

Impacts of Metabolism and Nutrition During the Transition Period on Fertility of Dairy Cows

J.E.P. Santos, R.S. Bisinotto, E.S. Ribeiro, F.S. Lima, and W.W. Thatcher
University of Florida, Gainesville
Email: jepsantos@ufl.edu

ABSTRACT

In early postpartum, high-producing dairy cows undergo a period of extensive tissue catabolism because of negative nutrient balance. Metabolic unbalances can lead to diseases, which are known to depress fertility in dairy cows. Negative nutrient balance has been associated with compromised immune and reproductive functions in dairy cows, and a compromised immune system leads to greater risk of diseases. Low circulating concentrations of glucose and insulin associated with elevated concentrations of non-esterified fatty acids (FA) and ketone bodies postpartum have detrimental effects on the oocyte, granulosa, and immune cells, disrupting their metabolism and reducing viability. Therefore, minimizing the extent and duration of negative nutrient balance in early lactation is expected to improve fertility. Reductions in circulating concentrations of Ca around parturition are also linked with impaired immune competence and have recently been linked with uterine diseases in dairy cows. Manipulation of the diet to influence metabolic health might benefit fertility. Dietary additives that influence rumen or intermediary metabolism to favor postpartum health, and supplementation with specific fatty acids during early lactation and the breeding period are potential alternatives to offset dietary insufficiencies and reestablish metabolic health in early lactation.

INTRODUCTION

Dairy cows must become pregnant and deliver a calf in order to undergo proper mammary development and lactogenesis. Reproduction is also critical to production because it determines when primiparous cows become multiparous leading to increments in milk yield, alters the average milk yield per day of lactation, affects the number of replacement animals available and the risk of culling, and influences the rate of genetic progress. It is well described that poor health during the transition period suppresses fertility in dairy cows. Perhaps the most debated aspect is the association between negative nutrient balance in early lactation and subsequent resumption of ovulatory cycles, and establishment and maintenance of pregnancy in high-producing dairy cows. During periods of negative energy balance (NEB), extensive lipolysis and products from fat metabolism seem to influence oocyte competence and subsequent embryo development. In addition, impaired metabolic health often leads to immunosuppression and the occurrence of diseases that further reduce fertility. Managing cows in the transition period in a proactive manner to minimize nutrient imbalances is mandatory for proper health, but it often benefits reproduction as well.

CLINICAL AND SUBCLINICAL DISEASES REDUCE FERTILITY OF DAIRY COWS

The transition from the nonlactating pregnant to nonpregnant lactating state requires the high-producing dairy cow to drastically adjust its metabolism so that nutrients can be partitioned to support milk synthesis, a process referred to as homeorrhexis. A sharp increase in nutrient requirements generally occurs when feed intake still is depressed in early lactation, 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 60 % of dairy cows across different levels of milk production, breeds, and management systems develop metabolic and infectious diseases in the first months of lactation (Ribeiro et al., 2011; Santos et al., 2011).

Calving related disorders and diseases that affect the reproductive tract are major contributors for depression of fertility. Dystocia, metritis, and clinical endometritis were observed in 14.6, 16.1, and 20.8 % of postpartum dairy cows in large US confinement herds, respectively (Santos et al., 2011). 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 (**VWP**), and 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 d of gestation than healthy cows.

The negative effects of reproductive disorders on subsequent fertility are also observed in dairy cows kept under grazing systems (Ribeiro et al., 2011). 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 d postpartum compared with unaffected herd mates. Cows affected with uterine diseases had marked depression in pregnancy per AI 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, lameness), subclinical, (subclinical hypocalcemia, subclinical ketosis, and severe NEB based on excessive plasma nonesterified fatty acids (**NEFA**)), or both, affected cows had increased anovulation, reduced pregnancy per AI, and increased pregnancy loss (Table 1; Ribeiro et al., 2011). These data strongly suggest that diseases in 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.

NUTRIENT BALANCE AND REPRODUCTION IN DAIRY COWS

Energy balance in early lactation has been positively associated with reproductive performance of dairy cows (Butler, 2003). The severity and length of NEB can be estimated through changes in body condition score (**BCS**). Cows that lost more body condition during the first 65 d postpartum were more likely to be anovular at the end of the VWP, had decreased pregnancy per AI, and increased risk of pregnancy loss after

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

Health problem	Estrous cyclic [*]	AOR (CI) ²	P
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
Health problem	Pregnant d 30 ^{*,¶}	AOR (CI)	P
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
Health problem	Pregnant d 65 ^{*,¶}	AOR (CI)	P
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

¹ Data from Ribeiro et al. (2011). Numbers for estrous cyclic, and pregnancy on d 30 and 65 after AI represent the proportion of cows affected from a population of 957 lactating cows on two dairy farms.

² AOR = adjusted odds ratio; CI = confidence interval.

^{a,b,c} Superscripts within a day of pregnancy differ ($P < 0.07$).

Contrasts: * Effect of uterine disease (Healthy vs. all others) $P < 0.05$;

¶ Additive effect of metritis and clinical endometritis (clinical endometritis only + metritis only vs. metritis and clinical endometritis) $P < 0.05$.

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 wk postpartum, Ribeiro et al. (2011) showed that cows under NEB (NEFA ≥ 0.7 mM) were less likely to resume ovarian cyclicity before 50 d postpartum and to become pregnant to the first AI of the breeding season. Others have reported similar results in dairy herds managed under confinement. The rate of pregnancy in the first 70 d of breeding was 16 % less for cows with blood NEFA ≥ 0.7 mM than for those with concentrations below this threshold in early lactation (Ospina et al., 2010a). Ketosis

resulting from extensive fat mobilization has also been associated with compromised fertility. Both the relative circulating concentration of beta-hydroxybutyrate (BHBA) and the duration of elevated BHBA concentrations were negatively associated 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 on wk 1 and 3 after calving, the proportion of pregnant cows was reduced by 2 and 3 %, respectively. Furthermore, the rate of pregnancy within 70 d after the end of the VWP was 13 % smaller among cows with blood BHBA ≥ 100 μ M compared with herdmates with

concentrations below 100 μM (Ospina et al., 2010a). Therefore, circulating concentrations of these metabolites can be used as indicators of excessive lipid mobilization that interfere with fertility. Furthermore, as the prevalence of cows with elevated concentrations of blood NEFA or BHBA increases, reproductive performance declines (Ospina et al., 2010b). In the latter study, the 21-d cycle pregnancy rate was reduced by 0.9 percentage units in herds in which more than 15 % of the sampled cows had NEFA ≥ 0.7 mM, and by 0.8 percentage units if more than 15 % of the sampled cows had BHBA ≥ 115 μM .

The reduction in 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 comparable with those observed in high-producing postpartum dairy cows (0.12 to 1 mM) has been shown to reduce function and viability. Increasing the concentration of NEFA in the culture media abridged 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 under severe NEB had increased NEFA and BHBA, 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 wk postpartum. They observed that several probes linked with inflammation and active immune response were still 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 in early postpartum had greater concentrations of NEFA and BHBA around calving than healthy cows (Hammon et al., 2006; Galvão et al., 2010). It is important to highlight that the occurrence of diseases early postpartum can further accentuate the adverse effects of NEB, as sick cows have reduced appetite and oftentimes lose more body weight than healthy cows.

ENERGY BALANCE, OVARIAN FUNCTION, AND OOCYTE COMPETENCE

Energy balance, to an extent, determines when normal reproduction resumes after parturition in most mammals (Schneider, 2004). In cattle, undernutrition has been linked with reduced frequency LH pulses by the pituitary gland (Schillo, 1992), which is

known to support follicle growth, maturation, and ovulation. Restricted supply of oxidizable fuels during NEB limits the ability of hypothalamic neurons to sustain the GnRH pulse generator (Schneider, 2004). This is thought to be mediated by glucose, which is a preferred substrate for neuron energy metabolism (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, in addition to oxidizable substrates, metabolic hormones such as insulin, insulin-like growth hormone-1 (**IGF-1**), and leptin have increased concentrations when nutrient intake is adequate, and all of them play a role in potentiating the secretion of GnRH and gonadotropins (Schneider, 2004).

Cows under NEB have limited hepatic expression of GH receptor 1A triggered by low circulating concentrations of insulin (Butler et al., 2003; 2004). This phenomenon uncouples the growth hormone (**GH**)/IGF-1 axis which reduces the synthesis of IGF-1 by the liver. Reduced concentrations of IGF-1 have been associated with diminished follicle sensitivity to LH, growth and steroidogenesis (Lucy et al., 1992; Butler et al., 2004). Conversely, the increase in circulating concentrations of insulin as energy balance improves seems to be one of the signals to re-establish the 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 delays resumption of ovulation postpartum and might compromise oocyte quality; which likely hampers estrous detection and pregnancy in dairy cows.

In addition to extensive nutrient shortages, high producing dairy cows also

undergo extensive ovarian steroid catabolism. This is thought to be mediated by the high dry matter intake and consequent increased splanchnic blood flow (Sangsritavong et al., 2002). Hepatic blood flow doubles in the first 3 mo postpartum averaging 1,147 L/h on the week preceding parturition and 2,437 L/h on the third month postpartum (Reynolds et al., 2003). The 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), which can have implications to oocyte quality and subsequent embryo development. Reduced circulating concentrations of estradiol, because of hepatic catabolism in cows with high dry matter intake, can result in shorter and less intense estrous periods (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., 2010).

A commonly discussed hypothesis put forth by Jack Britt suggested that developmental competence of the oocyte and the steroidogenic capacity of the follicle in dairy cows are influenced by the environment in which the follicles developed from the primary follicle to the pre-ovulatory state. Follicles from cows undergoing extensive NEB would suffer metabolic and biochemical changes that would influence the microenvironment to which they are exposed to and, consequently, the competence of their oocytes. This hypothesis has never been fully tested, although it is clear that the

catabolic state in early lactation influences follicle growth and oocyte quality.

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, intra-follicular glucose concentrations also decline 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) were shown to render 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 is has to be readily available for 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 in early lactation might compromise oocyte competence in dairy cows.

Extensive fat mobilization and the release of large amounts of NEFA into the bloodstream have been shown to exert a direct effect on 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, the addition of palmitic and stearic acids to the maturation media induced apoptosis and necrosis of cumulus cells, which was associated with impaired fertilization, cleavage, and development to the blastocyst stage (Leroy et al., 2005).

Changes in circulating concentrations of BHBA are promptly reflected in the follicular fluid (Leroy et al., 2004). However, *in vitro* models developed to study the effects of subclinical ketosis on fertility of dairy cows have failed to demonstrate a direct effect of BHBA on oocyte competence, which seems only to aggravate the responses to low concentrations of glucose during oocyte maturation (Leroy et al., 2006).

CALCIUM HOMEOSTASIS AND UTERINE HEALTH DURING EARLY POSTPARTUM

In early lactation, synthesis and secretion of colostrum depresses systemic concentrations of Ca, which often results in reduced availability of ionized Ca (Ca^{2+}) for cellular metabolism. Surveys in the US indicate that 25 % of primiparous and more than 41 % of multiparous cows are subclinically hypocalcemic ($\text{Ca} < 8.0$ mg/dL) in the first 48 h after calving (Reinhardt et al., 2011). Mild depressions in serum Ca concentrations postpartum have been implicated with uterine health. Martinez et al. (2011) observed that cows with serum $\text{Ca} < 8.59$ mg/dL in at least one of the first 3 d postpartum had reduced neutrophil phagocytic and killing activities *in vitro*, increased odds of developing fever (adjusted OR = 3.5; 95 % CI = 1.1-11.6) and metritis (adjusted OR = 4.5; 95 % CI = 1.3-14.9) and these associations were observed for both, cows considered to be of high or low risk of developing metritis based on calving problems. The authors concluded that the attributable risk for a cow to develop metritis because of low serum Ca was 75.3 % (Martinez et al., 2011).

Ionized Ca is an important second messenger in cellular signal transduction, and fluctuations in intracellular Ca^{2+}

concentrations are critical to activate immune cells (Lewis, 2001). Cows with milk fever have reduced intracellular stores of Ca^{2+} (Kimura et al., 2006), which might explain the increased risk of infectious diseases in these cows (Ribeiro et al., 2011). Collectively, these data suggest 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., 2011).

IMPROVING PERIPARTURIENT METABOLISM THROUGH MANAGEMENT AND NUTRITION

Cow Movement and Dry Period Length

Regrouping of cows induces social tensions that disturb feeding and resting patterns, and often causes a temporary depression in dry matter intake (von Keyserlingk et al., 2008). The changes in behavior with movement of cows between groups have led veterinarians and researchers to advise producers against regrouping when calving is imminent. It is thought that regrouping would further suppress intake and increase the risk of ketosis and fatty liver. However, it is unknown if regrouping when cows are moved to the close-up pen is detrimental to health and production in the subsequent lactation. Researchers at the University of Wisconsin have attempted to address this question and their findings refuted the concept that weekly addition of cows to the close-up group is detrimental to postpartum metabolism and production (Coonen et al., 2011). 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 length of the dry period. Reducing the dry period from 55 to 34 d increased BCS between wk 2 and 8 postpartum and reduced the concentrations of plasma NEFA at wk 3 postpartum (Watters et al., 2008), suggesting improved energy status postpartum. When energy balance was measured, cows subjected to a 28-d dry period experienced a less severe NEB postpartum, which resulted in reduced BCS and body weight losses compared with cows having the traditional 56 d dry period (Rastani et al., 2005). Some of the benefit to energy balance results from less milk production, particularly in cows starting their second lactation (Watters et al., 2008; Santschi et al., 2011a). The improved energy balance with short dry periods 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 cyclicity, cows with a dry period of 28 to 35 d had similar reproductive performance to those with a standard 8-wk dry period (Gümen et al., 2005; Watters et al., 2009; Santschi et al., 2011b). Nevertheless, in observational studies, extending the exposure of cows to the prepartum diet was associated with reduced days open (DO) and increased proportion of pregnant cows at wk 6 and 21 after the initiation of the breeding season (DeGaris et al., 2010).

Manipulating Prepartum Nutrient Intake by Diet Formulation

Altering caloric intake prepartum influences postpartum metabolism in dairy cows. *Ad libitum* nutrient intake during the entire dry period tends to increase body weight and BCS prepartum and predispose cows to increased lipid mobilization in 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. A summary of studies in which caloric intake was restricted in late gestation is depicted in Table 2. On average, cows consumed 14.6 and 19.8 Mcal/day for the low and high caloric intake, respectively. Restricting caloric intake prepartum, although suggested to improve metabolism (Douglas et al., 2006), reduced production of fat-corrected milk. The reduction in production averaged 2 kg/day. In the same data set, concentrations of BHBA in early lactation were mostly unaffected and averaged 6.8 mg/dL for both low and high caloric intake cows. Nevertheless, concentrations of BHBA were greater ($P < 0.05$) for high than low caloric intake cows in two studies (Douglas et al., 2006; Janovick and Drackley et al., 2011), but the opposite was

observed in another study in which low prepartum caloric intake resulted in greater concentrations of BHBA postpartum (Kanjapruithipong et al., 2010). Therefore, restricting caloric intake prepartum can be used to minimize lipid mobilization and triacylglycerol accumulation in the liver, but at the expense of milk production.

Altering the protein content of 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 dry matter with a high rumen undegradable protein source enhanced milk production in primiparous cows (Santos et al., 2001). Nonetheless, protein had negligible impacts on measures of reproduction. Time to resumption of ovulation postpartum, DO, and pregnancy per AI were all not affected by prepartum dietary protein concentration. Similarly, the incidence of diseases

Table 2. Effect of prepartum caloric intake on fat-corrected milk (kg/d)

Reference	Prepartum intake ¹		<i>P</i> ²
	Low caloric intake	High caloric intake	
Douglas et al., 2007	35.6	37.9	NS
Douglas et al., 2006	40.8	39.8	NS
Rabelo et al., 2003; 2005	38.5	40.4	0.59
Doepel et al., 2002	39.1	40.3	NS
Hayirli et al., 2011	33.7	35.2	0.27
Janovick and Drackley, 2011	40.5	46.1	0.09
Kanjapruithipong et al., 2010	26.1	28.4	0.04
Average	36.3	38.3	

¹ Prepartum caloric intake (net energy for lactation) averaged 14.6 and 19.8 Mcal/cow/day for the low and the high caloric intake, respectively.

² NS = not significant ($P > 0.10$).

postpartum was not affected by prepartum dietary protein. Therefore, diets for cows in 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 (NRC, 2001).

Feeding Postpartum Diets to Increase 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 parameters in dairy cattle. Feeding more dietary starch or enhancing the ruminal fermentability of starch in the diet usually results in increased plasma insulin concentrations. Insulin mediates recoupling of the GH/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 within the first 50 d 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 dry matter intake and offset any potential benefits of dietary manipulation on ovarian function.

Altering Metabolism by Supplementing Ionophores to Periparturient Dairy Cows

Another method to increase blood insulin is to supplement the diets of dairy cattle with ionophores. Monensin is

commonly used in cattle diets to selectively increase the microbial population in the rumen that favor propionate production and nitrogen conservation. Feeding monensin typically increases blood glucose and insulin and reduces NEFA and BHBA (Duffield et al., 2008a). Therefore, feeding monensin improves metabolic health, and these responses have resulted in reduced 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 ovulation postpartum, to reduce days to pregnancy, or to increase the rate of pregnancy in spite of consistent improvements in metabolic health (Abe et al., 1994; Duffield et al., 2008b).

Diet Manipulation to Improve Hepatic Lipid Metabolism

During periods of extensive fat mobilization, fat accumulates in the hepatic tissue. In early lactation cows with relatively low plasma NEFA concentrations (0.36 mM), the liver extracted 724 g of NEFA from blood over a 24 hr period (Reynolds et al., 2003). Thus, in cows with concentrations of NEFA above 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 these 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 triacylglycerols 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.

Supplementation of 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, the inclusion of supplemental choline on the diet from approximately 25 d before to 80 d after calving reduced loss of body condition postpartum and concentrations of BHBA, which resulted in less incidence of clinical and subclinical ketosis despite the increase in fat-corrected milk (Lima et al., 2011). 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 d of gestation.

Improving Ca Homeostasis Postpartum

The most common method to improve postpartum Ca status is the manipulation of the dietary cation-anion difference (**DCAD**) prepartum (Goff, 2004; Seifi et al., 2010). Reducing the DCAD by feeding salts with strong anions decreases blood pH and enhances the affinity of the parathyroid hormone (**PTH**) to the PTH receptor present

on cells in the bones, intestine, and kidneys (Goff, 2004). Although feeding strong anions reduces 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 and subclinical ketosis (Seifi et al., 2010). However, 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., 2011), interval 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 Ca postpartum on reproduction of dairy cows.

Fatty Acid Supplementation and Postpartum Health

Lipids are important molecules that serve as a source of energy and are critical components of the physical and functional structure of cells. They play important regulatory roles in cell metabolism and serve as molecules affecting transduction pathways that control cell activity and proliferation. Some fatty acids (**FA**) are considered essential as they cannot be synthesized by mammalian cells and have to be consumed in the diet. Using growing rats, Burr and Burr (1930) demonstrated that diets low in fat interfered with growth,

health, and ovulation; events then reversed after feeding the polyunsaturated FA C18:2 n-6 (linoleic acid) and C18:3 n-3 (α -linolenic acid; Burr and Burr, 1930).

The prostaglandin (PG) $F_{2\alpha}$ synthesized by the endometrium plays an important role in reproduction of dairy cows. During parturition, eicosanoids are produced in copious amounts and play an important role in regulating uterine contractions and expulsion of the placenta and uterine contents. Prostaglandin $F_{2\alpha}$ is an important eicosanoid that regulates CL lifespan and might influence uterine defense mechanisms. Feeding diets that differ in fatty acid profile influences the composition of the uterine tissues, which in turn affects the secretion of PGF $_{2\alpha}$ in the early puerperium (Cullens et al., 2004).

Santos et al. (2008) reviewed studies in which transition cows were fed diets differing in fatty acid profile. Prepartum supplementation with Ca salts of long chain FA rich in n-6 FA reduced the incidence of retained placenta, metritis, and mastitis compared with cows not fed fat prepartum (Cullens et al., 2004). Similarly, supplementing prepartum diets with 2 % Ca salts of either palm oil or a blend of C18:2 n-6 and trans-octadecenoic FA reduced the severity of uterine disease postpartum (Santos et al., 2008). It is known that polyunsaturated FA are capable of modulating immune response, and n-6 FA usually potentiate the inflammatory response; whereas n-3 FA tend to depress this response.

Recent work by our group evaluated cellular responses and innate immunity of dairy cows fed Ca salts containing mostly saturated/monounsaturated FA (palm oil), n-6 FA (safflower oil), or n-3 FA from fish oil (Silvestre et al., 2011b). Cows fed Ca salts

high in linoleic acid (n-6 FA) tended to have ($P < 0.10$) more linoleic acid in the caruncular tissue than cows fed palm oil. The n-6:n-3 ratio of FA was greater ($P < 0.05$) in caruncular tissue of cows fed Ca salts containing safflower compared with that of cows fed Ca salts of palm oil. Neutrophils from cows fed Ca salts of safflower oil rich in n-6 FA had increased ability to kill bacteria *in vitro*. Similarly, feeding more n-6 FA during the transition period increased the acute phase response and induced neutrophils to secrete an increased amount of pro-inflammatory cytokines (Silvestre et al., 2011b). On the other hand, when cows in the breeding period received either Ca salts of palm oil (saturated or monounsaturated FA) or of fish oil (n-3 FA), the latter induced a period of suppressed inflammatory responses. These data suggest that it is possible to manipulate the innate immunity by altering the fatty acid make up of the diet, although additional studies are needed to determine the exact combination of FA required to optimize postpartum health. Nevertheless, the combination of dietary FA that enhanced immune response around calving and suppressed it during breeding resulted in the highest proportion of pregnant cows following the first two postpartum inseminations (Silvestre et al., 2011a).

CONCLUSIONS

Dairy cows during the periparturient period undergo metabolic distress because of the initiation of lactation. In many cows, homeostatic controls are not able to sustain homeostasis and disease eventually develops. In fact, 30 to 50 % of the postpartum dairy cows are often diagnosed with disease event in the first 2 to 3 mo postpartum. Such problems are negatively associated with pregnancy success. In fact, diseases extend the period of anovulation,

reduce pregnancy per AI, and increase pregnancy loss. It is expected that implementation of nutritional and health programs that address the needs of dairy cows to minimize drastic changes in intermediary and mineral metabolism tend to favor metabolic health and subsequent reproduction. In many cases, these responses have not been demonstrated in controlled research. Nevertheless, common sense dictates that cows that undergo transition without health problems are more fertile.

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