potentially be improved through genetic selection (27). This is consistent with our findings in the current study; the NZ strain has been selected for survival in a grass-based system of production (12), and this selection for improved reproductive performance may be responsible for the superior embryo quality observed in this strain.

It has been previously reported that pregnancy rates are influenced by the size of the dominant follicle at ovulation, but the reports have been inconsistent. In a study with dairy cows comparing the efficiency of the Ovsynch protocol at different stages of the oestrous cycle, higher pregnancy rates were recorded when smaller follicles ovulated compared with larger follicles (28). Conversely, beef heifers with small ovulatory follicles (≤ 12 mm) had lower fertility (21). Recently, Lopes et al. (22) reported larger pre-ovulatory follicle diameters in lactating Holstein cows that subsequently became pregnant (22). Though differences between strains in embryo quality were observed in the present study, there was no difference between strains in size of the dominant follicle on the day of oestrus. Lopes et al. (22) also reported that plasma E2 levels on the day of insemination were greater in cows that subsequently became pregnant compared to cows that did not conceive, suggesting that follicle steroidogenic capacity and/or E2 clearance has an influence on subsequent pregnancy status. We did not observe any difference between the NA and NZ strains in preovulatory circulating E2 concentrations in the current study.

Many studies have indicated that a rapid increase in P4 concentrations post-insemination and elevated P4 concentrations on days 5 to 8 post-insemination are associated with improved likelihood of conception (9,21-23). Our understanding of the mechanism responsible for the delayed increase in progesterone in cows that fail to conceive has been increased in recent years. It has previously been reported that LH pulse characteristics, degree of luteal tissue vascularisation, and the steroidogenic capacity of luteal cells were not major factors responsible for inadequate P4 output by developing bovine corpora lutea (29). Similarly, a recent preliminary report comparing cows with high and low P4 concentrations at day 28 to 30 of pregnancy indicated no difference in either luteal P4 content or the mRNA abundance of genes involved in P4 synthesis and luteal function (30). Rather, those authors found that exogenous P4 was more rapidly cleared in cows that had low circulating P4 compared to cows that had high circulating P4. This is consistent with the results of Sangsritavong (31) who noted that liver blood flow and clearance of steroids (E2 and P4) were increased by greater dry matter intake. Greater liver clearance of P4 from blood has been posited as a potentially major cause of infertility in the high producing cow (32). We did not observe any differences between strains in circulating P4 concentrations on days 5 to 7 post-insemination, or in the P4AUC from day of oestrus to day 7 post oestrus. It should be noted that the samples collected for P4 analysis in the current study were collected during the early luteal phase of normal oestrous cycles without insemination at oestrus, and hence the results are not directly comparable with previous reports comparing circulating P4 in pregnant and non-pregnant cows (9,21-23).

It is generally accepted that EB and metabolic status influence ovarian activity; in particular the timing and severity of the negative EB (NEB) nadir has been

associated with the interval to resumption of ovarian activity (33) and elevated insulin and IGF-I concentrations increase circulating E2 concentrations (34). There was no difference between strains in circulating IGF-I concentrations during each superovulation and flushing protocol (mid to late lactation). This is in agreement with a recent study that reported circulating IGF-I concentrations during a full lactation in cows intensively selected for milk yield (select line) and cows of 1960's merit for milk production (control line) (35). Those authors observed that the select line cows had lower IGF-I in early lactation compared to the control line cows, but in late lactation both lines had similar IGF-I concentrations. It was recently reported that NZ cows had higher IGF-I in early lactation compared to NA cows (13).

The profiles of SCM yield and BCS change over the course of the lactation (Fig. 2) revealed differences between the two strains in the prioritisation of nutrient use with advancing stage of lactation. Both strains achieved similar levels of SCM production at peak yield, but as lactation advanced, the NA strain maintained higher levels of milk production. Conversely, both strains had similar BCS at the time of peak SCM yield, but the NZ strain replenished more body reserves in mid to late lactation. The BCS profile changes in the current study were broadly in agreement with previous reports where the NZ strain were observed to have a greater rate of BCS gain in mid to late lactation compared to the NA strain (36). Collectively, the results observed in the current study indicate a divergence in the prioritisation of nutrient use between the strains from the time of peak SCM yield through the end of the study period; the NA cows continued to preferentially partition nutrients to mammary milk synthesis, whereas the NZ cows commenced partitioning energy to replenishing body reserves. This long-term type of physiological regulation is consistent with the concept of homeorhesis, defined as the coordinated control in metabolism of body

tissues necessary to support a physiological state (37). It is well established that greater BCS improves likelihood of establishing a successful pregnancy in lactating dairy cows (38), and thus it is plausible that the inherent genetic drive of the NZ strain to commence partitioning energy to BCS gain after peak SCM yield could be responsible for the superior reproductive performance of this strain compared to their NA counterparts on pasture-based systems of production (2,3).

Precisely how greater BCS improves reproductive performance is not clear, but our results indicate that follicle diameter and periovulatory steroid concentrations are unlikely to play a major role. It has been demonstrated that oocytes recovered from cows of high genetic merit for milk yield exhibited lower rates of cleavage and blastocyst formation *in vitro* compared to cows of medium genetic merit for milk yield (39). Of note, the blastocyst formation rate was not affected by cow milk yield, but greater BCS significantly increased the blastocyst formation rate (39). The *in vitro* embryo development observations of previous studies (39), the *in vivo* embryo development observations in the present study, and the field observations of Horan et al. (2,3) confirm that genetic selection for increased milk yield has a negative effect on reproductive performance, and appear to indicate that differences in oocyte competence may be related to subsequent differences in embryonic development and likelihood of establishing a successful pregnancy. The molecular mechanisms that link increased milk production and reduced fertility in dairy cows is an area of growing interest. The signal transducer and activator of transcription 5A (STAT5A) gene plays a key role in cytokine and growth factor signalling. Recently, it was demonstrated that specific mutations in the STAT5A gene had significant associations with oocyte fertilization rate, embryo mortality and milk composition (40). Importantly, particular male-female allele combinations resulted in complete failure

of fertilization and embryo development. Identification of specific causative mutations associated with compromised reproductive performance (e.g., fertilization failure, early embryo mortality, late embryo mortality etc.) is an important area of research; incorporation of favourable mutations into progeny testing and genetic improvement programmes could have beneficial effects on dairy cow reproductive performance.

Conclusion

The results of this study indicate that the observed difference in conception rate of NZ Holstein-Friesian and the NA Holstein-Friesian dairy cows (2,3) may be related to a difference in embryo quality as early as Day 7 post-insemination. We did not identify any differences between strains in follicle or CL diameter, peak circulating concentrations of E2 prior to ovulation, postovulatory circulating P4 concentrations or circulating IGF-I concentrations during the study period. In contrast, the milk production and BCS data indicate that nutrient partitioning during the course of the study differed between the strains, allowing greater BCS replenishment in the NZ strain, and greater milk output in the NA strain. In conclusion, the NA and NZ strains of HF were selected in a different manner, as indicated by the pedigree indices for different traits. The degree of relatedness between the two strains is low, supporting the premise that there was a divergence in selection. The direction of genetic selection that occurred resulted in the genes responsible for early embryonic mortality having a greater effect in one strain than the other. Early embryo development plays a key role in the successful establishment and maintenance of pregnancy, and appears to be influenced by genetic background.

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 Brendan Horan and Pat Dillon for assistance in setting up this study. The technical assistance of Ms. N Hynes (University College Dublin) is also appreciated. National Development Plan funding is gratefully acknowledged.

References

1. Evans RD, Buckley F, Berry DP, Wallace M, Ducrocq V, Garrick DJ. Trends in milk production, calving rate and survival of cows in 14 Irish dairy herds as a result of the introgression of Holstein-Friesian genes. Anim Sci 2006;82: 423-433.
2. Horan B, Mee JF, Rath M, O'Connor P, Dillon P. The effect of strain of Holstein-Friesian cow and feeding system on reproductive performance in seasonal-calving milk production systems. . Anim Sci 2004;79: 453-467.
3. Horan B, Mee JF, O'Connor P, Rath M, Dillon P. The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. Theriogenology 2005;63: 950-971.
4. Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci 2002;85: 2803-2812.
5. Ayalon N. A review of embryonic mortality in cattle. J Reprod Fertil 1978;54: 483-493.
6. Diskin MG, Sreenan JM. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J Reprod Fertil 1980;59: 463-468.
7. Diskin MG, Murphy JJ, Sreenan JM. Embryo survival in dairy cows managed under pastoral conditions. Anim Reprod Sci 2006;96: 297-311.
8. Ahmad N, Beam SW, Butler WR, Deaver DR, Duby RT, Elder DR, Fortune JE, Griel LC, Jones LS, Milvae RA, Pate JL, Revah I, Schreiber DT,

Townsson DH, Tsang PCW, Inskeep EK. Relationship of fertility to pattern of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. J Anim Sci 1996;74: 1943-1952.

9. Stronge AJH, Sreenan JM, Diskin MG, Mee JF, Kenny DA, Morris DG. Post-insemination milk progesterone concentration and embryo survival in dairy cows. Theriogenology 2005;64: 1212-1224.

10. Garrett JE, Geisert RD, Zavy MT, Morgan GL. Evidence for maternal regulation of early conceptus growth and development in beef cattle. J Reprod Fertil 1988;84: 437-446.

11. Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 2001;121: 175-180.

12. Harris BL, Kolver ES. Review of Holsteinization of intensive pastoral dairy farming in New Zealand. J Dairy Sci 2001;84 (E Suppl.): E56-E61.

13. Patton JP, Murphy JJ, O'Mara FP, Butler ST. A comparison of energy balance and metabolic profiles of the New Zealand and North American strains of Holstein Frisian cow. Animal 2008; *In Press*.

14. Tyrrell HF, Reid JT. Prediction of the energy value of cow's milk. J Dairy Sci 1965;48: 1215-1223.

15. Mc Parland S, Kearney JF, Rath M, Berry DP. Inbreeding trends and pedigree analysis of Irish dairy and beef cattle populations. J Anim Sci 2007;85: 322-331.

16. Prendiville DJ, Enright WJ, Crowe MA, Vaughan L, Roche JF. Immunization of prepubertal beef heifers against gonadotropin-releasing hormone: immune, estrus, ovarian, and growth responses. J Anim Sci 1995;73: 2832-2839.

17. Enright WJ, Chapin LT, Moseley WM, Zinn SA, Kamdar MB, Krabill LF, Tucker HA. Effects of infusions of various doses of bovine growth hormone-releasing factor on blood hormones and metabolites in lactating Holstein cows. J Endocrinol 1989;122: 671-679.

18. Echternkamp SE, Spicer LJ, Gregory KE, Canning SF, Hammond JM. Concentration of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. Biol Reprod 1990;43: 463-468.

19. Wright JM. Photographic illustrations of embryo development stage and quality codes. Manual of the International Embryo Transfer Society, third ed IETS, Savoy, Il, 1998: 167-170.

20. Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using SAS procedures. J Anim Sci 1998;76: 1216-1231.

21. Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, Roberts AJ, Geary TW. Relationship between follicle size at insemination and pregnancy success. Proc Natl Acad Sci USA 2005;102: 5268-5273.

22. Lopes AS, Butler ST, Gilbert RO, Butler WR. Relationship of pre-ovulatory follicle size, estradiol concentration and season to pregnancy outcome in dairy cows. Anim Reprod Sci 2007;99: 34-43.

23. Larson SF, Butler WR, Currie WB. Reduced fertility associated with low progesterone post breeding and increased milk urea nitrogen in lactating cows. J Dairy Sci 1997;80: 1288-1295.

24. Monget PM, Martin GB. Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals. Hum Reprod 1997;12 (Suppl. 1): 33-52.

25. Taylor VJ, Cheng Z, Pushpakumara PG, Beever DE, Wathes DC. Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. . Vet Rec 2005;155: 583-588.
26. Wiebold JL. Embryonic mortality and the uterine environment in first-service lactating dairy cows. J Reprod Fertil 1988;84: 393-399.
27. Hayhurst CF, Firth M, Christie MF, Royal MD. Estimation of genetic variation in embryo quality: is there potential to genetically select cattle with the inherent ability to produce high quality embryos. . In: Proceedings of the British Society of Animal Science international conference “Fertility in Dairy Cows – bridging the gaps”, Liverpool, UK 2007;August 2007: 13.
28. Vasconcelos JLM, Silcox RW, Rosa GJM, Pursley JR, Wiltbank MC. Synchronisation rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the oestrous cycle in lactation dairy cows. Theriogenology 1999;52: 1067-1078.
29. Robinson RS, Hammond AJ, Nicklin LT, Schams D, Mann GE, Hunter MG. Endocrine and cellular characteristics of corpora lutea from cows with a delayed post-ovulatory progesterone rise. Dom Anim Endocrinol 2006;31: 154-172.
30. Rhinehart JD, Flores JA, Milvae RA, Inskeep EK. Luteal function at day 30 of pregnancy in relation to serum progesterone in dairy cows at risk for late embryonic or early fetal mortality. J Dairy Sci 2007;90 (Suppl. 1): 649.
31. Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol - 17β in dairy cattle. J Dairy Sci 2002;85: 2831-2842.

32. Lucy MC. Reproductive loss in high-producing dairy cattle: Where will it end? J Dairy Sci 2001;84: 1277-1293.
33. Beam SW, Butler WR. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J Reprod Fertil 1999;54 (Suppl): 411-424.
34. Butler ST, Pelton SH, Butler WR. Insulin increases 17 beta-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reproduction 2004;127: 537-545.
35. Weber WJ, Wallace CR, Hansen LB, Chester-Jones H, Crooker BA. Effects of genetic selection for milk yield on somatotrophin, insulin-like growth factor-I, and placental lactogen in Holstein cows. J Dairy Sci 2007;90: 3314-3325.
36. McCarthy S, Berry DP, Dillon P, Rath M, Horan B. Influence of Holstein-Friesian strain and feed system on body weight and body condition score lactation profiles. J Dairy Sci 2007;90: 1859-1869.
37. Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci 1980;63: 1514-1529.
38. Berry DP, Buckley F, Dillon P, Evans RD, Rath M, Veerkamp RF. Genetic relationships among body condition score, body weight, milk yield, and fertility in dairy cows. J Dairy Sci 2003;86: 2193-2204.
39. Snijders SEM, Dillon P, O'Callaghan D, Boland MP. Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. Theriogenology 2000;53: 981-989.

40. Khatib H, Monson RL, Schutzkus V, Kohl DM, Rosa GJ, Rutledge JJ. Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. J Dairy Sci 2008;91: 784-793.

Table 1. The mean pedigree indices of the North American and New Zealand strains of Holstein Friesian based on predicted transmitting abilities (and standard deviations) for milk production, calving interval and survival.

Trait	Strain[1] NA	NZ
Milk (kg)	+ 210 (117)	+ 1 (157)
Fat (kg)	+ 6.2 (3.5)	+ 6.5 (5.0)
Protein (kg)	+ 7.4 (4.4)	+ 3.7 (4.0)
Fat (g/kg)	+ 0.10 (1.4)	+ 1.13 (0.62)
Protein (g/kg)	+ 0.40 (0.32)	+ 0.75 (0.43)
Calving interval (days)	+ 0.99 (1.98)	- 2.86 (1.53)
Survival (%)	+ 0.04 (0.29)	+ 1.14 (0.48)

[1]NA = North American Holstein Friesian; NZ = New Zealand Holstein Friesian

[2]All predicted differences obtained from the February 2004 international evaluations of the INTERBULL Animal Centre (Uppsala, Sweden).

Table 2. FSH administration during superovulation protocol

Day	FSH dose (I.U./day[1])	FSH dose (mg/day)
1	210	120
2	175	100
3	105	60
4	70	40
Total	560	320

[1] I.U. = International Units. FSH was administered in equal doses 12 h apart on each day.

Table 3. Circulating peak pre-ovulatory oestradiol concentrations during a synchronized oestrus in NZ and NA strains of lactating dairy cows.

		Strain[1]			
	Ref Heat	NZ	NA	SEM	P-value
Peak E2[2]	1	4.42	3.98	0.83	0.6
	2	4.91	4.82	0.73	0.9
	3	4.58	4.05	0.71	0.5
Overall mean		**4.64**	**4.28**	**0.6**	**0.6**

[1]NZ = New Zealand Holstein-Friesian; NA = North American Holstein-Friesian; n = 10 cows/group at each reference heat.

[2]E2 = oestradiol

Table 4. Plasma progesterone area under the curve and circulating progesterone concentrations on Day 5 to 7 post-oestrus in NZ and NA strains of lactating dairy cows.

		Strain[1]			
	Ref Heat	NZ	NA	SEM	P-value
P4 AUC[2]	1	14.17	15.15	2.78	0.8
	2	18.17	15.53	2.33	0.4
	3	15.22	16.03	2.27	0.8
Overall mean		**15.57**	**15.85**	**1.65**	**0.9**
P4 Day 5 to 7 (ng/ml)[3]	1	1.20	1.03	0.16	0.5
	2	1.15	1.28	0.14	0.5
	3	1.23	0.96	0.12	0.16
Overall mean		**1.19**	**1.09**	**0.11**	**0.5**

[1]NZ = New Zealand Holstein-Friesian; NA = North American Holstein-Friesian; n = 10 cows/group at each reference heat.

[2]P4 AUC = progesterone area under the curve

[3]P4 Day 5 to 7 = mean circulating progesterone concentration on days 5 to 7 post-oestrus

Table 5. The effect of strain of Holstein-Friesian cow on embryo recovery, quality and stage of development on Day 7 post AI[1]

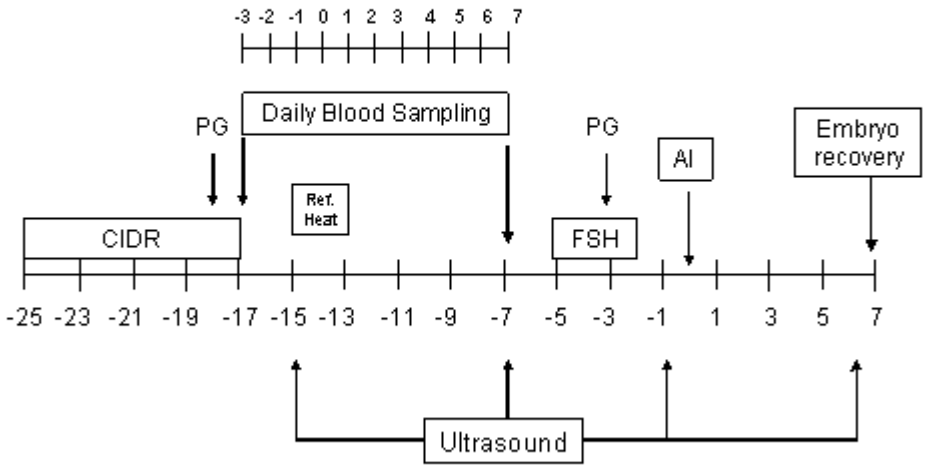
	NZ	NA	P-value
No. of cows	10	10	
No. of flushes recorded	15	14	
No. of corpora lutea (CL)	180	141	
No. of structures recovered	72	59	
Recovery rate[2]	0.40	0.42	
	Proportion (total no.)	Proportion (total no.)	
Transferable embryos	0.91 (63)	0.58 (42)	<0.01
Blastocysts	0.53 (45)	0.17 (10)	0.01
Morulae	0.37 (18)	0.41 (32)	0.7
Transferable-Blastocysts[3]	0.58	0.29	0.099
Transferable-Morula[4]	0.42	0.71	0.099
Non transferable structures	0.09 (9)	0.42 (17)	0.01
Degenerative embryos	(1)	(5)	
Unfertilised oocytes	(8)	(9)	
Empty zona's	(0)	(3)	

[1]NZ = New Zealand Holstein-Friesian; NA = North American Holstein-Friesian

[2]Recovery rate = no. of structures recovered/no. of CL

[3]The proportion of transferable embryos that were at the blastocyst stage

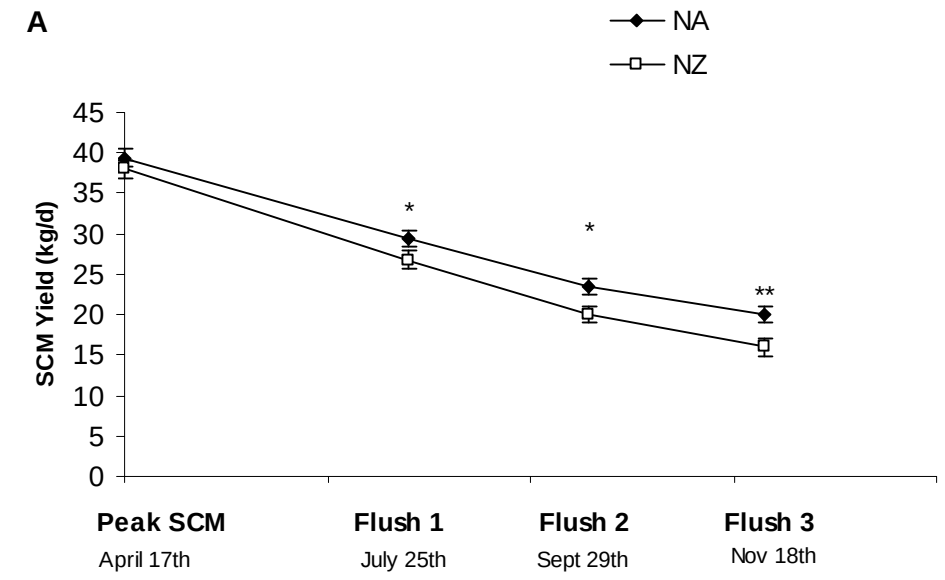
[4]The proportion of transferable embryos that were at the morula stage


-3 -2 -1 0 1 2 3 4 5 6 7
PG
Daily Blood Sampling
PG
AI
Embryo recovery
Ref. Heat
CIDR
FSH
-25 -23 -21 -19 -17 -15 -13 -11 -9 -7 -5 -3 -1 1 3 5 7
Ultrasound

1

Fig. 1. Diagram of the superovulation protocol that was used on 3 occasions between July and November.

CIDR = intravaginal P4-releasing device; PG = prostaglandin $F_{2\alpha}$ analogue; Ref. Heat = reference heat; FSH = follicle stimulating hormone; AI = artificial insemination


A
NA
NZ
SCM Yield (kg/d)
45
40
35
30
25
20
15
10
5
0
*
*
**
Peak SCM
April 17th
Flush 1
July 25th
Flush 2
Sept 29th
Flush 3
Nov 18th

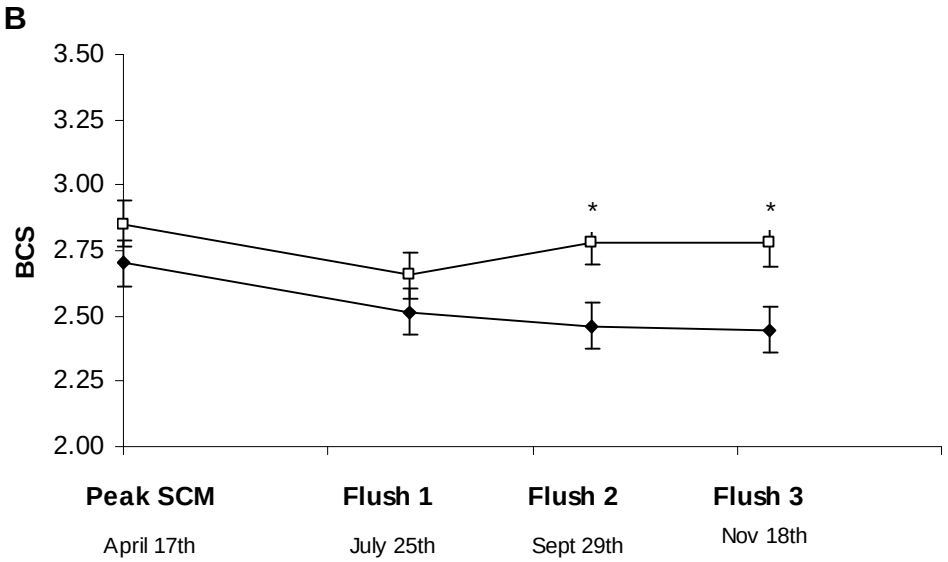
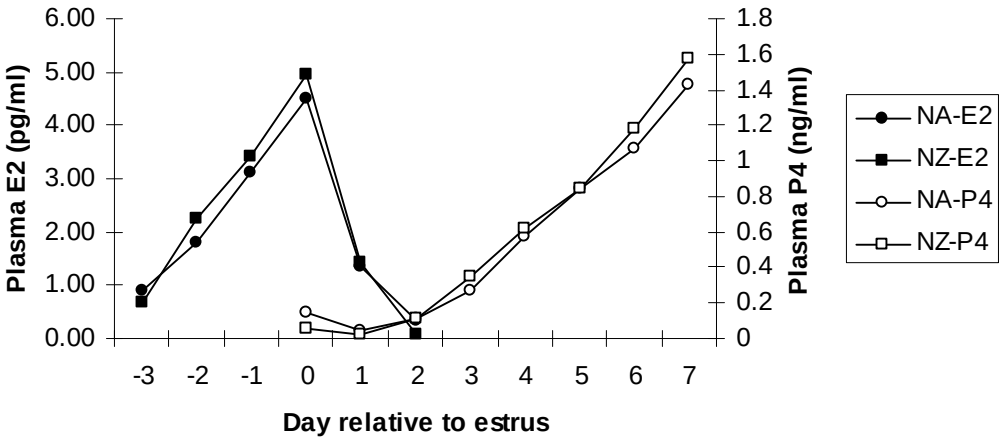

B
BCS
3.50
3.25
3.00
2.75
2.50
2.25
2.00
*
*
Peak SCM
April 17th
Flush 1
July 25th
Flush 2
Sept 29th
Flush 3
Nov 18th

Fig. 2. The effect of strain of Holstein-Friesian cow on solids-corrected milk (SCM) yield and body condition score (BCS). Panel A: A significant effect of strain on SCM yield was observed (P = 0.006; pooled SEM = 0.73 kg/day). Panel B: A significant effect of strain on BCS was observed (P = 0.049; pooled SEM = 0.06 BCS units). Differences between strains at each time point are depicted by symbols: * $P < 0.05$; ** $P<0.01$. n = 10 cows/group at each flush.

1


6.00
5.00
4.00
3.00
2.00
1.00
0.00
Plasma E2 (pg/ml)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Plasma P4 (ng/ml)
-3
-2
-1
0
1
2
3
4
5
6
7
Day relative to estrus
NA-E2
NZ-E2
NA-P4
NZ-P4

2

Fig. 3. Mean circulating E2 and P4 concentration in NZ and NA cows during the three synchronised oestrus cycles. The effect of strain on circulating E2 (P = 0.6; pooled SEM = 0.6 pg/ml) and P4 (P = 0.5; pooled SEM = 0.11 ng/ml) were not significant. n = 10 cows/group at each flush.

1