Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows

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ABSTRACT

The aim of this study was to examine the direct effect of lactation on the ability of the reproductive tract of postpartum dairy cows to support early embryo development. Twenty-one primiparous Holstein heifers were used. Immediately after calving, half of the cows were dried off (i.e., never milked), and the other half entered the milking herd and were milked twice daily. Jugular blood samples were taken twice per week from 15 d before calving to approximately 100 d postpartum to measure nonesterified fatty acids, β -hydroxybutyrate, glucose, insulin, and insulin-like growth factor-I. At the same time, body weight and body condition score were recorded for each cow. At approximately 60 d postpartum (experiment 1), approximately 65 two- to four-cell embryos, produced by in vitro maturation and fertilization, were endoscopically transferred to the oviduct ipsilateral to the corpus luteum of all cows on d 2 of the estrous cycle. Five days later (d 7), the oviduct and uterus were flushed nonsurgically and the number of embryos developing to the blastocyst stage was recorded. At approximately 90 d postpartum (experiment 2), the estrous cycles of the same cows were resynchronized and 15 to 20 in vitro-produced blastocysts were transferred to the uterus of each recipient on d 7. All cows were slaughtered on d 14 to assess embryo survival and dimensions. Body weight and body condition score were significantly different between groups for the entire postpartum period of the study. Concentrations of nonesterified fatty acids and β -hydroxybutyrate were higher and concentrations of glucose, insulin, and insulin-like growth factor-I were lower in lactating compared with nonlactating cows. Embryo recovery rates from lactating and dry cows were similar. In experiment 1, fewer embryos developed to the blastocyst stage in the lactating cows compared

with the nonlactating cows. In experiment 2, embryo survival and conceptus dimensions were not different between lactating and nonlactating cows. In conclusion, the data indicate that the reproductive tract of the lactating dairy cow is compromised in its ability to support early embryo development compared with that of matched dry cows and this may contribute to early embryo mortality observed in such animals.

Key words: fertility, conceptus, embryo transfer

INTRODUCTION

The physiological changes associated with high milk production are associated with poor reproductive inefficiency in commercial dairy herds (Lucy, 2001; Pryce et al., 2004). Decreasing (glucose, insulin, IGF-I) or increasing (NEFA, ketone bodies) circulating metabolites during nutrient partitioning associated with low BCS undoubtedly play a role in determining reproductive outcome. However, understanding the causes of infertility in dairy cattle is complex and may be attributable to compromised oocyte quality, a suboptimal reproductive tract environment incapable of supporting normal development, or a combination of both (Leroy et al., 2008a,b; Walsh et al., 2011).

Evidence for the contribution of poor oocyte quality to infertility comes from a variety of sources. First, data on nonsurgical flushing of unstimulated dairy cows (reviewed by Sartori et al., 2010) suggest that a significant proportion of embryos degenerate before the blastocyst stage. For example, in 3 studies by Cerri et al. (2009a,b,c), the proportion of viable embryos recovered on d 6 to 7 was approximately 50%. Given that the fertilization rate is estimated at 85 to 95%, this suggests that a significant proportion of embryos are lost as early as d 7. Second, several studies have reported higher pregnancy rates in lactating dairy cows after embryo transfer compared with AI (Putney et al., 1989; Ambrose et al., 1999; Drost et al., 1999; Rutledge, 2001; Al-Katanani et al., 2002; Vasconcelos et al., 2006; Demetrio et al., 2007). Third, exposure of oocytes in vitro to NEFA at physiological concentrations consis-

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tent with those measured in the preovulatory follicle of postpartum lactating cows is detrimental to oocyte development (Leroy et al., 2004, 2005).

In vitro studies examining the effect of lactation on oocyte quality have lead to equivocal results. For example, 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 compared with those from cows of average genetic merit. Rizos et al. (2005) reported no difference in the proportion of good quality oocytes undergoing fertilization and development to the blastocyst stage between lactating cows and heifers. Several studies from Virginia (Kendrick et al., 1999; Gwazdauskas et al., 2000; Walters et al., 2002) 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. A recent study from our group (Matoba et al., 2012) failed to demonstrate an effect of metabolic status postpartum on oocyte ability to undergo in vitro fertilization and develop to the blastocyst stage in vitro.

The reproductive tract (oviduct/uterus) clearly also plays a crucial role in providing an appropriate environment conducive to normal embryo development leading up to maternal recognition of pregnancy, a period around which a substantial part of embryo loss occurs (Diskin and Morris, 2008). Several studies from our group have emphasized the important role of progesterone in the first week after conception in establishing an optimum uterine milieu to support conceptus elongation around the time of maternal recognition (Clemente et al., 2009; Forde et al., 2009, 2011; Rizos et al., 2011). Embryo transfer studies allow us to test the ability of the reproductive tract to support development without the confounding effect of the cow's own, potentially compromised, oocyte. We recently reported that embryo development to d 7 in the reproductive tract of postpartum lactating cows was compromised compared with that in the tract of nulliparous heifers (Rizos et al., 2010), consistent with the data reviewed by Sartori et al. (2010). One justifiable criticism of that model is that a nulliparous heifer is not the same as a metabolically stressed postpartum cow in early lactation. To overcome this criticism in the current study, we used age-matched postpartum primiparous dairy cows that were either milked postcalving (i.e., lactating) or were dried off immediately at calving (i.e., never milked, nonlactating) to directly test the effects of lactation on postpartum fertility characteristics. The specific objectives of this study were to characterize

the direct effects of lactation on postpartum metabolic profiles and the ability of the reproductive tract to (1) support embryo development to the blastocyst stage, and (2) support elongation of the conceptus following transfer of a blastocyst at d 7.

MATERIALS AND METHODS

Animal 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, primiparous, autumn-calving cows were used ($n = 21$, mean age 3.9 ± 0.1 yr). The cows had been part of a previous fertility trial that involved 4 cycles of AI, pregnancy diagnosis, and prostaglandin-induced abortion, and were known to be of high fertility (~70% of 118 heifers pregnant at each cycle of AI; Parr et al., 2012). All cows were pregnant to a synchronized estrus following insemination with semen from the same bull. At calving, cows were randomly assigned to 1 of 2 treatment groups: lactating ($n = 11$) or nonlactating ($n = 10$). All cows were housed indoors on a slatted floor for the duration of the experiment. Lactating cows were offered a diet that consisted of 50:50 maize silage (DM 344 g/kg, CP 76 g/kg, and ME 11.8 MJ/kg of DM):grass forage (DM 239 g/kg, CP 101 g/kg, and ME 11.1 MJ/kg of DM) ad libitum plus 8 kg of concentrates (DM 883 g/kg, CP 281 g/kg, and ME 12.9 MJ/kg of DM) per day at milking (twice daily at 0700 and 1600 h). The nonlactating cows were offered the standard forage diet on an ad libitum basis. In the lactating group, milk production was recorded twice daily at each milking. A vaginal mucus score was taken at d 28 postpartum because evaluation of the character of vaginal mucus at this time has been shown to reflect the bacterial load within the uterine lumen (Williams et al., 2005); all cows scored 0 or 1 on a scale of 0 to 3, indicative of a normal bacterial load.

Body weight and BCS were recorded twice a week at approximately 2 wk before expected calving date, at calving, and then twice per week until the end of the experiment (approximately 95 d postpartum). Body condition 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) and Prendiville et al. (2009).

Plasma Hormone and Metabolite Analysis

To fully characterize the metabolic status of the cows, blood plasma samples were collected twice 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. Circulating progesterone concentrations were monitored following embryo transfer (see below).

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. Briefly, 200 μL of serum was pipette into an antibody-coated tube in duplicate and 1 mL of iodinated insulin tracer was subsequently added. All samples were incubated at room temperature (15 to 18°C) for 18 h, decanted, and counted for 90 s using the Wallac 1470 gamma counter (Wallac/Perkin Elmer, Waltham, MA). The inter- and intraassay CV for insulin were 12.0 and 12.6%, 14.2 and 13.6%, and 8.4 and 8.8% for low, medium, and high quality controls, respectively.

Serum IGF-1 concentrations were measured using a RIA as previously described (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 spun 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 2,000 rpm 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, Bordon, UK) added for a further 30-min incubation at room temperature. Finally, 250 μL of distilled water was added to each tube, samples spun at $1,500 \times g$ for 15 min and counted on the Wallac 1470 gamma counter (Wallac/Perkin Elmer). Intraassay CV for IGF-1 were 12.9, 5.2, and 9.2% for low, medium, and high standards, respectively. Interassay CV were 6.6, 4.2, and 8.1% for low, medium, and high standards, respectively.

Serum BHBA concentrations were measured using the Ranbut D-3-Hydroxybutyrate kit (Randox Laboratories Ltd., Crumlin, UK) using a kinetic enzymatic reaction with a sensitivity of 0.1 nmol/L. Twenty-five microliters of serum was added to each tube, 1,000 μL

of buffer mixed with enzyme/coenzyme was incubated for 60 s at 37°C, and the first reading was taken. Subsequent readings were taken after 1 and 2 min and mean absorbance change per minute was calculated. The inter- and intraassay CV were 1.9 and 1.9% for the low and 0.7 and 0.8% for the high quality controls, respectively.

Circulating NEFA concentrations were measured using the Randox NEFA enzyme assay with a sensitivity of 0.072 mmol/L. Serum samples (10 μL) were incubated with 200 μL of assay reagent 1 for 5 min at 37°C and 400 μL of reagent 2 incubated for a further 5 min at 37°C, and absorbance readings were taken. Inter- and intraassay CV for the medium and high NEFA quality controls were 1.0 and 0.8 (interassay CV) and 1.6 and 1.4 (intraassay CV), respectively.

Plasma glucose concentrations were measured using the automated Randox Glucose (Gluc-HK) hexokinase enzymatic method (sensitivity 0.662 mmol/L). Interassay CV were 1.1 and 0.7% for the low and high standards, and intraassay CV were 0.8 and 0.7% for the low and high standards, respectively.

Serum progesterone concentrations were measured using a competitive binding ^{125}I RIA Progesterone Coat-A-Count kit (Siemens Medical Solutions Diagnostics) as previously described (Forde et al., 2011). Briefly, 100 μL of serum was dispensed into antibody-coated tubes in duplicate. One milliliter of iodinated P4 tracer was added to each tube incubated for 3 h at room temperature (15 to 18°C) and counted for 90 s using the Wallac 1470 gamma counter (Wallac/Perkin Elmer). The low, medium, and high progesterone CV were 13.4, 3.6, and 5.4% for the interassay CV and 18.9, 8.8, and 7.5% for the intraassay CV respectively.

In Vitro Production of Bovine Embryos

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (Poole, UK). The techniques for producing embryos in vitro were described previously (Rizos et al., 2002). Immature cumulus oocyte complexes (COC) were obtained by aspirating follicles on bovine ovaries collected at slaughter. The COC were matured for 24 h in Tissue Culture Medium-199 supplemented with 10% (vol/vol) fetal calf serum and 10 ng/mL epidermal growth factor at 39°C under an atmosphere of 5% CO_2 in air with maximum humidity. For in vitro fertilization, matured COC were inseminated with frozen-thawed, Percoll-separated bull sperm at a concentration of 1×10^6 spermatozoa/mL. Gametes were co-incubated at 39°C under an atmosphere of 5% CO_2 in air with maximum humidity. At approximately 20 h postinsemination, presumptive zygotes were denuded,

divided into groups of 50, and transferred to 500- μ L culture wells. The basal medium for all embryo culture was synthetic oviduct fluid (SOF) supplemented with 5% fetal calf serum. Cleavage rate was recorded at 48 h postinsemination; only cleaved embryos were used for transfer for experiment 1 and only blastocysts on d 7 were used for transfer for experiment 2.

Experiment 1: Effect of Lactation on the Ability of the Reproductive Tract to Support Embryo Development from d 2 to 7

At approximately d 60 postpartum (60.9 ± 2.32), the estrous cycles of the cows ($n = 21$) were synchronized using a controlled internal drug release device (Pfizer, Sandwich, UK) for 8 d. One day before device removal, all animals received 0.5 mg of a PGF_{2 α} analog (Estrumate, Schering-Plough Animal Health, Hertfordshire, UK). Standing estrus was defined as d 0. Cleaved embryos produced in vitro were transferred ($n = 60$ –65 embryos per recipient) to the oviduct ipsilateral to the corpus luteum on d 2 of the estrous cycle as described previously (Havlicek et al., 2005; Rizos et al., 2010). For transfer, recipients were restrained and received an epidural anesthesia (3.5 mL of 2% lidocaine solution, Selectavet, Weyarn-Holzolleng, Germany). The tip of a bitubular endoscopic set (Storz, Tuttlingen, Germany) containing the endoscope and the transfer system was placed in the peritoneal cavity via the fornix of the vagina. After passive air movement into the cavity, the reproductive organs were assessed for suitability for embryo transfer. The transfer system consisted of a 1-mL syringe embedded into a manual dosimeter (IVFETflex.com, Graz, Austria) and connected to a perfusion tube (no. 701908, Braun, Melsungen, Germany). A fire-polished and curved 50- μ L glass capillary (Brand, Wertheim, Germany) was attached to the end of the perfusion tube. Embryos were loaded in SOF into the tip of the glass capillary and transferred via the infundibulum into the ampulla.

Five days later, on d 7 of the estrous cycle, embryos were recovered by endoscopic flushing of the oviduct and uterine horns. Accessing the oviduct was performed as described above. The glass capillary used for transfer was replaced by a silicon-covered flushing metal tube. Forty to 60 mL of PBS supplemented with 1% fetal calf serum were flushed through the oviducts and uterine horns and collected via an embryo-flushing catheter in an embryo filter (Em Con, no. 04135, Immuno Systems Inc., Spring Valley, WI). A further 300 to 400 mL was used for additional flushing of the uterine horns. Embryos were located under a stereo microscope. The number of embryos developing to the blastocyst stage was recorded immediately after recovery and follow-

ing overnight culture in SOF medium. A representative number ($n = 24$ per group) of d 7 blastocysts recovered from both groups were fixed in ethanol overnight and stained with Hoechst 33342 to assess cell number per blastocyst. A daily blood sample was taken from all animals by jugular venipuncture from d 0 to d 7 to establish the recipient endogenous P4 concentrations.

Experiment 2: Effect of Lactation on the Ability of the Reproductive Tract to Support Embryo Development from d 7 to 14

At approximately d 90 postpartum (93.9 ± 1.95), the same cows were resynchronized as described above. A total of 15 to 20 in vitro-produced blastocysts were transferred to each recipient on d 7 and all recipients were slaughtered on d 14 to assess embryo survival as described previously (Clemente et al., 2009). A daily blood sample was taken from all animals by jugular venipuncture from d 0 to 14 to establish the recipient endogenous P4 concentrations. Following slaughter, the reproductive tract was removed, sealed in a plastic bag, and placed in a sealed polystyrene box for transportation to the laboratory (within 60 min). After removal of the ovaries and the oviducts, the uterine horns were trimmed free of excess tissue before being flushed with 40 mL of PBS. Embryos were located under a stereomicroscope, measured, and then snap frozen individually in liquid nitrogen and stored at -80°C . The weight and dimensions of the corpora lutea (CL) were also recorded.

Statistical Analysis

Data were checked for normality and homogeneity of variance using histograms, qplots, and formal statistical tests in the UNIVARIATE procedure (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 SE of the nontransformed data are presented in the results for clarity. For all analyses, cow was the experimental unit. Characterization data (BW, BCS, and metabolic profiles) were analyzed using repeated measures with the MIXED procedure of SAS. Fixed effects included experimental treatment (lactating or nonlactating), day, and their interaction. The interaction term, if not statistically significant ($P > 0.10$), was subsequently excluded from the final model. Actual calving day was used as a linear covariate. Animal within treatment

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 (AIC). Models were run under compound symmetry, unstructured, autoregressive, or Toeplitz variance-covariance structures. The model with the least AIC value was selected. Embryo-related data (for experiments 1 and 2) were analyzed using the PROC MIXED procedure of SAS. The model had experimental treatment as a fixed effect and animal within treatment was included as a random effect. Differences between treatments 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.

RESULTS

Animal Characterization

Body weight, BCS, and metabolite profiles across the entire experimental period are shown in Figure 1. Cows in the lactating group had a mean milk yield across the study period of 25.04 ± 0.19 kg/d. Mean BW across the entire study period was 640.2 ± 15.2 kg and 688.3 ± 15.9 kg for lactating and nonlactating cows, respectively. Lactating and nonlactating cows had a similar BW before calving and for up to 2 wk postcalving. Subsequently, the BW of lactating cows declined and remained significantly lower ($P < 0.001$) than that of the nonlactating group throughout the remainder of the experimental period (Figure 1).

Mean BCS across the entire study period was 3.0 ± 0.05 and 3.6 ± 0.05 for lactating and nonlactating cows, respectively; BCS declined from approximately d 7 prepartum to d 7 postpartum in both groups. The BCS diverged significantly ($P < 0.001$) between lactating and nonlactating cows from d 7 onward, reaching a nadir at approximately d 32 in the lactating group. In the nonlactating group, BCS gradually increased from d 7 (3.0 ± 0.03) up to d 95 postpartum (4.2 ± 0.09), whereas in the lactating group, BCS remained low up to d 95 (3.0 ± 0.1).

Concentrations of NEFA diverged between lactating and nonlactating cows from d 7 postpartum and were higher ($P < 0.05$) in lactating cows from d 14 to 49 postpartum compared with in the nonlactating group, for which concentrations fell from a peak at calving to a nadir at approximately d 21, after which they remained relatively constant. Concentrations of BHBA were higher in lactating cows from calving throughout the experimental period compared with their nonlactating counterparts.

Concentrations of IGF-I declined dramatically from d 14 prepartum to a nadir on d 4 postpartum in lactating cows, after which they remained constant to d 95. In contrast, after an initial precalving decline from d -14 to calving, IGF-I concentrations increased markedly in nonlactating cows and maintained concentrations greater than double those in lactating cows throughout the study period. Similarly, insulin concentrations were significantly higher in nonlactating cows from calving throughout the experimental period.

We observed a dramatic spike in glucose concentrations coincident with calving in both groups, followed by a precipitous decline immediately postpartum. The extent of the postpartum decrease was less in lactating animals, resulting in divergent glucose concentrations between experimental groups from d 3 to approximately d 49 postpartum.

Experiment 1

Of the 11 cows in the lactating group, 7 exhibited standing estrus confirmed by the presence of an appropriately sized CL on d 2 at embryo transfer and on d 7 at embryo recovery; only data from these cows were included. In contrast, all 10 cows from the nonlactating group showed standing estrus, confirmed by the presence of a CL on the day of embryo transfer (d 2) and embryo recovery (d 7). Mean progesterone concentrations were similar between groups from d 0 to 7 (Figure 2).

For cows from which embryonic structures were recovered at d 7 (all except one), the recovery rate was similar for the lactating and nonlactating groups (65.6 ± 8.6 vs. 63.9 ± 7.2 , respectively). Of the structures recovered, $26.3 \pm 4.1\%$ had developed to the blastocyst stage in the lactating cows compared with $39.6 \pm 3.6\%$ in the nonlactating groups ($P < 0.05$; Table 1). Following overnight culture in vitro, these values increased to $32.6 \pm 4.4\%$ and $49.3 \pm 3.8\%$ for lactating and nonlactating cows, respectively ($P = 0.03$). Blastocysts were recovered from all cows in both groups (range: 12.8 to 36.4% per cow in the lactating group; range: 18.4 to 58.9% per cow in the nonlactating group). We found no evidence of a difference in blastocyst quality, as evidenced by total cell number in the blastocysts (63.1 ± 1.9 vs. 62.6 ± 1.9 , for lactating and nonlactating cows, respectively).

Experiment 2

All animals in both groups showed standing estrus following CIDR removal (d 0), which was confirmed by the presence of an active CL at embryo transfer

Figure 1. Characterization of postpartum lactating (▲) and nonlactating (■) dairy cows used in the study. Body weight and BCS (on a scale of 1 to 5) were recorded approximately 2 wk before expected calving date, at calving, and then weekly until the end of the experiment (approximately 95 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 and were analyzed for NEFA, BHBA, insulin, IGF-I, and glucose. Values refer to means $\pm$ SEM.

Table 1. Recovery and development of bovine embryos following endoscopic transfer to the oviducts of lactating or nonlactating postpartum dairy cows (experiment 1)

Cows	Embryos transferred (no.)	Recovery (no.; mean % $\pm$ SEM)	Day 7 blastocysts (no.; mean % $\pm$ SEM)	Total blastocysts ¹ (no.; mean % $\pm$ SEM)
Lactating	435	289 (65.6 $\pm$ 8.6)	75 (26.3 $\pm$ 4.1) ^a	97 (32.6 $\pm$ 4.4) ^a
Nonlactating	627	403 (63.9 $\pm$ 7.2)	165 (39.6 $\pm$ 3.6) ^b	203 (49.3 $\pm$ 3.8) ^b
<i>P</i> -value		0.88	0.05	0.03

^{a,b}Values in the same column with different letters differ significantly.

¹Following overnight culture.

on d 7 and at slaughter on d 14. One animal from the lactating group was excluded from the analysis because the elongated embryos recovered on d 14 were tangled and impossible to separate for measurement. Thus, 10 cows from both groups were used in the analysis. The recovery rate of embryos on d 14 was similar for both

groups (lactating: 39.8 $\pm$ 9.6%; nonlactating: 33.3 $\pm$ 9.6%; Table 2). Embryo dimensions (length, width, area) did not differ between groups (length: 1.6 $\pm$ 0.5 mm vs. 1.2 $\pm$ 0.5 mm; width: 0.7 $\pm$ 0.1 mm vs. 0.5 $\pm$ 0.1 mm; area: 1.5 $\pm$ 0.6 mm² vs. 0.9 $\pm$ 0.7 mm², for lactating and nonlactating cows, respectively; Table 2).

Figure 2. Progesterone concentrations (means $\pm$ SEM) in postpartum lactating (▲) and nonlactating (■) dairy cows used in the study during experiments 1 and 2. (A) Experiment 1: At approximately 60 d postpartum, approximately 65 two- to four-cell embryos, produced by in vitro maturation and fertilization of oocytes, were endoscopically transferred to the oviduct ipsilateral to the corpus luteum on d 2 of the estrous cycle. Five days later, on d 7, embryos were recovered by nonsurgically flushing the oviduct and uterus. (B) Experiment 2: At approximately 90 d postpartum, 15 to 20 in vitro-produced blastocysts were transferred to the uterus on d 7. All cows were slaughtered on d 14 to assess embryo survival and conceptus dimensions.

Table 2. Recovery rate and dimensions of d 14 embryos (mean $\pm$ SEM) recovered after the transfer of blastocysts on d 7 and corpus luteum (CL) diameter and weight at d 14 (experiment 2)

Cows	Embryos transferred (no.)	Recovery (no.; mean % $\pm$ SEM)	Length (mm)	Width (mm)	Area (mm ³)	CL diameter (mm)	CL weight (g)
Lactating	175	67 (39.8 $\pm$ 9.6)	1.6 $\pm$ 0.5	0.7 $\pm$ 0.1	1.5 $\pm$ 0.6	26.4 $\pm$ 1.5	10.4 $\pm$ 1.1 ^a
Nonlactating	175	65 (33.3 $\pm$ 9.6)	1.2 $\pm$ 0.5	0.5 $\pm$ 0.1	0.9 $\pm$ 0.7	24.1 $\pm$ 1.3	7.0 $\pm$ 0.8 ^b
<i>P</i> -value		0.64	0.63	0.21	0.51	0.2	<0.05

^{a,b}Values in the same column with different letters differ significantly.

Lactating cows had significantly greater progesterone concentrations from d 9 until slaughter at d 14 compared with nonlactating cows (Figure 2). That was consistent with a greater CL weight recorded at slaughter on d 14 (10.4 $\pm$ 1.1 g vs. 7.0 $\pm$ 0.8 g, for lactating and nonlactating, respectively; Table 2).

DISCUSSION

By comparing postpartum lactating and nonlactating (i.e., immediately dried off at calving) Holstein cows, this study is one of the first to address directly the influence of lactation and associated metabolic perturbation on embryo development in postpartum dairy cows. The main findings from the study were (1) that lactation induces a significant alteration in the pattern of many key metabolites associated with fertility in postpartum cows; (2) this is associated with an impairment in the ability of the reproductive tract of the postpartum lactating dairy cow to support early embryo development to the blastocyst stage around d 60 postpartum; and (3) by d 90 postpartum, despite some latent differences in metabolic profiles between groups, we did not find evidence for a deleterious effect of lactation on the ability of the uterus to support conceptus elongation.

Lactating dairy cows typically enter a state of negative energy balance (NEB) postpartum when the combined energy requirements for maintenance and milk production exceed dietary energy intake. The requirement for cows to conceive when they are in peak lactation in a 300-d lactation associated with a seasonally concentrated calving pattern, such as that in Ireland, often coincides with this period of NEB. Both the duration and severity of early postpartum NEB, and the associated reduced circulating concentrations of insulin, IGF-I, and glucose and elevated concentrations of NEFA and BHBA, have been linked to impaired reproductive performance (Butler and Smith, 1989; Lucy et al., 1991). The current study is one of the first to compare the metabolic profiles of lactating and nonlactating (immediately dried off at calving) cows and avoids the justifiable criticism of studies, including some of our own (Rizos et al., 2005, 2010), that compared lactating

cows to nulliparous heifers. The metabolite data from the lactating cows in the present study are entirely consistent with those from a recent study from our group (Matoba et al., 2012).

Associations of glucose, NEFA, and BHBA with energy balance in early lactation are well established, and they reflect enhanced mobilization of body reserves and partitioning of nutrients toward milk production. Negative energy balance is typically associated with a decrease in circulating concentrations of insulin, glucose, and IGF-I and increased concentrations of NEFA and BHBA, a product of tissue fatty acid catabolism (Grummer, 1995). Consistent with the literature, in both groups, NEFA concentrations rose in the 2 wk before calving. However, after an initial decline post-calving, concentrations were elevated in lactating cows from d 14 to 49 postpartum compared with those in nonlactating cows, for which concentrations fell from a peak at calving to a nadir at approximately d 21, after which they remained relatively constant. Concentrations of BHBA were higher in lactating cows from calving throughout the experimental period compared with their nonlactating counterparts. 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 reduce 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, 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. Recent data suggest that el-

evated NEFA in the follicle can result in compromised early embryo quality, viability, and metabolism (Van Hoeck et al., 2011). Data on NEFA concentrations in oviduct fluid in the postpartum period have not been reported but it is likely that they are elevated there also and may partly contribute to the reduced embryo development observed in lactating cows in the current study.

Glucose concentrations increased slightly during the prepartum period, increased dramatically at calving, and then decreased immediately postpartum, consistent with the published literature (Grummer, 1995; Butler et al., 2006; Patton et al., 2008). The extent of the postpartum decrease in glucose was less in lactating animals, resulting in divergent glucose concentrations between lactating and nonlactating cows from d 3 to approximately d 49 postpartum, presumably reflective of the dramatic increase in mammary glucose requirements associated with the onset of lactation. An increase in net glucose uptake, usually at the expense of pyruvate, is a feature of preimplantation embryo metabolism in all mammals studied, including cattle; glucose consumption is relatively low during early preimplantation development but increases dramatically at blastocyst formation, after which glucose is a major energy source (Tiffin et al., 1991; Rieger et al., 1992). The concentrations of glucose in oviduct (1.87 to 3.17 mM) and uterine fluid (3.78 to 4.54 mM) of nonlactating beef heifers are reported to remain relatively stable throughout the estrous cycle and to be approximately half those in plasma (Hugentobler et al., 2008). The peripheral glucose concentrations reported in the current study are consistent with these values; whether lactation-induced changes in oviduct fluid glucose concentrations exist that may directly affect embryo development is unknown.

The temporal pattern of plasma IGF-I concentration in lactating cows in the present study was similar to that in previous reports, with a decline at parturition and a gradual increase thereafter (McGuire et al., 1995; Patton et al., 2008; Matoba et al., 2012). In the current study, IGF-I concentrations declined dramatically from d -14 to a nadir on d 4 in lactating cows, after which they remained constant to d 95. In contrast, after a similar initial precalving decline, IGF-I concentrations increased markedly in nonlactating cows and maintained concentrations greater than double those in lactating cows throughout the study period. Similarly, insulin concentrations were significantly higher in nonlactating cows from calving throughout the experimental period. These data are consistent with the suggestion that plasma IGF-I concentrations in early lactation may be useful indicators of reproductive efficiency in dairy cattle (Patton et al., 2007). Addition

of IGF-1 to culture medium increases the proportion of bovine embryos that develop to the blastocyst stage in vitro and increases embryo survival following transfer to heat-stressed, lactating dairy cows (Block and Hansen, 2007), likely through alteration of gene expression in the embryo (Block et al., 2008). In addition to lower circulating IGF-I after calving in lactating cows, NEB may also influence IGF availability in the oviduct indirectly through changes in specific insulin-like growth factor binding protein expression (Fenwick et al., 2008).

As mentioned earlier, several studies have reported the outcome of embryo transfer at d 7 in dairy cows, thereby testing the ability of the reproductive tract to support development from d 7 onward independent of the oocyte (Putney et al., 1989; Ambrose et al., 1999; Drost et al., 1999; Rutledge, 2001; Al-Katanani et al., 2002; Vasconcelos et al., 2006; Demetrio et al., 2007). The consistently higher pregnancy rate following embryo transfer in those studies would suggest that impaired oocyte quality is a contributory factor to low fertility in dairy cows, but does not rule out a role for the embryo or reproductive tract environment. The recovery rate of approximately 65% in experiment 1 is entirely consistent with our previous study in postpartum dairy cows (Rizos et al., 2010) and is lower than that typically achieved with nulliparous heifers (~80%; Tesfaye et al., 2007; Rizos et al., 2010). The lower proportion of embryos developing to the blastocyst stage in lactating cows is also consistent with our previous study, in which lactating cows were compared with nulliparous heifers (Rizos et al., 2010), and suggests an impairment in the ability of the tract to support development, likely associated with the altered metabolic status as described above.

Using a previously validated multiple embryo transfer model (Clemente et al., 2009; Carter et al., 2010; Forde et al., 2011), the current study is the first to examine the ability of the postpartum reproductive tract of the dairy cow to support embryo development in the period encompassing the events between fertilization and d 7 and between d 7 and 14 independent of the confounding factors potentially associated with the endogenous oocyte. Somewhat surprisingly, we observed no difference in conceptus dimensions between lactating and nonlactating cows; indeed, the numerically greater conceptus length in the lactating group was associated with significantly higher progesterone concentrations from d 9 to 14 in that group. It is likely that by d 90, the deleterious influence of the lactation-induced perturbed metabolic status is significantly reduced compared with earlier postpartum time points.

Early luteal (d 4 to 5) concentrations of progesterone are a reasonable predictor of concentrations on d 7 and may provide the potential to identify animals at risk

of early embryo loss because of low concentrations of P4 and to selectively supplement such animals (Parr et al., 2012). Enhanced embryo development has been associated with elevated concentration of progesterone during the first week post conception (Carter et al., 2008; Clemente et al., 2009). Interestingly, progesterone concentrations from d 0 to 7 were not different in experiment 1, which was carried out at approximately 60 d postpartum, between lactating and nonlactating cows, contrasting with the hypothesis that lactation is associated with depressed steroid concentrations due to greater metabolic clearance associated with greater DMI (Sangsrivong et al., 2002). However, the lack of association between progesterone concentrations and blastocyst development is consistent with our previous data involving embryo recovery from inseminated beef heifers (Carter et al., 2008) and those following endoscopic transfer of 2- to 4-cell embryos to the oviducts of beef heifers with normal or elevated progesterone concentrations (Carter et al., 2010) but contrasts with a similar study comparing postpartum dairy cows and nulliparous heifers (Rizos et al., 2010). Consistent with experiment 1, in experiment 2 (carried out at approximately 90 d postpartum), progesterone concentrations were similar between lactating and nonlactating cows from d 0 to 8 but were significantly higher in lactating cows from d 9 to 14. These greater progesterone concentrations were associated with a significantly larger CL assessed at slaughter on d 14.

CONCLUSIONS

This study is one of the first to characterize the metabolic profile of postpartum dairy cows specifically induced by lactation and confirms our previous findings (Rizos et al., 2010) that lactation is associated with a compromised ability of the early postpartum reproductive tract to support early development to the blastocyst stage. These results are consistent with the results of several published studies indicating that a significant proportion (almost 50%) of embryos are not viable by d 6 to 7 (Sartori et al., 2010) and highlight the fact that although the oocyte is clearly a key player in explaining infertility in dairy cows, an important role for the embryo and reproductive tract and their interaction must also be considered.

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REFERENCES

- Al-Katanani, Y. M., M. Drost, R. L. Monson, J. J. Rutledge, C. E. Krininger 3rd, J. Block, W. W. Thatcher, and P. J. Hansen. 2002. Pregnancy rates following timed embryo transfer with fresh or vitrified in vitro produced embryos in lactating dairy cows under heat stress conditions. *Theriogenology* 58:171–182.
- Ambrose, J. D., M. Drost, R. L. Monson, J. J. Rutledge, M. L. Leibfried-Rutledge, M. J. Thatcher, T. Kassa, M. Binelli, P. J. Hansen, P. J. Chenoweth, and W. W. Thatcher. 1999. Efficacy of timed embryo transfer with fresh and frozen in vitro produced embryos to increase pregnancy rates in heat-stressed dairy cattle. *J. Dairy Sci.* 82:2369–2376.
- 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.
- Block, J., and P. J. Hansen. 2007. Interaction between season and culture with insulin-like growth factor-1 on survival of in vitro produced embryos following transfer to lactating dairy cows. *Theriogenology* 67:1518–1529.
- Block, J., C. Wrenzycki, H. Niemann, D. Herrmann, and P. J. Hansen. 2008. Effects of insulin-like growth factor-1 on cellular and molecular characteristics of bovine blastocysts produced in vitro. *Mol. Reprod. Dev.* 75:895–903.
- 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.
- Carter, F., N. Forde, P. Duffy, M. Wade, T. Fair, M. A. Crowe, A. C. Evans, D. A. Kenny, J. F. Roche, and P. Lonergan. 2008. Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. *Reprod. Fertil. Dev.* 20:368–375.
- Carter, F., F. Rings, S. Mamo, M. Holker, A. Kuzmany, U. Besenfelder, V. Havlicek, J. P. Mehta, D. Tesfaye, K. Schellander, and P. Lonergan. 2010. Effect of elevated circulating progesterone concentration on bovine blastocyst development and global transcriptome following endoscopic transfer of in vitro produced embryos to the bovine oviduct. *Biol. Reprod.* 83:707–719.
- Cerri, R. L., S. O. Juchem, R. C. Chebel, H. M. Rutigliano, R. G. Bruno, K. N. Galvao, W. W. Thatcher, and J. E. Santos. 2009a. Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *J. Dairy Sci.* 92:1520–1531.
- Cerri, R. L., H. M. Rutigliano, R. C. Chebel, and J. E. Santos. 2009b. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. *Reproduction* 137:813–823.
- Cerri, R. L., H. M. Rutigliano, F. S. Lima, D. B. Araujo, and J. E. Santos. 2009c. Effect of source of supplemental selenium on uterine health and embryo quality in high-producing dairy cows. *Theriogenology* 71:1127–1137.
- Clemente, M., J. de La Fuente, T. Fair, A. Al Naib, A. Gutierrez-Adan, J. F. Roche, D. Rizos, and P. Lonergan. 2009. Progesterone and conceptus elongation in cattle: A direct effect on the embryo or an indirect effect via the endometrium? *Reproduction* 138:507–517.
- Demetrio, D. G., R. M. Santos, C. G. Demetrio, and J. L. Vasconcelos. 2007. Factors affecting conception rates following artificial insemination or embryo transfer in lactating Holstein cows. *J. Dairy Sci.* 90:5073–5082.

- 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.
- Drost, M., J. D. Ambrose, M. J. Thatcher, C. K. Cantrell, K. E. Wolfson, J. F. Hasler, and W. W. Thatcher. 1999. Conception rates after artificial insemination or embryo transfer in lactating dairy cows during summer in Florida. *Theriogenology* 52:1161–1167.
- Fenwick, M. A., S. Llewellyn, R. Fitzpatrick, D. A. Kenny, J. J. Murphy, J. F. Patton, and D. C. Wathes. 2008. Negative energy balance in dairy cows is associated with specific changes in IGF-binding protein expression in the oviduct. *Reproduction* 135:63–75.
- Forde, N., M. E. Beltman, G. B. Duffy, P. Duffy, J. P. Mehta, P. O'Gaora, J. F. Roche, P. Lonergan, and M. A. Crowe. 2011. Changes in the endometrial transcriptome during the bovine oestrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. *Biol. Reprod.* 84:266–278.
- Forde, N., F. Carter, T. Fair, M. A. Crowe, A. C. Evans, T. E. Spencer, F. W. Bazer, R. McBride, M. P. Boland, P. O'Gaora, P. Lonergan, and J. F. Roche. 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. *Biol. Reprod.* 81:784–794.
- 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.
- Havlicek, V., F. Wetscher, T. Huber, G. Brem, M. Mueller, and U. Besenfelder. 2005. In vivo culture of IVM/IVF embryos in bovine oviducts by transvaginal endoscopy. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 52:94–98.
- Hugentobler, S. A., P. G. Humpherson, H. J. Leese, J. M. Sreenan, and D. G. Morris. 2008. Energy substrates in bovine oviduct and uterine fluid and blood plasma during the oestrous cycle. *Mol. Reprod. Dev.* 75:496–503.
- 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.
- Leroy, J. L., G. Opsomer, A. Van Soom, I. G. Goovaerts, and P. E. Bols. 2008a. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part I. The importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. *Reprod. Domest. Anim.* 43:612–622.
- Leroy, J. L., A. Van Soom, G. Opsomer, I. G. Goovaerts, and P. E. Bols. 2008b. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. *Reprod. Domest. Anim.* 43:623–632.
- 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., 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.
- Lucy, M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. *J. Dairy Sci.* 74:473–482.
- Matoba, S., L. O'Hara, F. Carter, A. K. Kelly, T. Fair, D. Rizos, and P. Lonergan. 2012. The association between metabolic parameters and oocyte quality in postpartum Holstein dairy cows. *J. Dairy Sci.* 95:1257–1266.
- 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.
- Parr, M. H., M. P. Mullen, M. A. Crowe, J. F. Roche, P. Lonergan, A. C. O. Evans, and M. G. Diskin. 2012. Relationship between early luteal progesterone concentrations and embryo survival and establishment of repeatability estimates for embryo survival and circulating progesterone concentrations in Holstein Friesian heifers. *J. Dairy Sci.* 95:2390–2396.
- 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 $\times$ 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.
- Putney, D. J., M. Drost, and W. W. Thatcher. 1989. Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. *Theriogenology* 31:765–778.
- Rieger, D., N. M. Loskutoff, and K. J. Betteridge. 1992. Developmentally related changes in the metabolism of glucose and glutamine by cattle embryos produced and co-cultured in vitro. *J. Reprod. Fertil.* 95:585–595.
- 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.
- Rizos, D., F. Carter, U. Besenfelder, V. Havlicek, and P. Lonergan. 2010. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J. Dairy Sci.* 93:1022–1029.
- Rizos, D., S. Scully, A. K. Kelly, A. D. Ealy, R. Moros, P. Duffy, A. Al Naib, N. Forde, and P. Lonergan. 2011. Effect of administration of human chorionic gonadotropin on Day 5 post oestrus on corpus luteum characteristics, circulating progesterone and conceptus elongation in cattle. *Reprod. Fertil. Dev.* 24:472–481.
- Rizos, D., F. Ward, P. Duffy, M. P. Boland, and P. Lonergan. 2002. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: Implications for blastocyst yield and blastocyst quality. *Mol. Reprod. Dev.* 61:234–248.
- Rutledge, J. J. 2001. Use of embryo transfer and IVF to bypass effects of heat stress. *Theriogenology* 55:105–111.
- Sangsritavong, S., D. K. Combs, R. Sartori, L. E. Armentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17 β in dairy cattle. *J. Dairy Sci.* 85:2831–2842.
- 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.
- Tesfaye, D., P. Lonergan, M. Hoelker, F. Rings, K. Nganvongpanit, V. Havlicek, U. Besenfelder, D. Jennen, E. Tholen, and K. Schellander. 2007. Suppression of connexin 43 and E-cadherin transcripts in

- in vitro derived bovine embryos following culture in vitro or in vivo in the homologous bovine oviduct. *Mol. Reprod. Dev.* 74:978–988.
- Tiffin, G. J., D. Rieger, K. J. Betteridge, B. R. Yadav, and W. A. King. 1991. Glucose and glutamine metabolism in pre-attachment cattle embryo in relation to sex and stage of development. *J. Reprod. Fertil.* 93:125–132.
- Van Hoeck, V., R. G. Sturmey, P. Bermejo-Alvarez, D. Rizos, A. Gutierrez-Adan, H. J. Leese, P. E. Bols, and J. L. Leroy. 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. *PLoS One* 6:e23183.
- Vanholder, T., J. L. Leroy, A. V. 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.
- Vasconcelos, J. L., D. G. Demetrio, R. M. Santos, J. R. Chiari, C. A. Rodrigues, and O. G. Sa Filho. 2006. Factors potentially affecting fertility of lactating dairy cow recipients. *Theriogenology* 65:192–200.
- Walsh, S. W., E. J. Williams, and A. C. Evans. 2011. A review of the causes of poor fertility in high milk producing dairy cows. *Anim. Reprod. Sci.* 123:127–138.
- 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.
- Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. *Theriogenology* 63:102–117.