



Impact of animal health on reproduction of dairy cows

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Abstract

Many of the diseases that affect dairy cattle either in confinement or pasture-based systems typically occur in the first two months of lactation, before the first postpartum insemination. The increased susceptibility to metabolic and infectious diseases with parturition and the onset of lactation poses a major challenge to reproduction. A wealth of information in the scientific literature is available linking diseases with depressed reproduction in dairy cows. Unfortunately, only few studies have established a causal relationship between a specific disease and fertility, and little is known about the mechanisms that underlie the decrease in pregnancy in dairy cows that had disease in early lactation. It is clear that dairy cows that suffer from disease processes have impaired resumption of postpartum ovulation, compromised fertilization and pre- and peri-implantation conceptus development, altered conceptus gene expression, increased pregnancy loss and, ultimately reduced pregnancy per insemination that causes an extension in time to pregnancy. Because mechanisms are poorly understood, no target intervention is available at this time to reverse the poor reproduction in cows that develop periparturient diseases, except methods to induce cyclicity in anovular cows or to improve insemination rate in cows not detected in estrus. Regardless of a better understanding of the underlying biology of poor fertility in diseased cows, a pivotal approach is to implement strategies that mitigate the risk factors that predispose cows to disease. Such interventions include, but are not limited to, improving transition cow management and grouping, proper dietary formulation to prevent periparturient diseases associated with intermediary and mineral metabolism, strategies for reducing calving-related disorders, and methods to prevent mastitis and lameness. Future developments in target strategies to improve reproduction of cows suffering from peripartum diseases will require a better understanding of the impaired biological processes that compromise establishment and maintenance of pregnancy in this subfertile population of cows.

Keywords: dairy cattle, disease, embryo, pregnancy.

Introduction

Adequate reproductive performance of the lactating herd is a major component of profitability in

dairy farms (Ribeiro *et al.*, 2012). Improved reproduction shortens the transition from being a primiparous to becoming a multiparous cow leading to increments in milk yield, increases the average milk yield per day of calving interval by reducing the days in milk of the lactating herd, increases the number of replacement animals available, influences culling decisions, and accelerates the rate of genetic progress. Unfortunately, improving fertility is not trivial. Establishment and maintenance of a pregnancy to term are affected by several genetic, physiological, and environmental factors that can be manipulated in order to sustain high fertility. Although causality is not always proven, it is well established that diseases negatively influence reproduction in dairy cows.

During early lactation, dairy cows undergo a period of extensive tissue catabolism because of negative nutrient balance. The latter has been linked to unrestrained metabolic disturbances that often lead to diseases which, in turn, dramatically decrease both productive and reproductive performance. Negative nutrient balance has been associated with compromised immune and reproductive functions in dairy cows. Two of the most common clinical diseases in dairy cattle are metritis and mastitis, both of which have been negatively associated with subsequent reproductive performance.

In addition, dairy cows develop the so called subclinical disorders, such as subclinical ketosis and hypocalcemia. The first, being more an adaptation to inadequate caloric intake, has been linked to reduced fertility but, to date, little evidence exists to establish causation between elevated ketones and animal performance. Improper adaptation to increased Ca demands for lactation results in suboptimal concentrations of Ca in blood and increased risk of uterine diseases which impairs fertility. Subclinical and clinical hypocalcemia reduces cytosolic ionized Ca (Ca^{2+}) in immune cells and compromise innate and, possibly, acquired immunity. Establishing nutritional and management methods to minimize the incidence of diseases in early lactation is one of the multiple steps to improve fertility in a dairy herd.

Prevalence of postpartum diseases and impact on fertility of dairy cows

Transition from the dry period (nonlactating pregnant state) to lactation (nonpregnant lactating state) requires the high-producing dairy cow to drastically adjust her metabolism so that nutrients can be

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partitioned to support milk synthesis, a process referred to as homeorrhexis. A sharp increase in nutrient requirements generally occurs at the onset of lactation, when feed intake is usually depressed, which causes extensive mobilization of body tissues, particularly body fat, but also amino acids, minerals and vitamins. Despite tight homeostatic controls and homeorhetic adjustments to cope with the changes in metabolism caused by milk production, 45 to 71% of dairy cows across different levels of milk production, breeds and management systems develop metabolic and infectious diseases in the first months of lactation (Santos *et al.*, 2010a; Ribeiro *et al.*, 2013).

Calving-related disorders and diseases that affect the reproductive tract are major contributors to depression of fertility. Dystocia, metritis, and clinical endometritis were observed in 14.6, 16.1, and 20.8% of postpartum dairy cows in large U.S. confinement herds, respectively (Santos *et al.*, 2010a). Cows that presented at least one of the aforementioned disorders were 50 to 63% less likely to resume ovarian cyclicity by the end of the voluntary waiting period, and were 25 to 38% less likely to become pregnant following the first artificial insemination (AI) postpartum compared with healthy cows. Moreover, cows with dystocia and those diagnosed with clinical endometritis were 67 and 55% more likely

to lose their pregnancies during the first 60 days of gestation compared with healthy cows. The negative effects of reproductive disorders on subsequent fertility are also observed in dairy cows kept under grazing systems (Ribeiro *et al.*, 2013). Even though the prevalence of dystocia, metritis, and clinical endometritis are numerically less in grazing-based herds (8.2, 5.7, and 14.7%, respectively), cows with metritis had 2.7-fold increased odds of being anovular at 50 days postpartum compared with unaffected herd mates. Cows affected uterine diseases had marked depression in pregnancy at the first postpartum AI and increased risk of pregnancy loss. In fact, when diseases were classified as clinical (calving problem, metritis, clinical endometritis, mastitis, pneumonia, digestive problems, and lameness), subclinical [subclinical hypocalcemia, subclinical ketosis, and severe negative energy balance (NEB) based on excessive plasma non-esterified fatty acids (NEFA)], or both, affected cows had increased anovulation and reduced pregnancy per AI (Table 1; Ribeiro *et al.*, 2013). These data strongly indicate that diseases during early lactation have a profound impact on fertility of dairy cows. Maintaining metabolic health to minimize the risk of clinical and subclinical health problems are expected to benefit fertility of dairy cows.

Table 1. Association among clinical and subclinical diseases and fertility responses in grazing dairy cows.

Item	Incidence	AOR (CI) ¹	P value	Contrast ²	
				C1	C2
Estrous cyclic on day 49 postpartum				< 0.01	0.02
Healthy	95.6 ^a	1.00	...		
Subclinical disease only	88.9 ^{b,c}	0.35 (0.16-0.76)	<0.01		
Clinical disease only	93.0 ^{a,b}	0.63 (0.23-1.75)	0.37		
Subclinical and clinical disease	83.5 ^c	0.23 (0.10-0.50)	<0.01		
Pregnant day 30				< 0.01	0.10
Healthy	73.5 ^a	1.00	...		
Subclinical disease only	63.1 ^b	0.67 (0.44-0.99)	0.05		
Clinical disease only	54.8 ^{b,c}	0.44 (0.26-0.75)	<0.01		
Subclinical and clinical disease	50.0 ^c	0.39 (0.24-0.61)	<0.01		
Pregnant day 65				< 0.01	0.07
Healthy	66.2 ^a	1.00	...		
Subclinical disease only	57.1 ^{a,b}	0.72 (0.49-1.05)	0.09		
Clinical disease only	46.3 ^{b,c}	0.45 (0.26-0.76)	<0.01		
Subclinical and clinical disease	42.1 ^c	0.39 (0.25-0.61)	<0.01		

^{a,b,c}Superscript letters within item estrous cycle, pregnant on day 30, and pregnant on day 60 differ ($P < 0.05$).

¹AOR = adjusted odds ratio; CI = confidence interval. ²Contrasts: C1 = effect of disease (healthy vs. all others); C2 = effect of having both clinical and subclinical diseases combined versus only clinical or subclinical (subclinical and clinical disease vs. subclinical disease only + clinical disease only). Data from Ribeiro *et al.* (2013).

Diseases are associated with impaired embryo development

Mechanisms by which diseases in the periparturient period impair reproduction are not clearly

understood. Most studies are of epidemiological nature and the overwhelming majority associates negative effects of diseases during early lactation with reduced pregnancy per AI or extended intervals to pregnancy. In general, cattle affected by diseases have reduced



appetite, increased body weight loss, altered partitioned of nutrients, and exacerbated immune response (Gifford *et al.*, 2012). Inflammatory diseases create an acute phase response that partitions more nutrients, particularly amino acids, for synthesis of hepatic acute phase proteins (Gifford *et al.*, 2012). This response to contain invading pathogens, although desired, alters the partition of nutrients away from productive functions. Moreover, inflammatory mediators produced during activation of the immune system can reach the reproductive tract and influence uterine function, follicle growth, oocyte quality, and subsequent embryo development (Turner *et al.*, 2012). To evaluate this idea,

we conducted retrospective analyses of data from multiple studies in which day 5 to 6 and day 15 to 16 embryos were collected from single ovulating dairy cows to determine if peripartum diseases were associated with reduced embryo quality and impaired development in lactating dairy cows (Ribeiro *et al.*, 2014c). Embryos-oocytes collected from 419 cows on day 5 to 6 after AI were evaluated for fertilization and early cleavage, and grade quality (Table 2; Ribeiro *et al.*, 2014c). It is clear that cows suffering from at least 1 case of clinical disease had reduced fertilization, compromised embryo quality, and reduced embryo development as early as 5 to 6 days after insemination.

Table 2. Impact of health problems during early lactation on embryo quality in dairy cows.

Item	Group ¹			p ²	
	Healthy	Single disease	Multiple diseases	Disease	Number of diseases
Embryos-Ova					
Number	252	87	80	---	---
Fertilized, %	86.1	81.6	73.8	0.03	0.22
Grades 1-3, %	73.4	62.1	51.3	<0.01	0.16
Grades 1-2, %	61.9	50.6	41.3	<0.01	0.23
Embryos					
Grades 1-3, %	85.3	76.1	69.5	<0.01	0.40
Grades 1-2, %	71.9	62.0	55.9	0.01	0.49
Cell number	38.8	35.6	33.3	0.04	0.49

¹Healthy = no diagnosis of clinical disease; Single disease = diagnosis of a single clinical disease in early lactation; Multiple diseases = diagnosis of more than one clinical disease event in early lactation. ²Orthogonal contrasts. Effect of disease: healthy *vs.* single disease + multiple diseases; Effect of number of diseases: single disease *vs.* multiple diseases. Data from Ribeiro *et al.* (2014c).

After ovulation and fertilization of the oocyte in the oviduct, embryonic cells derive from cleavages of the zygote and stay enclosed the zona pellucida, forming a morula by day 4 of development (Spencer *et al.*, 2007). These early events are dependent on oocyte inherited molecules, and glucose and amino acids uptake from the oviduct (Gardner, 1998; Duranthon *et al.*, 2008). It is also during this period that the zygote's genome is activated, more precisely at 8-16-cell stage transition in ruminants (Duranthon *et al.*, 2008). The morula becomes compacted and enters the uterus, where the totipotent blastomeres undergo the first cell differentiation. Therefore, it is plausible to suggest that diseases influence oocyte competence and/or oviductal/endometrial support for fertilization and early embryo development in dairy cows.

In a process dependent of cell adhesion, polarity, and expression of specific transcription factors, the blastomeres from morula differentiate in either inner

cell mass or trophectoderm cells, forming the blastocyst (Duranthon *et al.*, 2008). The spherical blastocyst will then expand and hatch from the zona pellucida by day 8 of development (Spencer *et al.*, 2007). After blastocyst shedding from the zona pellucida, trophectoderm cells of the spherical blastocyst proliferate and elongate along the uterine lumen prior of the initiation of implantation (Spencer *et al.*, 2007). In a first moment, the spherical embryo stays free-floating into the uterine lumen and cell proliferation leads to formation of an ovoid conceptus (embryo and associated extra-embryonic membranes) by day 13. Up to this point, endometrial physiology is coordinated mainly by progesterone and there is no distinction between the endometrium of a pregnant and a nonpregnant female (Bauersachs and Wolf, 2013). Around day 14, however, the 1-mm ovoid conceptus starts to elongate by intensive proliferation of trophoblast cells and become a 12-cm filamentous structure by day 17. This process of conceptus



elongation is dependent of histotroph secretion by the glandular epithelium of the endometrium (Gray *et al.*, 2000). Concurrent with conceptus elongation, the highly active trophoblast cells secrete bioactive products that affect endometrial physiology, establishing a complex crosstalk between the two tissues that coordinate critical events for pregnancy establishment, formation of a functional placenta, and pregnancy survival to term, including: 1) maternal recognition of pregnancy by secretion of interferon- τ ; 2) establishment of a servomechanism of conceptus nourishment; 3) differentiation of binucleated trophoblast cells; and 4) immunomodulation of the maternal immune system in the endometrium to avoid conceptus rejection (Spencer *et al.*, 2007; Bauersachs and Wolf, 2013). These aforementioned events highlight the importance, complexity, and potential reasons for developmental

failures during conceptus elongation. Not surprisingly, on average 33% of viable blastocysts fail to elongate and establish a healthy pregnancy in dairy cows (Ribeiro *et al.*, 2014c).

Results from conceptuses collected on day 15 to 16 after AI from 198 lactating dairy cows that had a synchronized ovulation (progesterone <1 ng/mL on the day of AI, and >1 ng/mL on days 7 and 15 after AI) indicated that cows with clinical diseases had similar pregnancy, but marked reduction in development (Table 3; Ribeiro *et al.*, 2014c). Conceptuses of cows with clinical disease were less developed and secreted less interferon- τ in the uterine lumen compared with those from healthy cows, which suggests impaired signaling for maternal recognition of pregnancy and establishment of crosstalk between conceptus and endometrium for pregnancy establishment.

Table 3. Impact of health problems during early lactation on embryo quality in dairy cows.

Item	Group ¹			p ²	
	Healthy	Single disease	Multiple diseases	Disease	Number of diseases
Number	114	64	20	---	---
Pregnant day 15, %	47.7	52.9	53.9	0.58	0.88
Interferon- τ , pg/mL	404.9	184.3	29.2	< 0.01	0.06
Size, mm	25.1	17.5	16.9	< 0.01	0.23
Progesterone day 7, ng/mL	3.3	3.5	3.3	0.84	0.71

¹Healthy = no diagnosis of clinical disease; Single disease = diagnosis of a single clinical disease in early lactation; Multiple diseases = diagnosis of more than one clinical disease event in early lactation. ²Orthogonal contrasts. Effect of disease: healthy vs. single disease + multiple diseases; Effect of number of diseases: single disease vs. multiple diseases. Data from Ribeiro *et al.* (2014c).

To investigate the carryover effects of diseases on the biology of trophectoderm cells during elongation, 22 conceptuses, 12 from day 15 and 10 from day 16 after AI, were subjected to transcriptome analysis using the Affymetrix GeneChip Bovine Genome Array (Ribeiro *et al.*, 2014b). Half of the conceptuses on each day were recovered from cows affected by diseases postpartum and the other half from cows that were not affected by diseases from parturition until the day of uterine flushing. Conceptuses subjected to this analysis were similar in size, and the analysis revealed few changes in transcript expression, although some of the transcripts differently expressed are likely important for conceptus elongation and maintenance of pregnancy that could explain the phenotypic differences observed in embryo development and pregnancy outcome.

On day 15 after AI, only 7 transcripts were differently expressed (Ribeiro *et al.*, 2014b). Among them, the fatty acid translocase cluster of differentiation (CD) 36 had the greatest difference in mRNA abundance. The trophectoderm cells of conceptuses recovered from cows

affected by diseases postpartum did not express CD36, but the same gene was relatively highly expressed in conceptuses from healthy cows. CD36 is a scavenger receptor related to cell adhesion and fatty acid uptake, two important cellular events during conceptus elongation (Ribeiro *et al.*, 2014b). In fact, lipid metabolism seems to be an essential cellular function in conceptus elongation (Ribeiro *et al.*, 2014b). Thus, the lack of expression of CD36 in conceptuses recovered from cows that suffered diseases might be related with their impaired elongation. On day 16 after AI, 35 transcripts were differently expressed (Ribeiro *et al.*, 2014b). Among those, there was upregulation of transcripts of major histocompatibility complex (MHC) I and II, and proteins associated with inflammatory process such as lactotransferrin, serum amyloid A3, and S100 calcium binding protein A12 in conceptus recovered from cows affected by diseases. Transcript expression of MHC has been reported to be reduced as the conceptus elongate (Ribeiro *et al.*, 2014b), which could be a mechanism to minimize the presentation of paternal alloantigens and the risk of tissue rejection by



the maternal immune system as reported in other species (Bainbridge, 2000). In fact, bovine clone embryos have been reported to have greater expression of MHC, which is believed to be one of the reasons for the greater incidence of pregnancy losses when transferred into recipients (Davies *et al.*, 2004). Thus, greater expression of MHC molecules might be related to the greater pregnancy loss observed in cows affected by clinical diseases. On the other hand, the greater expression of inflammatory proteins might be related to altered uterine environment caused by diseases, but consequent importance and physiological responses remain unclear.

The gestation period between early implantation and early pregnancy diagnosis is not well studied in cattle. The lack of detailed information is probably caused by the inability to examine conceptuses at this period without slaughtering cows. Measuring peripheral responses to pregnancy might be a non-invasive alternative for comparative studies with large number of animals (Ribeiro *et al.*, 2014a). Recent studies demonstrated that interferon- τ produced by trophoblast cells reaches the maternal circulation (Oliveira *et al.*, 2008) and induces expression of interferon-stimulated genes (ISGs) in leukocytes (Ott and Gifford, 2010), which parallels the total amount of interferon- τ in utero (Matsuyama *et al.*, 2012). In addition, pregnancy associated glycoproteins (PAG) secreted by binucleated cells in early placentation such as pregnancy-specific protein B (PSPB) are abundantly expressed and can be detected in peripheral blood (Sasser *et al.*, 1986). Both expression of ISGs in leukocytes and concentration of PSPB in plasma during the peri-implantation period have been associated positively with pregnancy establishment and maintenance in dairy cows and can be used as non-invasive methods to study the bovine pregnancy at peri-implantation stages (Ribeiro *et al.*, 2014a).

To investigate the effect of diseases on peripheral responses to pregnancy, blood samples from 481 lactating cows were collected on days 19, 26, 30 and 37 after first insemination postpartum. Samples from day 19 had leukocytes isolated for analysis of gene expression, and the remaining samples had plasma harvested for measurement of PSPB concentration. Incidence of diseases was recorded and cows were classified as having or not suffered from clinical diseases from parturition until pregnancy diagnosis on day 34 after AI (Ribeiro and Santos, 2014, Department of Animal Sciences, University of Florida, Gainesville, FL, USA, unpublished results). There were no evident differences in concentrations of PSPB in plasma of healthy cows and those affected by clinical diseases. However, transcriptome analysis of leukocytes from 36 lactating cows, half healthy and half diseased, revealed distinct responses to pregnancy. There were 14 and 10 transcripts differently expressed between pregnant and nonpregnant cows in the healthy and diseased groups, respectively, and only one interferon

stimulated gene (IFI6, Interferon alpha-inducible protein 6) was common. In pregnant cows, disease influenced the expression of 12 transcripts, whereas in nonpregnant cows, disease influenced the abundance of 3 transcripts in leukocytes. Pregnancy in healthy cows upregulated transcripts commonly reported to be affected by the conceptus such as RTP4, MX1, MX2, OAS1, whereas a different set of genes was upregulated by pregnancy in the disease group. These findings are likely related with distinct conceptus elongation and secretion of interferon- τ described previously and the relevance of these findings need further investigation.

Collectively, it is clear that a negative association between health problems and early embryo development exists. Fertilization and cleavage, morula development, conceptus elongation and embryo survival are negatively affected by diseases. These processes involve changes in the transcriptome of the conceptus and cells influenced by the conceptus, but likely in many other reproductive tissues.

Negative nutrient balance impacts health and reproduction in dairy cows

Increased nutrient needs associated with suppression of appetite generally drive dairy cows into a state of NEB, which is often observed during the last week of gestation and the first 1 to 2 months postpartum. Under normal conditions, dry matter intake (DMI) increases from 9.6 kg/day during the week preceding parturition to more than 22 kg/day at 11 weeks postpartum (Reynolds *et al.*, 2003). In contrast, caloric requirements are only partially met by feed consumption during the first weeks postpartum. Consequently, high-producing dairy cows experience NEB during the first 4 to 6 weeks postpartum, which often averages -5 Mcal of net energy/day, the equivalent of approximately 1 kg of body weight loss/day (i.e. 30 to 50 kg of body weight loss in the first 6 weeks of lactation), mostly from adipose tissue. Reduced circulating concentrations of glucose and insulin up-regulate the lipolytic signals that result in hydrolysis of stored triglycerides in adipose tissue and increase availability of NEFA to be used as an energy source. The liver removes a fraction of the NEFA from blood and uptake of NEFA depends on the type of fatty acid present in the circulation (Mashek and Grummer, 2003). Re-esterification to triglycerides in the hepatocytes and ketogenesis increase when uptake of NEFA by the hepatic tissue is excessive.

Energy balance during early lactation has been positively associated with reproductive performance of dairy cows (Butler, 2003). Severity and duration of NEB can be estimated by changes in body condition score (BCS). Cows losing more body condition during the first 65 days postpartum were more likely to be anovular at the end of the voluntary waiting period, had decreased pregnancy per AI, and increased risk of



pregnancy loss after the first AI postpartum (Santos *et al.*, 2009). Using circulating concentration of NEFA as an indicator of the energetic status of grazing dairy cows in the first 2 weeks postpartum, Ribeiro *et al.* (2013) reported that cows in NEB (NEFA ≥ 0.7 mM) were less likely to resume ovarian cyclicity before 50 days postpartum and to become pregnant to the first AI of the breeding season. Others have reported similar results in dairy herds managed in confinement (Walsh *et al.*, 2007; Santos *et al.*, 2010a; Ospina *et al.*, 2010b).

Rate of pregnancy during the first 70 days of breeding was 16% less for cows with blood NEFA ≥ 0.7 mM than for those with concentrations below this threshold (Ospina *et al.*, 2010b). Ketosis resulting from extensive fat mobilization also has been associated with compromised fertility. Both the relative circulating concentration of β -OH-butyrate (BHBA) and the duration of elevated BHBA concentrations were associated negatively with the probability of pregnancy following the first postpartum AI (Walsh *et al.*, 2007). In fact, for every 100 μ M increase in BHBA concentration during the first and second weeks after calving, the proportions of pregnant cows at first AI were reduced by 2 and 3%, respectively. Furthermore, rate of pregnancy by 70 days after the end of the voluntary waiting period was 13% less among cows with blood BHBA concentration $\geq \sim 1.0$ mM compared with herd mates with BHBA below 1.0 mM (Ospina *et al.*, 2010b). In fact, as the prevalence of cows with elevated concentrations of serum NEFA or BHBA increased, reproductive performance declined (Ospina *et al.*, 2010a). In the latter study, the 21-day cycle pregnancy rate declined by 0.9 percentage units in herds in which more than 15% of the sampled cows had serum NEFA concentration ≥ 0.7 mM, and by 0.8 percentage units if more than 15% of the sampled cows had serum BHBA concentrations ≥ 1.15 mM. Therefore, circulating concentrations of these metabolites can be used as indicators of excessive lipid mobilization, which impairs fertility.

Reduced fertility associated with low nutrient intake and NEB is, at least in part, mediated by the damaging effects on immunity and postpartum health. Exposing immune cells *in vitro* to NEFA at concentrations compatible with those observed in high-producing postpartum dairy cows (0.12 to 1 mM) reduced function and viability. Increasing the concentration of NEFA in the culture media impaired the synthesis of interferon- γ and IgM by peripheral blood mononuclear cells (Lacetera *et al.*, 2004). Furthermore, NEFA reduced phagocytosis-dependent oxidative burst in polymorphonuclear leucocytes (Scalia *et al.*, 2006). When concentrations of NEFA in the culture medium were further increased to 2 mM, polymorphonuclear oxidative burst was not altered, but more leukocytes underwent necrosis, thereby impairing function. Not only NEFA, but also BHBA has been implicated with immunosuppression in postpartum dairy cows. Incubation of bovine neutrophils with

increasing concentrations of BHBA reduced phagocytosis, extracellular trap formation, and killing (Grinberg *et al.*, 2008). *In vivo* observations support the immunosuppressive effects of NEB. Cows in severe NEB had increased concentrations of NEFA and BHBA in plasma, which was associated with decreased leukocyte numbers (Wathes *et al.*, 2009). It is likely that cows that are unable to recover feed consumption after parturition, and therefore, remain in more severe NEB, are more susceptible to diseases. It is known that reduced nutrient intake and NEB even before calving are associated with poor uterine recovery from parturition and the occurrence of uterine diseases (Hammon *et al.*, 2006). These observations seem to be linked with changes in patterns of endometrium gene expression mediated by the energetic status of the cows. Wathes *et al.* (2009) evaluated global gene expression of the endometrium of cows at 2 weeks postpartum. They reported that several transcripts linked with inflammation and active immune response were upregulated in cows undergoing severe NEB compared with those exhibiting a more modest caloric deficit, suggesting a delay in uterine involution. In addition, cows that developed uterine diseases early postpartum had greater concentrations of NEFA and BHBA in blood around calving than healthy cows (Hammon *et al.*, 2006; Galvão *et al.*, 2010). It is important to highlight that occurrence of diseases early postpartum can further accentuate the adverse effects of NEB, as sick cows have reduced appetite and often times lose more body weight than healthy cows.

In addition to the changes in energy balance, circulating concentrations of antioxidants such as β -carotene, and vitamins A (retinol) and E (α -tocopherol) also are regulated temporally and decrease during the periparturient period (Goff *et al.*, 2002). As these compounds play important roles in immune function, low concentrations of these vitamins have been associated with increased susceptibility to disease and, potentially, with reduced fertility in dairy cows. Prepartum circulating β -carotene and, more importantly, vitamin E were reduced in cows that retained their placenta than for healthy cows (LeBlanc *et al.*, 2004). In fact, for every 1 μ g/mL increase in circulating vitamin E during the week preceding parturition, the risk of retained placenta decreased by 21%. Furthermore, the decrease in circulating concentrations of β -carotene, vitamin A, and vitamin E associated with parturition was more accentuated among cows that developed mastitis during the first 30 days postpartum than among healthy cows (LeBlanc *et al.*, 2004). During the last week prepartum, a 100 ng/mL increase in circulating vitamin A concentration was associated with a 60% decrease in the risk of clinical mastitis (LeBlanc *et al.*, 2004).

Impact of energy balance on oocyte competence

During lactation, most of the glucose produced by the liver is used for synthesis of lactose to support



milk production. A transient insulin resistance early postpartum diminishes utilization of glucose by peripheral tissues to secure its availability for the mammary gland. Although the follicle is capable of controlling fluctuations in glucose availability, which generally results in concentrations in the follicular fluid greater than those observed in blood, intrafollicular glucose concentrations also decrease around parturition (Leroy *et al.*, 2004). It has been shown that glucose is critical for adequate oocyte maturation, affecting cumulus expansion, nuclear maturation, cleavage, and subsequent blastocyst development. In fact, glucose concentrations compatible with those observed in cows suffering from clinical ketosis (1.4 mM) reduced rates of cell cleavage and the proportion of embryos developing to blastocysts (Leroy *et al.*, 2006). Although the oocyte does not directly use glucose as an energy source, it must be readily available to cumulus cells for glycolysis to provide pyruvate and lactate, oocyte's preferred substrates for ATP production (Cetica *et al.*, 2002). Therefore, it is possible that hypoglycemia during early lactation might compromise oocyte competence in dairy cows.

Follicular fluid is derived from blood originating from capillaries in the theca cells by osmotic pressure (Rodgers and Irving-Rodgers, 2010). Production of hyaluronan and proteoglycan by granulosa cells creates an osmotic gradient that draws fluid from the thecal vasculature through the thecal interstitium, the follicular basal lamina and the mural granulosa cells (Rodgers and Irving-Rodgers, 2010). As fluid accumulates in the antrum, it bathes the cumulus cells and the oocyte. Changes in nutrient supply that lead to either hypo- or hyperglycemia may influence lipid metabolism and alter composition of follicular fluid. For instance, hyperglycemic insults influence the composition of the follicular fluid, which may lead to long-term negative effects on oocytes by altering nuclear maturation (Jungheim *et al.*, 2010; Sutton-McDowall *et al.*, 2010).

Extensive fat mobilization and the release of large amounts of NEFA into the bloodstream have been shown to be associated with fertility of postpartum dairy cows. Concentrations of NEFA in the follicular fluid parallel those of serum, and they increase around parturition (Leroy *et al.*, 2005). Maturation of oocytes *in vitro* in the presence of saturated fatty acids reduced oocyte competence and compromised the initial development of embryos. Specifically, addition of palmitic and stearic acids to the maturation media induced apoptosis and necrosis of cumulus cells, impaired fertilization, cleavage, and development to the blastocyst stage (Leroy *et al.*, 2005). Changes in circulating concentrations of BHBA are promptly reflected in follicular fluid (Leroy *et al.*, 2004). *In vitro* models developed to study the effects of subclinical ketosis on fertility of dairy cows, however, have failed to demonstrate a direct effect of BHBA on oocyte

competence, which seems only to aggravate responses to low concentrations of glucose during oocyte maturation (Leroy *et al.*, 2006). Therefore, it is proposed that the oocyte is vulnerable to potential harmful effects of an altered biochemical milieu in the follicular micro-environment (Leroy *et al.*, 2012).

Energy balance and ovarian function postpartum

The stage set by NEB modulates the activity of the hypothalamic-pituitary-ovarian axis. Undernutrition has been linked to the inability of the hypothalamus to sustain high frequency of luteinizing hormone (LH) pulses by the pituitary gland (Schillo, 1992). Indeed, LH pulse frequency was shown to be positively correlated with energy balance and negatively correlated with blood NEFA concentration (Kadokawa *et al.*, 2006). The underlying mechanism by which NEB reduces LH release is likely to involve the supply of oxidizable fuels to neurons and hormonal modulation of hypothalamic and pituitary cells (Schneider, 2004). Glucose is a preferred substrate for neuron energy metabolism and inadequate supply of glucose inhibits the GnRH pulse generator (Schneider, 2004). Under a favorable nutritional status, the hormonal milieu to which the hypothalamus and pituitary gland are exposed favors the release of GnRH and gonadotropins. For instance, leptin, a hormone known to have increased concentrations during positive energy balance, stimulates release of GnRH by the hypothalamus, and blood leptin was found to be strongly correlated with both LH pulse frequency and amplitude (Kadokawa *et al.*, 2006). In addition to low LH support, cows in NEB have limited hepatic expression of growth hormone (GH) receptor 1A triggered by low circulating concentrations of insulin (Butler *et al.*, 2003, 2004). This phenomenon uncouples the GH insulin-like growth factor (IGF) 1 axis, which reduces the synthesis of IGF-1 by the liver. Reduced concentrations of IGF-1 in blood have been associated with diminished follicle sensitivity to LH, follicle growth, and steroidogenesis (Lucy *et al.*, 1992; Butler *et al.*, 2004). Conversely, increase in circulating concentrations of insulin as energy balance improves seems to be one of the signals to reestablish GH receptor expression in the liver and restore IGF-1 synthesis in dairy cows (Butler *et al.*, 2003). Restricting follicular growth and synthesis of estradiol delay resumption of postpartum ovulation and might compromise oocyte quality, which likely hampers expression of estrus and pregnancy in dairy cows.

In addition to extensive nutrient shortage, high producing dairy cows also undergo extensive ovarian steroid catabolism. This is thought to be mediated by the high DMI and subsequent increased splanchnic blood flow (Sangsritavong *et al.*, 2002). Hepatic blood flow doubles during the first 3 months postpartum averaging 1,147 L/h in the week preceding parturition and 2,437 L/h in the third month postpartum (Reynolds



et al., 2003). Increased clearance of ovarian steroids can have important implications to the reproductive biology of dairy cows and indirectly influence follicle development (Wiltbank *et al.*, 2006), therefore, affecting oocyte quality and subsequent embryo development. Progesterone-induced uterine histotroph secretion is critical for the nourishment and elongation of the bovine conceptus (Robinson *et al.*, 2006). Therefore, an increase in the rate of progesterone clearance is expected to result in a slower rise in progesterone concentrations after insemination, reducing embryo development (Robinson *et al.*, 2006), which has implications for pregnancy maintenance. Similarly, reduced circulating concentrations of estradiol because of hepatic catabolism in cows with high DMI can result in a shorter and less intense estrus period (Lopez *et al.*, 2004). In addition, estradiol catabolism requires follicles to grow for longer periods of time to be able to trigger estrus and ovulation (Sartori *et al.*, 2004; Wiltbank *et al.*, 2006). Longer periods of follicular dominance reduce embryo quality (Cerri *et al.*, 2009a) and pregnancy per AI in cows inseminated on estrus (Bleach *et al.*, 2004) or following timed AI (Santos *et al.*, 2010b).

Calcium homeostasis and uterine health early postpartum

Control of blood concentrations of total Ca and Ca^{2+} is critical to maintain normal muscle contractility, transmission of nerve impulses, and immune function. Nonetheless, homeostatic controls during early lactation might not prevent decreases in Ca concentrations during the first week postpartum. Amount of Ca secreted in colostrum on the day of calving is almost 8 to 10 times the entire serum Ca pool in a dairy cow (Goff, 2004). Therefore, it is no surprise that most cows undergo a period of subclinical hypocalcemia and a proportion of them develop milk fever. In fact, surveys in the US indicate that 25, 41, 49, 51, 54, and 42% of cows in their first through sixth lactation are hypocalcemic (Ca <8 mg/dL or 2 mM) during the first 48 h after calving (Reinhardt *et al.*, 2011). In order to maintain postpartum serum total Ca and Ca^{2+} concentrations, dairy cows must increase bone remodeling for Ca resorption or increase intestinal Ca absorption.

Impact of milk fever on the health of dairy cows is very conspicuous, as it can result in downer cows and death if left untreated. Nevertheless, milder depressions of serum Ca concentrations are often not diagnosed and can have a pronounced negative effect on postpartum health and fertility. Recently, Martinez *et al.* (2012) observed that cows with serum Ca <8.59 mg/dL during at least 1 of the first 3 days postpartum had reduced neutrophil phagocytic and killing activities *in vitro*, increased odds of developing fever (adjusted odds ratio [OR] = 3.5; 95% confidence interval [CI] = 1.1 to

11.6) and metritis (adjusted OR = 4.5; 95% CI = 1.3 to 14.9). These associations were observed for cows considered to be of large and small risk of developing metritis based on calving problems (Martinez *et al.*, 2012). Ionized Ca is an important second messenger in cellular signal transduction. Fluctuations in intracellular Ca^{2+} concentrations are critical to activate immune cells (Lewis, 2001). Cows with retained placenta have reduced neutrophil function (Kimura *et al.*, 2002). Intracellular stores and flux of Ca^{2+} in response to cell activation are reduced in lymphocytes of dairy cows with milk fever (Kimura *et al.*, 2006).

To reiterate the findings by Kimura *et al.* (2006), recent work by our group (Martinez *et al.*, 2014b) demonstrated that induction of subclinical hypocalcemia compromises innate immunity (Fig. 1). Holstein dry cows were subjected to a normocalcemic (Ca^{2+} >1.1 mM) or a subclinical hypocalcemic (Ca^{2+} <1.0 mM) treatment for 24 h. The induction of subclinical hypocalcemia was accomplished by continuous infusion of a solution containing 5% ethylene glycol tetraacetic acid (EGTA), a specific chelating agent for Ca^{2+} . Normocalcemic cows received saline i.v. and an oral bolus of 43 g of Ca at 0 and 12 h after initiating the infusion. Heart and respiratory rates, rectal temperature, and rumen contractions were measured during and after infusion at 6- to 12-h intervals. Ionized Ca, K, Mg, and blood pH were evaluated at 0 h, hourly during the infusion period, and at 24, 48 and 72 h after the infusion to monitor Ca^{2+} . In addition, DMI, neutrophil function, and white blood cell differential count were evaluated at 0, 24, 48 and 72 h after treatments. As expected, infusion of a 5% EGTA solution successfully induced subclinical hypocalcemia in cows (0.78 ± 0.01 vs. 1.27 ± 0.01 mM Ca^{2+}) during 23 h. No differences were detected in heart and respiratory rates, rectal temperature, and white blood cell counts between subclinical hypocalcemia and normocalcemic cows. On the day of infusion, cows induced to have subclinical hypocalcemia had lesser K (2.92 ± 0.07 vs. 3.47 ± 0.07 mM) and greater Mg (0.94 ± 0.03 vs. 0.68 ± 0.03 mM) in blood. The decrease in blood Mg was likely caused by supplemental oral Ca in normocalcemic cows. Subclinical hypocalcemic cows had reduced ($P < 0.01$) DMI on the day of infusion (5.1 vs. 10.0 kg/d) and decreased ($P = 0.01$) rumen contractions every 2 min (1.7 vs. 2.7) during the second half of the infusion period. Cows induced to have subclinical hypocalcemia had a reduced percentage of neutrophils with phagocytosis (79.9 ± 8.8 vs. 119.2 ± 13.0 , % baseline) and oxidative burst (80.2 ± 17.9 vs. 140.3 ± 17.9 , % baseline), evident at 24 h after the end of the infusion (Fig. 1; Martinez *et al.*, 2014b). It was concluded that subclinical hypocalcemia compromises DMI, rumen function, and innate immunity, all of which likely related to the immunosuppression observed in cows at calving and increased risk of uterine diseases in cows with marginal blood Ca (Martinez *et al.*, 2012).

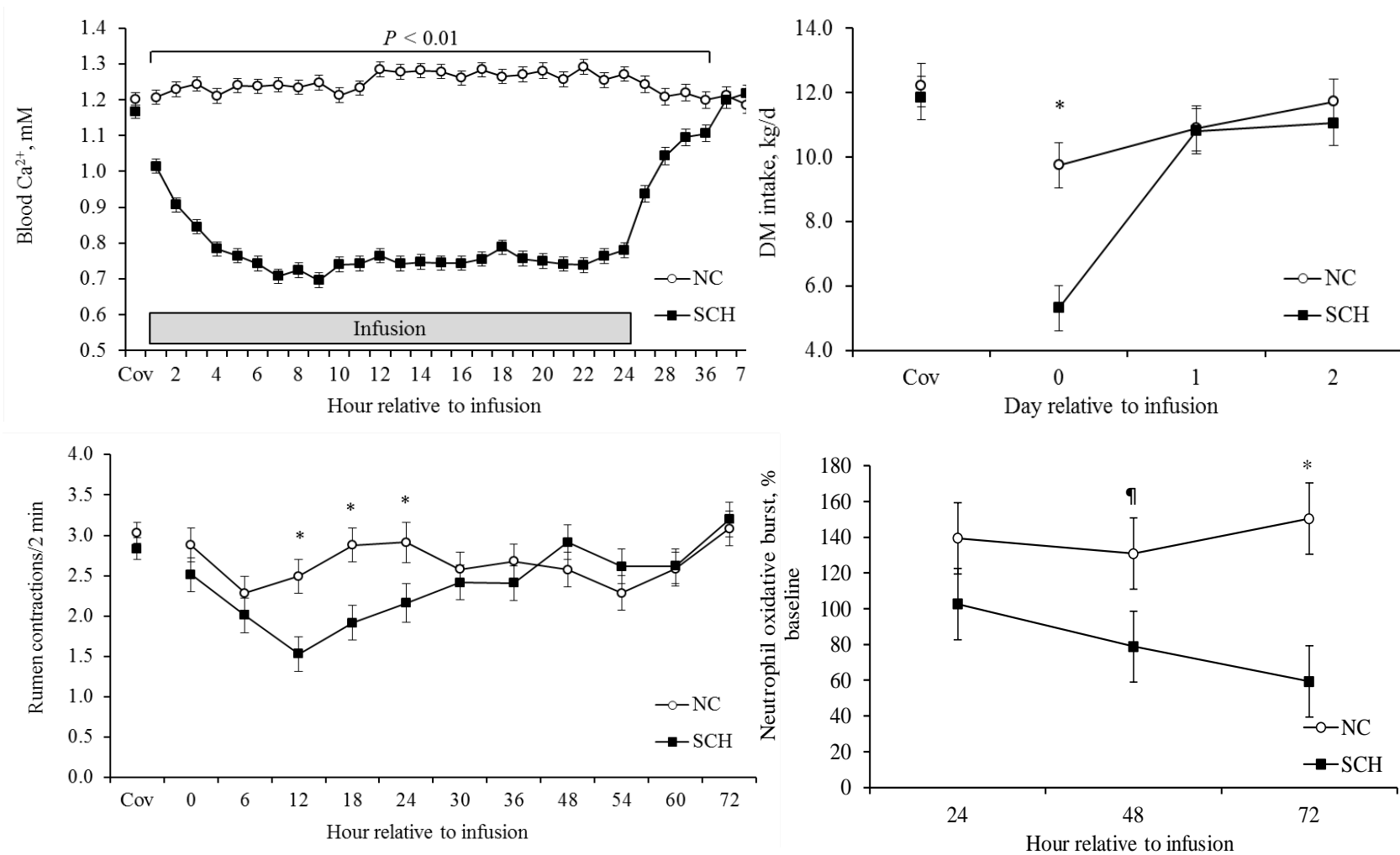


Figure 1. Blood Ca^{2+} concentrations (upper left), dry matter intake (upper right), rumen contractions (bottom left), and neutrophil killing of *Escherichia coli* (bottom right) of cows subjected to normocalcemia (NC; n = 10) or induced subclinical hypocalcemia (SCH; n = 10). Cov = mean of measurements taken during 48 h preceding treatments and used for covariate adjustment of data during statistical analyses. * = within day or hour treatments differ ($P < 0.05$); = within day or hour treatments tend ($P < 0.10$) to differ. Data from Martinez *et al.* (2014b).



Collectively, these data indicate that Ca status is linked with immune cell function and plays a role in the risk of uterine diseases of dairy cows. Cows suffering from uterine diseases have delayed postpartum ovulation, reduced pregnancy per AI, and increased pregnancy loss (Santos *et al.*, 2010a). In fact, reduced serum Ca concentrations immediately before or after calving reduced pregnancy at first AI in lactating dairy cows (Chapinal *et al.*, 2012), and impaired pregnancy rate (Martinez *et al.*, 2012).

Management of transition cows to improve periparturient health and fertility

The multifactorial nature of reproduction requires a “holistic” and integrated approach to management from housing to feeding and breeding, such that risk of periparturient diseases are reduced and pregnancy is improved.

Cow movement and dry period duration

Regrouping of cows induces social behaviors that oftentimes disturb feeding and resting patterns, thereby resulting in a temporary increase in aggression concurrently with a reduction in DMI (von Keyserlingk *et al.*, 2008). Therefore, regrouping cows upon imminent calving is not advised as it would further suppress intake and increase the risk of ketosis and fatty liver. The question of when cows can and cannot be moved, however, still remains. Recent work from Wisconsin refuted the concept that weekly addition of cows to the close-up group is detrimental to postpartum metabolism and production (Coonen *et al.*, 2011). A recent study by the Minnesota group (Silva *et al.*, 2013) reinforced the findings of Coonen *et al.* (2011) and indicated that weekly regrouping of cows had no impact on subsequent lactation so long as stall availability, bunk space, and 3 to 4 weeks in the close-up group were offered to cows. It seems that when appropriate feedbunk space and number of stalls are available, transition cows can adapt to the weekly regrouping.

A strategy to improve postpartum intermediary metabolism is to manipulate the duration of the dry period. Reducing the dry period from 55 to 34 days increased BCS between 2 and 8 weeks postpartum and reduced the concentrations of plasma NEFA at week 3 postpartum (Watters *et al.*, 2008), suggesting improved postpartum energy status. When energy balance was measured, cows subjected to a 28-day dry period had a less severe NEB postpartum, which resulted in reduced BCS and body weight losses compared with cows having the traditional 56-day dry period (Rastani *et al.*, 2005). Some of the benefit to a less NEB is the result of less milk production, particularly in cows starting their second lactation (Watters *et al.*, 2008; Santschi *et al.*, 2011a). Improved energy balance with a short dry period likely explains the earlier first postpartum

ovulation and reduction in anovular cows (Gümen *et al.*, 2005; Watters *et al.*, 2009). Despite changes in energy status and an earlier resumption of estrous cycles, cows with dry periods of 28 to 35 days had similar reproductive performance to those with a standard 8-week dry period (Gümen *et al.*, 2005; Watters *et al.*, 2009; Santschi *et al.*, 2011b). Nevertheless, in observational studies, extending exposure of cows to the prepartum diet was associated with reduced number of days open and increased proportion of pregnant cows at weeks 6 and 21 after the initiation of the breeding season (DeGaris *et al.*, 2010).

Prepartum diet formulation

Altering prepartum caloric intake influences postpartum metabolism in dairy cows. Ad libitum nutrient intake during the entire dry period tended to increase prepartum body weight and BCS and predispose cows to increased lipid mobilization during early lactation (Douglas *et al.*, 2006). Several studies have evaluated the impact of manipulating the energy density of the prepartum diet on postpartum performance. In some cases, nutrient intake was restricted not by altering the diet formulation but by limiting the amount of feed offered. Bisinotto *et al.* (2011) summarized data from several studies in which the caloric intake prepartum was manipulated. In general, restricting nutrient intake resulted in an average reduction of 2 kg/day of fat-corrected milk, with minor effects on plasma concentrations of BHBA. In some studies, high caloric intake resulted in greater triacylglycerol accumulation in the liver (Douglas *et al.*, 2006; Janovick *et al.*, 2011) because of greater fat mobilization measured as plasma NEFA. The increased postpartum lipid mobilization is likely the result of increased milk yield without a concurrent increase in DMI. Therefore, restricting prepartum caloric intake can be used to minimize lipid mobilization and triacylglycerol accumulation in the liver, but at the expense of milk production.

Altering protein content of the prepartum diet has little impact on performance of postpartum multiparous cows; however, increasing prepartum dietary protein from 12.7 to 14.7% of the diet DM with a high ruminally undegradable protein source enhanced milk production in primiparous cows (Santos *et al.*, 2001). Nonetheless, dietary protein fed prepartum had negligible impacts on measures of reproduction. Time to resumption of postpartum ovulation, days open, and pregnancy per AI were not affected by prepartum dietary protein concentration. Similarly, incidence of diseases postpartum was not affected by prepartum dietary protein. Therefore, diets for cows during the last weeks of gestation should contain between 12% (multiparous cows) and 15% (primigravid cows) crude protein to result in an estimated 1 kg/day of metabolizable protein intake (National Research Council, 2001).



Increasing postpartum blood insulin

A number of studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on reproductive traits in dairy cattle. Feeding more dietary starch or enhancing ruminal fermentability of starch in the diet usually results in increased plasma insulin concentrations. Insulin mediates recoupling of the GH and IGF-1 axis (Butler *et al.*, 2003), which is important for follicle development and ovulation. Gong *et al.* (2002) fed cows of low- and high-genetic merit isocaloric diets that differed in the ability to induce high or low insulin concentrations in plasma. Feeding the high-starch diet reduced the interval to first postpartum ovulation and resulted in a greater proportion of estrous cyclic cows by 50 days postpartum. Nevertheless, this response has not been consistent (Garnsworthy *et al.*, 2009). It is important to remember that although diets high in starch favor increases in plasma insulin, excessive amounts of readily fermentable starch has the potential to suppress DMI and offset any potential benefits of dietary manipulation on ovarian function.

Altering hepatic lipid metabolism

During periods of extensive fat mobilization, fat accumulates in the hepatic tissue. In early lactating cows with relatively low plasma NEFA concentrations (0.36 mM), the liver extracted 724 g of NEFA from blood during a 24-h period (Reynolds *et al.*, 2003). Thus, in cows with concentrations of NEFA >1 mM, as those with extensive lipid mobilization immediately after calving, the liver might remove as much as 2 kg of NEFA per day, the equivalent of 20% of its weight. Most of the NEFA reaching the liver are oxidized for energy production or converted into BHBA, with a smaller contribution for synthesis of very low-density lipoprotein (VLDL). The bovine liver has limited capacity to synthesize and secrete VLDL, thereby compromising export of triacylglycerol during periods of extensive hepatic NEFA uptake. The resulting hepatic lipidosis has been associated with retained placenta, ketosis, displaced abomasum, and impaired immune function and reproduction (Jorritsma *et al.*, 2000; Bobe *et al.*, 2004). Thus, reducing the risk of lipid-related disorders might improve reproduction of dairy cows. Supplementing periparturient dairy cows with rumen-protected choline has been used as a strategy to improve lipid metabolism and alleviate hepatic lipidosis. When feed intake was restricted to 30% of the maintenance to simulate a period of NEB and induce hepatic lipidosis, the supplementation of rumen-protected choline reduced triacylglycerol accumulation in the liver (Cooke *et al.*, 2007). Furthermore, inclusion of supplemental choline in the diet from approximately 25 days before to 80 days after calving reduced loss of postpartum body condition and

concentrations of BHBA in plasma, which resulted in lower incidence of clinical and subclinical ketosis despite the increase in fat-corrected milk (Lima *et al.*, 2012). Although feeding rumen-protected choline reduced morbidity, and improved metabolic health, no benefits were observed for reproduction. Supplemental rumen-protected choline did not affect the resumption of postpartum estrous cyclicity, pregnancy per AI at the first and second inseminations, or maintenance of pregnancy in the first 60 days of gestation.

Supplementing ionophores to periparturient dairy cows

Ionophores are lipophilic molecules involved with ionic transport across cell membranes. Monensin is a carboxylic polyether ionophore that has been used in animal nutrition because it selectively inhibits gram-positive bacteria. The shift in the ruminal microbiota caused by monensin favors propionate production and N conservation by reducing ruminal proteolysis. Feeding monensin typically increases blood glucose and insulin and reduces the concentrations of NEFA and BHBA in blood (Duffield *et al.*, 2008a). In association with improved metabolic health, monensin was effective in reducing the incidence of ketosis, displaced abomasum, and mastitis (Duffield *et al.*, 2008b). When monensin was supplemented as a controlled-release capsule, it reduced the incidence of metritis (Duffield *et al.*, 2008b). Surprisingly, feeding monensin to dairy cows during the transition period has not been shown to hasten resumption of postpartum ovulation, reduce days to pregnancy, or increase the rate of pregnancy in spite of consistent improvements in metabolic health (Abe *et al.*, 1994; Duffield *et al.*, 2008b).

Improving postpartum calcium homeostasis

Improving serum concentrations of Ca during early lactation is achieved by enhancing bone mineral resorption, intestinal absorption of dietary Ca, and by increasing the ionized Ca fraction in blood. A common method to improve Ca homeostasis is to manipulate the dietary cation-anion difference (DCAD) prepartum (Goff *et al.*, 1991; Goff, 2004; Seifi *et al.*, 2010). Reducing the DCAD by feeding salts containing strong anions decreases blood pH and enhances the affinity of the parathyroid hormone (PTH) for the PTH receptor present on cells in the bones, intestine, and kidneys (Goff, 2004). Although altering the DCAD of the diet by feeding strong anions can reduce feed intake during supplementation, the improved postpartum Ca metabolism often results in greater postpartum feed intake (DeGroot *et al.*, 2010). Feeding acidogenic diets prepartum did not reduce the incidences of retained placenta, lameness, or subclinical ketosis (Seifi *et al.*, 2010). In contrast, supplementing cows with calcium chloride in a gel formulation 12 h before the expected calving and at 0, 12, and 24 h after calving reduced the



incidence of clinical and subclinical hypocalcemia, and displacement of abomasum (Oetzel, 1996). Despite the benefits of feeding acidogenic diets on Ca homeostasis and the link between serum Ca and uterine diseases and reproduction in dairy cows (Martinez *et al.*, 2012), intervals to first insemination and pregnancy were not affected by feeding a low DCAD diet prepartum (Seifi *et al.*, 2010). Additional research is needed with properly powered experiments to critically evaluate the impact of reducing subclinical hypocalcemia by manipulating the DCAD of prepartum diets or supplementing postpartum Ca on reproductive traits of dairy cows.

Our group has attempted to increase serum total Ca and Ca^{2+} by supplementing Ca orally as boluses containing 50% of the Ca as calcium chloride (CaCl_2) and 50% as calcium sulfate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). The amount of supplemental Ca needed to have appreciable changes in blood total and Ca^{2+} were at least 86 g/day when such boluses were used and the increase in blood Ca lasted for no longer than 8 h (Martinez *et al.*, 2014a). Therefore, if postpartum Ca supplementation is used, it is likely that cows must receive at least 80 g/day for 3 to 4 days to minimize risk of subclinical hypocalcemia with the goal of preventing the development of uterine diseases. Because reproduction is impaired in cows with subclinical hypocalcemia (Martinez *et al.*, 2012), it is plausible to suggest that manipulation of the prepartum diets to avoid low serum Ca and postpartum supplementation of Ca may likely improve uterine health and subsequent fertility.

Feeding antioxidants to influence health and reproduction

During the immediate postpartum period, the cow's immune system is challenged severely, and the innate and humoral defenses are suppressed (Martinez *et al.*, 2012). Incidence of diseases and disorders can be elevated during this phase of the lactation cycle and they have several negative impacts on reproductive performance. Reduction in adaptive and innate immunity at parturition increases the risk of health disorders such as retained placenta, metritis, and mastitis.

Selenium has long been associated with immunity. Cattle supplemented with Se-yeast had an 18% increase of Se in plasma in comparison with cows fed sodium selenite in some studies (Weiss, 2003). Depending soil type and content, plants can be deficient in Se, which reflects in the supply of this mineral to cattle. Under the conditions of a Se inadequacy during the heat stress season in Florida, supplementing dairy cows with an organic source of Se in the form of selenized yeast elevated plasma Se concentrations compared with sodium selenite (Silvestre *et al.*, 2007). Conversely, in 2 subsequent experiments, when the same supplementation scheme was applied to cows in a

Se adequate area, Se concentrations in plasma did not differ (Rutigliano, 2006; Rutigliano *et al.*, 2008; Cerri *et al.*, 2009b). Measures of innate and humoral immune responses, embryo quality, and fertility of dairy cows were unaltered by source of Se in the Se-adequate area (Rutigliano *et al.*, 2008; Cerri *et al.*, 2009b). Nevertheless, selenized yeast improved neutrophil function, serum titers against ovalbumin, and uterine health in cows in the Se-deficient area (Silvestre *et al.*, 2007). These findings indicate that responses to supplemental antioxidants such as Se in a more bioavailable form depend on the Se status of the animal.

Conclusions

It is accepted that reproduction is important for the profitability of dairy farms, and health of dairy cows during the peripartum period is one of the many determinants of reproductive success. Cows that experience periparturient problems have delayed return to ovulation, reduced pregnancy per AI, and increased pregnancy loss. The negative effects on fertility occur at multiple stages of gestation, with reduction in fertilization, hindered morula and day 15 conceptus development, and altered pattern of gene expression in conceptus and peripheral tissues influenced by the conceptus, which ultimately compromise establishment and maintenance of pregnancy in dairy cows. Because our understanding of the underlying biology of subfertility in cows with diseases is poor, methods to mitigate depression in pregnancy have to be holistic and attain to minimizing the risk factors that predispose cows to diseases.

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