Genetic strain and reproductive status affect endometrial fatty acid concentrations

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ABSTRACT

Poor reproductive performance limits cow longevity in seasonal, pasture-based dairy systems. Few differences in ovarian dynamics have been reported in different strains of Holstein-Friesian cows, implying that the uterine environment may be a key component determining reproductive success. To test the hypothesis that the uterine environment differs among genetic strains of the Holstein-Friesian cow, endometrial fatty acids (FA) were analyzed from New Zealand (NZ), and North American (NA) Holstein-Friesian cows. The effect of reproductive status was also investigated, with cows from both Holstein-Friesian strains slaughtered on either d 17 of the estrous cycle (termed cyclic) or d 17 of pregnancy (after embryo transfer; termed pregnant). Endometrial tissues were collected from 22 cows (NZ pregnant, $n = 6$; NZ cyclic, $n = 4$; NA pregnant, $n = 6$; NA cyclic, $n = 6$), and FA composition was analyzed. Daily plasma progesterone concentrations, milk production, milk FA composition, body weight, and body condition score were determined. Milk yield (4% fat-corrected milk) was similar for the NZ (28.5 kg/d) and NA (29.3 kg/d; SE 2.07 kg/d) cows, but NZ cows had a greater mean milk fat percentage. Mean plasma progesterone concentrations were significantly greater in NZ cows. Plasma progesterone concentrations were similar in the pregnant and cyclic groups. Mean length of the trophoblast recovered from the pregnant cows (NZ: 20.8 ± 2.84 cm; NA: 27.9 ± 10.23 cm) was not affected by genetic strain. Endometrial tissues from NZ cows contained greater concentrations of C17:0, C20:3n-3, and total polyunsaturated FA. The endometria from pregnant cows contained greater concentrations of C17:0, C20:2, and C20:3n-6, and less C20:1, C20:2, C20:5n-3. The observed changes in endometrial FA between Holstein-Friesian cows of different genetic origins or reproductive states may reflect differences

in endometrial function and may affect reproductive function.

Key words: endometrium, fatty acid, Holstein-Friesian strain, pregnancy

INTRODUCTION

Pasture-based dairying is inherently seasonal in its calving pattern, driven by pasture growth patterns and the need to align peak pasture demand with peak pasture availability (Holmes et al., 2002; Verkerk, 2003). Breeding objectives aligned with specific dairy systems have resulted in the establishment of different genetic strains of the Holstein-Friesian cow. Strains selected in nonseasonal systems are less suited to pasture-based systems, with less farm profit (McCarthy et al., 2007) resulting from lower conception rates (Horan et al., 2005; Kolver et al., 2007; Macdonald et al., 2008).

Many aspects of the reproductive process may result in a lower conception rate, including follicle and oocyte quality, early embryo growth, and the uterine environment. Recent research reported that the interovulatory interval was longer in North American compared with New Zealand cows (Fahey et al., 2003), yet only small effects on follicular and corpus luteal development have been demonstrated (Verkerk et al., 2000). In comparison, research focused on embryo development has reported that the proportion of blastocyst-to-morula stage embryos recovered after superovulation was lower in North American cows (de Feu et al., 2008). These data indicate that differences in oocyte and embryo quality may contribute to the reduced conception rates reported in these animals (Horan et al., 2005; Macdonald et al., 2008). Differences in the uterine environment may be the key to improving the conception rates of North American cows.

Specific fatty acids (FA) are able to alter physiological processes, modifying both gene expression and cellular function (Hostetler et al., 2005; Coyne et al., 2008). One strategy being investigated to improve conception rate is the use of dietary n-3 FA to modify endometrial FA concentrations (Bilby et al., 2006; Childs et al., 2008) and reduce uterine $\text{PGF}_{2\alpha}$ synthesis during the

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preimplantation period (Thatcher et al., 2006; Wathes et al., 2007). This strategy aims to reduce PGF_{2α} during the critical periods of pregnancy recognition and maintenance (Bazer et al., 1997; Arosh et al., 2004). The aim of this study was to investigate the effect of genetic strain and pregnancy status on endometrial FA concentrations.

MATERIALS AND METHODS

Animals and Management

All procedures were undertaken with the approval of the Ruakura Animal Ethics Committee (Hamilton, New Zealand). Twenty-two lactating Holstein-Friesian cows (in second to fifth lactation) were enrolled. Cows were representative of 2 genetic strains [$<23\%$ North American genetics (NZ, $n = 10$) or $>92\%$ North American genetics (NA, $n = 12$)] in 2 reproductive states [d 17 of the estrous cycle (termed cyclic) or pregnancy (pregnant to embryo transfer; termed pregnant)]. All cows were managed as a single herd, with fresh pasture grazed in an intensive rotational manner similar to that described previously (Roche et al., 2006). Grazing cows were allocated a pasture allowance of >40 kg of DM/cow per day (measured to ground level). Pasture grazing residuals were used to ensure adequate pasture allowance: postgrazing residuals of greater than 1,800 kg of DM/ha were targeted during spring.

An average BW was calculated from daily BW measures taken for at least 4 consecutive days leading up to slaughter. An average BCS was calculated from individual BCS taken at 6 body sites (on a scale of 1 to 10, with 1 being emaciated, and 10 being obese; Roche et al., 2004).

Milk production and milk composition were analyzed in the 24-h period before slaughter, with milk samples from an afternoon milking and the following morning milking combined. Milk yield, milk fat, CP, true protein, CN, lactose, and TS were measured (FT120, Foss Electric, Hillerød, Denmark). A composite sample (20 mL) was stored at -70°C until fat extraction and FA analyses.

Synchronization Protocol

All animals enrolled in the study were free of uterine infection (Metricheck score of 0 or 1; McDougall et al., 2007) and had not been treated for mastitis before slaughter. Animals were grouped by calving date, and estrous cycles were synchronized at similar DIM (NZ: 60.0 DIM; NA: 58.2 DIM; cyclic: 57.7 DIM, pregnant: 60.0 DIM; SE 4.56 d) by using a controlled intravaginal drug-release (CIDR) device containing progesterone

(1.38 g; CIDR-B, Pfizer Animal Health Group, Auckland, New Zealand) for 8 d (day of insertion, d -8). On the day of CIDR-B insertion, the cows were injected with 2 mL of estradiol benzoate (2 mg i.m., Cidirol, Bomac Laboratories Limited, Auckland, New Zealand). All animals received two 2-mL injections of sodium cloprostenol (500 μg ; EstroPlan, Parnell Laboratories NZ Ltd., Auckland, New Zealand) on d 6 (an injection morning and afternoon to reduce the variability in luteal regression) and a 2.5-mL injection of GnRH analog buserelin (10 μg ; Receptal, Intervet Limited, Auckland, New Zealand) 24 h after CIDR device removal. Estrous was detected using tail paint (Macmillan et al., 1988), with the day after GnRH injection designated d 1 of the estrous cycle.

Embryo Production and Embryo Transfer

Embryos were created using ovaries collected at local abattoirs (Thompson, 2000). Cumulus-oocyte complexes (COCS) were aspirated from follicles 3 to 8 mm in diameter. Cumulus-oocyte complexes were washed in HEPES buffered tissue culture medium-199 supplemented with 10% fetal calf serum and cultured in 50- μL drops (10 COCS per drop) of maturation medium under mineral oil for 24 h at 39°C and 5% CO_2 in humidified air. The medium used for maturation was tissue culture medium-199, supplemented with 10% fetal calf serum, 10 $\mu\text{g}/\text{mL}$ of FSH, 10 $\mu\text{g}/\text{mL}$ of LH, and 1 $\mu\text{g}/\text{mL}$ of estradiol-17 β . After 22 to 24 h of maturation, COCS were washed twice in HEPES synthetic oviduct fluid (Thompson, 2000) and transferred to fertilization drops.

After separation on a Percoll discontinuous density gradient (45 to 90%), spermatozoa were added to the fertilization drops (5 oocytes per 50- μL drop under mineral oil) to give a final concentration of 1×10^6 sperm/mL. After 20 to 24 h of fertilization, cumulus cells were removed, and the zygotes were cultured in 20- μL drops (10 zygotes per drop) of modified HEPES synthetic oviduct fluid under mineral oil. Oocytes were fertilized using semen from a bull routinely used for in vitro fertilization (Karl Dustin, Friesian 92304, LIC, Hamilton, New Zealand). Embryo culture was performed in a modular incubation chamber in a humidified atmosphere of 5% CO_2 , 7% O_2 , and 87% N_2 for 7 d (d 0 = day of insemination). On d 5, the cleavage stage of the embryo was recorded before being transferred to fresh media. Cows allocated to the pregnant group received high-quality (grade 1) blastocysts and expanded blastocysts by nonsurgical embryo transfer into the uterine horn ipsilateral to the palpable corpus luteum 7 d after GnRH injection.

Blood Sampling and Plasma Progesterone Concentrations

Blood was sampled daily from d 1 to the day of slaughter (d 17); samples were collected from the coccygeal vein into evacuated tubes (Becton & Dickinson, Franklin Lakes, NJ) containing sodium heparin anticoagulant. Blood samples were immediately placed in ice water and centrifuged within 1 h (12 min at $1,500 \times g$). Aspirated plasma fractions were stored at -20°C . Progesterone concentrations in frozen plasma were determined in a single RIA by using a commercially available kit (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). The intraassay coefficients of variation for low (0.4 ng/mL), medium (3.3 ng/mL), and high (5.1 ng/mL) standards were 8.9, 9.7, and 7.1%, respectively. The minimum detectable concentration was 0.04 ng/mL.

Tissue Collection

Animals were slaughtered at a commercial abattoir (AgResearch Abattoir, Hamilton, New Zealand) on d 17 of the reproductive cycle ($n = 22$). Average DIM at slaughter for the 4 groups were as follows: NA, 86.8 d; NZ, 86.4 d (SE 4.27d); cyclic, 86.3 d; pregnant, 86.9 d (SE 4.13 d). The uterus was dissected within 1 to 1.5 h of slaughter. Each uterine horn was isolated before being flushed with 20 mL of saline. The conceptuses and uterine flushings were collected before dissection. Each uterine horn was dissected separately, with intercaruncular endometrial samples taken from the middle section of each uterine horn. Samples of endometrial tissues (between 0.5 and 1.0 g) were immediately transferred into cryotubes, frozen in liquid N_2 , and stored at -70°C .

Endometrial Fat Extraction and FA Analyses

Endometrial tissues from each uterine horn underwent a combined fat extraction and transmethylation, using a modified direct transmethylation method (Sukhija and Palmquist, 1988). After freeze-drying, an internal standard (23:0 methyl ester) was added for use in quantitative determination, and the methylation reagents were added (5 mL; 1:1 2% H_2SO_4 in methanol:toluene). The reactions were undertaken at 80°C for 2 h under N_2 . Once the sample had cooled, 5 mL of saturated salt solution and the sample were mixed and then centrifuged at $2,500 \times g$ for 10 min. The toluene was removed, before an additional 2 mL of toluene, and combined with the first extract.

Endometrial FA methyl esters (**FAME**) were analyzed by injecting 1 μL onto a Solgel wax column (split

100:1; 30 m $\times$ 0.25 mm i.d., with a 0.25- μm film thickness; SGE, Victoria, Australia). The initial column temperature was set to 210°C for 8 min, and then increased to 240°C at $10^{\circ}\text{C}/\text{min}$ and held for 3 min. The carrier gas was H_2 , with an average linear velocity of 60 cm/s in a constant flow mode.

Standards for FA were obtained from Matreya Inc. (Pleasant Gap, PA) and the conjugated linoleic acid isomer mixes were obtained from Sigma Chemicals (St. Louis, MO), and Nu-Chek Prep (Elysian, MN). Standards GLC10, GLC20, GLC30, GLC40, GLC50, GLC70, GLC80, GLC90, GLC100, PUFA No. 1 (Marine Source), PUFA No. 2 (Animal Source), and Supelco 37 Component FAME Mix (Supelco, Sigma Aldrich, St. Louis, MO) were used as qualitative methyl ester references. Detector responses were corrected using theoretical response factors according to AOCS official method Ce 1e-91 (AOCS, 1998).

Endometrial FA concentrations are expressed as the percentage of total FA. The factor used to convert milligrams of FAME to milligrams of FA was the molecular weight of FA divided by the molecular weight of the FAME. Total FA, expressed as triglycerides (mg/g of tissue, wt/wt) was calculated as follows: triglycerides = $\Sigma(3 \times \text{FA})$, after applying a correction factor for molecular weight + molecular weight of glycerol/molecular weight of $3 \times \text{FA}$ (AOAC official method 996.06; AOAC, 2002).

Statistical Analyses

Statistical analyses were undertaken using the GenStat Release 11 statistical package (Payne et al., 2008). Data are presented as means $\pm$ standard error of the difference (**SED**) unless otherwise stated. Significance is represented using $P < 0.05$, whereas $P \leq 0.10$ was considered to indicate a tendency toward statistical significance.

Data from 19 cows (NZ pregnant, $n = 4$; NZ cyclic, $n = 3$; NA pregnant, $n = 6$; NA cyclic, $n = 6$) with endometrial tissue from both uterine horns were included in the subsequent analyses. The 3 cows that were excluded (NZ pregnant, $n = 2$; and 1 cow from the NZ cyclic group) had only a single endometrium sample available from 1 uterine horn. All analyses used linear models with REML methods, which included strain, reproductive status, and their interactions as fixed effects and cow as a random effect.

Plasma progesterone data from the 19 cows were log-transformed and analyzed using splines and linear models, including strain, reproductive status, and their interactions as fixed effects and cow as the random effect. A uniform covariance structure was used for repeated measurements through time, and heterogeneity

Table 1. Effect of genetic strain of Holstein-Friesian cow and reproductive status on cow BW, BCS, milk yield (4% FCM yield), and milk composition (fat, protein, CN, and lactose percentages)

Item	Strain ¹		Reproductive status ³				<i>P</i> -value ⁴		
	NA	NZ	SED ²	Cyclic	Pregnant	SED	Strain	Status	S × RS
Cows (n)	12	7	—	9	10	—	—	—	—
BW (kg)	551	509	30.8	536	536	29.8	0.19	1.00	0.39
BCS (units)	4.0	3.9	0.13	3.9	4.1	0.13	0.54	0.18	0.28
4% FCM (kg/d)	29.3	27.3	2.37	28.0	29.2	2.29	0.39	0.59	0.19
Milk fat (%)	3.79	4.42	0.352	3.99	4.05	0.340	0.08	0.86	0.14
Milk protein ⁵ (%)	3.47	3.59	0.088	3.52	3.52	0.085	0.21	0.98	0.68
Milk CN (%)	2.72	2.79	0.072	2.75	2.74	0.070	0.34	0.86	0.64
Milk lactose ⁶ (%)	4.94	4.98	0.074	4.94	4.96	0.071	0.55	0.77	0.03

¹NA = North American, NZ = New Zealand.²SED = standard error of the difference.³Cyclic = d 17 of the estrous cycle; pregnant = d 17 of pregnancy.⁴Strain × reproductive status interactions (S × RS).⁵Milk protein % = CP %.⁶Milk lactose (%) S × RS: NA-cyclic 4.87%, NA-pregnant 5.00%, NZ-cyclic 5.08%, NZ-pregnant 4.91% (SED 0.092%).

of variance over time was allowed for. These analyses indicated an interaction ($P < 0.01$) of strain with day, and log progesterone data analyzed for each day separately.

RESULTS

Cow Production, Plasma Progesterone, and Trophoblast Length

Strain and reproductive status effects on BW, BCS, milk production, and milk composition are presented in Table 1. Cow BW, BCS, milk yield (4% FCM), milk fat percentage, protein percentage, and CN percentage were similar between strain and reproductive status. A strain × reproductive status interaction ($P < 0.05$) was observed for milk lactose, with milk lactose percentage being greatest in NZ cyclic (5.08%), and then NA pregnant (5.00%), NZ pregnant (4.91%), and NA cyclic (4.87%; SED 0.092%). Average daily progesterone during the experimental period was greater ($P < 0.05$) in NZ than NA cows (NZ: 3.58 ng/mL; NA: 3.15 ng/mL, SED 0.314 ng/mL), primarily because of greater peak progesterone concentrations between d 11 and 16. There was no effect of pregnancy status ($P = 0.41$; pregnant: 3.26 ng/mL, cyclic: 3.36 mg/mL, SED 3.03 ng/mL). Average trophoblast size (mean ± SEM) was not affected by genetic strain, but there was a 3.6-fold greater variation in the trophoblast length of embryos in NA recipients (NZ: 20.8 ± 2.84 cm; NA: 27.9 ± 10.23 cm).

Endometrial FA Concentrations

The effects of cow strain and reproductive status on concentrations of endometrial FA (% of total FA) are

summarized in Table 2. The most abundant FA in endometrial tissue were C18:1 (~20%), C18:0 (~17.5%), C16:0 (~12.5%), and C20:4n-6 (~7.2%). Concentrations of C17:0 and C20:3n-3 were higher ($P < 0.05$) in the endometrial tissues from NZ cows, and there was a tendency ($P < 0.06$) for higher total concentrations of polyunsaturated FA (PUFA). In comparison, concentrations of C18:1, C20:2, and total concentrations of monounsaturated FA tended ($P < 0.07$) to be less in NZ cows. Concentrations of total n-6 and n-3 FA, and the ratio of n-6 to n-3 were not affected by genetic strain.

Total triglyceride concentrations tended to be greater ($P < 0.10$) in endometrial tissues from pregnant cows (pregnant: 6.4 mg/g of tissue; cyclic: 6.0 mg/g of tissue; SED 0.24 mg/g of tissue). In addition, endometrial tissue from pregnant cows contained higher ($P < 0.01$) concentrations of C17:0 and C20:3n-6 FA, and less C20:2 ($P < 0.05$), C20:1 ($P = 0.06$), and C20:5n-3 ($P = 0.10$). Saturated FA, monounsaturated FA, PUFA, total n-6 and n-3 FA, and the ratio of n-6 to n-3 were not affected by reproductive status. No interactions between genetic strain and reproductive status were evident. Correlations between endometrial FA and the length of the trophoblast were not significant (Table 3).

DISCUSSION

This study showed that few individual FA differed across the strains examined, although there was a tendency ($P = 0.06$) for total PUFA to be elevated in NZ cows. Endometrial tissues from NZ cows also tended ($P = 0.07$) to contain greater concentrations of n-3 FA, but the ratio of n-6 to n-3 was not affected. Few differences

Table 2. Mean [$\pm$ standard error of the difference (SED)] fatty acid concentrations (% of total fatty acids) in endometrial tissue in North American (NA) and New Zealand (NZ) Holstein-Friesian cows, and on d 17 of the estrous cycle (cyclic) or of pregnancy (pregnant)

Endometrium (% of total fatty acids)	Strain		Reproductive status		SED	P-value ¹		
	NA	NZ	Cyclic	Pregnant		Strain	Status	S $\times$ RS
Cows (n)	12	7	9	10	—	—	—	—
Individual fatty acid								
C14:0	0.37	0.33	0.35	0.35	0.041	0.30	0.91	0.67
C14:1	0.15	0.16	0.15	0.15	0.012	0.47	0.76	0.35
C15:0	0.39	0.39	0.40	0.38	0.017	0.91	0.56	0.31
C16:0	12.42	12.47	12.24	12.65	0.270	0.83	0.12	0.97
C16:1	1.42	1.57	1.54	1.45	0.150	0.31	0.57	0.70
C17:0	0.94	1.01	0.94	1.01	0.024	≤ 0.01	≤ 0.01	0.94
C18:0	17.45	17.52	17.48	17.49	0.253	0.76	0.97	0.82
C18:1	21.10	19.69	20.58	20.21	0.754	0.07	0.57	0.85
C18:2n-6	6.72	7.18	6.79	7.11	0.304	0.17	0.19	0.39
C18:3n-3	1.72	2.02	1.97	1.77	0.167	0.11	0.36	0.25
C20:0	0.60	0.58	0.59	0.59	0.017	0.28	0.54	0.14
C20:1	0.18	0.11	0.19	0.11	0.042	0.15	0.06	0.95
C20:2	0.30	0.22	0.30	0.22	0.039	0.06	< 0.05	0.19
C20:3n-6	2.08	2.14	1.98	2.24	0.099	0.51	≤ 0.01	0.85
C20:4n-6	7.04	7.21	7.29	6.96	0.339	0.56	0.20	0.29
C20:3n-3	0.18	0.23	0.19	0.21	0.020	< 0.05	0.31	0.86
C20:5n-3	1.68	1.73	1.79	1.62	0.099	0.60	0.10	0.73
C22:0	1.25	1.17	1.16	1.27	0.080	0.38	0.31	0.23
C22:5n-3	3.37	3.49	3.46	3.41	0.155	0.48	0.88	0.57
C24:0	1.29	1.16	1.13	1.33	0.099	0.27	0.12	0.16
C22:6n-3	3.08	3.27	3.15	3.19	0.232	0.41	0.91	0.82
C24:1	0.52	0.48	0.45	0.55	0.065	0.69	0.28	0.11
Unidentified	16.21	16.29	16.23	16.27	0.452	0.90	0.80	0.58
Fatty acid group								
SFA ²	34.71	34.63	34.28	35.06	0.432	0.90	0.11	0.49
MUFA ³	23.37	22.01	22.91	22.47	0.716	0.07	0.47	0.77
PUFA ⁴	26.16	27.48	26.92	26.73	0.681	0.06	0.80	0.89
n-6 fatty acids ⁵	15.83	16.52	16.05	16.30	0.434	0.11	0.60	0.78
n-3 fatty acids ⁶	10.03	10.74	10.57	10.20	0.364	0.07	0.40	0.46
n-6:n-3 ⁷	1.59	1.54	1.53	1.60	0.046	0.42	0.20	0.31

¹Strain $\times$ reproductive status interactions (S $\times$ RS).²Saturated fatty acids (SFA) = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0.³Monounsaturated fatty acids (MUFA) = C14:1 + C16:1 + C18:1 + C20:1 + C24:1.⁴Polyunsaturated fatty acids (PUFA) = C18:2n-6 + C18:3n-3 + C20:2 + C20:3n-6 + C20:4n-6 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.⁵n-6 fatty acids = C18:2n-6 + C20:3n-6 + C20:4n-6.⁶n-3 fatty acids = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.⁷n-6:n-3 ratio = (C18:2n-6 + C20:3n-6 + C20:4n-6)/(C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).

in endometrial FA from pregnant and nonpregnant cows were evident, with several individual FA (C17:0, C18:1, C18:2, and C20:3n-6) being affected.

Genetic Strain

The n-6:n-3 FA ratios reported in the current study for both strains (1.5 to 1.6) were similar to those observed in cows fed medium and high levels of fish oil (1.45 to 1.80; Childs et al., 2008), but were much lower those that observed in cows fed a concentrate diet (3.6; Childs et al., 2008; Coyne et al., 2008). Because greater concentrations of n-3 FA are thought to be beneficial for reproductive function (Thatcher et al., 2006; Wathes et al., 2007), fresh pasture may provide an advantage.

When compared with TMR, pasture contains 5-fold more C18:3 FA (pasture: 57 g/100 g of FA, TMR: 11 g/100 g of FA; Kay et al., 2005).

Because diets were similar across strains in the current study, the differences observed in endometrial FA between NZ and NA cows may be a result of the greater plasma progesterone concentration in NZ cows. Progesterone is involved in the accumulation of uterine lipids in ovine endometrial cells (Brinsfield and Hawk, 1973; Boshier et al., 1987). However, the biological significance of the observed difference in endometrial FA is unclear. Small changes in specific FA have been observed to modulate gene expression in some tissues. For example, saturated FA can induce proinflammatory gene products, whereas unsaturated FA reduce the

Table 3. Regression coefficients (R^2) between endometrial fatty acid concentrations (% of total fatty acids) and trophoblast length ($n = 10$)

Item	R^2	P -value
Individual fatty acid		
C14:0	0.0006	0.95
C14:1	0.0253	0.66
C15:0	0.0409	0.58
C16:0	0.0554	0.51
C16:1	0.0734	0.45
C17:0	0.0324	0.62
C18:0	0.2755	0.12
C18:1	0.0002	0.97
C18:2n-6	0.1060	0.36
C18:3n-3	0.0598	0.49
C20:0	0.0006	0.95
C20:1	0.0007	0.94
C20:2	0.0078	0.81
C20:3n-6	0.0448	0.56
C20:4n-6	0.1994	0.19
C20:3n-3	0.0184	0.71
C20:5n-3	0.0776	0.44
C22:0	0.0141	0.74
C22:5n-3	0.1269	0.31
C24:0	0.0093	0.79
C22:6n-3	0.0679	0.47
C24:1	0.0092	0.79
Fatty acid group		
SFA ¹	0.0508	0.53
MUFA ²	0.0076	0.81
PUFA ³	0.0239	0.67
n-6 fatty acids ⁴	0.0016	0.91
n-3 fatty acids ⁵	0.0779	0.44
n-6:n-3 ⁶	0.1340	0.30

¹Saturated fatty acids (SFA) = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0.

²Monounsaturated fatty acids (MUFA) = C14:1 + C16:1 + C18:1 + C20:1 + C24:1.

³Polyunsaturated fatty acids (PUFA) = C18:2n-6 + C18:3n-3 + C20:2 + C20:3n-6 + C20:4n-6 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.

⁴n-6 fatty acids = C18:2n-6 + C20:3n-6 + C20:4n-6.

⁵n-3 fatty acids = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3.

⁶n-6:n-3 ratio = (C18:2n-6 + C20:3n-6 + C20:4n-6)/(C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).

expression of these proinflammatory genes (Lee and Hwang, 2006). Similarly, PUFA have been reported to alter gene expression in bovine endometrial tissues, increasing the mRNA abundance for the peroxisome proliferator-activator receptor prostaglandin E synthase while reducing the abundance of phospholipase A₂ mRNA (Bilby et al., 2006; Coyne et al., 2008). Therefore, the greater concentrations of PUFA in the endometrium of NZ cows may lead to as yet unidentified changes in endometrial function.

Reproductive Status

Research examining the effect of fish oils or fishmeals on endometrial FA concentrations, and their potential

effect on endometrial physiology has been of interest for some time. However, detailed studies of changes to endometrial FA during different reproductive states in cattle have not been reported. The current study reports significant differences in a small number of individual FA between d 17 of the estrous cycle and pregnancy. These changes in endometrial FA during pregnancy may reflect differences in endometrial FA metabolism and may alter gene expression.

Concentrations of arachidonic acid (C20:4n-6) have been reported to decline from d 7 to 15 of the reproductive cycle in the guinea pig (Leaver and Poyser, 1980, 1981) and during the estrous cycle in sheep (Meier et al., 1997). Fatty acids that act as precursors for prostaglandin synthesis (arachidonic acid and C20:3n-6) were greater in pregnant endometria. Changes in endometrial arachidonic acid and arachidonic acid precursor FA, such as C20:3n-6, are reportedly associated with changes in endometrial prostaglandin synthesis (Leaver and Poyser, 1980, 1981; Meier et al., 1997). Mean daily plasma progesterone concentrations were elevated in both pregnant and nonpregnant cows, an indication that functional luteal regression had not been initiated at d 17. Therefore, the observed differences in C20:3n-6 FA and, to a lesser extent, arachidonic acid concentrations in pregnant and nonpregnant cows are not associated with greater luteolytic PGF_{2α} synthesis in the nonpregnant cows. More saturated FA (C20:1 and C20:2) were less abundant in pregnant endometrial tissues. It is possible that the changes in endometrial FA were due to the presence of the preimplantation embryo. Although a direct effect of early trophoblast on endometrial FA has not been shown, the close contact of the trophoblast and endometrial surface (Wathes and Wooding, 1980; Simon et al., 2000) may facilitate such as effect. This mechanism may result in changes in the endometrial FA, such as the reduction of C20:1 and C20:2 in pregnant endometrial tissue, to facilitate the synthesis of prostaglandins and eicosanoids by the trophoblast (Hwang et al., 1988; Lewis, 1989). The implications of these opposing changes in arachidonic acid precursor FA (upregulation of C20:3n-6 and downregulation of C20:1 and C20:2) in the endometrial tissues of pregnant cows are not clear.

Although mean embryo size was not different in NZ and NA cows, the range of embryo size was 3.6 times greater in NA cows. Few animals provided data, but the greater variation in trophoblast size in NA cows would support the hypothesis that embryo development is affected by cow genetic strain (de Feu et al., 2008). Poor embryo development and early embryo death are key components of reduced fertility (Diskin and Morris, 2008), and factors that contribute to early embryo death include poor oocyte quality and effects of both

the oviduct and uterine environments on the preimplantation embryo. In the current study, the use of embryo transfer removed the influence of the follicle and oocyte development as possible contributing factors; the data therefore imply a genetic effect on the uterine environment that may influence the ability of the maternal environment to support embryo development. Investigating differences in uterine and endometrial function in cows of different genetic strains will provide new insights into how selection for increased milk production has affected the uterus and early embryo development.

CONCLUSIONS

Results demonstrate that genetic strain and reproductive status alter endometrial FA concentrations. The changes in endometrial FA during pregnancy may reflect maternal-embryonic interactions during the establishment of pregnancy in cows. Results support the hypothesis that endometrial tissues are different in divergent strains of Holstein-Friesian cows. Before suitable tools targeting increased conception rates of high-production dairy cows can be developed, a greater understanding is required of the effect of genetics on uterine and endometrial function, and the association with conception rates.

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