A COMPARATIVE STUDY OF OVARIAN FOLLICLE DYNAMICS AND IGF-I CONCENTRATION DURING AN OESTRUS CYCLE IN TWO GENOTYPES OF LACTATING HOLSTEIN-FRIESIAN COWS OFFERED PASTURE OR A TOTAL MIXED RATION

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SUMMARY
The objective was to characterize ovarian follicle dynamics and changes in systemic concentration of IGF-I during an oestrous cycle. The four treatment groups were New Zealand Holstein-Friesian (NZHF) Pasture (n=6) and TMR (n=10) and Overseas Holstein-Friesian (OSHF) Pasture (n=7) and TMR (n=6). From Day 17 following a synchronised oestrus, blood samples were collected and ovarian structures were monitored by ultrasound until the end of the subsequent cycle (ovulation). Every cow in the study had two waves of follicle development. NZHF cows had shorter (P<0.05) oestrous cycles than OSHF cows. Genotype or feeding system had no effect on emergence, selection, growth rate, maximum diameter or duration of dominance of the anovulatory or ovulatory follicles. Mean (±se) plasma IGF-I concentrations were higher (57.5±3.3 v 37.1±3.9 ng/mL; P<0.01) for cows offered TMR compared to pasture and also for NZHF compared to OSHF cows (56.3±3.6 v 38.3±3.6 ng/mL; P<0.01). In conclusion, this study has identified substantial differences in circulating IGF-I concentrations between dairy cow genotypes and between the feeding systems under which such cows are managed.

Keywords: dairy cow, IGF, follicle, pasture, total mixed ration

INTRODUCTION
Dairy farming systems in New Zealand are characterised by low cost, seasonal milk production based on pasture feeding and high reproductive efficiency. There is evidence that reproductive performance and survival is declining in dairy cattle in New Zealand as the usage of overseas Holstein-Friesian genetics has increased (Harris and Kolver 2001). In 1998 a study was undertaken to evaluate the performance of Holstein-Friesian cows with either 100% overseas ancestry (OSHF) or those derived from New Zealand selection lines (NZHF) with <12.5% OSHF genes, but of equivalent genetic merit for milk production (Kolver et al. 2000). The objective of this study, a continuation of the previous study, was to examine the effect of genotype and feeding system on selected reproductive characteristics during an oestrous cycle.

MATERIALS AND METHODS
Details of the management of animals in the present study during previous years, starting in 1998 has been described (Kolver et al. 2000; 2002). In a 2 x 2 factorial designed experiment, 50 cows of both OSHF (100% overseas ancestry) and NZHF (<12.5% OSHF genes but with equivalent genetic merit for milk production) were allocated to either a total mixed ration (TMR) based on feed ingredients typical of the North American or European systems in which the OSHF genetics had been selected, or
Using IGF-I

high quality perennial ryegrass and white clover pasture (Pasture) offered at a generous allowance and low stocking rate (>60 kg dry matter/cow/day; approximately 2.2-2.5 cows/ha).

Synchronisation of oestrous. On Day 27±3.0 post-partum (range 20-32) cows received an 8-day controlled intravaginal drug-releasing device containing 1.38g of progesterone (CIDR®, Pharmacia, Auckland, New Zealand), and an intramuscular injection (i.m.) of 10µg busrelin, a GnRH analogue (Receptal®, Intervet Ltd.). At 24hrs before CIDR device removal each cow received an i.m. injection of a prostaglandin analogue, cloprostenol (Lutylase, Parnell Laboratories New Zealand Ltd.). At 24 hrs post-CIDR device removal every cow was injected with 1mg of estradiol benzoate (CIDIROL®, InterAG, Hamilton, New Zealand).

Ultrasonography. Transrectal ultrasonography of the ovarian structures was conducted after morning milking using a real-time ultrasound scanner equipped with a 7.5 MHz linear array transducer (Aloka). From Day 17 of a synchronised oestrous cycle, ovarian structures (individual follicles ≥ 4 mm) were measured and their relative positions were recorded on ovarian maps drawn during the examination.

Blood sampling. Blood was collected into heparinized vaccutainers via coccygeal venipuncture immediately before each ultrasound examination. Samples were placed on ice and centrifuged (2500 x g for 12 minutes) within 2 hours of collection. Plasma was withdrawn and then stored frozen (-20°C) until time of assay.

IGF-I assay. Concentration of IGF-I in extracted (acid-ethanol cryoprecipitation) plasma was determined at 3-day intervals during the oestrous cycle and/or on day of ovulation using a double antibody radioimmunoassay. Sensitivity of the assay was 11.79 ng/mL. Inter-assay coefficients of variation were 6.9%, 7.4% and 20.8% for standard concentrations of 125.7, 32.9 and 12.0 ng/mL, respectively.

Statistical analyses. Results are reported as least square means with the average standard error of the difference (SED). IGF-I and follicular data were analysed using the repeated measures PROC MIXED procedure with REML estimation (SAS 1996). The model included age, genotype, feeding system and their interaction as fixed effects. Sire of cow within genotype was included as a random effect. Chi-square analysis was used to determine proportionate differences in oestrous cycle length. Intra-class correlations were used to assess the proportion of the total variation in plasma IGF-I concentrations that was due to between cow variation.

RESULTS AND DISCUSSION
There were 29 of the 50 cows enrolled in the study which cycled normally from day of ovulation (day 63±7.1 post partum) to a subsequent ovulation. Based on the continued presence or absence of luteal tissue at ultrasonography, 17 cows failed to respond to the synchrony treatment and remained anoestrous while another 4 cows had persistent corpora lutea. Effect of genotype and feeding system on inter-ovulatory intervals (Table 1) and IGF-I concentration (Table 1; Figure 1) are presented. All cows in the study had two waves of follicular development. NZHF cows (range 19-24 days) had
shorter (P<0.05) oestrous cycles than OSHF cows (range 20-25 days). The proportion of oestrous cycles between 19 and 20 days was higher (P<0.01) in NZHF (8/16; 50%) compared with OSHF (1/13; 8%) cows. The proportions of oestrous cycles between 21 and 22 days (NZHF (5/16; 31%) v OSHF (6/13; 46%)) and between 23 and 25 days (NZHF (3/16; 19%) v OSHF (6/13; 46%)) were not different (P>0.05) between genotypes.

Table 1. Inter-ovulatory intervals and IGF-I concentration in plasma during an oestrus cycle in New Zealand (NZHF) and Overseas (OSHF) Holstein-Friesian cows offered pasture or a total mixed ration (TMR)

<table>
<thead>
<tr>
<th>Genotype (G)</th>
<th>Feed system (F)</th>
<th>NZHF</th>
<th>OSHF</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pasture TMR</td>
<td>SED</td>
<td>G</td>
<td>F</td>
</tr>
<tr>
<td>Number of cows</td>
<td>6 10</td>
<td>7</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>Inter-ovulatory interval (days)</td>
<td>21.8 21.1</td>
<td>22.5 23.0</td>
<td>0.66</td>
<td>0.01</td>
</tr>
<tr>
<td>IGF-I (ng/mL)</td>
<td>47.9 64.8</td>
<td>26.4 50.2</td>
<td>4.99</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Genotype or feeding system had no effect on emergence, selection, growth rate, maximum diameter or duration of dominance of the ovulatory or anovulatory follicles. Mean plasma IGF-I concentration was higher (57.5±3.3 v 37.1±3.9 ng/mL; P<0.01) for cows offered TMR compared to pasture and also for NZHF compared to OSHF cows (56.3±3.6 v 38.3±3.6 ng/mL; P<0.01). The pooled intraclass correlation based on the residuals (sample minus group mean) was 0.79. Thus 79% of the total variation in IGF-I concentrations was due to between-cow variation indicating the high repeatability of sampling IGF-I on a within-cow basis.
Using IGF-1

Figure 1. Changes in least square mean (±SEM) plasma IGF-I concentrations during an oestrus cycle in New Zealand (NZHF) and Overseas (OSHF) Holstein-Friesian cows offered pasture or a total mixed ration (TMR).

Decreased fertility ensues when follicles are persistent and oestradiol is elevated for prolonged periods (Mihm et al. 1994). In the present study, although the inter-ovulatory intervals were longer for OSHF cows, the interval from emergence of the ovulatory follicle to ovulation was not different between genotypes. Similar differences in inter-ovulatory intervals between NZHF and OSHF have also been reported previously (Bilby et al. 1998). Nutritional status is known to influence IGF-I concentration (Breier et al. 1986) and IGF-I itself is a known regulator of follicular development and hence reproductive function in cattle (Spicer and Echternkamp 1995). Selection for higher milk yield has been achieved through changes in the growth hormone–IGF axis (Lucy and Crooker 1999). In agreement, the present study reports that cows selected almost exclusively for milk yield (OSHF) had circulating IGF-I concentrations that were 32% lower than cows not selected exclusively for milk yield (NZHF). These divergences in IGF-I concentrations between genotype can be further increased by dietary management. OSHF cows offered exclusively pasture diets had IGF-I concentrations almost 60% lower than NZHF cows offered TMR. IGF-I concentrations within the follicle are positively correlated with those in circulation (Echternkamp et al. 1990). This suggests that OSHF cows offered pasture in the present study were possibly disadvantaged in terms of ovarian function via lower IGF-I concentration. IGF-I is a suggested regulator of embryo development (Wathes et al. 2001) which, if too slow may result in embryo mortality (Mann et al. 1999). It is likely that low IGF-I concentrations are associated with the poor conception rates observed in OSHF cows offered pasture diets in previous studies (Kolver et al. 2000; 2002). In relation to IGF-I concentrations the design of the present study is also worth considering as only cows that cycled normally were
monitored for the study period. Thus the cows not monitored mainly for reasons of anovulatory anoestrous would likely have had even lower IGF-I concentrations, and this is because energy balance, which largely defines the degree and duration of anovulation, is positively correlated with IGF-I (Beam and Butler 1998).

CONCLUSION
In conclusion, this study has identified differences in the length of the inter-ovulatory interval between Holstein-Friesian cows with either 100% overseas ancestry (OSHF) or those derived from New Zealand selection lines (NZHF) with <12.5% OSHF genes, but of equivalent genetic merit for milk production. Circulating IGF-I concentrations were strongly influenced by both the genotype of the cow and the feeding system in which such cows were managed.

REFERENCES
Harris, B.L. and Kolver, E.S. (2001) J. Dairy Sci. 84:56.