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Influences of nutrition and metabolism on fertility of dairy cows

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Abstract

During early postpartum, high-producing dairy cows undergo a period of extensive tissue catabolism because of negative nutrient balance. Homeorrhetic controls assure that nutrients are partitioned to favor lactation at the same time that homeostasis secures survival. However, unrestrained metabolic disturbances 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. Low circulating concentrations of glucose and insulin associated with elevated concentrations of non-esterified fatty acids and ketone bodies postpartum have disruptive and detrimental effects on the oocyte, granulosa and immune cells. Negative nutrient balance is associated with changes in the pattern of ovarian follicle growth which can indirectly affect oocyte quality. Some of this disruption seems to be the result of endocrine and biochemical changes that alter the micro-environment of the growing and maturing oocyte. In addition, cows under negative nutrient balance have extended periods of anovulation. Postpartum anestrus, as well as infertility, is magnified by losses of body condition during the early postpartum period. The underlying mechanism for resumption of ovulatory cycles seems to be associated with metabolic signals and regulatory hormones primarily insulin and insulin-like growth factor (IGF)-1, which link nutritional status with gonadotropin secretion, recoupling of the growth hormone-IGF system, and follicle maturation and ovulation. Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation. Furthermore, fertility in postpartum cows is also determined by uterine health. Reductions in circulating concentrations of Ca and antioxidant vitamins around parturition are also linked with impaired immune competence and result in greater risk of uterine diseases that impair reproduction. Specific nutrients and dietary ingredients have been implicated to affect reproduction in cattle. Excess intake of dietary protein has been suggested as detrimental to fertility, although feeding excess of dietary protein can no longer be justified. Addition of moderate amounts of supplemental fat to the diet improves caloric intake, modulates prostaglandin F2α secretion by the uterus, affects ovarian dynamics, enhances luteal function and embryo quality, and has moderate positive effects on fertility. More specifically, some fatty acids might impact fertilization rate and embryo quality in dairy cows. On the contrary, some dietary ingredients, such as gossypol, when ingested in large quantities decrease fertility of dairy cows because of its negative effects on embryo quality and pregnancy maintenance.

Keywords: dairy cow, embryo, metabolism, reproduction.

Introduction

Reproductive efficiency of the lactating herd is a major component of profitability in dairy farms. Reproduction determines when primiparous cows become multiparous leading to increments in milk yield, alters the average milk yield per day of calving, affects the number of replacement animals available and the risk of culling, and influences the rate of genetic progress. Unfortunately, improving fertility is not trivial. The 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 established, it is well described that poor nutritional status and metabolic health negatively influence reproduction in dairy cows. The energetic status of a cow modulates the secretion of hormones that play key roles in growth of ovarian follicles, ovulation, corpus luteum (CL) formation, and oocyte competence. Furthermore, extensive lipolysis and products from fat metabolism may be detrimental to oocyte competence and subsequent embryo development. In addition, impaired metabolic health often leads to immunosuppression and the occurrence of diseases that further reduce fertility.

Prevalence of diseases postpartum and impact on fertility of dairy cows

The transition from the nonlactating pregnant state to nonpregnant lactating 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 homeorrhesis. A sharp increase in nutrient requirements generally occurs when feed intake 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 homeorrhetic adjustments to cope with the changes in metabolism

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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 (Santos *et al*., 2010a; Ribeiro *et al*., 2011).

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 US 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 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 lower 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 herdmates. Cows affected with 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, 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.

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

Health problem	Estrous cyclic* (%)	AOR (CI)[1]	P-value
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 day 30* (%)¶	AOR (CI)	P-value
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 day 65* (%)	AOR (CI)	P-value
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] 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), $P < 0.05$. Data from Ribeiro *et al*. (2011).

Negative nutrient balance impacts health and reproduction of dairy cows

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

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 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.* (2011) reported that cows under NEB (NEFA ≥0.7 mM) were less likely to resume ovarian cyclicity before 50 days postpartum and to become pregnant from 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). The rate of pregnancy in 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 in early lactation (Ospina *et al*., 2010b). Ketosis resultant from extensive fat mobilization has also 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 μM increase in BHBA concentration in weeks 1 and 2 after calving, the proportion of pregnant cows at first AI was reduced by 2 and 3%, respectively. Furthermore, the rate of pregnancy within 70 days after the end of the voluntary waiting period was 13% less among cows with blood BHBA concentration ≥ ~1.0 mM compared with herdmates with BHBA <1.0 mM (Ospina *et al*., 2010b). Furthermore, as the prevalence of cows with elevated concentrations of serum NEFA or BHBA increases, reproductive performance declines (Ospina *et al*., 2010a). In the latter study, the 21-day cycle pregnancy rate was reduced 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 that interfere with fertility.

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 compatible 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 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 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 in blood around calving than healthy cows (Hammon *et al*., 2006; Galvão *et al*., 2010). It is important to highlight that the occurrence of diseases in 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.

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 around parturition (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 lower for cows that retained their placenta than for healthy cows (LeBlanc *et al*., 2004). In fact, for every 1 µg/ml increase in circulating vitamin E during the week preceding parturition, the risk of retained placenta decreased by 21%. Furthermore, the decline 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). In 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 the 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 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) reduced 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 has to 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 in early lactation might compromise oocyte competence in dairy cows.

The follicular fluid is derived from blood originating from capillaries in the theca cells through osmotic pressure (Rodgers *et al*., 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 *et al*., 2010). As fluid accumulates in the antrum, it bathes the cumulus cells and the oocyte. Changes in nutrient supply that leads to either hypo- or hyperglycemia can influence lipid metabolism and alter the composition of the 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 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 FA 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, 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). Therefore, it is proposed that the oocyte is vulnerable to potential harmful effects of an altered biochemical milieu in the follicular microenvironment (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 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 under positive energy balance, stimulates the 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 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)/insulin-like growth factor-1 (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, 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 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 ovulation postpartum and might compromise oocyte quality, which likely hampers estrous detection 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 dry matter intake and consequent increased splanchnic blood flow (Sangsritavong *et al*., 2002). Hepatic blood flow doubles in 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). 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 for 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. Similarly, reduced circulating concentrations of estradiol because of hepatic catabolism in cows with high dry matter intake 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*., 2009b) 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 during early postpartum

The control of blood concentrations of Ca is critical to maintain normal muscle contractility, transmission of nerve impulses, and immune function. Nonetheless, homeostatic controls in early lactation might not prevent declines in Ca concentrations in the first week postpartum. The 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 portion of them develop milk fever. In fact, surveys in the US indicate that 25, 41, 49, 51, 54, and 42% of 1st to 6th lactation cows are hypocalcemic (Ca <8 mg/dl) in the first 48 h after calving (Reinhardt *et al*., 2011). In order to maintain serum total and ionized Ca (Ca^{2+}) concentrations postpartum, dairy cows have to increase bone remodeling for Ca resorption or increase intestinal Ca absorption.

The 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 in at least one of the first 3 days 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). 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*., 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). 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*., 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.

Cow movement and dry period length

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 dry matter intake (von Keyserlingk *et al*., 2008). Therefore, regrouping cows at the imminence of calving is not advised as it would further suppress intake and increase the risk of ketosis and fatty liver. However, the question of when cows can and cannot be moved 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). 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 days increased BCS between weeks 2 and 8 postpartum and reduced the concentrations of plasma NEFA at week 3 postpartum (Watters *et al*., 2008), suggesting improved energy status postpartum. When energy balance was measured, cows subjected to a 28-day dry period experienced 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). The 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 cyclicity, cows with a dry period 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 the 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 caloric intake prepartum influences postpartum metabolism in dairy cows. *Ad libitum* nutrient intake during the entire dry period tended 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. 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 and Drackley, 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 dry matter intake. 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 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 dry matter 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 ovulation postpartum, days open and pregnancy per AI were not affected by prepartum dietary protein concentration. Similarly, the incidence of diseases 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 (National Research Council - NRC, 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 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 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 dry matter intake 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 lactation cows with relatively low plasma NEFA concentrations

(0.36 mM), the liver extracted 724 g of NEFA from blood over a 24-h 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 in the diet from approximately 25 days before to 80 days after calving reduced loss of body condition postpartum 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 ovarian 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 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).

Improving Ca homeostasis postpartum

Improving serum concentrations of Ca in 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 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*., 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 Ca postpartum on reproduction of dairy cows. 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 will likely improve uterine health and subsequent fertility.

Fatty acid supplementation and reproduction of dairy cows

Fatty acids are structural and functional lipid components of cells capable of modulating membrane fluidity and permeability, and carrying out cell signaling, in addition to providing calories for tissue energy metabolism. Therefore, fat supplementation to dairy cattle diets provides benefits that go beyond the provision of calories, and these effects are influenced by the profile of FA of the fat source.

Prostaglandin (PG) F2α synthesized by the endometrium plays an important role in reproduction of dairy cows. Dietary FA supplementation has been shown to influence tissue FA composition. This makes it possible to manipulate arachidonic acid concentration in the endometrium, the precursor for PGF2α synthesis (Bilby *et al.,* 2006b). In fact, the feeding of supplemental fat prepartum (30% of FA as C18:2 n-6) enhanced uterine secretion of PGF2α (Cullens *et al*., 2004). 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). Altering the FA supply to dairy cows not only alters the FA composition of uterine tissues, but has also the potential to influence immune function (Silvestre *et al*., 2011a).

Although the incorporation of supplemental fat enriched in polyunsaturated FA has been shown to influence follicle growth (Santos *et al*., 2008), it is unclear whether supplemental fats differing in FA profile have any effect on resumption of estrous cyclicity. In general, type of FA does not change the proportion of estrous cyclic cows at around 60 days postpartum (Santos *et al*., 2008). Even though the amount of lipids in the oocyte of ruminants is greater than that in most studied species, which would lead to the potential to change FA profile to influence oocyte competence, feeding polyunsaturated FA did not impact oocyte quality based on subsequent embryo development *in vitro* (Bilby *et al*., 2006a). Nevertheless, studies *in vivo* support the concept that altering the FA profile of the diet influences fertilization and embryo quality in lactating dairy cows (Santos *et al*., 2008; Cerri *et al*., 2009a). Furthermore, manipulating the sequence of FA fed during the transition and breeding periods influence fertility of dairy cows by improving pregnancy per AI because of reduced pregnancy loss (Silvestre *et al.,* 2011b). Some of the effects of specific FA promoting improved reproduction are thought to be mediated by changes in innate immunity during the transition and breeding periods (Silvestre *et al*., 2011a). Collectively, results from studies on the effects of fat supplementation on fertility of dairy cows suggest that incorporating fat into dairy cattle rations improves pregnancy per AI (Santos *et al*., 2008). Furthermore, the type of FA seems to play a role in the establishment and maintenance of pregnancy in cattle (Santos *et al*., 2008).

Feeding antioxidants to influence reproduction

During the immediate postpartum period, the cow's immune system is challenged severely, and the innate and humoral defenses are suppressed (Silvestre *et al*., 2011a; Martinez *et al*., 2012). Incidence of diseases and disorders can be high 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 to cows fed sodium selenite in some studies (Weiss, 2003). Depending on the soil, 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 two subsequent experiments, when the same supplementation scheme was applied to a Se adequate area, Se concentrations in plasma did not differ (Rutigliano, 2006; Rutigliano *et al*., 2008; Cerri *et al*., 2009c). 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 (Cerri *et al*., 2009c; Rutigliano *et al*., 2008). Nevertheless, selenized yeast improved neutrophil function, serum titers against ovalbumin, and uterine health 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.

Nutritional interventions that may depress fertility

Some dietary components can influence reproduction in a negative manner. By-products of the metabolism of proteins and toxic compounds contained in cotton products have the potential to interfere with gamete viability, and the uterine environment, all of which impair establishment and maintenance of pregnancy.

Protein and reproduction

Lactating dairy cattle require large quantities of metabolizable amino acids for synthesis of milk protein. It is typical for lactating rations to contain crude protein between 16 and 18% of its total dry matter. Diets with limited crude protein can compromise microbial growth and fermentation in the rumen, which often reflects in declines in feed intake and milk production. On the other hand, feeding protein in excess to what is needed by the cows has been implicated in increased ammonia and urea concentrations in blood and milk, which have been used as markers for reduced fertility (Butler, 1998). The suggested decline in fertility of cattle fed excess of protein is caused by alterations in uterine physiology with a decline in uterine pH during the early luteal phase of the estrous cycle (Butler, 1998). A more acidic uterine environment is less conducive with maintenance of pregnancy in cattle (Ocon and Hansen, 2003). This effect seems to be restricted to the early stages of embryo development (Rhoads *et al*., 2006).

Because high-producing lactating dairy cows are more efficient in utilizing protein sources when diets are moderate in crude protein and are balanced for the supplies of metabolizable protein and limiting amino acids (Noftsger and St. Pierre, 2003), it is not justified to feed diets with protein concentrations that will increase urea N and harm fertility.

Consumption of gossypol from cotton byproducts and reproduction

Gossypol was first discovered by Chinese scientists after noticing that no children were born for more than a decade in a village where people cooked food with cottonseed oil. Since then, innumerous reports in the literature have confirmed the anti-fertility effect of gossypol in mammals. Gossypol disrupts cell membrane metabolism, affects glycolysis, influences mitochondrial and energy metabolism in the cell, and increases fragility of cell membranes, such as red blood cells. In fact, erythrocyte fragility has been one of the indicators of potential gossypol toxicosis.

Risco *et al*. (1992) were one of the first to show that gossypol can be toxic and even kill growing cattle. They fed rations with 200, 400 or 800 mg/kg of free gossypol (FG) to bull calves for 120 days. The diets with 400 and 800 mg/kg of FG were considered to be toxic and could potentially cause the death of growing ruminants. Baby calves have little ability to detoxify gossypol; therefore toxicity can be easily induced by feeding cotton products.

A series of experiments by our group demonstrated that consumption of up to 40 mg of FG/kg of body weight did not influence follicle and luteal development in dairy heifers, but feeding a diet with 40 mg of FG/kg of body weight reduced embryo quality and development *in vivo* and *in vitro* (Coscioni *et al*., 2003a, b; Villaseñor *et al*., 2008). These effects likely explain the reduced risk of pregnancy in dairy cows with high plasma gossypol concentrations (Santos *et al*., 2003) and compromised embryo survival after transfer (Galvão *et al*., 2006). Therefore, it is prudent to feed lactating dairy cows amounts of cottonseed that result in low plasma gossypol concentrations.

Conclusions

It is accepted that reproduction is important for the profitability of dairy farms, and nutritional status and metabolic health are both associated with successful reproduction. Cows that experience periparturient problems have delayed return to ovulation, lower pregnancy per insemination, and increased pregnancy loss. Therefore, implementing nutritional and health programs that reduce the risk of metabolic disturbances are expected to not only improve cow health, but also enhance fertility. Strong evidence suggests that management of cows during the prepartum period affects uterine health. Inadequate intake of nutrients prepartum and altered feeding behavior increases the risk of metritis in dairy cows. Strategies to manipulate peripartum metabolic health involve dietary formulation to minimize the degree and extent of negative nutrient balance, improve Ca homeostasis, and minimize the severity of immunosuppression around and immediately after calving. Supplementation with unsaturated FA of the n-3 and n-6 families usually improves fertility, as long as it does not interfere with rumen microbial metabolism. Source of supplemental Se might influence health and reproduction of dairy cows, but response seems to be dependent upon the Se concentrations in dietary ingredients. Lastly, although lactating dairy cows can consume substantial amounts of gossypol with no detrimental effects on health and lactation, when plasma gossypol concentrations exceed 5 µg/ml, embryo development and establishment and maintenance of pregnancy are compromised.

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