# Applying nutrition and physiology to improve reproduction in dairy cattle

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The establishment and maintenance of pregnancy in lactating dairy cows is a complex biological event that is influenced by a multitude of factors, from the reproductive biology of the cow to managerial aspects of the dairy farm. It is often mentioned in the scientific literature that fertility in dairy cows has declined concurrent with major advances in milk production. Some of this decline is attributed to the negative genetic correlation between milk production and reproduction. In the United States, yearly production per cow has increased steadily at a rate of 1.3% in the last decade and it is likely that this trend will continue in the years to come. At this rate, the average cow in the United States will be producing over 14 tons of milk per year in 2050 and technologies will have to be developed to allow these cows to reproduce to maintain the sustainability of dairy production. Despite high production, it is not uncommon for dairy herds with rolling herd averages for milk yield above 11,000 kg to overcome the challenges of reproduction and obtain satisfactory reproductive performance. Among other things, those herds have been able to mitigate some of the mechanisms that suppress reproduction in dairy cows such as extended postpartum anovulatory period, poor estrous detection, low pregnancy per insemination and, to a lesser extent, the high pregnancy loss. The success of those farms comes from an integrated approach to fertility that includes adequate cow comfort, elaborated transition cow management and nutrition, aggressive postpartum health monitoring program with preventative and curative measures to mitigate the negative effects of diseases on reproduction, and a sound reproductive program that includes manipulation of the ovarian cycle to allow for increased insemination rate. More recently, introduction of fertility traits in selection programs have created new opportunities for improved reproduction without neglecting economically important production traits.

## Introduction

Reproductive efficiency is a major component of economic success in dairy herds. The establishment of a pregnancy as well as the loss of pregnancy are extremely valuable particularly

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because they determine milk production per day of calving interval and the risk of a cow to be removed from the herd (De Vries, 2006). Unfortunately, fertility in dairy cows involves extremely complex biological events that are influenced by a multitude of environmental and biological factors, as well as the genetic makeup of the dairy cow.

The physiological and environmental stresses faced by high-producing dairy cows under the current production systems compromise estrous detection and their ability to conceive and deliver a viable offspring. Some of the limitations to high fertility are related to the reproductive biology of the high-producing cow which, through homeorrhetic controls, has resulted in adaptations to the increased nutrient needs for milk synthesis and metabolic rate. Although reproduction is critical for perpetuation of the species, from the individual point of view, reproduction is considered an expendable process that has low priority under nutrient restrictions (Wade and Jones, 2004). Considering the nutrient requirements of a high-producing dairy cow which often consumes 4 to 6 times its maintenance needs, it is plausible to suggest that nutrient extraction by the ovaries and the early pregnant uterus is negligible. Nonetheless, signals from splanchnic and adipose tissues coordinate the release of gonadotropins and the support to resumption of ovulation and pregnancy, such that under periods of nutrient restriction, ovulation is impaired and establishment and maintenance of pregnancy compromised. Some of the many limitations to high fertility in dairy cows include: incidence of periparturient diseases, anovulation, reduced estrous behavior, and compromised embryo quality and development; managerial and environmental factors such as nutrition, comfort offered by facilities, and thermal stress; and genetics of the cow. Because of these factors and the complexity of reproduction, the solutions for improving fertility will include both short- and long-term components that address the biology of the cow as well as the environmental events and managerial procedures critical for successful reproduction. In spite of the link suggested between high-production and compromised fertility, it is unlikely that the rate of increase in milk yield per cow will diminish in the following years or decades. In fact, the ongoing trend for increasing milk production and the need for improvements in efficiency of use of resources with higher producing cows (Capper et al., 2009) will make the production of today's cow to be overshadowed by that of future generations. The intent of this manuscript is to present some of the current strategies to overcome poor reproduction in dairy herds.

## The paradox of milk yield and fertility

Almost every article on dairy cattle fertility in the scientific literature mentions that the increment in milk production observed in the last 50 years, as consequence of genetic selection and improvements in nutrition and management, has coincided with a corresponding decline in fertility. Geneticists have demonstrated a negative genetic correlation between milk yield and fertility in dairy cattle (Roxström et al., 2001; Hansen et al., 1983), although some have also suggested that the low heritability of fertility traits precludes major antagonisms between selection for milk yield and reduction in reproduction (Hansen, 2000). Because environmental factors and lactation have major impacts on the reproductive efficiency of dairy herds, it is likely that the negative associations between the genetics for production traits and fertility are only observed after the onset of lactation and the consequent shifts in nutrient partition to favor the mammary gland (Bauman and Currie, 1980).

The genetic potential for milk production today for the dairy cow was established in 1998 when LA-Foster Blackstar Lucy 607 completed a 365-day lactation producing 34,144 kg of milk, more than 750 kg of fat, and almost 980 kg of protein (Holstein World, 1999). This was Lucy's fifth lactation at the age of 6 years and 3 months, thereby indicating that this phenomenal cow was able to reproduce on a yearly basis in spite of the massive production of milk. Lucy's production at peak was 116 kg/d, which is the equivalent to 2.5 to 3 times the peak production of the average high-producing Holstein cow today. It is obvious that, today, Lucy is an aberration of the population, but it is also clear that the right genetic selection and environment can result in tremendous production. In fact, studies conducted at the University of Minnesota with a control and a selected line of Holstein cows for milk yield starting in 1964 clearly demonstrated the marked increase in milk yield in 1998 for an entire lactation (10,959 vs. 6,454 kg/year; Hansen, 2000). The selected line of cows has not experienced a depression in fertility, although health traits have been compromised (Hansen et al., 2000).

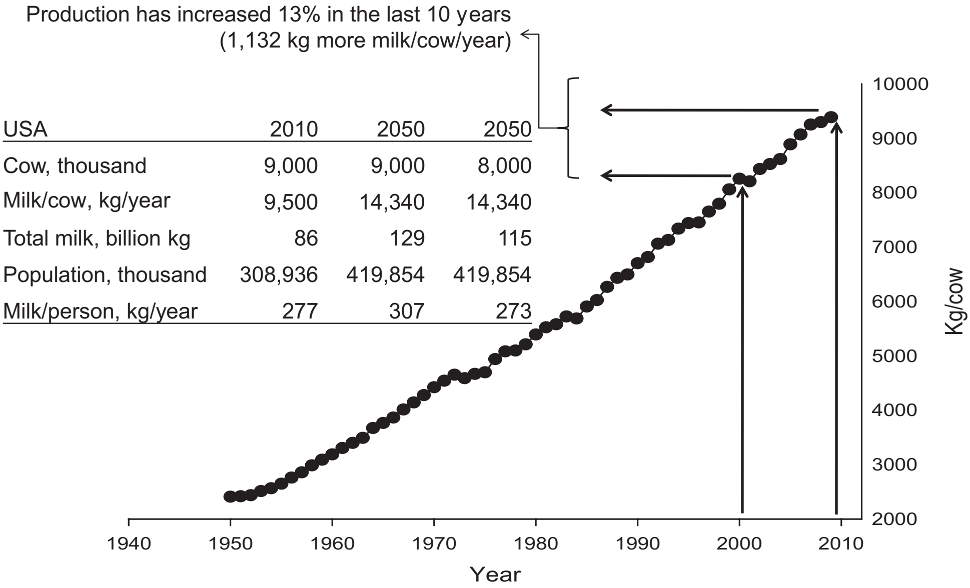
### *The rationale for increased production*

The major reason for emphasis on production traits is because most of the cash receipts of a dairy farm come from sales of milk, and only a minor portion results from sales of animals, even those destined for dairy production. Using data 2000 to 2007 from two dairy farms with 1,500 cows, each with an annualized mortality of lactating cows of 5.3%/year and an average milk yield of 12,400 kg/cow/year, approximately 88% of the cash receipts were obtained through the sales of milk, 9.1% from sales of prepartum or early lactation cows for dairy purposes, 2.4% from sales of cows destined to slaughter, and only 0.4% from sales of newborn male calves. Historical facts and conventional wisdom maintain that growth and consolidation resulting in large family-owned farms seem to be the present and future of the dairy industry in the United States (LaDue et al., 2003) and other leading dairy countries. In fact, the number of dairy farms with fewer than 100 cows will decline by 92% between 2000 and 2020 (LaDue et al., 2003). This consolidation poses new challenges for reproduction; one of them is the ratio of cows/personnel, thereby resulting in less individual attention and a more group-basis approach for health and reproduction activities. This consolidation is concurrent with the steady increase in the yearly milk production per cow in the United States (Fig. 1). Assuming the same increase in yearly milk yield per cow of 1.3% and the projections for population growth in the United States from the United States Census Bureau (http://www.census.gov/population/www/), it will be possible to reduce the dairy cow population by 11.1% in the next 40 years and still maintain the same milk availability per capita of approximately 270 kg/year.

In addition to the economic component, adoption of technology and modernization of dairy practices to enhance production have substantially reduced the resources needed to produce milk. Capper et al. (2009) estimated that a farm in the US today produces the same amount of milk with 21% of the animals, 23% of the feedstuffs, and 10% of the land required in 1944. Greater milk production per cow dilutes the needs for maintenance and the trend for increased milk per cow will likely continue given the need to sustain a growing population with no additional use of land and other natural resources.

### *Genotype and fertility*

It is no question that genotype and environment need to be matched to optimize production and fertility (Macdonald et al., 2008). Cows with greater potential for production but with restricted nutrient intake suffer greater body weight losses (Macdonald et al., 2008), which depresses fertility (Santos et al., 2009). In fact, cows of greater genetic potential subjected to grazing conditions had reduced pregnancy at the end of the breeding period (Macdonald et al., 2008). It was later demonstrated that cows selected for higher milk production suffered uncoupling of the somatotropic axis characterized by reduced expression of growth hormone (GH)



| USA | 2010 | 2050 | 2050 |
|---|---|---|---|
| Cow, thousand | 9,000 | 9,000 | 8,000 |
| Milk/cow, kg/year | 9,500 | 14,340 | 14,340 |
| Total milk, billion kg | 86 | 129 | 115 |
| Population, thousand | 308,936 | 419,854 | 419,854 |
| Milk/person, kg/year | 277 | 307 | 273 |

**Fig. 1**. Continuous increase in the yearly milk production per cow in the United States from 1950 to 2009 and projected production and milk availability per person based on the steady increase of 1.3%/year and the current (2010) and expected population in 2050. It is projected that the average cow in the United States will produce 14,340 kg/year in 2050, which will allow for a reduction in the dairy cow population from 9 to 8 million and still maintain the same per capita milk availability. Information compiled from the population division of the United States Census Bureau and from the United States Department of Agriculture.

receptor in the liver (Lucy et al., 2009). Re-coupling of the GH and insulin-like growth factor-1 (IGF-1) system has a pivotal role to reestablish follicular steroidogenesis and the ovulatory process in dairy cows, which might be related to fertility. As feed intake increases and energy balance improves, the concentrations of insulin in plasma increase because of the greater flux of propionate and synthesis of glucose by the liver, and the increments in plasma insulin and energy balance seem to be some of the signals to reestablish the GH receptor population in the liver of dairy cows (Butler et al., 2003). This re-couples the somatotropic axis and results in substantial increases in plasma concentrations of IGF-1 and in the steroidogenic capacity of ovarian follicles (Butler et al., 2004).

Because selective partition of nutrients favoring the mammary gland can have major implications to the energy reserves of dairy cows and impact future fertility, one could conclude that an obvious solution is to suppress milk yield; however, little of the variation in energy balance of cows is determined by the amount of energy secreted in milk and a much greater proportion is determined by energy intake (**Fig. 2**). Therefore, selecting for less milk yield with no changes in nutrient intake is unlikely to be the most productive method to improve energy status of early lactation dairy cows. Completely abolishing the dry period resulted in a dramatic decline in milk yield in the subsequent lactation with minor effects on nutrient intake (Rastani et al., 2005). Cows devoid of a dry period produced substantially less milk (5.7 to 9 kg/day) than their counterparts with a 28 or 56-day dry period (Rastani et al., 2005). Interestingly, continuous milking resulted in cows undergoing negligible negative energy balance in early lactation and they experienced early postpartum ovulation (Gümen et al., 2005). It is unlikely that such measures will be adopted by dairy producers in an attempt to improve reproduction considering the substantial losses of production. A more reasonable alternative is to develop markers that identify cows that consume more feed in the first weeks of lactation and determine whether this might have a genetic link. It has been suggested that energy balance in early lactation has a genetic component (Friggens et al. 2007); therefore, it might be a possible to select sires of daughters that undergo less exacerbated negative nutrient balance but, at the same time, maintain high production.

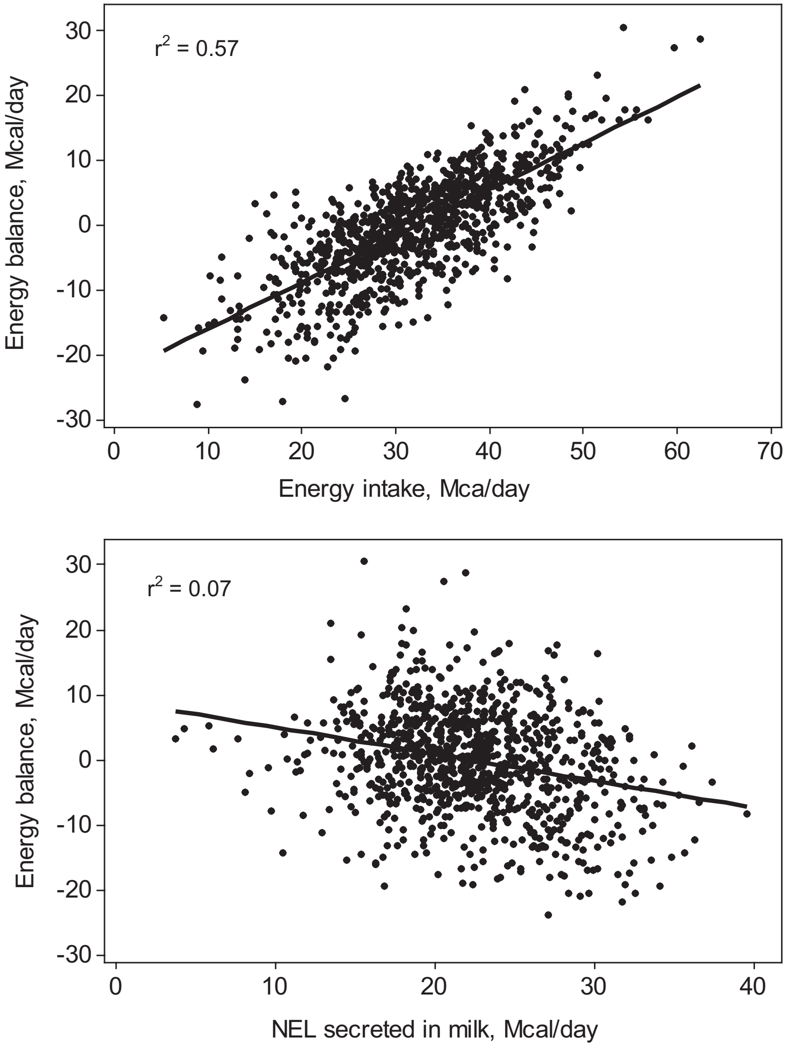


**Fig. 2.** Regression of energy balance (Mcal/day of net energy for lactation, NEL) and net energy intake (Mcal/day) or NEL secreted in milk in a group of 136 Holstein cows in the first 6 weeks postpartum. Adjusted coefficients of determination ($r^2$) with energy balance were 0.57 and 0.07 for energy intake and energy secretion in milk, respectively.

### *Selecting for improved fertility without compromising production*

One of the topics often discussed is the possibility of selecting cows for improved fertility at the same time that lactation performance is not compromised. Although heritability values for fertility and some health traits are generally low, there is surprisingly large genetic variation in these traits that allow for selection of cattle (Weigel and Rekaya, 2000). In fact, since the adoption of productive life in the selection program in the United States in the 90's, breeding value of daughters compiled by the Animal Improvement Programs Laboratory of the United States Department of Agriculture has no longer declined and has shown some signs of improvement in the last decade.

Because of the interest for selection of cattle for fertility traits, a fertility index designated as daughter pregnancy rate (DPR) was developed (VanRaden et al., 2004) and incorporated in the selection program in February of 2003 in the United States. This index is derived from days open, which to no surprise has low heritability, approximately 0.04. The calculation is based on the formula: pregnancy rate = 21/(days open - voluntary waiting period + 11), of which 21 represents the length of the estrous cycle in which a cow has the opportunity to become pregnant, days open is the interval between calving and pregnancy, the voluntary waiting period is a determined value of 60 days, and 11 is the midpoint of 21 days for the length of the estrous cycle. The standard 60-day voluntary waiting period has been prone to criticism because this parameter varies widely between and within farms (Chang et al., 2007), and a delay in the eligibility to first insemination would compromise predictions of DPR. Recently, Chang et al. (2007) evaluated 44,901 lactation records to study the number of 21-day opportunity periods required to achieve pregnancy. The duration of the voluntary waiting period ranged from 28 to 74 days and, despite this variability, the predicted transmitting ability (PTA) of the sires using a voluntary waiting period either fixed at 60 days or according to the farm value resulted in very high correlation (0.98), although some sires were ranked differently according to the model used. The same study observed that the daughters of sires with greater PTA for DPR, as computed by the Animal Improvement Programs Laboratory, required fewer 21-day periods to become pregnant (Chang et al., 2007). The relationship between PTA values of DPR and days open is not linear (VanRaden et al., 2004), but most cow populations become pregnant on average after two to five 21-day breeding opportunities. Within that range, the relationship becomes linear, and each point in DPR represents a change in 4 days open (VanRaden et al., 2004).

Data from 626 Holstein bulls that are proven sires included in the active AI list were collected from the August 2010 summaries from the database of the Animal Improvement Programs Laboratory (http://www.aipl.arsusda.gov). Predicted transmitting ability values for production traits, net merit, and DPR were merged with data for bull fertility based on sire conception rate. Simple regression analysis of data from these 626 bulls indicate a weak negative relationship ($r^2 = 0.09$) between milk yield and DPR. On the other hand, if selection is based on net merit, which takes into account productive, health and reproductive traits, then the relationship became positive with better prediction (**Fig. 3**, panel A). Part of that is because 11% of the value of net merit is attributed to DPR.

Of the 626 Holstein bulls, 382 had data on sire conception rate. No relationship was observed between sire conception rate and DPR; in other words, the fertility of the sire had no relationship with the fertility of its daughters (**Fig. 3**, panel B). Nevertheless, when sires were categorized as low ($\leq 1$) or high ($> 1$) sire conception rate, a total of 213 of the 382 bulls were classified as high fertility. There was still a wide variety of bulls to select from with high sire conception rate that resulted in considerable positive PTAs for both net merit and DPR (**Fig. 3**, panel C). The changes in DPR are substantial considering that the range for most of the Holstein sire population goes from -3 to +3, a 6 percentage point value that represents a spread of 24 days open. These data indicate that selection for productive traits such as yield of fat and protein does not need to be accomplished at the expense of current and future fertility of the dairy herd.

## Health, body condition, and fertility in dairy cows

Although major emphasis has been given to high milk yield as a potential suppressor of fertility in dairy cows, little or no association has been observed between milk production in early lactation and the risk of anovulation, pregnancy, and pregnancy loss in high-producing dairy cows (Santos et al., 2009). However, a major issue facing dairy cows under intensive systems is the high incidence of health problems, particularly those that affect the reproductive tract and those of metabolic origin.

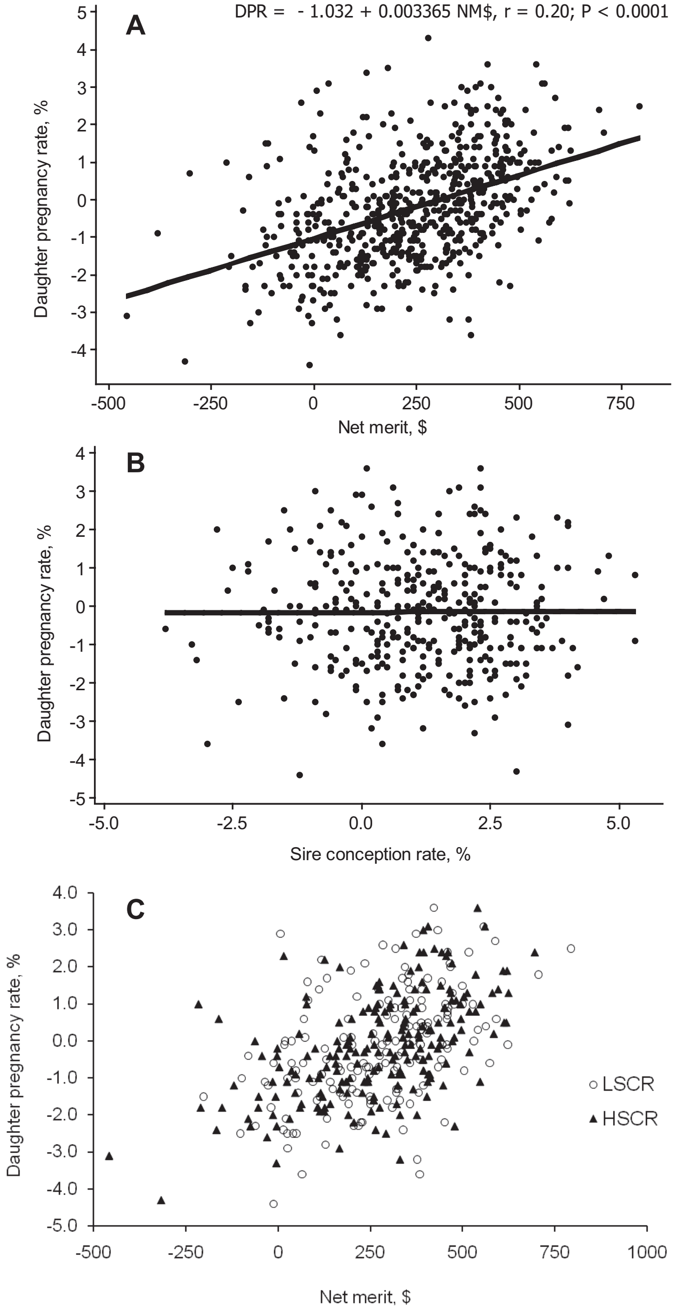


**Fig. 3**. Relationship between daughter pregnancy rate (DPR) and other selection traits in a population of Holstein bulls that are proven sires included in the active AI list from the august 2010 summaries from the database of the Animal Improvement Programs Laboratory (http://www.aipl.arsusda.gov/). Panel A indicates that selection for net merit results in daughters with better pregnancy rate. Panel B depicts no relationship between sire and daughter fertility. Panel C segregates bulls according to sire conception rate (SCR) as low (< 1; range, -3.8 to 1) or high (> 1; range, 1.1 to 5.3).

Data from 5,719 postpartum dairy cows evaluated daily for health disorders from eight experiments conducted by our group on seven dairy farms were compiled. All cows were evaluated for cyclicity at 65 days postpartum by sequential progesterone analyses in plasma 12 to 14 days apart. These cows were subjected to presynchronized timed AI programs using variations of the Ovsynch protocol. Of the 5,719 dairy cows evaluated, only 55.8% of them were considered healthy and did not develop clinical disease in the first 60 days postpartum. Incidence of diseases (calving related problems, 14.6%; metritis, 16.1%; clinical endometritis, 20.8%; fever, 21.0%; mastitis, 12.2%; ketosis, 10.4%; lameness, 6.8%; digestive problems, 2.8%; pneumonia, 2.0%) was high and 27.0% of the cows were diagnosed with a single disease event, whereas 17.2% had at least 2 disease events in the first 2 months of lactation. At this point, either the genetics of high production has lead to increased risk of health problems or producers have not been able to offer cows the proper preventative health program through nutrition, cow comfort, and general management to minimize the risk of diseases. It is likely that a mismatch between genotype and management conditions at the farm occur in many instances. Interestingly, the diagnosis of diseases in the first 60 days postpartum did not influence milk yield in that lactation, and the 305-day milk yields were 10,919, 11,041, and 10,858 kg for cows considered healthy, those with a single disease, and those with multiple diseases, respectively. In spite of the similar milk yield, cows diagnosed with health problems were less likely to be cyclic at 65 days postpartum (**Table 1**). Calving related disorders and those that affect the reproductive tract were the major contributors for depressed cyclicity.

**Table 1.** Impact of health problems in the first 60 d postpartum on resumption of estrous cyclicity by 65 d postpartum in dairy cows[1]

| Health status | Cyclic, % | Adjusted OR (95% CI)[2] | P |
|---|---|---|---|
| Health problem | | | |
| Healthy | 84.1 | 1.00 | --- |
| 1 case of disease | 80.0 | 0.97 (0.72 – 1.30) | 0.83 |
| > 1 case of disease | 70.7 | 0.60 (0.44 – 0.82) | 0.001 |
| Type of health problem[3] | | | |
| Calving problem | 70.5 | 0.52 (0.40 – 0.68) | < 0.001 |
| Metritis | 63.8 | 0.37 (0.28 – 0.50) | < 0.001 |
| Clinical endometritis | 68.9 | 0.51 (0.37 – 0.71) | < 0.001 |
| Fever postpartum | 80.0 | 0.55 (0.40 – 0.74) | < 0.001 |
| Mastitis | 81.5 | 0.87 (0.55 – 1.36) | 0.53 |
| Clinical ketosis | 77.7 | 0.71 (0.47 – 1.07 | 0.10 |
| Lameness | 85.0 | 0.82 (0.52 – 1.30) | 0.40 |
| Pneumonia | 88.9 | 1.78 (0.22 – 14.34) | 0.59 |
| Digestive problem | 60.7 | 0.54 (0.25 – 1.17) | 0.12 |

[1] 5,719 postpartum dairy cows evaluated daily for health disorders on seven dairy farms in the United States.

[2] OR = odds ratio; CI = confidence interval.

[3] Calving problem = includes dystocia, twin birth, stillbirth, and retained placenta, which was characterized by presence of fetal membranes 24 h after calving; Metritis = watery fetid uterine discharge in the first 14 days postpartum; Clinical endometritis = vaginal mucus score > 2 (> 10% pus in the mucus); Fever = rectal temperature > 39.5 °C in the first 14 days postpartum; Mastitis = presence of abnormal milk in one of the quarters; Clinical ketosis = lack of appetite and presence of ketonuria using test strips; Pneumonia = increased lung sounds and respiratory frequency concurrent with fever; Digestive problem = indigestion caused by displacement of abomasum, bloat or diarrhea.

Similar to the depression in cyclicity, diagnosis of health disorders in early lactation markedly reduced pregnancy at the first postpartum AI (**Table 2**), and increased the risk of pregnancy loss in the first 60 days of gestation (**Table 3**). On the contrary, healthy cows achieved very high fertility with 51.4% pregnancy per AI at first postpartum insemination (**Table 2**). These data indicate that reduction in morbidity by preventing periparturient diseases has the potential to enhance fertility of dairy cows by improving resumption of postpartum ovulation, increasing pregnancy per AI, and minimizing pregnancy loss.

**Table 2.** Impact of health problems in the first 60 d postpartum on pregnancy at first postpartum AI of dairy cows[1]

| Health status | Pregnant, % | Adjusted OR (95% CI)[2] | *P* |
|---|---|---|---|
| Health problem | | | |
| Healthy | 51.4 | 1.00 | |
| 1 case of disease | 43.3 | 0.79 (0.69 – 0.91) | 0.001 |
| > 1 case of disease | 34.7 | 0.57 (0.48 – 0.69) | < 0.001 |
| Type of health problem[3] | | | |
| Calving problem | 40.3 | 0.75 (0.63 – 0.88) | < 0.001 |
| Metritis | 37.8 | 0.66 (0.56 – 0.78) | < 0.001 |
| Clinical endometritis | 38.7 | 0.62 (0.52 – 0.74) | < 0.001 |
| Fever postpartum | 39.8 | 0.60 (0.48 – 0.65) | < 0.001 |
| Mastitis | 39.4 | 0.84 (0.64 – 1.10) | 0.20 |
| Clinical ketosis | 28.8 | 0.50 (0.36 – 0.68) | < 0.001 |
| Lameness | 33.3 | 0.57 (0.41 – 0.78) | < 0.001 |
| Pneumonia | 32.4 | 0.63 (0.32 – 1.27) | 0.20 |
| Digestive problem | 36.7 | 0.78 (0.46 – 1.34) | 0.38 |

[1] 5,719 postpartum dairy cows evaluated daily for health disorders on seven dairy farms in the United States.

[2] OR = odds ratio; CI = confidence interval.

[3] Calving problem = includes dystocia, twin birth, stillbirth, and retained placenta, which was characterized by presence of fetal membranes 24 h after calving; Metritis = watery fetid uterine discharge in the first 14 days postpartum; Clinical endometritis = vaginal mucus score > 2 (> 10% pus in the mucus); Fever = rectal temperature > 39.5 °C in the first 14 days postpartum; Mastitis = presence of abnormal milk in one of the quarters; Clinical ketosis = lack of appetite and presence of ketonuria using test strips; Pneumonia = increased lung sounds and respiratory frequency concurrent with fever; Digestive problem = indigestion caused by displacement of abomasum, bloat or diarrhea.

One of the consequences of diseases is that cows have reduced appetite and oftentimes lose more body weight. Nutrient intake is the major driver of energy balance (**Fig. 2**), and energy balance is associated with fertility in dairy cows (Butler, 2003). In fact, cows that lost more body condition in the first 65 days postpartum were more likely to be anovular, had decreased pregnancy per AI, and increased risk of pregnancy loss (Santos et al., 2009). In addition, health disorders might have direct impacts on the reproductive tract, particularly uterine diseases and those that result in inflammatory responses such as mastitis and pneumonia. Therefore, it is critical that management of early lactation dairy cows include methods to minimize negative nutrient balance and excessive losses of body condition. Dairy form has a genetic correlation of -0.73 with body condition score (Dechow et al., 2004). Because of this negative genetic correlation, selection indexes now include a penalty for dairy form in an attempt to preserve body condition and improve fertility.

**Table 3.** Impact of health problems in the first 60 d postpartum on risk of pregnancy loss in the first 60 d of gestation in dairy cows[1]

| Health status | Pregnancy loss, % | Adjusted OR (95% CI)[2] | P |
|---|---|---|---|
| Health problem | | | |
| Healthy | 8.9 | 1.00 | --- |
| 1 case of disease | 13.9 | 1.73 (1.25 – 2.39) | < 0.001 |
| > 1 case of disease | 15.8 | 2.08 (1.36 – 3.17) | < 0.001 |
| Type of health problem[3] | | | |
| Calving problem | 15.9 | 1.67 (1.16 – 2.40) | < 0.01 |
| Metritis | 11.3 | 1.01 (0.71 – 1.60) | 0.76 |
| Clinical endometritis | 15.1 | 1.55 (1.04 – 2.32) | 0.03 |
| Fever postpartum | 18.0 | 2.00 (1.24 – 3.14) | < 0.01 |
| Mastitis | 19.8 | 2.62 (1.48 – 4.64) | < 0.001 |
| Clinical ketosis | 14.6 | 1.64 (0.75 – 3.59) | 0.22 |
| Lameness | 26.4 | 2.67 (1.38 – 5.12) | < 0.01 |
| Pneumonia | 16.7 | 1.87 (0.40 – 8.69) | 0.42 |
| Digestive problem | 15.8 | 1.81 (0.52 – 6.32) | 0.35 |

[1] 5,719 postpartum dairy cows evaluated daily for health disorders on seven dairy farms in the United States.

[2] OR = odds ratio; CI = confidence interval.

[3] Calving problem = includes dystocia, twin birth, stillbirth, and retained placenta, which was characterized by presence of fetal membranes 24 h after calving; Metritis = watery fetid uterine discharge in the first 14 days postpartum; Clinical endometritis = vaginal mucus score > 2 (> 10% pus in the mucus); Fever = rectal temperature > 39.5 °C in the first 14 days postpartum; Mastitis = presence of abnormal milk in one of the quarters; Clinical ketosis = lack of appetite and presence of ketonuria using test strips; Pneumonia = increased lung sounds and respiratory frequency concurrent with fever; Digestive problem = indigestion caused by displacement of abomasum, bloat or diarrhea.

## Optimization of reproductive programs as a platform to evaluate nutrition and health effects on fertility

Major advancements in manipulation of the estrous cycle have been achieved to optimize fertility of dairy cows subjected to synchronization of ovulation programs since the advent of the Ovsynch protocol in 1995. These programs were originally designed to improve insemination rate because of the challenges with estrous detection on dairy farms (Lopez et al., 2004); however, new knowledge of the reproductive biology of the dairy cow and the ability to manipulate follicle growth and luteal lifespan has created opportunities to optimize fertility at the same time that insemination is assured.

It is no surprise that adoption of timed AI programs for routine management of reproduction in dairy herds has been widespread, and benefits to reproductive efficiency also have translated into economic advantages to the producer. The average value of a pregnancy has been estimated at $278.00 for farms in the United States (de Vries, 2006). Under intensive systems, implementation of timed AI has been considered economically advantageous to treat anovular cows compared with methods based on detection of estrus (De Vries et al., 2006). When compared with a well-managed natural service breeding program, timed AI resulted in similar reproductive performance (Lima et al., 2009), but greater economic return (Lima et al., 2010). More recently, work from New Zealand in a grazing system clearly demonstrated the economic benefit of timed AI programs at the beginning of the breeding season in cyclic and anovular dairy cows (McDougall, 2010).

### *Optimizing timed AI programs*

Optimizing these programs is important to the reproduction of dairy herds, but also to serve as a platform to test other concepts such as the impact of nutrition on embryo quality and pregnancy (Cerri et al., 2009a). Response to the Ovsynch protocol improves when cows ovulate to the first GnRH of the program and when a responsive CL is present at the $PGF_{2\alpha}$ treatment (Chebel et al., 2006; Moreira et al., 2001; Vasconcelos et al., 1999). These responses benefit pregnancy per AI (Chebel et al., 2006; El-Zarkouny et al., 2004; Moreira et al., 2001) in part because ovulation to the initial GnRH reduces the length of dominance and improves embryo quality (Cerri et al., 2009b). Furthermore, initiating the Ovsynch protocol in early diestrus minimizes the risk of spontaneous regression of the CL and ovulation before completion of the program (El-Zarkouny et al., 2004; Moreira et al., 2001; Vasconcelos et al., 1999). A common method to presynchronize the estrous cycle is the use of $PGF_{2\alpha}$ injections administered 14 days apart with the Ovsynch initiated 12 days later (Moreira et al., 2001; El-Zarkouny et al., 2004). Respecting the interval between presynchronization and initiation of Ovsynch is critical to optimizing ovulation to the initial GnRH treatment and pregnancy (Galvão et al., 2007).

The benefits of this program to fertility go beyond synchronization of the estrous cycle. A large proportion of dairy cows suffer from uterine diseases (Galvão et al., 2009), and intra-uterine antimicrobial therapy has been shown to improve reproduction of cows with endometritis (Leblanc et al., 2002). The issue of milk and tissue residue with antimicrobials is of concern particularly when 15 to 30% of the cows would need to be treated upon diagnosis of endometritis. When cows received routine $PGF_{2\alpha}$ treatments starting after 30 days postpartum, the use of intrauterine cephalosporin after 40 days postpartum had no benefit to uterine health, pregnancy at first AI, pregnancy loss, and the rate of pregnancy in the first 300 days postpartum (Galvão et al., 2009). Therefore, routine use $PGF_{2\alpha}$ helps eliminate uterine infections and inflammation, particularly in cows with a responsive CL, synchronizes estrus for insemination, and presynchronizes the estrous cycle to improve response to timed AI programs.

In many herds, the prevalence of anovular cows is high (Santos et al., 2009), and these cows unlikely benefit from presynchronization with $PGF_{2\alpha}$ (Moreira et al., 2001). A potentially more promising system to improve fertility of anovular cows is the use of GnRH for presynchronization such as in the double Ovsynch program, which has been shown to increase pregnancy per AI of primiparous cows (Souza et al., 2008), which are known to be more prone to anovulation (Santos et al., 2009).

Another aspect is the timing of induction of ovulation after luteolysis and subsequent interval to insemination in these programs. Brusveen et al. (2008) demonstrated that maintaining an interval of 16 hours between induction of ovulation and AI, when GnRH is given at 56 hours after luteolysis, optimized pregnancy per insemination in dairy cows. Finally, optimizing the length of ovulatory follicle dominance by reducing the interval between follicle recruitment and ovulation has been shown to benefit fertility (Santos et al., 2010).

The encouraging aspect of these optimized programs is that pregnancy per AI in high-producing cows is now above 40% and, in some cases, as high as 50%. Furthermore, these programs allow producers to optimize insemination rate with high fertility, which improves the overall pregnancy rate of the herd. Even when no estrous detection is used and cows are inseminated only after timed AI, overall pregnancy rate is high (Lima et al., 2010). These programs do not necessarily solve the underlying problems causing reduced fertility in lactating dairy cows, but they have offered producers an alternative to circumvent the changes in the reproductive biology of the high-producing dairy cow that seem to compromise reproductive performance. They also offer a platform with tightly controlled ovarian cycles to test nutritional interventions that might benefit fertility.

## Incorporating nutritional programs to enhance fertility

The transition period from late gestation to early lactation is the most turbulent time in the life of a cow (Drackley, 1999). The endocrine and metabolic adaptations to lactation associated with the decline in feed intake in late gestation contribute to exacerbation in negative nutrient balance in early lactation. Concurrently, this same period is characterized by a high risk of diseases that oftentimes compromise fertility (**Tables 1-3**). From a biological and evolutionary point of view, it is logical that cows prioritize milk production to sustain their offspring rather than resumption of reproductive cycles to reestablish fertility. From a production point of view, both components milk yield and reproduction must be optimized to enhance sustainability of dairy farming. During periods of energy restriction, oxidizable fuels consumed in the diet are prioritized toward essential processes that sustain life (Wade and Jones, 2004). Homeorrhetic controls in early lactation assure that body tissue, primarily adipose stores, is mobilized in support of milk production (Bauman and Currie, 1980). Therefore, the early lactation cow, that is unable to consume enough energy-yielding nutrients to meet the needs of production and maintenance, will sustain high yields of milk components at the expense of body tissues. This poses a problem to reproduction, as energy status is linked with delayed ovulation (Butler, 2003). Energy deprivation reduces the frequency of LH pulses, thereby impairing follicle maturation and ovulation. Furthermore, undernutrition inhibits estrous behavior partly because of reduced estrogen receptor-alpha abundance in the brain (Hileman et al., 1999).

The most important component of energy balance is dry matter intake and not the changes in milk energy secretion (**Fig. 3**). In addition to the obvious influence of the environment, recent studies have proposed that energy balance of early lactation cows has a genetic component (Friggens et al. 2007). The challenge of manipulating energy balance is to understand the mechanisms that control appetite of cows in early lactation (Allen et al., 2009; Drackley, 1999). This becomes particularly important given the notion that glucogenic diets and those with greater energy density might favor resumption of postpartum ovulation in dairy cows (Garnsworthy et al., 2009). This is critical as these diets might suppress dry matter intake because of their effects on satiety (Allen et al., 2009).

### *Glucogenic diets*

Diet composition can be manipulated in an attempt to influence ovarian recrudescence and restoration of fertility in dairy cows. The first postpartum ovulation in dairy cattle occurs approximately 2 weeks after the nadir of negative energy balance (Butler, 2003). Severe weight and body condition losses are associated with anovulation in dairy cattle (Santos et al., 2009), which compromises reproductive performance at first postpartum insemination. Perhaps the major underlying factor for delayed ovulation is the low LH pulsatility that compromises the development of the dominant follicle, its steroidogenesis, and acquisition of ovulatory capacity. Intense catabolism of adipose tissue and lack of adequate concentrations of metabolic cues might further compromise fertility because of potential implications to oocyte competence (Leroy et al., 2008).

Although some cows develop follicles to diameters compatible with those of ovulatory follicles during periods of extensive tissue catabolism, many lose their dominance and regress (Gümen et al., 2003). It is suggested that the re-coupling of the somatotropic axis has a pivotal role to reestablish follicular steroidogenesis and ovulation in dairy cows. Insulin seems to be one of the signals to reestablish the GH receptor population in the liver of cows (Butler et al., 2003), which re-couples the GH/IGF-1 axis causing substantial elevation in plasma IGF-1 and enhancement of the steroidogenic capacity of ovarian follicles (Butler et al., 2004).

Gong et al. (2002) demonstrated that diets rich in starch, also called glucogenic, increased the concentrations of insulin in early lactation and expedited first postpartum ovulation. Van Knegsel et al. (2007) substantiated the positive effects of a glucogenic diet to hasten recrudescence of first postpartum ovulation in dairy cows. Similarly, findings by Garnsworthy et al. (2009) suggested that a combination of a glucogenic diet before the first postpartum ovulation, followed by a diet enriched with lipids resulted in the best reproductive performance. These findings warrant further investigations with large number of cows to confirm that the benefits to first ovulation are also observed for pregnancy. Nevertheless, Garnsworthy et al. (2009) suggested that the benefits of glucogenic diets early in lactation might be detrimental during the breeding period because hyperinsulinemia might compromise oocyte quality and embryo development typically observed in heifers and nonlactating cows (Santos et al., 2008b). Whether this is also true for lactating cows with high nutrient needs remains to be demonstrated.

Caution is needed when excess of fermentable carbohydrates are fed because propionate is a known powerful hypophagic agent in ruminants (Allen et al., 2009). The net flux of propionate from the portal-drained viscera increases immediately after feed consumption (Benson et al., 2002), and propionate is thought to increase oxidative pathways in the hepatocytes that alter firing of the vagus nerve and influence appetite. Intake is determined by meal size (satiety) and inter-meal interval (hunger), and diets high in rapidly fermentable starch stimulate satiety and result in smaller meals (Allen et al., 2009). It is possible that the benefits of an altered diet formulation in the first few weeks postpartum in an attempt to stimulate early recrudescence of ovarian activity might be negated if at the expense of dry matter intake. Therefore, it is important that early lactation diets promote high energy intake, primarily from diets containing substrates that stimulate gluconeogenesis to enhance plasma glucose and insulin. Probably, the critical point is to ensure that every cow has access to feed and is capable of consuming the largest quantity of diet possible, at the same time that a disease prevention and treatment program is implemented to control and treat diseases that suppress appetite.

### *Supplementation with lipids*

Feeding fat to dairy cattle has been shown to improve pregnancy per AI in some, but not all cases (Santos et al., 2008a), and this improvement is likely attributed to the non-caloric effects of certain fatty acids (de Veth et al., 2009; Santos et al., 2008a; Staples et al., 1998). One of the limitations to fatty acid feeding is the extensive rumen biohydrogenation of unsaturated fatty acids; however, even when fatty acids are biohydrogenated, the resulting *trans* fatty acids produced in the rumen might also benefit fertility (de Veth et al., 2009).

Feeding flaxseed, a source rich in linolenic acid (C18:3 n-3), reduced pregnancy loss in dairy cows (Ambrose et al., 2006). Juchem et al. (2010) demonstrated that cows fed a supplement rich in unsaturated fatty acids had 1.5 times greater odds to be pregnant compared with cows fed more saturated fatty acids. Feeding more unsaturated fatty acids increased fertilization and improved embryo quality (Cerri et al., 2009a). In fact, changes in the fatty acid composition of the follicular fluid during early lactation have been associated with alterations in oocyte competence in dairy cows (Leroy et al., 2008). Furthermore, when embryos were produced *in vivo* from super-stimulated dairy cows, those fed fat sources rich in polyunsaturated fatty acids produced more developed embryos (Thangavelu et al., 2007).

Some fatty acids have the ability to modulate the uterine secretion of $PGF_{2\alpha}$ (Staples et al., 1998), which has been proposed as an alternative to improve embryonic survival in cattle (Santos et al., 2008a; Staples et al., 1998). In 3 of 5 experiments described by Santos et al. (2008a), feeding n-3 fatty acids to lactating dairy cows reduced pregnancy losses. More recently, Silvestre et al. (2010a) demonstrated that sequential feeding of a fat supplement containing n-6 fatty acids during the transition period followed by feeding n-3 fatty acids during the breeding period maximized the cumulative proportion of pregnant cows after the first two postpartum inseminations (**Fig. 4**). It was suggested that during remodeling of tissues in early lactation, supplying more n-6 fatty acids might enhance immune response and favor tissue repair (Silvestre et al., 2010b), which can then favor reproduction (Juchem et al., 2010). On the other hand, during the breeding period, attenuating the immune system with n-3 fatty acids might benefit embryonic survival (Silvestre et al., 2010a; Santos et al., 2008a; Santos et al., 2008b). Collectively, these data suggest that feeding moderate amounts of supplemental fat to dairy cows generally improves fertility and responses are mediated by the supply of specific fatty acids for absorption.

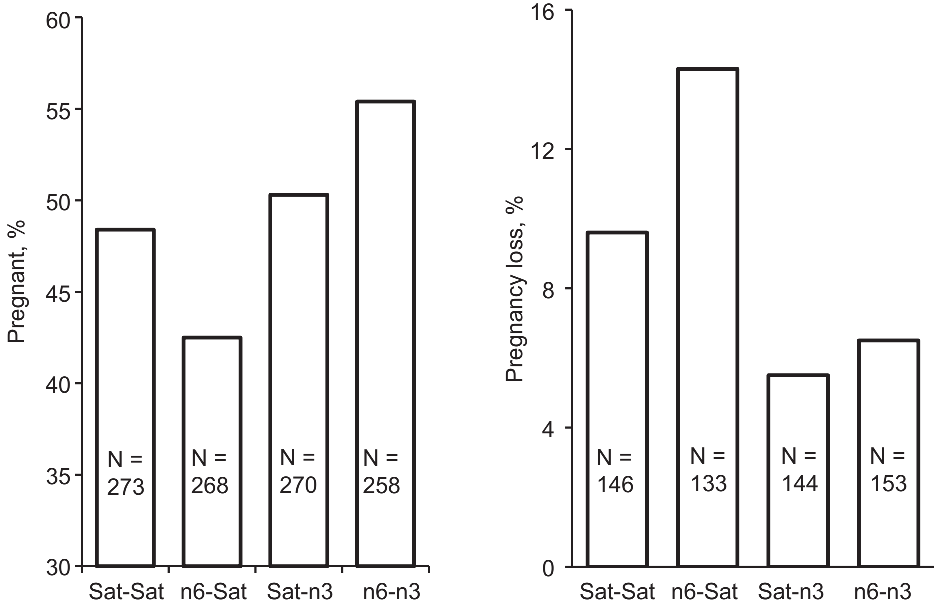


**Fig. 4**. Effect of feeding Ca salts of either mostly saturated (Sat), mostly omega 6 (n6) or mostly omega 3 (n3) fatty acids during the transition (-30 to 30 days postpartum) or the breeding periods (31 to 160 days postpartum) on pregnancy on day 60 after the first and second AI or pregnancy loss between 32 and 60 days of gestation. Treatments are depicted according to the sequence of fat fed during the transition and breeding periods. Cows fed n3 had greater 52.9 vs. 45.5%, $P < 0.01$ pregnancy per AI than those fed Sat primarily as a result of reduced 6.1 vs. 11.8%, $P < 0.01$ pregnancy loss (Silvestre et al., 2010a).

## Conclusions

It is clear that good reproduction in dairy herds require a team approach to the issues facing the high-producing dairy cow. Increased production partitions more nutrients to the mammary gland, which results in less priority to expendable biological processes for the individual cow, one of them being reproduction. Tissue catabolism in early lactation has been implicated in the depression of fertility and it is no surprise that major losses in body weight, particularly body fat, compromise resumption of postpartum ovulation and pregnancy in dairy cows. These effects become even more pronounced following the occurrence of periparturient diseases. A holistic approach to fertility is needed and it is unlikely that a single strategy will solve reproductive problems in dairy herds. Immediate solutions have been developed and optimized to circumvent the challenges with low estrous expression and reduced insemination rates and these programs now allow for high pregnancy per insemination because of improved periovulatory reproductive events of follicle dominance, CL regression and ovulation. Because postpartum health has a dramatic impact on fertility of dairy cows, it is pivotal that nutrition and health programs be designed to minimize the risk and reduce the prevalence of periparturient diseases. Furthermore, specific nutrients targeted at defined periods of the lactation cycle influence signals that favor recrudescence of postpartum ovulation, and establishment and maintenance of pregnancy. The high-yielding cow of today will soon be surpassed by more productive cows, and the gain in production should be concurrent with gains in fertility and health. Matching the genotype with proper management, nutrition and health programs, combined with selection for fertility and the use of breeding technologies will allow producers to cope with the reproductive challenges faced by dairy cows producing well over 11 tons of milk a year.

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