Perspective on Physiological/Endocrine and Nutritional Factors Influencing Fertility in Post-partum Dairy Cows

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Contents
Increasing reproductive performance of post-partum lactating dairy cows is a multi-factorial challenge involving disciplines of production medicine, nutrition, physiology and herd management. Systems of programmed timed insemination have been fine-tuned to achieve pregnancy per artificial inseminations (AI) approximating 45%. Systems have optimized follicle development, integrated follicle development with timing of induced corpus luteum regression and fine-tuned sequential timing of induced ovulation and AI. Use of programmes for insemination have identified occurrence of anovulatory ovarian status, body condition, uterine health and seasonal summer stress as factors contributing to reduced herd fertility. Furthermore, programmes of timed insemination provide a platform to evaluate efficacy of nutritional and herd health systems targeted to the transition and post-partum periods. The homeothermic periparturient period, as cows deal with decreases in dry matter intake, results in a negative energy balance and is associated with a period of immunosuppression. Cows that transition well will cycle earlier and have a greater risk of becoming pregnant earlier post-partum. The innate arms of the immune system (acute and adaptive) are suppressed during the periparturient period. Cows experiencing the sequential complex of disorders such as dystocia, puerperal metritis, metritis, endometritis and subclinical endometritis are subsequently less fertile. Targeted strategies of providing specific nutraceuticals that provide pro- and anti-inflammatory effects, such as polyunsaturated fatty acids (e.g., linoleic, eicosapentaenoic/docosahexaenoic, conjugated linoleic acid), sequential glycogenic and lipogenic enrichment of diets, and organic selenium appear to differentially regulate and improve the immune and reproductive systems to benefit an earlier restoration of ovarian activity and increased fertility.

Introduction
Intensive genetic selection for milk production without attention to reproductive performance has contributed to an inverse relationship between milk production and reproduction (Lucy 2001). A recent review documented the multiplicity of factors contributing to low reproductive performance of dairy cows (Rodriguez-Martinez et al. 2008). Various factors identified with low fertility were insufficient weight within genetic selection programmes for longevity, health and fertility; interrelated factors such as negative energy balance (NEBAL), level of milk production, dystocia, retained placenta, twinning, stillbirths and endometritis that reduce the risk for pregnancy; as well as inadequate attention to body confirmation, nutrition and reproductive management, infectious diseases, animal comfort and housing. Various short-term and long-term strategies were described to improve fertility that may or may not sustain high levels of milk production.

Reproductive management of the lactating dairy cow has been a challenge because of poor expression of oestrus and low fertility to insemination at a detected oestrus. The duration of oestrus is reduced as milk production increases, and the frequency of double ovulations and subsequent occurrence of twins also are increased in cows with high levels of milk production at the time of the breeding period (Lopez et al. 2005). The high-producing dairy cow (≥39.1 kg/day) of today expresses oestrus for approximately 7 h during which time an average of 6.5 standing events take place with an accumulative period of standing of 20 s (i.e., 3 s per standing event; Lopez et al. 2004). The poor reproductive performance of lactating dairy cows is the result of a multiplicity of factors that need to be segmented into a sequential series of physiological and metabolic windows from late pregnancy through the first two artificial inseminations (AI) of the designated breeding period. The objective of this manuscript is to provide a perspective as to the physiological constraints and opportunities to increase reproductive performance of lactating dairy cows.

Programming the Insemination Period of Post-partum Dairy Cows
If the assumption is that healthy post-partum lactating cows are cycling, then one can address what are the components of the insemination programme that need to be optimized to achieve maximal pregnancy per AI. This will then allow addressing the transitional and post-partum events that contribute to the reductions in fertility of dairy cows. A timed AI programme is a fixed timed artificial insemination prior to an induced ovulation without detection of oestrus, which follows the optimal sequential programming of follicular development and turnover of the corpus luteum (CL). Because of the impact of high milk production on reduced expression of oestrus, a timed AI programme for a successful service is a suitable reproductive management strategy. It is essential that producers and veterinarians understand the physiological underlying reasons why certain components of the reproductive management programme are able to improve reproductive performance, or conversely why a misunderstanding of the programme can lead to catastrophic pregnancy results. No single reproductive breeding programme is practical and economically optimal for all dairy production units because of the differences in available facilities, size of the unit, labour that places reproduction as a high priority and a functionally dynamic record system.

© 2010 Blackwell Verlag GmbH
Factors Influencing Fertility of Post-partum Dairy Cows

The original Ovsynch protocol (two injections of GnRH administered 7 days before and 48 h after an injection of PGF$_{2\alpha}$, and cows inseminated 16–20 h after the second injection of GnRH; Pursley et al. 1997) preceded by a PGF$_{2\alpha}$ pre-synchronization programme (Presynch–Ovsynch) has become the nucleus programme for reproductive management in the US dairy industry (Moreira et al. 2001; El-Zarkouny et al. 2004). Successful use of such a programme is dependent highly upon obtaining good compliance in implementing all component parts of the protocol. Optimization of stage of the oestrous cycle (i.e., days 5–9) at the onset of the Ovsynch protocol is important to achieve synchronized ovulations to the first and second injections of GnRH preceding the timed AI and to insure there is an active CL and luteal concentrations of progesterone throughout the period of ovulatory follicle development. The first injection of GnRH will induce ovulation of a first-wave follicle and recruitment of a new follicle wave, which upon induction of ovulation to the second GnRH increases the probability of producing a viable oocyte for fertilization and a robust CL. The continual exposure to progesterone between the first injection of GnRH and then PGF$_{2\alpha}$ is important for sequentially programming the brain, oviduct and uterus with the appropriate changes in hormones, receptors and secretions, leading to an induced ovulation, fertilization and development of an embryo capable of maintaining a pregnancy with minimal embryonic and foetal losses. The original Presynch–Ovsynch programme entailed two injections of PGF$_{2\alpha}$ given 14 days apart with the Ovsynch protocol initiated 12 days after the second injection of PGF$_{2\alpha}$ for pre-synchronization (Moreira et al. 2001). A recent study indicated that an 11-day interval after pre-synchronization (i.e., cows would be predominately on days 5–8 of the oestrous cycle) was better than a 14-day interval to begin the timed AI protocol (Galvão et al. 2007). The overall ovulation rate to the first injection of GnRH was greater for an 11-day than a 14-day interval (62.0 > 44.7%). This was attributed to GnRH being given at 11 days when the first-wave follicle will ovulate, whereas the 14-day interval increased the proportion of cows injected early in the second follicle wave a time the follicle was developed insufficiently to ovulate in response to GnRH. The latter follicle would continue to develop and undergo a slight extension in the period of dominance compared to the newly recruited follicle from the day 11 injection interval for GnRH. Indeed, pregnancy per AI was 6.6% units greater for the 11-day interval (40.1 > 33.5% at day 38 after timed AI).

Producers often favour the convenience of carrying out a timed AI when the first injection of GnRH is administered (i.e., referred to as a Cosynch programme) to reduce the number of times cows need to be handled. Alternatively, some producers prefer to perform timed AI on the following day at approximately 24–28 h after the GnRH injection for convenience. Either option will likely reduce the probability of pregnancy to AI. The importance of the correct timing is indicated by a study completed at the University of Wisconsin (Brusven et al. 2008). All cows were pre-synchronized with two injections of PGF$_{2\alpha}$, and the Ovsynch protocol was started 11 days later. The optimal timing programme was to inject GnRH 56 h after injection of PGF$_{2\alpha}$ (i.e., allowing additional time for development of the ovulatory follicle) and inseminate the cows 16 h after GnRH, which was 72 h after the injection of PGF$_{2\alpha}$. Per cent pregnant to AI was 36.1% compared with the Cosynch 48 -h (26.7%) or 72 -h (27.3%) programmes. The latter two programmes injected GnRH and performed the timed AI concurrently at 48 h or 72 h, respectively. Clearly, subtle changes in timing of the GnRH injection and time of insemination result in significant differences in per cent pregnant to an insemination.

High-producing lactating dairy cows have a greater incidence of two waves of follicle growth during the oestrous cycle compared with growing heifers that are more likely to have three follicular waves (Savio et al. 1988). The interval from follicle emergence to oestrus is approximately 3.5 days greater for cows with two follicular waves than for those with three follicular waves (Bleich et al. 2004). In a comparison of Ovsynch programmes initiated at day 3 or 6 of the oestrous cycle, Cerri et al. (2009b) reported that ovulatory follicles with shorter length of dominance (5–6 days) yielded a greater proportion of better quality (grades 1 and 2) embryos, whereas cows with a longer period of dominance had increased proportion of poor quality and degenerated embryos. Fertilization rate remained unaffected by the period of dominance.

One means of reducing the period of ovulatory follicle dominance is to shorten the interval from follicle recruitment to luteal regression (i.e., implement a 5-day interval between GnRH and PGF$_{2\alpha}$ injection of the TAI protocol) to possibly increase pregnancy per timed AI in lactating dairy cows. Following two injections of PGF$_{2\alpha}$ at 36 and 50 days post-partum, Santos et al. (2010) randomly assigned 933 cows to a Cosynch 72-h protocol (CoS72: day 61 GnRH, day 68 PGF$_{2\alpha}$, day 71 GnRH) or to a 5-day Cosynch 72 h with two injections of PGF$_{2\alpha}$ (5-day CoS2: day 61 GnRH, day 66 and 67 PGF$_{2\alpha}$, day 69 GnRH). Blood was sampled for progesterone analyses at the first GnRH, first PGF$_{2\alpha}$, second GnRH and 7 day after timed AI. Greater rates of CL regression (96.3% vs. 91.5%) and pregnancy per AI (37.9% vs. 30.9%) occurred for 5-day CoS2 than CoS72, respectively. It was essential to inject two doses of PGF$_{2\alpha}$ given 24 h apart (i.e., days 66 and 67) to ensure complete regression of the CL.

Following two injections of PGF$_{2\alpha}$ at 46 and 60 days in milk, Bisinotto et al. (2010) randomly assigned 1227 lactating dairy cows to a 5-day Ovsynch 56 h or to a 5-day Cosynch 72 h as depicted in Fig. 1. Pregnancy per AI did not differ between treatments when evaluated on either days 32 or 60 after timed AI. Indeed, overall pregnancy per AI of 45.9% on day 32 was excellent for US conditions (Santos et al. 2004, 2009) with a pregnancy loss between gestation days 32 and 60 of 13.1% (73/557). Thus, the 5-day Cosynch 72 -h programme with two injections of PGF$_{2\alpha}$ is an efficient synchronization programme to optimize fertility of dairy cows.

Collectively, these experimental approaches that have fine-tuned the dynamics of follicle development, CL development and regression, and timing of ovulation and insemination result in good on farm responses of
Periparturient and Post-partum Factors Associated with Subsequent Reproductive Performance

Analyses of herd reproductive performance

Several studies have examined various factors associated with cyclicity and pregnancy per AI utilizing reproductive management platforms such as oestrous or ovulation synchronization protocols initiated after pre-synchronization (Santos et al. 2009) or presence of a CL (López-Gatius 2003). In a study involving 6396 Holstein cows from four dairy farms in California, relationships among parity, body condition score (BCS) at calving and at AI, season of year when cows calved and milk yield on resumption of post-partum oestrous cycles by 65 days post-partum, as well as effect of these variables on pregnancy per AI and pregnancy loss to the first post-partum insemination, were evaluated (Santos et al. 2009). Cows had their oestrous cycle pre-synchronized with two PGF2α injections given 14 days apart and were inseminated between 69 and 82 days post-partum following either an oestrous or ovulation synchronization protocol initiated 12–14 days after pre-synchronization.

Blood was sampled for progesterone determination at the time of the second PGF2α injection of the pre-synchronization protocol and again at initiation of the oestrous or ovulation synchronization programmes 12–14 days later. Progesterone concentrations determined whether cows had initiated oestrous cycles after parturition. Cows were scored for body condition in the week after parturition, and again at AI, between 69 and 82 days post-partum. Pregnancy was diagnosed at 30 ± 3 and 58 ± 3 days after AI. Pregnancy per AI was lower in the warm season (27.4 < 44.4%). During the warm season, P/AI decreased 6% units for every 1000 -kg increase in annual milk production, whereas during the cool season, P/AI did not appear to be affected by increases in annual milk production. Furthermore, the incidence of inactive ovaries (i.e., detection of no CL or ovarian cyst at two consecutive transrectal palpations 7 days apart) was higher in the warm season (i.e., warm vs. cool season, 12.9 > 1.2% and 12.3 > 2.4%, respectively). These findings confirm on a farm basis that aberrant follicle development occurs during the warm season. Reductions in plasma inhibin concentrations were detected in heat-stressed lactating dairy cows (Wolfenson et al. 1995; Roth et al. 2000). Furthermore, concentrations of plasma inhibin were reduced in the summer for cyclic buffaloes (Palta et al. 1997). Collectively, these findings suggest that depression of follicular dominance during periods of heat stress (Wolfenson et al. 1995; Roth et al. 2000) involves a decrease in inhibin secretion by granulosa cells and subsequent alterations in FSH that leads to an increase in development of large follicles (e.g., persistent follicles). This may account for the increase in twinning rate between May and July, following insemination of cows during the previous summer and fall (Ryan and Boland 1991). An increase in numbers of large follicles and double ovulations occur in summer; however, because of elevated body temperature, early embryo losses occur and pregnancies are not sustained. When cows body temperature decline with lower ambient temperatures in early fall, they undergo a transitory recovery of follicle dominance, which results in increased incidence of double ovulations and twin pregnancies, eventually observed in the calvings, occurring the following summer (Ryan and Boland 1991; Roth et al. 2000).

The impact of season, more specifically heat stress, on fertility cannot be overlooked in arid, tropical and subtropical areas of the world. Even in areas of temperate climate, decreases in fertility are observed during the summer, and this trend likely is to continue or worsen as a consequence of global warming, which is likely to impact animal production systems of the 21st century.

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Post-partum endocrine and ovarian function

The critical transition period occurs from 3 week before to 3 week after parturition (Drackley 1999). During the 2 weeks pre-partum, feed intake decreases in association with significant decreases in progesterone, increases in estradiol and enhanced foetal size, which may contribute to a NEBAL that is exacerbated with the onset of lactation. This delicate hormonal transition is further confounded by the stress of the parturition process and environmental herd management. The NEBAL reaches a nadir within 2–3 weeks post-partum and undergoes a restoration reaching a zero energy balance at approximately 8–10 weeks. A major component of energy balance is the inherent dry matter intake relative to changes in milk production that varies markedly among animals. Although non-genetic effects obviously influence energy balance, long-term studies that minimized environmental effects indicated that dynamics in energy balance in early lactation appear to be somewhat genetically driven (Friggens et al. 2007). Understanding the factors controlling energy balance and their influence on ovarian recrudescence and restoration of fertility is an important area of investigation.

Major alterations in both lipid and carbohydrate metabolism occur early post-partum to partition dietary nutrients and nutrients from tissues towards the mammary gland. The importance of growth hormone (GH), as a principal coordinator of these activities, in the high-producing dairy cow is clear (Lucy 2003). In addition to directly stimulating liver, muscle, and fat to release metabolites such as glucose and non-esterified fatty acids (NEFA), GH acts on the liver and other organs, such as the pancreas and gut, to promote the release of several hormones such as insulin-like growth factor I (IGF-I) and insulin that act on the brain and also the reproductive tract. During the post-partum period when dairy cows undergo NEBAL, the somatotrophic axis (comprised of GH, the GH receptor and IGF-I) becomes uncoupled in the liver, and there is elevated GH and decreased IGF-I in the circulation. The increase in GH and NEFA antagonizes insulin action and creates a state of insulin resistance in post-partum cows (Lucy 2008).

The normal post-partum reduction in insulin action enhances the lipolytic signals of GH and catecholamines, resulting in increased concentrations of NEFA. Post-absorptive carbohydrate metabolism is altered by the reduced insulin action during NEBAL, with the net effect of reduced glucose uptake by peripheral tissues (i.e. muscle and adipose), but increased uptake by the mammary gland. The reduction in insulin-mediated glucose uptake by non-mammary tissues prioritizes the mammary gland for synthesis of milk and milk components (Lucy 2008). The reduced nutrient uptake coupled with the net release of nutrients (i.e. amino acids and NEFA) from storage sites is a key homeorhetic mechanism implemented by cows in NEBAL to support lactation (Bauman and Currie 1980). The reduced concentrations of insulin and their subsequent restoration during this period appear to alter follicle development. As the GH receptor becomes coupled, liver secretion of IGF-1 increases, which decreases pituitary secretion of GH and leads to a greater responsiveness to insulin. Insulin and IGF-1 act directly on the ovary to increase the sensitivity of the ovary to LH and FSH, as well as eliciting effects on the uterus and embryo (Lucy 2008). Infusion of insulin early post-partum, while concurrently maintaining normal concentrations of blood glucose, stimulated plasma IGF-1, size of the first-wave dominant follicle and plasma concentrations of estradiol (Butler et al. 2004).

Holstein dairy cows have the first wave of follicle growth beginning at 5–7 days post-partum regardless of NEBAL. This early growth of follicles is caused by increased concentrations of FSH in blood. However, the functional outcome of the first-wave follicle varies depending upon the associated metabolic and hormonal changes of the cow. Approximately 40–50% of the cows ovulate the first-wave-dominant follicle, which have increased steroidogenic capacity in association with greater concentrations of insulin and IGF-I. Such follicles are able to stimulate the release of LH and undergo ovulation. Another 30–40% of the cows will have the first-wave follicle turnover without ovulating and will ovulate a follicle from subsequent follicular waves later in the post-partum period, and the remaining 10–30% of the cows remain anovulatory by 50 days post-partum (Beam and Butler 1997, 1998). As described earlier, resumption of ovarian cyclicity before the first AI has been shown to be associated with a higher fertility at first AI. Lactating cows that ovulate early in lactation have better reproductive outcomes than cows that ovulate later (Darwash et al. 1997; McCoy et al. 2006). Furthermore, cows that are cyclic before the first post-partum AI have a greater pregnancy per AI than anovular cows (Santos et al. 2009). Increased fertility in cows that ovulate or express oestrus early in lactation is believed to be influenced by the number of oestrous cycles before first insemination (Thatcher and Wilcox 1973; Darwash et al. 1997), which would provide progesterone priming and evacuation of uterine contents at each oestrus. Cows ovulating the dominant follicle of the first post-partum follicular wave appear to have less severe NEBAL and a shorter interval to the nadir in NEBAL (Beam and Butler 1997, 1998). Indirect monitors of energy balance, such as concentrations of NEFA and beta-hydroxybutyrate (BHBA) in plasma, are positively associated with uterine diseases such as clinical metritis and subclinical endometritis (Hammon et al. 2006), which leads to long-term reductions in fertility (Gilbert et al. 2005). Galvão et al. (2009a) demonstrated that cows ovulating by 21, 49 days, or non-ovulating by 49 days had a frequency of subclinical endometritis of 25.9%, 53.1% and 21.0%, respectively. Of importance was that early ovulation by 21 days post-partum resulted in a greater first-service pregnancy per AI than cows cycling or non-cycling by 49 days post-partum (38.6%, 28.1% and 23.6%, respectively). The advantage of early cyclicity among the groups was evident with a shorter interval to insemination and increased hazard of pregnancy up to 30 days post-partum. These associations support the concept that early ovulation may be an indicator of good overall health in cows with a better energy balance during.
transition. Such a state decreases the odds of having clinical and subclinical uterine disease, leading to better overall reproductive efficiency.

**Post-partum immune function**

Dry matter intake is reduced greatly a few days before parturition and remains low for a few days after parturition at a time lactational demands contribute to a pronounced NEBAL. This period often represents a time when the immune system of the cow is suppressed severely, making them particularly vulnerable to diseases such as metritis and mastitis (Kimura et al. 2002; Goff 2006; Hammon et al. 2006). Observations in mastectomized vs intact cows during the periparturient period indicate that the initiation of lactation and milk synthesis are factors contributing to immunosuppression in cows (Kimura et al. 1999). Neutrophil function declined in both intact and mastectomized cows as parturition approached, but rebounded very quickly in mastectomized cows after parturition. Neutrophil function remained depressed for several weeks after parturition in milk-producing cows. The implication of these observations is that metabolic challenges experienced by the dairy cow at the onset of milk production impaired immune cell function. Indeed, cows with puерperal metritis and clinical endometritis or subclinical endometritis had lower dry matter intake and higher concentrations of NEFA in plasma during the periparturient period, and higher BHBA during early lactation compared to cows with normal uterine health (Hammon et al. 2006).

The development of post-partum uterine disease depends on the immune response of the cow and type of bacteria that colonize the uterus (Sheldon et al. 2006). Post-partum endometritis and subclinical endometritis are common reproductive diseases that has been associated with a reduction in pregnancy per AI and extended interval to pregnancy in lactating dairy cows (Kasimanickam et al. 2004; Gilbert et al. 2005; Rutigliano et al. 2008; Galvão et al. 2009a). A sequence of periparturient events leads to development of metritis in the first 2 weeks post-partum, clinical endometritis after 3 weeks post-partum and establishment of subclinical endometritis after 4 weeks post-partum, in which the subclinical inflammation of the endometrium is a chronic localized inflammatory process affecting 20–50% of the dairy cows in the first 60–80 days of lactation. It has been characterized by the presence of increased proportion of neutrophils in uterine cytology (Kasimanickam et al. 2004; Gilbert et al. 2005). Suppression of immune function post-partum (Kehrli et al. 1989) is associated with increased risk of endometritis (Hammon et al. 2006). Early in lactation, bacteria contaminate the uterus of >90% of dairy cows. Although most cows eliminate this bacterial contamination in the subsequent 5 weeks, a persistent inflammatory response continues to affect the uterus as either clinical or subclinical endometritis (Galvão et al. 2009b). Sensitively designed experiments to examine therapies for endometritis and subclinical endometritis are limited relative to important production outcomes such as subsequent probabilities of increased pregnancy and timing of pregnancy events (LeBlanc 2008). Many experiments suffer from small number of experimental units and concurrent negative controls. Two large field studies reported significantly improved reproductive performance in cows with validated cases of clinical endometritis (LeBlanc et al. 2002) or with risk factors for endometritis (McDougall 2001) that were treated IU with one dose of an antimicrobial containing cephapirin benzathine. In the former study (LeBlanc et al. 2002), cows with clinical endometritis were assigned randomly to receive cepha- pirin, PGF2α or no treatment. Cows that received cephapirin between 27 and 33 DIM became pregnant 60% faster than untreated controls. In the study comprised of cows with prior risk factors for endome- tritis, candidate cows were assigned randomly at 41 ± 14 DIM to receive cephapirin IU or remain untreated (McDougall 2001). Among cows that had delivered a dead calf, had RP or had purulent discharge from the vulva observed after 13 days post-partum, those treated with cephapirin were approximately 2–3 times more likely to be pregnant by 56 days into the breeding season. When examining pregnancy responses, cows with subclinical endometritis have been treated unsuccessfully either with PGF2α or intrauterine administration of antibiotics after already receiving PGF2α. (Galvão et al. 2009b,c). Alternative strategies of regu- lating immune function perhaps through the diet warrant investigation.

The immune system is comprised of two responsive component systems that work in a sequential, complementary manner to prevent infections. These are termed the innate and the acquired immune systems. The innate immune system is comprised of immediately available mechanisms that fight the first stages of infection associated with pathogens such as bacteria. Via implementation of this initial defensive response of the innate system, there is time gained to allow the acquired system to develop an antibody response against a specific pathogen to mediate specific cytotoxic effects for protection.

Innate immunity prevents infection by targeting general properties of pathogens as opposed to modifying cellular structure in response to the type of pathogen (Carroll and Forsberg 2007; Murphy et al. 2008). The phagocytic cells of the innate immune system identify pathogens by recognizing distinct pathogen-associated molecular patterns (PAMPs). For example, lipopolysaccharide from the gram-negative cell wall of bacteria is recognized by Toll-like receptors 2 and 4 on the surface of innate immune cells. Binding of PAMPs to Toll-like receptors initiates killing mechanisms by the neutrophils and macrophages.

The acute-phase protein (APP) response is stimulated by the release of proinflammatory cytokines (IL-1, IL-6 and TNF-α) from macrophages and monocytes at the site of inflammation or infection. The initial release of proinflammatory cytokines is amplified by their para- crine actions, which causes their further release and eventually results in a systemic increase in cytokines. The proinflammatory cytokines, such as IL-1, IL-6 and TNF-α, mediate the hepatocyte production and secre- tion of the APPs. The APPs have various biological functions, such as proteinase inhibitors, enzymes,
coagulation proteins, metal-binding proteins and transport proteins. The APPs become important mediators of immunological functions and play an active role in pathogen trapping, tissue repair and remodelling. Consequently, they may be secreted in a dynamic manner during the periparturient period of lactating dairy cows that undergo marked changes in uterine involution, exposure to pathogens and major potential alterations in microbial rumen function because of transitional changes in feed intake.

The second arm of the immune system is termed the acquired or adaptive system and is characterized by the production of antibodies and effector T cells that target cells displaying specific antigens. Pathogens can be phagocytosed and digested by antigen-presenting cells (e.g., macrophages, B lymphocytes and dendritic cells). Digested pieces of pathogens are presented on the surface of the antigen-presenting cells to two large groups of T cells characterized by a co-receptor CD8 (i.e., these cells differentiate into CD8 cytotoxic T cells) or the co-receptor CD4 T effector cells defined as TH1, TH2 or TH17. The TH1 cells can further activate macrophages to increase intracellular bacterial death; TH2 cells can activate antigen-specific B lymphocytes that further differentiate into plasma cells that secrete specific antibodies or stimulate clonal expansion of a B-cell lineage (i.e., memory cells that provide immune memory for future pathogenic insults). The TH 17 cells induce localized epithelial and stromal cells to produce chemokines that recruit neutrophils to sites of infection early in the adaptive immune response. Depending upon the antigen-presenting cell (e.g., macrophages, B lymphocytes and dendritic cells), a variety of cytokines may be secreted that cause differential development of effector T cells (i.e., IL-12 and interferon-gamma induce TH1 cells; IL-4 induces TH2 cells; TGF-β induces TH17 cells).

Sheldon et al. (2009) integrated the mechanisms of infection and immunity in the female reproductive tract of cattle that ultimately regulates reproductive efficiency. Cows post-partum that have uterine infections (metritis) are less likely to ovulate because growth of the dominant follicle is slower and there are lower concentrations of plasma estradiol. Furthermore, intrauterine infusion of Escherichia coli endotoxin suppressed the pre-ovulatory surge of LH in heifers. If cows do ovulate, then endometrial cytokines may alter steroidogenesis of luteal cells that contribute to a lower secretion of progesterone or increase the PGE2/PGF2 ratio that may extend the luteal phase.

Nutraceutical Regulation of Post-partum Reproductive Function

A nutraceutical is defined as a product isolated or purified from feeds that is demonstrated to have a physiological benefit or provide protection against chronic disease. A nutritional approach to improve post-partum uterine health is a conducive alternative to reduce the use of antibiotics and provide a nutrient management programme to the platform of preventive medicine. This would be a pro-active strategy conducive to producers and the public. Immunosuppression in the pre- and post-partum periods is associated with decreased dry matter intake and occurrence of NEBAL. Therefore, it is quite logical to increase energy density of the diet. Feeding a high-energy diet (i.e., enriched in starch and non-fibre carbohydrates) vs a low-energy diet failed to alter TNF-α production ex vivo of whole blood in response to E. coli lipopolysaccharide and did not alter CD14 + monocyte number and CD14 expression level on monocytes (Ronvet et al. 2005).

Differential feeding of antioxidants minerals, supplemental lipids and carbohydrates during the transition, post-partum and breeding periods are potential strategies to accommodate metabolic adaptations associated with restoration of post-partum health and optimization of fertility. Based upon a clearer understanding of the metabolic and hormonal factors regulating the adaptations of cows as they transition from pregnancy to pregnancy, with intervening peripartum and lactation periods, several approaches of targeted feeding to optimize lactating cow health and performance have been undertaken. These include feeding of by-pass fat supplements (Santos et al. 2008) that increase both energy density and potent polysaturated fatty acids that can exert either pro- or anti-inflammatory effects. This has been further extended to sequential feeding of high carbohydrate/insulin-stimulating to high fat/insulin-depressing diets (Garnsworthy et al. 2009).

Feeding organic selenium

A nutraceutical management strategy involves feeding organic selenium (Se; Se-yeast [SY; Sel-Plex®, Alltech, Nicholasville, KY, USA]) during the pre- and early post-partum periods. Glutathione peroxidase (Se-GPx) is one of the selenoenzymes capable of protecting the cell against oxidative injury. The Se-GPx catalyses the reduction of H2O2 to water and organic hydroperoxides to alcohols while utilizing the peptide glutathione as a cofactor. It is important for example that neutrophils provide a high oxidizing intracellular environment to kill phagocytosed bacteria, but it is essential that neutrophils regulate the balance between reactive oxygen metabolites (superoxide [O2·−] and hydrogen peroxide [H2O2]) in order not to damage the cell organelles and membranes leading to its death.

An experiment was conducted that fed organic selenium as SY during the pre- and post-partum periods (Silvestre et al. 2006a,b). Objectives were to evaluate effects of organic Se on pregnancy per AI at the first and second post-partum services, uterine health, immune status and milk yield during the summer heat stress period. Cows were assigned at 23 ± 8 days pre-partum to diets of organic Se ([SY; n = 289] or inorganic sodium selenite [SS; n = 285]) fed at 0.3 mg/kg of diet dry matter for > 81 days post-partum. Rectal temperature was recorded each morning for 10 days post-partum. Vaginoscopies were performed at 5 and 10 days post-partum. Cows were programmed for timed AI for the first and second post-partum inseminations following a pre-synchronized Ovsynch protocol at first AI, followed by resynchronization starting 7 days before pregnancy diagnosis on day 30 after AI. Pregnancy was diagnosed by transrectal ultrasonography conducted at 30 days after first AI. Strategic blood sampling deter-
tracted anovulatory status at Ovsynch and ovulatory response after the first timed AI. The pregnancy per AI at second service was determined by transrectal palpation at approximately 42 days after insemination.

Blood was sampled for Se (n = 20 cows/diet) at −25, 0, 7, 14, 21 and 37 days post-partum. Plasma Se increased in SY-fed cows (0.087 > 0.069 ± 0.004 μg/ml; p < 0.01). Diet altered frequency of multiparous cows detected with >1 event of fever (rectal temperature >39.5°C; SY, 13.3% [25/188] < SS, 25.5% [46/181]; p < 0.05) but the SY effect was not observed in primiparous cows which had a much higher frequency of fever (40.5%). Mean vaginoscopy scores of 5 and 10 days post-partum for SY and SS groups were clear (47.1% vs 35.0%), mucopurulent (43.4% vs 47.8%) and purulent (9.3% vs 17.1%) discharge scores, respectively (p < 0.05). The frequency of cows with a purulent discharge was reduced, and the proportion of cows with a clean discharge was increased. This is additional support that feeding the organic selenium (i.e., SY) improved the uterine environment.

Measures of innate immunity were evaluated by phagocytic and oxidative burst capacity of neutrophils in whole blood using a dual colour flow cytometric method. Samples were collected from a subsample of 36 cows at −26 and 40 cows at 0, 7, 14, 21 and 37 days post-partum and analysed for neutrophil function. Adaptive immunity (ability to induce an antibody response) was monitored with anti-IgG to ovalbumin following vaccination with 1 mg of ovalbumin dissolved in either an E. coli J5 vaccine at −60 and −22 ± 6 days pre-partum or in PBS and adjuvant at calving. Serum samples were collected on days of immunization and at 21 and 42 days post-partum.

Percentage of gated neutrophils that phagocytized E. coli and underwent oxidative burst capacity of neutrophils in whole blood using a dual colour flow cytometric method. Samples were collected from a subsample of 36 cows at −26 and 40 cows at 0, 7, 14, 21 and 37 days post-partum and analysed for neutrophil function. Adaptive immunity (ability to induce an antibody response) was monitored with anti-IgG to ovalbumin following vaccination with 1 mg of ovalbumin dissolved in either an E. coli J5 vaccine at −60 and −22 ± 6 days pre-partum or in PBS and adjuvant at calving. Serum samples were collected on days of immunization and at 21 and 42 days post-partum.

Percentage of gated neutrophils that phagocytized E. coli and underwent oxidative burst did not differ between dietary groups at −26 days post-partum (44.6 ± 4.6%). For subsequent samples, a diet by parity by day interaction was detected (p < 0.05); namely, SY improved neutrophil function at parturition in multiparous cows (42 ± 6.14% vs 24.3 ± 7.2%) but did not differ at 7, 14, 21 and 37 days post-partum. In primiparous cows, neutrophil function was enhanced at 7, 14 and 37 days post-partum (53.9 vs 30.7%, 58.6 vs 41.9%, and 53.4 vs 34.8%, respectively; pooled SE = 6.8%). Neutrophil function was suppressed in primiparous cows at the time of parturition and restored with SY supplementation at 7–14 days post-partum. In spite of an increase in neutrophil function on the day of parturition, the multiparous cows did not have a post-partum restoration in neutrophil function until 21–37 days post-partum for both SY and SE treatments. Organic Se improved phagocytosis and killing activity of neutrophils in both multiparous and primiparous cows. However, the primiparous cows seemed to be more responsive in that SY stimulated neutrophil function on 7 and 14 days post-partum, whereas SY stimulation in multiparous cows was evident on only the day of parturition.

Anti-IgG to ovalbumin did not differ between dietary groups at −60 and −22 days pre-partum (0.18 ± 0.01 and 0.97 ± 0.04 optical density). A diet by parity interaction was detected because IgG concentration did not differ between diet groups in primiparous cows across all days of sample collection (1.37 ± 0.08, 1.43 ± 0.07; p > 0.10); however, it was higher in SY multiparous cows at 21 and 42 days post-partum (1.91 ± 0.1 > 1.24 ± 0.07, 1.44 ± 0.7 > 0.99 ± 0.07; p < 0.01). Thus, our measurement of adaptive immunity was improved in multiparous dairy cows in response to SY but not in primiparous cows. Our findings indicated that feeding Se as organic Se beginning at 26 days pre-partum elevated plasma Se concentrations, increased neutrophil function at the time of parturition, improved immuno-responsiveness in multiparous cows, and improved uterine health during summer in a region (Florida) that is typically Se deficient.

Diet failed to alter first-service pregnancy per AI on day 30 after insemination (SY, 24.9% [62/249] and SS, 23.6% [62/262]) or pregnancy losses between 30 and 55 days after insemination (SY, 39.3% and SS, 37.1%). These low probabilities of pregnancy and high embryonic losses were typical of cows managed during the summer heat stress period of Florida. Diet did indeed alter second-service pregnancy per AI [SY, 17.1% (34/199) vs SS, 11.3% (24/211); p < 0.03]. We hypothesize that cows of the SY group were better able to re-establish an embryotrophic environment at second service following either early or late embryonic losses. For example, cows presented for second service may not have been pregnant to the first service by 30 days at the ultrasound diagnosis or were pregnant and underwent embryonic loss and required a second service. Indeed, pregnancy per AI to the second service for cows that had lost an embryo was 22.7% (5/22) for the SY vs 4.2% (1/24) in the control or SS group (p < 0.09); pregnancy per AI to the second service for cows that were not diagnosed pregnant at first service and were re-inseminated did not differ 16.3% (29/177) for SY vs 12.3% (23/187) for SS.

Under the conditions of selenium deficiency during the summer heat stress season, selenium yeast fed in the ration (Sel-Plex®; 0.33 mg/kg), beginning at 26 days pre-partum, elevated plasma Se concentrations, increased neutrophil function at the time of parturition, improved immuno-responsiveness in multiparous cows, improved uterine health and increased second-service pregnancy per AI. A replicated experiment conducted concurrently in California failed to detect effects of organic selenium on immune function, uterine health and pregnancy per AI because of a high background level of selenium in the diets (Rutigliano et al. 2008).

**Feeding ruminally inert calcium salts of polysaturated fatty acids**

Feeding supplemental fatty acids (e.g., C18:2n-6 linoleic acid) during the periparturient period is a strategy to increase the energy density of the diet, but fatty acids could act also as a precursor for the biosynthesis of prostaglandins of the two series that exert a pro-inflammatory effect that may benefit post-partum health of the cow. After the main post-partum period of immune-suppression and uterine involution, it may be reasonable to feed fatty acids (e.g., C20:5n-3 eicosapentaenoic) that lead to suppression in the biosynthesis of...
inflammatory molecules. This would reduce possible residual inflammatory responses in the uterus associated with carry-over effects of subclinical endometritis or as reported in repeat breeder cows, and also reduce the potential luteolytic peaks at the time that the conceptus is suppressing PGF$_{2\alpha}$ secretion to maintain the CL for pregnancy maintenance.

In a recent Florida study, Silvestre et al. (2008a,b) and Silvestre (2008c) randomly allocated cows (n = 1582) into two experimental transition diets beginning at approximately 30 days before the expected date of parturition and continued until 30 days post-partum. After 30 days, cows within each transition diet were allocated randomly into the experimental breeding diets that were fed until 160 days post-partum. Experimental transition and breeding diets differed only in the source of supplemental fatty acids. Transition diets consisted of calcium salts of palm oil (PO; EnerGII; 47% C16:0 palmitic) or calcium salts of safflower oil (SO; Prequel 21; 64% C18:2n-6), and breeding diets consisted of calcium salts PO or calcium salts of fish oil (FO, StrataG; 11% of C20:5n-3 + C22:6n-3 docosahexaenoic acid). All calcium salts of fatty acids contained 82% of fatty acids and were manufactured by Virtus Nutrition (Corcoran, CA, USA) and supplemented at 1.5% of the dietary dry matter. Diets were formulated to meet or exceed NRC (2001) nutrient requirements for net energy of lactation, crude protein, fibre, mineral and vitamins and fed to obtain intakes of 200 and 400 g/day of calcium salts of FAs, for pre- and post-partum cows, respectively. Diets were fed as a total mixed ration twice daily targeting 5% refusals.

Blood samples were collected from subsamples of cows at enrolment (n = 18) and in the post-partum period (n = 47) at parturition (i.e., 2.8 ± 1.8 h after delivery), 4 and 7 days post-partum for analyses of neutrophil activity and abundance of adhesion molecules using flow cytometry. Number of bacteria (E. coli and Staphylococcus aureus) phagocytized per neutrophil was greater (p < 0.01) for cows in the SO at 4 days post-partum associated with a greater (p < 0.05) intensity of H$_2$O$_2$ produced per neutrophil at 4 and 7 days post-partum in cows fed SO fat supplement (Fig. 2). Neutrophil abundance of L-selectin (arbitrary units) was increased (p < 0.01) after parturition (752.75) and was greater (p < 0.05) at 4 and 7 days post-partum for SO (1205.3 and 1134.2; SE = 96.2) compared with PO (862.5 and 892.8; SE = 95.8)-supplemented cows, respectively. No effects of diet or day were observed in (862.5 and 892.8; SE = 95.8)-supplemented cows, (1205.3 and 1134.2; SE = 96.2) compared with PO (752.75) and was greater (p < 0.05; Fig. 3) for SO (0.034 OD and 0.07) to be greater for cows fed SO (0.07) to be greater for cows fed SO (9.16 ± 0.73) compared with PO (7.16 ± 0.73) supplements.

Blood samples were collected from PO (n = 15) and SO (n = 17) cows daily from parturition to 10 days post-partum and continued thrice weekly until 35 days post-partum for analyses of plasma concentrations of APPs (i.e., haptoglobin and fibrinogen), respectively. Plasma concentrations of haptoglobin and fibrinogen were higher (p < 0.05; Fig. 3) for SO (0.034 OD and 248.8 mg/dl, respectively) compared with PO (0.02 OD and 205.3 mg/dl, respectively). The haptoglobin and fibrinogen response for the 32 cows included six cows that experienced metritis during the first 10 days post-partum. When these cows were deleted from the analyses, the higher basal concentrations in cows fed the SO diet were higher (p < 0.01) than those fed the PO diet. Consequently, this amplification of acute protein concentrations in plasma possibly reflects a greater sensitivity and stimulus in chronic secretion manifested by the linoleic enriched diet that perhaps play an important role in tissue repair and remodelling such as uterine regression during the period up to 30–35 days.

Although feeding SO improved aspects of innate immunity (i.e., neutrophil function and acute-phase

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**Fig. 2.** Least squares means (± SE) of neutrophil mean fluorescence intensity (MFI) for number of bacteria phagocytized per neutrophil (bars) and for intensity of H$_2$O$_2$ produced per neutrophil (lines) in whole blood stimulated with E. coli (a) or S. aureus (b). Cows were supplemented with palm oil (PO; n = 23) or safflower oil (SO; n = 24) during the transition period. *p < 0.05 and **p < 0.01.
response), SO and PO cows had similar frequency distributions of mucupurulent (10.0% and 14.4%) and purulent (30.4% and 28.0%) cervical discharges evaluated once between 8 and 10 days post-partum.

Collectively, feeding a rumen-inert source of fatty acids rich in linoleic fatty acid, beginning approximately 3 weeks pre-partum to 30 days post-partum, changed fatty acid profiles of tissues placing the cow in a "pro-inflammatory state". Such a state involves a lower threshold for initiation of an inflammatory response and increased sensitivity of cells upon stimuli. Inflammation is the first step for initiation of an immune response.

Cows at 43 days post-partum had their ovulation synchronized with the Presynch–Ovsynch protocol for first AI. All cows received a controlled internal drug-releasing device (CIDR) containing 1.38 g of progesterone at 18 days after the first timed AI followed 7 days later by removal of the CIDR device and an 100 μg injection of GnRH. At 32 days after first AI, cows were examined for pregnancy by transrectal ultrasonography to identify presence of an embryo and an embryonic heart beat. Non-pregnant cows were injected with 25 mg of PGF$_{2\alpha}$ and then injected with 100 μg of GnRH 56 h later. Insemination was performed 16 h after the last GnRH for the second service. Cows were examined for pregnancy by per-rectum ultrasonography at 32 days after second service. All cows diagnosed pregnant after first and second services were re-examined by per-rectum ultrasonography at 60 days after insemination to determine pregnancy losses.

Transition, breeding and interaction of diets did not affect pregnancy per AI at 32 and 60 days after the first AI (Table 1). However, pregnancy loss from day 32 to 60 of gestation was less (p < 0.05) in FO- compared with PO-supplemented cows during the breeding period. For second service, both breeding diet and a transition by breeding diet interaction (p < 0.05) were detected for the 60 day pregnancy per AI response in which FO stimulated pregnancy rate per AI but the response to FO was greater in cows fed the SO transition diet (Table 1).

Yet, there was no increase in pregnancy per AI in cows fed the PO breeding diet regardless of transition diet.

Neutrophil cytokine production and profiles of FAs were measured in a subsample of cows (n = 28) at 85 days post-partum at a time when cows were fed the breeding diets (i.e., PO or FO) for approximately 35 days. Culture procedures for neutrophils were as described for the sampling at 35 days post-partum described previously. Mean concentration of TNF-α, but not IL-1β, in supernatants of isolated neutrophils was less (p < 0.01) for cows supplemented with FO (42.55 pg/ml and 0.6 ng/ml) compared with PO (82.68 pg/ml and 0.78 ng/ml) in response to lipopolysaccharide, respectively. Concurrently, The neutrophil content of C20:5n-3 (1.5% and 0.30%), C22:5n-3 (3.48% and 2.33%) and C22:6n-3 (1.65% and 0.11%) fatty acids were all greater (p < 0.01) in cows fed FO compared with PO diets, respectively. Consequently, the ratio n-6

![Graph](Image)

Fig. 3. Least squares means (±SE) for plasma concentration of haptoglobin (a) and fibrinogen (b) for cows fed calcium salts of palm oil (n = 15) or safflower oil (n = 17) during the pre-partum period (at least 20 days) to 35 days post-partum. *p < 0.01

Table 1. First- and second-service pregnancies per artificial inseminations at 32 and 60 days after insemination and pregnancy loss for experimental diets

<table>
<thead>
<tr>
<th>Diets</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>First service % (n = 15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 32</td>
<td>38.7 (107/276)</td>
<td>35.8 (96/268)</td>
<td>39.1 (103/263)</td>
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<tr>
<td>Day 60</td>
<td>33.7 (92/273)</td>
<td>29.7 (79/266)</td>
<td>37.0 (97/262)</td>
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<tr>
<td>Loss</td>
<td>11.5 (12/104)</td>
<td>15.9 (15/94)</td>
<td>4.9 (3/102)</td>
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<tr>
<td>Second service % (n = 15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 32</td>
<td>27.7 (43/155)</td>
<td>26.7 (41/154)</td>
<td>30.3 (44/154)</td>
</tr>
<tr>
<td>Day 60</td>
<td>21.0 (38/152)</td>
<td>22.5 (34/151)</td>
<td>27.3 (39/143)</td>
</tr>
<tr>
<td>Loss</td>
<td>5.0 (2/40)</td>
<td>10.0 (4/38)</td>
<td>7.1 (3/42)</td>
</tr>
</tbody>
</table>

*Cows at 43 days post-partum had their ovulation synchronized with the Presynch–Ovsynch protocol for first AI. All cows received a controlled internal drug-releasing device (CIDR) containing 1.38 g of progesterone at 18 days after the first timed AI followed 7 days later by removal of the CIDR device and an 100 μg injection of GnRH. At 32 days after first AI, cows were examined for pregnancy by transrectal ultrasonography to identify presence of an embryo and an embryonic heart beat. Non-pregnant cows were injected with 25 mg of PGF$_{2\alpha}$ and then injected with 100 μg of GnRH 56 h later. Insemination was performed 16 h after the last GnRH for the second service. Cows were examined for pregnancy by per-rectum ultrasonography at 32 days after second service. All cows diagnosed pregnant after first and second services were re-examined by per-rectum ultrasonography at 60 days after insemination to determine pregnancy losses.

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(C18:2 + C22:4) / n-3 (C18:3 + C20:5 + C22:6) of fatty acids was less (p < 0.01) in cows fed FO (3.75) compared with PO (8.48). These responses indicate that at the time of conducting inseminations, neutrophils available to reproductive tissues were under a greater anti-inflammatory response, which may complement the immune-suppressive effects of the conceptus in early pregnancy.

Collectively, the strategic feeding of pro-inflammatory and anti-inflammatory calcium salts of polyunsaturated fatty acids has clear effects on post-partum immune and reproductive functions. The threshold for triggering an immune response (i.e., creating a pro-inflammatory state that can respond greatly upon challenge) because of feeding FAs precursors of pro-inflammatory eicosanoids can benefit the post-partum immunity (innate immunity and secretion of APPs) of dairy cows. Conversely, following a health transition period, supplementation of FO can increase the threshold for triggering an anti-immune response during the breeding period by exerting an anti-inflammatory state that may attenuate immune responses in early pregnancy to benefit pregnancy rate and survival of embryos.

The role of conjugated linoleic acid (CLA) isomers on reproductive function in dairy cows has been investigated. These bioactive fatty acids are produced as intermediates during rumen biohydrogenation of polyunsaturated fatty acids, and certain CLA isomers cause a reduction in milk fat synthesis. This may alleviate energy demands during early lactation. Castañeda-Gutiérrez et al. (2005) evaluated lactational performance, net energy balance and reproductive response of dairy cows supplemented with two doses of CLA from 2 weeks before expected calving until 9 weeks post-partum. Experimental responses were indicative that CLA may enhance an earlier occurrence of ovulation post-partum and increase pregnancy rate independent of energy balance. Supplementation with greater amounts of trans-10, cis-12 CLA increased plasma concentrations of IGF-I and progesterone, while tendency to increase the estradiol / progesterone ratio in ovarian follicles but did not alter uterine prostaglandin response to oxytocin challenge. The mechanism through which CLA affects reproduction may involve improved ovarian follicular and luteal steroidogenesis and increased circulating concentrations of IGF-I (Castañeda-Gutiérrez et al. 2007). A multi-study verified an improvement in reproductive performance with optimal supplementation of trans-10, cis-12 CLA (i.e., 10.1 g/day) that was associated with a reduction in the interval to first post-partum ovulation and a decreased median time to pregnancy of 34 days in high-producing cows (de Veth et al. 2009).

Juchem et al. (2010) evaluated the effect of feeding calcium salts of PO or a blend of C18:2n-6 and trans-octadecenoic fatty acids to cows both pre- and post-partum. Risk of uterine disease was similar between sources of fatty acids, but cows fed the blend of C18:2n-6 and trans-octadecenoic fatty acids had a reduced risk of puerperal metritis (8.8% vs 15.1%). Cows fed unsaturated fatty acids were 1.5 times more likely to be pregnant at 27 or 41 days after AI compared with cows fed PO. Improvements in pregnancy when cows were fed Ca salts of a mix of C18:2n-6 and trans-octadecenoic FA were supported by increased fertilization (p ≤ 0.10) and embryo quality (p ≤ 0.02) in non-supervolatized lactating dairy cows (Cerri et al. 2009a). Feeding fat to dairy cows improves post-partum health and fertility. The type of fatty acid fed appears to have differential effects on immune and fertility (i.e., earlier post-partum restoration of ovarian activity, pregnancy per AI and pregnancy losses).

Sequential feeding of glucogenic–lipogenic diets

A series of investigations, integrating nutrition, metabolism and fertility, indicates that feeding a high-starch diet will enhance circulating insulin concentrations, stimulate follicle development and increase incidence of ovulations prior to 50 days of lactation (Gong et al. 2002 and Garnsworthy et al. 2008a). However, diets designed to increase plasma insulin concentration had negative effects on blastocyst development following in vitro maturation and fertilization (Fouladi-Nashtha et al. 2005). In contrast, a high-fat diet increased blastocyst rate compared to a low-fat diet (Fouladi-Nashtha et al. 2007) and lowered insulin in plasma (Garnsworthy et al. 2008b). When all cows were fed supplemental fats (ruminal inert, enriched linoleic, enriched linolenic), no differences in blastocyst development were detected among the different fat diets (Fouladi-Nashtha et al., 2009). Consequently, fat feeding may benefit embryo development and also decrease insulin secretion.

This has led to the sequential feeding of a glucogenic diet to stimulate both follicle development and onset of ovarian cycles and then feeding a lipogenic diet during the breeding period to attenuate insulin secretion and increase fatty acid availability (Garnsworthy et al. 2009). The ultimate objective of practical interest was to evaluate dietary feeding sequences on pregnancy outcomes. Lactating cows were fed from parturition until a first rise in progesterone either a diet (H; n = 30) to promote high insulin concentrations in plasma (i.e., high starch and low fat contents) or a diet (L; n = 30) to promote low insulin concentrations in plasma (i.e., low starch and high fat contents). At the time of the first-detected progesterone rise, cows in each diet group (H and L) were allocated randomly to receive either the H or L diets until 120 days post-partum so that there were four dietary sequence treatments (HH, HL, LH and LL; n = 15 per group). Treatments did not affect dry matter intake, milk yield and metabolizable energy balance. Plasma insulin concentration was elevated in cows fed on H compared with cows fed on L. Treatment did not affect days to first progesterone rise, first oestrus or first insemination. At 120 days post-partum, 27% of cows on each of treatments HH, LL and LH were pregnant compared to 60% of the cows on treatment HL (p < 0.02). Pregnancy response to sequential feeding is exciting and brings to bear several potential implications and/or questions. The investigators noted that the experiment was performed under carefully controlled research conditions and that larger studies will be required to test the concepts under field conditions.
conditions with greater numbers of cows before they can be applied to commercial practice. High insulin concentrations fed early post-partum may stimulate ovarian follicle development but it did not result in any earlier occurrence of ovulation, oestrus or timing of first insemination. However, the initial stimulation of the reproductive system because of enhanced exposure of insulin may exert a carry-over effect on responsiveness of the cows to high fat feeding (HL). In HL fed cows, perhaps insulin-primed follicles have oocytes that are more responsive to maturation and fertilization processes, and subsequent blastocyst development and/or embryo survival are enhanced by the high fat feeding. The concept that an early post-partum glyceogen diet may influence subsequent response to fat feeding can be extended to the specific class of fatty acids that are predominant in the fat (e.g. omega 3 or omega 6 polyunsaturated fatty acids). The recent observations that linolenic acid (Marei et al. 2009) added during in vitro maturation of bovine cumulus-oocyte complexes increased subsequent in vitro fertilization and blastocyst development would support a biological response that may potentially benefit pregnancy per AI under field conditions with targeted feeding of specific lipids.

The integration of the disciplines of ruminant nutrition, reproductive physiology, immunology and clinical medicine has the potential to provide useful alternatives to improve post-partum health and fertility in dairy cows in a scenario of increasing milk production.

Conflict of interest

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Author contributions

W.W. Thatcher: Wrote the manuscript, principle investigator on several of the experiments, data analysis and interpretation; J.E.P. Santos principle investigator on several of the experiments, data analysis, interpretation, and critical revision of the manuscript; E.T. Silvestre participated in data acquisition and analysis, and interpretation of data; I.H. Kim involved in data acquisition and review of literature; C.R. Staples involved in diet formulations, analysis of dietary components and data analysis.

References


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