The association between metabolic parameters and oocyte quality early and late postpartum in Holstein dairy cows

S. Matoba,*¹ L. O'Hara,* F. Carter,* A. K. Kelly,* T. Fair,* D. Rizos,† and P. Lonergan*²

*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

†Departamento de Reproducción Animal, INIA, 28040 Madrid, Spain

ABSTRACT

The objective of this was to study the association between metabolic parameters and oocyte quality in postpartum lactating dairy cows as assessed by oocyte morphology and development after fertilization and culture in vitro. Holstein-Friesian spring-calving cows were used ($n = 16$, parity 3.0 ± 0.36 , weight at calving 611 ± 16.2 kg, previous 305-d milk yield $6,454.0 \pm 276.4$ kg). Bodyweight (BW) and body condition score were recorded at approximately 2 wk before expected calving date, at calving, and then weekly until the end of the experiment (approximately 80 d postpartum). Blood plasma samples were collected weekly, starting 2 wk before the expected calving date and continuing until the end of the experiment and were analyzed for nonesterified fatty acids (NEFA), β -hydroxybutyrate (BHBA), insulin, insulin-like growth factor-I, and glucose. Transvaginal oocyte recovery was carried out twice weekly on each cow for a period of approximately 12 wk starting 14 d after calving until approximately 80 d postpartum. A linear decrease in BW was observed from calving (d 0) to d 28, after which it remained stable. Body condition score decreased from 14 d pre-calving, reaching a nadir at approximately d 35 to 42, after which it increased to the end of the period. Non-esterified fatty acid concentrations were significantly elevated from the week before calving until d 42 post-calving, whereas BHBA concentration was significantly elevated from calving to d 49 postcalving. Insulin-like growth factor-I concentration dramatically decreased from d -14 to a nadir on d 7. A significant increase in glucose concentration occurred from d -7 to d 0, followed by a precipitous decrease to d 7. Based on the metabolic profiles (particularly NEFA and BHBA concentrations), data from d 0 to 42 postpartum (period 1) were compared with corresponding data from d 42 to 80 (period 2). Apart from body condition score, all

of the physiological parameters measured (milk yield, BW, and blood metabolites) differed significantly between the 2 periods. In particular, insulin-like growth factor-I, insulin, and glucose concentrations were higher post-d 42, whereas BHBA and NEFA were lower compared with pre-d 42 postpartum. The number of oocytes recovered per session and oocyte quality grade did not differ between periods. Positive associations of follicles aspirated and insulin, BHBA, and NEFA were detected. The number of oocytes recovered was positively associated with milk yield, BW, and glucose and NEFA concentrations. The number of cleaved oocytes was positively associated with BW and NEFA concentration. In conclusion, the data do not provide evidence of an effect of lactation-induced metabolic stress on oocyte developmental competence in the postpartum dairy cow assessed in terms of morphological quality and ability to develop following in vitro fertilization.

Key words: oocyte developmental competence, dairy cow fertility, ovum pick-up, in vitro fertilization

INTRODUCTION

Reproductive inefficiency has a major effect on profitability in commercial dairy herds. Substantial evidence exists of an association between high milk production and the decrease in reproductive efficiency (Lucy, 2001; Pryce et al., 2004), although the evidence for a direct causal relationship is less clear (Buckley et al., 2003; Patton et al., 2007; LeBlanc, 2010). One of the disadvantages of the 300-d lactation associated with seasonally concentrated calving patterns, such as that operated in Ireland, is the requirement for cows to conceive when they are in peak lactation, which often coincides with a state of negative energy balance (NEB). Dairy cows typically enter a state of NEB postpartum when the combined energy requirements for maintenance and milk production exceed dietary energy intake. This deficit arises as cows generally achieve peak milk production at an earlier stage than maximal feed intake. This results in increased mobilization of body reserves to overcome the shortfall in dietary intake. Both the duration and severity of early

Received June 23, 2011.

Accepted November 2, 2011.

¹Current address: National Livestock Breeding Center, Odakurahara 1, Nishigo, Fukushima 961-8511, Japan.

²Corresponding author: pat.lonergan@ucd.ie

postpartum NEB are correlated with the interval to resumption of ovulatory activity following parturition and the associated decreased circulating concentrations of insulin, IGF-I, and glucose, and elevated concentrations of NEFA and BHBA are linked with impaired reproductive performance (Butler and Smith, 1989).

Infertility in dairy cattle is a multifactorial problem, which may be due to suboptimal follicle development associated with poor estrus exhibition and, consequently, poor detection, suboptimal oocyte quality, altered sperm transport, problems at fertilization, a suboptimal reproductive tract environment to support embryo development, or a combination of these factors. One way of experimentally separating issues surrounding the follicle or oocyte, or both, from those relating to the reproductive tract environment is to use transvaginal oocyte recovery coupled with in vitro fertilization (IVF). Differences in development seen in this scenario would, by definition, not be related to post-ovulation issues (i.e., the reproductive tract), but rather reflect the intrinsic quality of the oocyte. Although several authors have reported development of ovum-pick up-IVF embryos in dairy cows (e.g., Fouladi-Nashta et al., 2007), few have compared development from lactating dairy cows and nonlactating cows or heifers. In one study from our group (Rizos et al., 2005), no difference was observed in the proportion of good-quality oocytes undergoing fertilization and development to the blastocyst stage between lactating cows and heifers. Snijders et al. (2000) found that a lower proportion of oocytes recovered from dairy cows with a higher genetic merit for milk production underwent cleavage or developed to the blastocyst stage in vitro than those from cows of average genetic merit. Several studies from Virginia (Kendrick et al., 1999; Gwazdauskas et al., 2000; Walters et al., 2002) have demonstrated that conditions related to early lactation have a negative effect on oocyte quality and endocrine measures in dairy cattle. However, in these papers, oocyte quality was assessed based solely on morphology, which may be of limited value.

The environment in which the oocyte develops can have an effect on its subsequent developmental competence. Britt (1992) hypothesized that follicles grown during the period of NEB could be affected by unfavorable metabolic changes, resulting in a latent effect on the quality of the oocyte offered for fertilization some 60 to 80 d later; in other words, conditions to which the oocyte is exposed early in lactation may be as or more important than those immediately preceding insemination. This is based on the fact that it takes several months (60–80 d) for the oocyte to grow from the early preantral stage to the mature preovulatory follicle (Lussier et al., 1987; Britt, 1992; Fair, 2010).

Evidence also exists for a negative association between contemporaneous follicular environment and follicle and oocyte function. Elevated NEFA concentrations have been shown to be toxic for bovine (Vanholder et al., 2005) and human (Mu et al., 2001) granulosa cell growth and function. Furthermore, oocyte maturation in vitro in the presence of NEB-associated concentrations of palmitic and stearic acids is hampered, leading to lower cleavage and blastocyst development (Leroy et al., 2005). Consistent with these findings, Bender et al. (2010) reported significant differences in the metabolomic profiles of preovulatory follicular fluid from postpartum dairy cows and heifers. Of particular interest, 24 fatty acids and 9 aqueous metabolites were significantly different; concentrations of saturated fatty acids (palmitic and stearic) were higher in follicular fluid from postpartum cows compared with heifers.

To study the immediate and prolonged effects of NEB on oocyte quality, the aim of this study was to examine oocyte morphology and the ability to undergo fertilization and embryo development to the blastocyst stage when collected from dairy cows at varying stages postpartum from calving until approximately d 80 when such cows would normally be bred.

MATERIALS AND METHODS

Cows and Management

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland, in accordance with the Cruelty to Animals Act, 1897, and the European Community Directive 86/609/EC. All procedures were sanctioned by the University College Dublin, Ireland Animals Research Ethics Committee.

Holstein-Friesian spring-calving cows were used ($n = 16$, parity 3.0 ± 0.36 , weight at calving 611 ± 16.2 kg, previous 305-d milk yield $6,454.0 \pm 276.4$ kg). All cows were housed indoors on a slatted floor for the duration of the experiment (around 3 mo) and were fed a standard ration consisting of 50:50 maize silage:grass forage ad libitum plus 8 kg of concentrates per day at milking (twice daily at 0700 and 1600 h).

Body weight and BCS were recorded at approximately 2 wk before expected calving date, at calving, and then weekly until the end of the experiment (approximately 80 d postpartum). Body condition score was assessed by the same person based on a scale of 1 to 5 (with 1 being extremely thin and 5 being extremely fat) with increments of 0.25, based on the scoring described by (Lowman et al., 1976; Prendiville et al., 2009). Milk yield was recorded daily for each cow during the experimental period.

Plasma Hormone and Metabolite Analysis

To fully characterize the cows, blood plasma samples were collected weekly, starting 2 wk before the expected calving date and continuing until the end of the experiment and were analyzed for NEFA, BHBA, IGF-I, insulin, and glucose.

Insulin concentrations in serum were measured using a solid-phase ^{125}I RIA Insulin Coat-A-Count kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with a sensitivity of 1.2 μL U/mL. Intra-assay coefficients of variation for insulin were 6.4, 3.6, and 2.5% for the low, medium, and high standards, respectively. Corresponding interassay coefficients of variation were 6.3, 3.5, and 2.5%.

Serum IGF-1 concentrations were measured using an RIA assay as described previously (Beltman et al., 2010). Serum samples (100 μL) were extracted with 400 μL of ethanol, acetone, and acetic acid in a 60:30:10 ratio at 4°C for 16 h. All samples were centrifuged at $1,500 \times g$ for 30 min and 100 μL of supernatant was removed, neutralized with 100 μL of Tris (0.855 M), and centrifuged again at $1,500 \times g$ for a further 30 min. This supernatant was diluted 1:10 with 900 μL of assay buffer in polypropylene tubes. Fifty microliters of a 1:750,000 dilution of the primary antibody (anti-human IGF-I, National Hormone and Peptide Program, Torrance, CA) was added to each tube and incubated at room temperature for 1 h. Following addition of 100 μL of iodinated IGF-1 (approximately 10,000 cpm) to each tube, samples were incubated overnight at 4°C and 50 μL of secondary antibody (anti-rabbit IgG; Immunodiagnostic Systems, Bolden, UK) added for a further 30-min incubation at room temperature (15–18°C). Finally, 250 μL of distilled water was added to each tube, samples centrifuged at $1,500 \times g$ for 15 min and counted on the Wallac 1470 gamma counter (Wallac/Perkin Elmer, Waltham, MA). Intra-assay coefficients of variation for IGF-I were 12.4, 11.5, and 7.1% for low, medium, and high standards, respectively. Interassay coefficients of variation were 12.3, 11.9, and 7.1% for low, medium, and high standards, respectively.

Serum BHBA concentrations were measured using the Ranbut D-3-hydroxybutyrate (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK), using a kinetic enzymatic reaction with a sensitivity of 0.1 nmol/L. Circulating NEFA concentrations were measured using the Randox NEFA enzyme assay with a sensitivity of 0.072 mmol/L. Plasma glucose concentrations were measured using the automated Randox Glucose (Gluc HK) hexokinase enzymatic method (sensitivity 0.662 mmol/L). Intra-assay coefficients of variation for the metabolites ranged from 2.88 to 4.58% and the interassay coefficients of variation ranged from 2.32 to 6.52%.

Oocyte Recovery

Transvaginal oocyte recovery was carried out twice weekly on each cow for a period of approximately 12 wk starting 14 d after calving until approximately 80 d postpartum, as described previously by Imai et al. (2006). Briefly, ovum pick-up (OPU) was conducted using an ultrasound scanner (SSD-500; Aloka, Tokyo, Japan) and a 5.0 MHz convex array transducer (UST-974-5; Aloka) attached to a 17 G $\times$ 540 mm stainless steel needle guide (modification of an embryo transfer gun). All visible follicles ≥ 3 mm were aspirated with a vacuum pressure of 30 mmHg (aspiration rates are 75 mL/min; VMAR-5000 COOK; William a Cook Pty. Ltd., Queensland, Australia). The follicular contents were aspirated into a 50-mL conical tube containing PBS supplemented with heparin (400 IU/mL) and BSA (3 mg/mL) held at 37°C in a heat block. Prior to puncture, epidural anesthesia (20 mg/mL of lidocaine hydrochloride = 5 mL of Norocaine; Norbrook Laboratories Ltd., Newry, Northern Ireland) was administered to each animal. The size distribution of the follicles available for puncture was recorded for each animal. Once all visible follicles were punctured for an individual animal, the tube containing follicular material was transported to the laboratory and filtered through an EmCon filter (Veterinary Concepts Inc., Spring Valley, WI). Cumulus oocyte complexes (COC) were identified under a stereomicroscope and classified into 4 morphological categories based on their cumulus cells investment: grade 1, compact cumulus cells; grade 2, 2 to 3 layers of cumulus cells; grade 3, 1 layer of cumulus cells or denuded oocytes; or grade 4, oocytes with expanded cumulus cells (de Loos et al., 1989).

In Vitro Embryo Production

All COC were matured, fertilized, and cultured in vitro in an individually identifiable manner as described previously (Matoba et al., 2010). Briefly, COC were washed in PBS and cultured in groups of 20 in 100- μL droplets of maturation medium (TCM 199 supplemented with 10% fetal calf serum and 10 ng/mL of epidermal growth factor) covered with mineral oil in a polyester mesh (Sefar Petex; Sefar Ltd., Bury, UK; 500- μm opening and 220- μm filament diameter) at 39°C for 24 h in an atmosphere of 5% CO_2 in humidified air. After maturation, COC were washed in the same droplet in IVF medium [Tyrode medium supplemented with 25 mM bicarbonate, 22 mM Na-lactate, 1 mM Na-pyruvate, 6 mg/mL of fatty acid-free BSA, and 10 μg /mL of heparin-sodium salt (184 U/mg of heparin; Calbiochem Inc., San Diego, CA)] and inseminated with frozen-thawed Percoll-separated spermatozoa, co-

incubated for 18 to 20 h at 39°C in an atmosphere of 5% CO₂ in humidified air.

In preparation for in vitro culture, presumptive zygotes were individually denuded by vortexing for 1.5 min and gentle pipetting with a fine glass pipette, washed twice in PBS and twice in culture medium (synthetic oviduct fluid supplemented with 5% fetal calf serum) before being cultured in 100-μL droplets of the same medium individually using a smaller polyester mesh of 170-μm opening and 96-μm filament diameter. Culture took place at 39°C in an atmosphere of 5% CO₂, 5% O₂, and 90% N₂ in humidified air. Cleavage rate and blastocyst development were assessed on d 2 and d 7 to 8, respectively (day of IVF = d 0).

Statistical Analysis

Data were checked for normality and homogeneity of variance using histograms, Q plots, and formal statistical tests in the UNIVARIATE procedure of SAS (version 9.1.3; SAS Institute Inc., Cary, NC). Data that were not normally distributed were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS. The transformed data were used to calculate *P*-values. The corresponding least squares means and standard errors of the nontransformed data are presented in the results for clarity.

Based on the fact that cows had returned to normal NEFA and BHBA profiles by approximately d 42 postpartum, metabolic, body composition, and in vitro fertility (number of oocytes that cleaved and reached blastocyst stage) data from d 0 to 42 postpartum (period 1) were compared with corresponding data from d 42 to 80 (period 2) using repeated measures with the MIXED procedure of SAS. The model included terms for period (1 or 2) and puncture session or measurement within period. Lactation number and previous milk yield of the animal were included as adjustment variables but were removed because they were not significant. Cow was included as a random effect in the model with the most appropriate covariance structure between records within cow determined by minimizing the Akaike information criterion. Models were run under compound symmetry, unstructured, autoregressive, or Toeplitz variance-covariance structures. Differences between periods were determined by F-tests using Type III sums of squares. The PDIF command, incorporating the Tukey test, was applied to evaluate pairwise comparisons between treatment means. Pearson correlation coefficients among traits were determined using the CORR procedure of SAS. Correlation coefficients were classified as strong ($r > 0.6$), moderate (r between

0.4 and 0.6), or weak ($r < 0.4$), respectively. Change in body compositing traits during the test period for each animal was computed as the coefficient of the linear regression of measurements upon time (day) using the REG procedure of SAS.

RESULTS

The descriptive statistics of milk production, BW, and BCS, and plasma analytes of all animals across the period of the study (d -14 prepartum to d 80 postpartum) are presented in Table 1. Cows in the study had a mean milk yield across the period of the study of 30.3 ± 4.8 kg/d. Milk fat and protein were measured on a monthly basis. Mean fat and protein values over the course of the experiment were 4.61% (SD 0.62) and 3.60 (SD 0.42), respectively. The mean BW across the entire study period was 572.0 ± 54.5 kg and the mean BCS was 2.8 ± 0.4 . A linear decrease in BW was observed from calving (d 0) to d 28, after which it remained stable (Figure 1). The mean BCS across the period was 2.79 ± 0.38 . The BCS decreased from 14 d precalving, reaching a nadir at approximately d 35 to 42, after which it increased to the end of the period.

Nonesterified fatty acid concentrations were significantly elevated from the week before calving until d 42 postcalving, whereas BHBA concentration was significantly elevated from calving to d 49 postcalving. Insulin-like growth factor-I concentration dramatically decreased from d -14 to a nadir on d 7, after which it increased gradually across time. Similarly, insulin concentration decreased from d -14 to a nadir at approximately d 21, and then increased gradually. A significant increase in glucose concentration occurred from d -7 to d 0, followed by a precipitous decrease to d 7, after which it slowly increased.

Based on the fact that the concentrations of NEFA and BHBA had returned to prepartum levels by approximately d 42 postpartum (Figure 1), data from d 0 to 42 postpartum (period 1) were compared with corresponding data from d 42 to 80 (period 2). This separation is consistent findings of Whitaker (1997), who described the NEB cut-off point for plasma NEFA for lactating cows in early lactation as 0.7 mmol/L and the BHBA cut-off value for milking cows has been estimated as 1.0 mmol/L.

Apart from BCS, all of the physiological parameters measured (MY, BW, and blood metabolites) differed significantly between the 2 periods (Table 2). In particular, IGF-I ($P = 0.003$), insulin ($P < 0.0001$), and glucose ($P < 0.0001$) concentrations were higher post-d 42, whereas BHBA and NEFA concentrations were lower ($P < 0.001$) compared with those pre-d 42 postpartum.

Table 1. Descriptive statistics (mean, SD, 95% CI, minimum, and maximum) in lactating dairy cows for milk yield, BW, BCS, blood metabolites, and oocyte recovery and development parameters

Variable	Mean	SD	LCLM ¹	UCLM ²	Minimum	Maximum
Milk yield (kg)	30.29	4.79	29.57	31.01	15.97	41.11
BW (kg)	571.96	54.47	564.02	579.91	410.00	706.00
Δ BW	−0.663	1.160	−1.081	−0.245	−3.102	1.846
BCS (scale 0–5)	2.79	0.38	2.74	2.85	2.00	4.00
Δ BCS	0.000	0.013	−0.005	0.005	−0.024	0.024
IGF-I (ng/mL)	81.91	34.66	76.84	86.98	24.00	186.45
Insulin (μIU/mL)	2.37	1.73	2.10	2.64	0.08	9.59
Glucose (mmol/L)	3.25	0.48	3.18	3.32	1.80	5.90
BHBA (mmol/L)	0.78	0.41	0.72	0.84	0.28	2.58
NEFA (mmol/L)	0.49	0.34	0.44	0.53	0.07	1.99
Follicles aspirated	10.96	4.84	10.40	11.53	3	32
Oocytes recovered	7.07	4.19	6.58	7.56	0	24
Oocytes grade 1 and 2	5.18	3.33	4.79	5.57	0	21
Oocytes grade 3 and 4	1.21	1.38	1.05	1.37	0	8
Oocytes degenerated	0.69	0.96	0.58	0.80	0	6
Cleaved oocytes	2.85	2.29	2.55	3.15	0	10
Cleavage %	45.8	32.1	41.5	50.0	0	100
Cleavage grade 1 and 2%	87.0	24	83.4	90.5	0	1.5
Blastocyst total	0.90	1.27	0.73	1.07	0	6
Blastocyst %	14.9	21.4	12.3	17.7	0	100
Blastocyst % (of cleaved)	29.3	31.5	25	33.9	0	100
Blastocyst grade 1 and 2%	93.2	21.7	88.9	97.4	0	100

¹Lower confidence limit for the mean.²Upper confidence limit for the mean.

The number of follicles aspirated per animal per session averaged 11.0 ± 4.8 across the entire period and was affected by period postpartum ($P < 0.01$; Table 3). The number of oocytes recovered per session and oocyte quality grade did not differ between periods. The effect of period postpartum on oocyte cleavage and blastocyst development in lactating dairy cows is shown in Table 4 and representative images of the same batch of immature bovine COC matured, fertilized, and cultured to d 7 in a polyester mesh in an individually identifiable manner are shown in Figure 2.

Correlation coefficients between milk yield, BW, BCS, and blood metabolites and oocyte recovery and embryo development parameters are shown in Table 5. Positive associations ($P < 0.05$) of follicles aspirated and insulin ($r = 0.2$), BHBA ($r = 0.22$), and NEFA ($r = 0.36$) were detected. The number of oocytes recovered was positively associated with milk yield ($r = 0.18$, $P < 0.05$), BW ($r = 0.17$, $P < 0.05$), glucose ($r = 0.2$, $P < 0.05$), and NEFA ($r = 0.25$, $P < 0.01$). The number of cleaved oocytes was positively associated with BW ($r = 0.36$, $P < 0.01$) and NEFA ($r = 0.23$, $P < 0.05$). Blastocyst number was positively associated with BW ($r = 0.48$, $P < 0.001$).

DISCUSSION

The main finding of this study is that despite the increase in NEFA and BHBA and other metabolic changes occurring in association with calving and at-

tainment of peak lactation in the weeks following parturition, oocyte quality as assessed morphologically and in terms of ability to undergo fertilization and develop to the blastocyst stage was not affected by postpartum metabolic status.

It is generally accepted that most embryonic mortality in cattle occurs before maternal recognition of pregnancy (approximately d 16 post-conception; Diskin and Morris, 2008). Although data from single-ovulating lactating dairy cows are scarce, recent publications suggest that despite a fertilization rate of $>85\%$, only approximately 50% of embryos are viable at d 7 (reviewed by Sartori et al., 2010). Such early failures in development could implicate oocyte quality rather than failures of the reproductive tract to support development.

Pregnancy, decreased DMI during late gestation, lactogenesis, and parturition have dramatic effects on metabolism in dairy cows during the transition period from 3 wk before to 3 wk after calving. Increases in plasma NEFA occur during the 10 d before calving and may precede the decrease in feed intake. Plasma NEFA concentrations are highest at calving and decrease rapidly after calving. Plasma glucose concentrations remain stable or increase slightly during the prepartum transition period, increase dramatically at calving, and then decrease immediately postpartum (Grummer, 1995). Associations between glucose, NEFA, and BHBA and energy balance in early lactation are well established and reflect enhanced mobilization of body reserves

Figure 1. Characterization of postpartum lactating dairy cows used in the study. Body weight and BCS (on a scale of 1 to 5) were recorded at approximately 2 wk before expected calving date, at calving, and then weekly until the end of the experiment (approximately 80 d postpartum). Milk yield was recorded daily for each cow during the experimental period. Blood plasma samples were collected weekly, starting 2 wk before the expected calving date and continuing until the end of the experiment (approximately d 80 postpartum), and were analyzed for NEFA, BHBA, insulin, IGF-I, and glucose. Error bars indicate standard error. Color version available in the online PDF.

and partitioning of nutrients toward milk production. Negative energy balance is typically associated with a decrease in circulating concentrations of insulin, glu-

cose, and IGF-I and increased concentrations of NEFA and BHBA, a product of tissue fatty acid catabolism (Grummer, 1995).

Table 2. Effect of period postpartum on descriptive statistics in lactating dairy cows for milk yield, BW, BCS, and blood metabolites

Variable	Period		SEM	<i>P</i> -value
	Pre-d 42	Post-d 42		
Milk yield (kg)	29.39	31.29	0.73	0.0002
BW (kg)	579.79	558.54	10.72	<0.0001
Δ BW	−1.34	0.01	0.23	0.0013
BCS (scale 0–5)	2.79	2.80	0.085	0.8026
Δ BCS	−0.008	0.008	0.003	0.001
IGF-I (ng/mL)	69.27	100.71	6.42	<0.0001
Insulin (μIU/mL)	2.02	2.79	0.23	0.0028
Glucose (mmol/L)	3.15	3.39	0.05	<0.0001
BHBA (mmol/L)	0.91	0.57	0.06	<0.0001
NEFA (mmol/L)	0.61	0.30	0.04	<0.0001

Fluctuating concentrations of metabolites in the early postpartum period may offer potential explanations for latent effects of early postpartum energy balance on subsequent fertility. Although associations between these metabolites and early lactation energy balance are well established and reflect enhanced mobilization of body reserves and partitioning of nutrients toward milk production, precision of estimation of energy balance for individual cows from plasma metabolite profiles is poor (Reist et al., 2002). In support of this, plasma concentrations of glucose, NEFA, and BHBA were not associated with resumption of cyclicity or conception rate (Patton et al., 2007). In that study, DMI during the first 28 d postpartum was identified as the principal component of energy balance influencing subsequent fertility. The same authors reported that milk protein content and plasma IGF-I concentration in early lactation may be useful indicators of reproductive efficiency (Patton et al., 2007).

The temporal pattern of plasma IGF-I concentration in the present study was similar to previous reports, with a decrease at parturition and a gradual increase thereafter (McGuire et al., 1995; Patton et al., 2008). The pattern of glucose in plasma was consistent with previous reports (Butler et al., 2006; Patton et al., 2008) with an abrupt rise in the week before calving and marked decrease immediately postcalving to concentrations lower than those prepartum and is reflective

of the dramatic increase in mammary glucose requirements associated with the onset of lactation.

It has been suggested that metabolic alterations associated with postpartum NEB affect the oocyte; some studies have indicated that increased concentrations of NEFA and BHBA in follicular fluid adversely affect oocyte quality (Leroy et al., 2004, 2005). In addition, NEFA have been shown to decrease steroidogenesis and proliferation in follicular thecal cells (Vanholder et al., 2005). Leroy et al. (2005) determined the NEFA concentration and composition in follicular fluid of high-yielding dairy cows in relation to serum early and late postpartum and subsequently added the 3 predominant NEFA (oleic, palmitic, and stearic) in follicular fluid during oocyte maturation *in vitro*. Both palmitic and stearic acid had a negative effect on meiotic maturation, fertilization, and blastocyst formation. In agreement, Bender et al. (2010) compared the metabolomic profiles of preovulatory follicular fluid from heifers and postpartum dairy cows and found higher concentrations of saturated fatty acids (palmitic and stearic) in follicular fluid from postpartum cows compared with heifers.

Objective measures of oocyte quality in dairy cows are relatively sparse. Snijders et al. (2000) reported that oocytes recovered from high genetic merit cows had lower cleavage and blastocyst development than those from medium merit cows. Kendrick et al. (1999) examined the effects of energy balance on hormonal

Table 3. Effect of period postpartum on number of follicles aspirated, oocytes recovered, and oocyte quality in terms of morphology in lactating dairy cows

Variable	Period		SEM	<i>P</i> -value
	Pre-d 42	Post-d 42		
Follicles aspirated	11.42	10.39	0.99	0.01
Oocytes recovered	6.92	7.07	0.77	0.69
Oocytes grade 1 and 2	5.01	5.22	0.60	0.49
Oocytes grade 3 and 4	1.27	1.14	0.15	0.41
Oocytes degenerated	0.65	0.73	0.11	0.48

Figure 2. Representative images of the same batch of immature bovine cumulus oocytes complexes (COC) recovered by transvaginal follicle puncture and matured, fertilized, and cultured to d 7 in a polyester mesh in an individually identifiable manner. Twenty oocytes were placed in the mesh on the bottom of the culture dish in a 100- μ L droplet of medium under mineral oil. A) Immature COC, and B) d-7 embryos. Oocytes/embryos from 3 cows are shown enclosed in boxes indicating oocytes/embryos from the same animal; Δ indicates blastocysts. Note: A) and B) are not to the same scale. Color version available in the online PDF.

Table 4. Effect of period postpartum on oocyte cleavage and blastocyst development in lactating dairy cows

Variable	Period		SEM	P-value
	Pre-d 42	Post-d 42		
Oocytes in culture	6.04	6.04	0.69	0.99
Cleaved oocytes	3.06	2.56	0.37	0.1
Cleavage %	50.7	42.5		
Cleavage grade 1 and 2% ¹	82.5	89.0		
Blastocyst total	0.93	0.86	0.18	0.68
Blastocyst %	15.4	14.3		
Blastocyst % (of cleaved)	30.3	33.6		
Blastocyst grade 1 and 2%	90.2	95.4		

¹Based on grade 1 and 2 oocytes.

secretion patterns and the structure of recovered oocytes. Oocyte pick-up was conducted twice weekly from d 30 to 100 of lactation. Total oocytes increased linearly from d 30 to 100 postpartum. Regardless of treatment, oocyte quality was highest at 30 d. In a subsequent study from the same group (Walters et al., 2002), a single aspiration was performed on each of 120 cows during wk 1 to 12 postpartum. Oocyte quality score was affected by the quadratic days postpartum $\times$ parity interaction; first- and second-parity cattle had greater oocyte quality scores from d 20 to 70 postpartum compared with third-parity cattle, but third parity cattle had greater quality oocytes near the end of the 12-wk period. In agreement with the current findings, Roth et al. (2008) reported that the steroid content of preovulatory follicular fluid, the proportion of morphologically good quality oocytes, cleavage rate, and development to blastocyst stage (assessed after activation) did not differ between nulliparous heifers, cows around the time of first AI (60–95 d postpartum), and cows in midlactation (120–225 d). Rizos et al. (2005) reported that although the number of oocytes aspirated and collected from 43-d postpartum lactating dairy cows was lower than in nulliparous heifers, the cleavage rate and blastocyst development in vitro did not differ.

In conclusion, the data from the current study do not provide evidence of an effect of lactation-induced metabolic stress on oocyte developmental competence in the postpartum dairy cow assessed in terms of morphological quality and ability to develop following IVF. The results should be viewed in the context that developmental rates to the blastocyst stage using IVF in cattle are typically on the order of 30 to 40% and the sensitivity of the assay may be insufficient to separate subtle differences in oocyte quality.

ACKNOWLEDGMENTS

This publication has emanated from research conducted with the financial support of Science Foundation Ireland (Grant Number SFI/07/SRC/B1156 to P. Lonergan) and of the Spanish Ministry of Science and Innovation (Grants AGL2009–11810 to D. Rizos). The authors thank M. Wade and P. Duffy (University College Dublin, Ireland) for their excellent technical assistance; P. Furney, N. Hynes, and M. Garrett (University College Dublin) for the plasma assay; and the staff of University College Dublin Lyons Research Farm (Newcastle, Ireland) for management, access, and help to experimental animals. The authors are also thankful

Table 5. Associations (correlation coefficients) among measures of milk yield (MY), BW, BCS, and blood metabolites and oocyte recovery and embryo development parameters in postpartum lactating dairy cows (d 0 to 80 postpartum)¹

Variable	MY	BW	BCS	IGF-I	Insulin	GLU	GI	BHBA	NEFA
Follicles aspirated	0.15	0.15	−0.03	−0.20*	0.20*	0.17†	−0.21*	0.22**	0.36***
Oocytes recovered	0.18*	0.17*	−0.01	−0.12	0.15†	0.20*	−0.15†	0.03	0.25**
Oocytes grade 1 and 2	0.06	0.17	0.10	−0.05	0.15	0.26	−0.14	0.03	0.20*
Oocytes grade 3 and 4	0.27**	0.02	−0.28**	−0.20*	0.08	−0.07	−0.10	0.05	0.18*
Oocytes degenerated	0.23**	0.17	0.01	−0.11	0.02	0.04	−0.01	0.00	0.15†
Cleaved oocytes	−0.03	0.36**	0.11	−0.07	0.09	0.08	−0.12	0.07	0.23*
Cleaved grade 1 and 2	−0.06	0.29**	0.08	−0.11	0.03	0.09	−0.06	0.08	0.15
Cleaved grade 3 and 4	−0.03	0.16	0.07	−0.01	0.10	−0.01	−0.11	−0.02	0.16
Blastocyst total	0.00	0.50***	0.13	−0.06	0.04	0.09	−0.04	0.02	0.13
Blastocyst grade 1 and 2	0.02	0.48***	0.13	−0.05	0.06	0.12	−0.06	−0.01	0.09
Blastocyst grade 3 and 4	−0.15	0.19	0.07	−0.05	−0.08	−0.18	0.08	0.19†	0.24*

¹GLU = glucose; GI = glucose:insulin ratio. Values in bold are significant.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

to H. Misawa (Misawa Medical Industry Co. Ltd., Tokyo, Japan) for the modification of an oocyte pick-up (OPU) needle.

REFERENCES

- Beltman, M. E., N. Forde, P. Furney, F. Carter, J. F. Roche, P. Lonergan, and M. A. Crowe. 2010. Characterisation of endometrial gene expression and metabolic parameters in beef heifers yielding viable or non-viable embryos on Day 7 after insemination. *Reprod. Fertil. Dev.* 22:987–999.
- Bender, K., S. Walsh, A. C. Evans, T. Fair, and L. Brennan. 2010. Metabolite concentrations in follicular fluid may explain differences in fertility between heifers and lactating cows. *Reproduction* 139:1047–1055.
- Britt, J. H. 1992. Impacts of early postpartum metabolism on follicular development and fertility. *Bovine Pract.* 24:39–43.
- Buckley, F., K. O'Sullivan, J. F. Mee, R. D. Evans, and P. Dillon. 2003. Relationships among milk yield, body condition, cow weight, and reproduction in spring-calving Holstein-Friesians. *J. Dairy Sci.* 86:2308–2319.
- Butler, S. T., S. H. Pelton, and W. R. Butler. 2006. Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. *J. Dairy Sci.* 89:2938–2951.
- Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy-balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767–783.
- de Loos, F., C. van Vliet, P. van Maurik, and T. A. Kruip. 1989. Morphology of immature bovine oocytes. *Gamete Res.* 24:197–204.
- Diskin, M. G., and D. G. Morris. 2008. Embryonic and early foetal losses in cattle and other ruminants. *Reprod. Domest. Anim.* 43(Suppl. 2):260–267.
- Fair, T. 2010. Mammalian oocyte development: Checkpoints for competence. *Reprod. Fertil. Dev.* 22:13–20.
- Fouladi-Nashta, A. A., C. G. Gutierrez, J. G. Gong, P. C. Garnsworthy, and R. Webb. 2007. Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. *Biol. Reprod.* 77:9–17.
- Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.* 73:2820–2833.
- Gwazdauskas, F. C., K. W. Kendrick, A. W. Pryor, and T. L. Bailey. 2000. Impact of follicular aspiration on folliculogenesis as influenced by dietary energy and stage of lactation. *J. Dairy Sci.* 83:1625–1634.
- Imai, K., M. Tagawa, H. Yoshioka, S. Matoba, M. Narita, Y. Inaba, Y. Aikawa, M. Ohtake, and S. Kobayashi. 2006. The efficiency of embryo production by ovum pick-up and in vitro fertilization in cattle. *J. Reprod. Dev.* 52(Suppl.):S19–S29.
- Kendrick, K. W., T. L. Bailey, A. S. Garst, A. W. Pryor, A. Ahmadzadeh, R. M. Akers, W. E. Eyestone, R. E. Pearson, and F. C. Gwazdauskas. 1999. Effects of energy balance of hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. *J. Dairy Sci.* 82:1731–1741.
- LeBlanc, S. 2010. Assessing the association of the level of milk production with reproductive performance in dairy cattle. *J. Reprod. Dev.* 56(Suppl.):S1–S7.
- Leroy, J. L., T. Vanholder, J. R. Delanghe, G. Opsomer, A. Van Soom, P. E. Bols, J. Dewulf, and A. de Kruif. 2004. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. *Theriogenology* 62:1131–1143.
- Leroy, J. L., M. R., T. Vanholder, B. Mateusen, A. Christophe, G. Opsomer, A. de Kruif, G. Genicot, and A. Van Soom. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction* 130:485–495.
- Lowman, B. G., N. Scott, and S. Somerville. 1976. Condition scoring of cattle. Rev. ed. E. Bulletin No. 6. East of Scotland College of Agriculture, Edinburgh, UK.
- Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: Where will it end? *J. Dairy Sci.* 84:1277–1293.
- Lussier, J. G., P. Matton, and J. J. Dufour. 1987. Growth rates of follicles in the ovary of the cow. *J. Reprod. Fertil.* 81:301–307.
- Matoba, S., T. Fair, and P. Lonergan. 2010. Maturation, fertilisation and culture of bovine oocytes and embryos in an individually identifiable manner: A tool for studying oocyte developmental competence. *Reprod. Fertil. Dev.* 22:839–851.
- McGuire, M. A., D. E. Bauman, D. A. Dwyer, and W. S. Cohick. 1995. Nutritional modulation of the somatotropin/insulin-like growth factor system: Response to feed deprivation in lactating cows. *J. Nutr.* 125:493–502.
- Mu, Y.-M., T. Yanase, Y. Nishi, A. Tanaka, M. Saito, C.-H. Jin, C. Mukasa, T. Okabe, M. Nomura, K. Goto, and H. Nawata. 2001. Saturated FFAs, palmitic acid and stearic acid, induce apoptosis in human granulosa cells. *Endocrinology* 142:3590–3597.
- Patton, J., D. A. Kenny, S. McNamara, J. F. Mee, F. P. O'Mara, M. G. Diskin, and J. J. Murphy. 2007. Relationships among milk production, energy balance, plasma analytes, and reproduction in Holstein-Friesian cows. *J. Dairy Sci.* 90:649–658.
- Patton, J., J. J. Murphy, F. P. O'Mara, and S. T. Butler. 2008. A comparison of energy balance and metabolic profiles of the New Zealand and North American strains of Holstein Friesian dairy cow. *Animal* 2:969–978.
- Prendiville, R., K. M. Pierce, and F. Buckley. 2009. An evaluation of production efficiencies among lactating Holstein-Friesian, Jersey, and Jersey × Holstein-Friesian cows at pasture. *J. Dairy Sci.* 92:6176–6185.
- Pryce, J. E., M. D. Royal, P. C. Garnsworthy, and I. L. Mao. 2004. Fertility in the high-producing dairy cow. *Livest. Prod. Sci.* 86:125–135.
- Reist, M., D. Erdin, D. von Euw, K. Tschuemperlin, H. Leuenberger, Y. Chilliard, H. M. Hammon, C. Morel, C. Philipona, Y. Zbinden, N. Kuenzi, and J. W. Blum. 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. *J. Dairy Sci.* 85:3314–3327.
- Rizos, D., L. Burke, P. Duffy, M. Wade, J. F. Mee, K. J. O'Farrell, M. MacSiurtain, M. P. Boland, and P. Lonergan. 2005. Comparisons between nulliparous heifers and cows as oocyte donors for embryo production in vitro. *Theriogenology* 63:939–949.
- Roth, Z., G. Inbar, and A. Arav. 2008. Comparison of oocyte developmental competence and follicular steroid content of nulliparous heifers and cows at different stages of lactation. *Theriogenology* 69:932–939.
- Sartori, R., M. R. Bastos, and M. C. Wiltbank. 2010. Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle. *Reprod. Fertil. Dev.* 22:151–158.
- Snijders, S. E., P. Dillon, D. O'Callaghan, and M. P. Boland. 2000. Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. *Theriogenology* 53:981–989.
- Vanholder, T., J. L. M. R. Leroy, A. Van Soom, G. Opsomer, D. Maes, M. Coryn, and A. de Kruif. 2005. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. *Anim. Reprod. Sci.* 87:33–44.
- Walters, A. H., T. L. Bailey, R. E. Pearson, and F. C. Gwazdauskas. 2002. Parity-related changes in bovine follicle and oocyte populations, oocyte quality, and hormones to 90 days postpartum. *J. Dairy Sci.* 85:824–832.
- Whitaker, D. A. 1997. Interpretation of metabolic profiles in dairy cows. *Cattle Pract.* 5:57–60.