## Impacts of nutrition on reproduction of dairy cattle

J.E.P. Santos Department of Animal Sciences, University of Florida, Gainesville, FL

## Abstract

Nutrition has many important impacts on the reproductive performance of cattle. Inadequate caloric intake in adult cattle has a detrimental impact on reproductive activity of the female bovine. Cows under negative energy balance have extended periods of anovulation. Postpartum anestrus, as well as infertility, is magnified by losses of body condition during the early postpartum period. Resumption of ovulatory cycles is associated with energy balance, and the underlying mechanisms seem to be associated with metabolic signals and regulatory hormones primarily insulin and insulin-like growth factor (IGF) I, which link nutritional status with gonadotropin secretion, re-coupling of the growth hormone-IGF system, and follicle maturation and ovulation. Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation. Nevertheless, feeding excess of starch to promote increases in insulin and glucose might suppress intake of early lactating dairy cows. thereby precluding benefits to cyclicity. Feeding behavior of dairy cows during the transition period, particularly a decline in feed intake before calving, is associated with risk of postpartum uterine disease. Because metritis and more chronic forms of uterine diseases have profound negative effects on pregnancy in dairy cows, providing adequate bunk space and an environment to maximize feed intake might potentially improve fertility of dairy cows. Specific nutrients and dietary ingredients have been implicated in reproduction in cattle. Excess of dietary protein has been suggested as detrimental to fertility, although the evidence is weak. Addition of moderate amounts of supplemental fat to the diet improves caloric intake, modulates prostaglandin $F_2\alpha$  (PGF) secretion by the uterus, affects ovarian dynamics, enhances luteal function and embryo guality, and has moderate positive effects on fertility. More specifically, some fatty acids (FA) might impact fertilization rate and embryo quality in dairy cows. On the contrary, some dietary ingredients, such as gossypol, when ingested in large quantities decrease fertility of dairy cows because of negative effects on embryo quality and pregnancy maintenance.

Keywords: Nutrition, dairy cow, periparturient disease, infertility, gossypol

### Introduction

Selection of dairy cattle for milk yield has linked the endocrine and metabolic controls of nutrient balance and reproductive events so that reproduction in dairy cattle is compromised during periods of nutrient shortage, such as in early lactation. The energy costs to synthesize and secrete hormones, ovulate a follicle, and sustain an early developing embryo are probably minimal compared to the caloric needs for maintenance and lactation. However, the metabolic and endocrine cues associated with negative energy balance (NEB) impair resumption of ovulatory cycles, oocyte and embryo quality, and establishment and maintenance of pregnancy in dairy cattle.

As the demands for milk synthesis increase, reproductive functions may be depressed when no compensatory intake of nutrients is achieved. Numerous recent studies have found that reproductive performance is compromised by the nutrient demands associated with high levels of production. Milk yield increases at a faster rate in the first four to six weeks after parturition than caloric intake, consequently high yielding cows will experience some degree of negative balance of nutrients during the early postpartum period. When cows experience a period of NEB, the blood concentrations of nonesterified fatty acids (NEFA) increase, at the same time that IGF-I, glucose, and insulin are low. These shifts in blood metabolites and hormones might compromise ovarian function and fertility. It has also been reported that energy balance and dry matter intake (DMI) might affect plasma concentrations of progesterone, which may interfere with follicle development and maintenance of pregnancy.<sup>1,2</sup>

During the last decades, genetic selection and improved management of herds have dramatically increased milk production of dairy cows, at the same time that fertility has decreased.<sup>3</sup> Selection for higher milk production in dairy cattle has changed endocrine profiles of cows so that blood concentrations

of bovine somatotropin and prolactin have increased; whereas insulin has decreased.<sup>4</sup> These hormonal changes and the increased nutrient demands for production might negatively impact reproduction of dairy cows. However, adequate nutrition and sound management have been shown to offset depression of fertility in herds with average milk production exceeding 12,000 kg/cow/yr.<sup>5,6</sup>

Several nutritional strategies have been proposed to improve reproduction of dairy cattle with no detrimental effect on lactational performance. Maximizing DMI during the transition period, minimizing the incidence of periparturient problems, feeding of diets that promote increased concentrations of insulin in early lactation, adding supplemental fat to diets, and manipulating the FA content of fat sources are expected to benefit reproduction in dairy cattle. However, factors such as high incidence of metabolic diseases early postpartum, poor body condition score (BCS) at first insemination, and excessive gossypol concentrations in plasma are detrimental to fertility of dairy cattle.

# Nutrition and postpartum uterine health and fertility

Epidemiological studies have clearly demonstrated strong relationships between postparturient diseases and subsequent reproductive performance in dairy cattle. Cows diagnosed with clinical hypocalcemia were 3.2 times more likely to experience retained placenta (RP) than cows that did not have clinical hypocalcemia.<sup>7</sup> Whiteford and Sheldon also found that hypocalcemia was associated with occurrence of uterine disease in lactating dairy cows.<sup>8</sup> Markusfeld reported that 80% of cows with ketonuria developed metritis.<sup>9</sup>

A major risk factor for uterine disease is RP. Generally, cows with RP have increased risk of developing metritis compared with cows not experiencing RP. Both metritis and RP double the risk of cows remaining with uterine inflammation at the time of first postpartum insemination.<sup>10</sup> In the US, a recent USDA study indicated that the incidence of RP in dairy cows was  $7.8 \pm 0.2\%$ .<sup>11</sup> A 2006 study on five dairy farms in Israel observed that RP was diagnosed in 13.1% (9.4 to 18.1%) and 9.2% (3.6 to 13.8%) of multiparous and primiparous cows, respectively.<sup>12</sup> In the same study, metritis affected 18.6% (15.2 to 23.5%) and 30% (19.4 to 42.3%) of the multiparous and primiparous cows, respectively. Both RP and metritis can have devastating effects on reproductive efficiency in lactating dairy cows, with reduced conception rates and extended intervals to pregnancy.<sup>12</sup> In fact, not only does the clinical disease negatively affect fertility of dairy cows; but subclinical endometritis, a disease characterized by increased proportion of neutrophils in uterine cytology without the presence of clinical signs of inflammation of the uterus, has major deleterious effects on conception rates of lactating dairy cows at first postpartum insemination.

A rising story suggests that feed intake and feeding behavior around parturition might mediate some of the increased risk for uterine diseases in dairy cattle.<sup>13-15</sup> Hammon et al observed that cows developing uterine disease postpartum experienced reduced DMI beginning one week before calving.<sup>13</sup> Similarly, cows diagnosed with severe metritis after calving were already consuming less dry matter two weeks prior to calving.<sup>14</sup> In the same study, even cows that subsequently developed mild metritis had reduced DMI one week before calving compared with cows with healthy uteri. The same group observed that cows subsequently developing metritis spent significantly less time eating before and after calving than cows that did not develop metritis.<sup>15</sup> These data indicate that suppressed intake of nutrients or alterations in feeding behavior prior to calving are major risk factors for development of metritis postpartum.

A potential link between nutrient intake and development of uterine diseases may be the immune status of the cow. Kimura et al evaluated neutrophil function in 142 periparturient dairy cows from two herds by evaluating chemotaxic and killing activity of those cells.<sup>16</sup> The authors observed that 14.1% of the cows developed RP. Neutrophils isolated from blood of cows with RP had reduced ability to migrate to placental tissue and reduced myeloperoxidase activity, a marker for oxidative burst and killing activity of neutrophils. Interestingly, the reduced neutrophil function was observed between one and two weeks prior to calving, which suggests that the reduced innate immune function may be part of the cause of RP rather than a consequence of the disease. In fact, cows that developed uterine disease, either clinical metritis or subclinical endometritis, experienced reduced DMI and neutrophil function prior to calving.<sup>13</sup>

These data strongly suggest that inadequate nutrient intake before calving might predispose cows to impaired immune function; and, subsequently, increased risk for uterine diseases that negatively affect reproduction.

Because intake of nutrients seems to influence energy status and immune function of dairy cows, both of which seem to be related to risk of uterine diseases; it is prudent to suggest that nutritional and management strategies that optimize nutrient intake around parturition should improve uterine health and subsequent fertility of dairy cows. Perhaps, of equal or greater importance than the diet composition is the environment to which the preparturient cow is subjected. Inadequate cow comfort, competition for space, and hierarchical status can influence the ability of the cow to consume nutrients; which can consequently predispose her to uterine disease.<sup>13-15</sup>

# **Resumption of postpartum estrous cycles**

The onset of lactation creates an enormous drain of nutrients in high producing dairy cows; which, in many cases, antagonizes the resumption of ovulatory cycles. During early postpartum, reproduction is deferred in favor of individual survival. Therefore, in the case of the dairy cow, lactation becomes a priority to the detriment of reproductive functions.

During periods of energy restriction, oxidizable fuels consumed in the diet are prioritized toward essential processes such as cell maintenance, circulation, and neural activity. Homeorhetic controls in early lactation assure that body tissue, primarily adipose stores, will be mobilized in support of milk production. Therefore, the early lactation dairy cow that is unable to consume enough energy-yielding nutrients to meet the needs of production and maintenance, will sustain high yields of milk and milk components at the expense of body tissues. This poses a problem to reproduction, as delayed ovulation has been linked repeatedly with energy status.<sup>3</sup> Caloric deprivation reduces the frequency of pulses of luteinizing hormone (LH); thereby impairing follicle maturation and ovulation. Furthermore, undernutrition inhibits estrous behavior by reducing responsiveness of the central nervous system to estradiol by reducing the estrogen receptor  $\alpha$  content in the brain.<sup>18</sup>

Generally, the first postpartum ovulation in dairy cattle occurs 10 to 14 days after the nadir of NEB.<sup>3</sup> Severe weight and BCS losses caused by inadequate feeding or illnesses are associated with anovulation and anestrus in dairy cattle. In fact, cows with low BCS at 65 days postpartum are more likely to be anovular, which compromises reproductive performance at first postpartum insemination.<sup>19</sup>

Prolonged postpartum anovulation or anestrus extends the period from calving to first artificial insemination (AI) and reduces fertility during the first postpartum service.<sup>19</sup> In fact, anovular cows not only have reduced estrous detection and conception rates, but also have compromised embryo survival.<sup>20</sup> On the other hand, an early return to cyclicity is important in regard to early conception. The timing of the first postpartum ovulation determines and limits the number of estrous cycles occurring prior to the beginning of the insemination period. Typically, in most dairy herds, fewer than 20% of cows should be anovulatory by 60 days postpartum.<sup>19</sup> Estrous expression, conception rate, and embryo survival improved when cows were cycling prior to an estrous synchronization program for first postpartum insemination.<sup>20,21</sup>

Resumption of ovarian activity in high producing dairy cows is determined by energy status of the animal. Therefore, feeding management that minimizes loss of body condition during the early postpartum period and incidence of metabolic disorders during early lactation should increase the number of cows experiencing a first ovulation during the first four to six weeks postpartum.

#### **Protein and reproduction**

Lactating dairy cattle require large quantities of metabolizable amino acids for synthesis of milk protein. It is typical for lactating rations to contain crude protein between 16 and 18% of total dry matter. Diets with limited crude protein can compromise microbial growth and rumen fermentation, which often reflects in declines in feed intake and milk production. On the other hand, feeding protein in excess to what is needed by the cows has been implicated in increased in ammonia and urea concentrations in blood and milk, which have been used as markers for reduced fertility.<sup>22</sup> The suggested decline in fertility of

cattle fed excess protein is caused by alterations in uterine physiology with a decline in uterine pH during the early luteal phase of the estrous cycle.<sup>22</sup> A more acidic uterine environment is less conducive with maintenance of pregnancy in cattle.<sup>23</sup> This effect seems to be restricted to the early stages of embryo development.<sup>24</sup>

Because high-producing lactating dairy cows are more efficient in utilizing protein sources when diets are moderate in crude protein and are balanced for the supplies of metabolizable protein and limiting amino acids, it is not justified to feed diets with protein concentrations that will increase urea N and harm fertility.<sup>25</sup>

## Caloric intake and reproduction

Caloric intake appears to have the greatest impact on energy status of lactating dairy cows. Santos et al reported that most of the variation in energy balance in early postpartum high-producing Holstein cows is determined by caloric intake and little by the amount of calories secreted in milk.<sup>26</sup> Therefore, selecting for less milk yield with no changes in nutrient intake is unlikely to be a productive method to improve energy status of early lactation dairy cows. Therefore, differences among cows in the severity of NEB are more related with how much energy they consume than with how much milk they produce. During periods of NEB, blood concentrations of glucose, insulin, and IGF-I are low; as well as the pulse frequency of gonadotroin releasing hormone (GnRH) and LH. Plasma progesterone concentrations are also affected by the energy balance of dairy cows. These metabolites and hormones have been shown to affect folliculogenesis, ovulation, and steroid production *in vitro* and *in vivo*. The exact mechanism by which energy status affects secretion of releasing hormones and gonadotropins is not well defined; but it is clear that lower levels of blood glucose, IGF-I, and insulin may mediate this process.

It has been suggested that NEB influences reproduction of dairy cows by impacting the quality and viability of the oocyte of the ovulatory follicle and the corpus luteum (CL) resultant of the ovulation of that follicle. Because there is substantial evidence that metabolic factors can influence early follicular development, it is conceivable that changes in metabolism during periods of NEB could influence preantral follicles destined to ovulate weeks later during the breeding period. To test this hypothesis, Kendrick et al randomly assigned 20 dairy cows to one of two treatments formulated so that cows consumed either 3.6% (high energy) or 3.2% (low energy) of their body weight.<sup>27</sup> Follicles were transvaginally aspirated twice weekly and oocytes were graded based upon cumulus density and ooplasm homogeneity. Cows in better energy balance (high energy) had greater intrafollicular IGF-I and plasma progesterone levels and tended to produce more oocytes graded as good. Therefore, NEB not only delays resumption of ovulatory cycles, but it might also influence the quality of occytes once cows are inseminated.

### Nutritional manipulation to increase nutrient intake

Nutritional efforts to minimize the extent and duration of NEB may improve reproductive performance. The first and most important factor that affects caloric intake in dairy cows is feed availability.<sup>28</sup> Therefore, dairy cows should have continual access to a high quality, palatable diet to assure maximum DMI. However, DMI is limited during late gestation and early lactation, which can compromise total caloric intake and reproductive performance. Several nutritional management strategies have been proposed to increase caloric intake during early lactation. Feeding high quality forages, increasing the concentrate:forage ratio, or adding supplemental fat to diets are some of the most common ways to improve caloric intake in cows.

A number of studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on reproductive parameters in dairy cattle. In early postpartum dairy cattle under NEB, reduced expression of hepatic growth hormone receptor 1A (GHR-1A) is thought to be responsible for the lower concentrations of IGF-I in plasma of cows.<sup>29</sup> Because IGF-I is an important hormonal signal that influences reproductive events such as stimulation of cell mitogenesis, hormonal production, and embryo development, among other functions; increasing concentrations of IGF-I early

postpartum are important for early resumption of cyclicity and establishment of pregnancy. It is interesting to note that insulin mediates the expression of GHR-1A in dairy cows, which results in increased concentrations of IGF-I in plasma.<sup>30,31</sup> Because IGF-I and insulin are important for reproduction in cattle, feeding diets that promote greater insulin concentrations should benefit fertility. Gong et al fed cows of low and high genetic merit isocaloric diets, that differed in the ability to induce high or low insulin concentrations in plasma.<sup>31</sup> The diets that induced high insulin reduced the interval to first postpartum ovulation and increased the proportion of cows ovulating in the first 50 d postpartum.

Feeding fat and fatty acids to influence reproduction

Feeding fat to dairy cattle usually improved the risk for pregnancy, although responses have not been consistent.<sup>33</sup> When fat feeding improved production and increased body weight loss, primiparous cows experienced reduced pregnancy risk at first AI;<sup>34</sup> although pregnancy to AI was extremely high in the unsuplemented cows. However, Ferguson et al observed a 2.2 fold increased risk of pregnancy at first AI and all AI in lactating cows fed 0.5 kg/d of fat, which tended (P = 0.08) to enhance the proportion of pregnant cows at the end of the study (93 vs. 86.2%).<sup>35</sup>

In grazing cows, supplementation with 0.35 kg of FA improved the risk of pregnancy after the first postpartum AI; although a similar proportion of cows were pregnant at the end of the study.<sup>36</sup> Feeding calcium salts of long chain fatty acids (Ca-LCFA) of palm oil improved pregnancy of dairy cows,<sup>37</sup> although the authors did not report statistical significance. On the other hand, others did not observe improvements in fertility of dairy cows supplemented with Ca-LCFA<sup>38,39</sup> or oilseeds;<sup>40</sup> which might be attributed to increased milk yield and body weight losses.<sup>34,39</sup>

Because the benefits of feeding fat may originate from specific FA,<sup>41,42</sup> others have evaluated whether feeding FA differing in the degree of saturation might influence fertility of cows. The essential FA of the n-6 and n-3 families are available in much smaller supply to ruminants than nonruminants because of microbial biohydrogenation of FA in the rumen,<sup>43</sup> suggesting that their supplementation may benefit reproduction.<sup>33,42</sup>

Three recent studies explored the role of n-6 and n-3 FA supplementation to lactating dairy cows on risk of pregnancy after the first postpartum AI.<sup>44-46</sup> When cows were fed 0.75 kg of fat from flaxseed, a source rich in C18:3 n-3, or sunflower seed, a source rich in C18:2 n-6; pregnancy tended (P = 0.07) to be greater for cows fed n-3 FA. However, a similar response was not observed by others when cows were fed flaxseed as the source of n-3 FA.<sup>45,46</sup> When cows received a sequence of FA feeding of Ca salts rich in n-6 during the transition period and n-3 during the breeding period, the cumulative first and second AI pregnancy was improved primarily because cows fed n-3 FA during the breeding period had less pregnancy loss.<sup>47</sup>

Juchem et al evaluated the effect of feeding cows pre- and postpartum Ca-LCFA of either mostly saturated and monounsaturated FA or a blend of C18:2 n-6 and trans-octadecenoic FA.<sup>48</sup> They observed that cows fed unsaturated FA had 1.5 times greater risk of pregnancy either at 27 or 41 days after AI compared with cows fed mostly saturated FA. Improvements in pregnancy risk when cows were fed C18:2 n-6 and trans-octadecenoic FA were supported by improved fertilization and embryo quality in non-superovulated lactating dairy cows.<sup>49</sup>

Because n-3 FA can suppress uterine secretion of  $PGF_2\alpha$ ,<sup>50-52</sup> it is thought that they have the potential to improve embryonic survival in cattle.<sup>53</sup> In three of five experiments, feeding n-3 FA either as flaxseed rich in C18:3 n-3<sup>44,46</sup> or fish oil rich in eicosapentanoic acid (EPA) and docosahexanoic acid (DHA)<sup>47</sup> reduced pregnancy losses in lactating dairy cows after the first postpartum AI. On the other hand, when n-6 FA were fed as Ca-LCFA, pregnancy losses were similar to those observed for cows fed Ca-LCFA of palm oil.<sup>47,48</sup>

Collectively, these data suggest that feeding fat to dairy cows generally improves fertility and responses are observed when the caloric density of the ration increased with fat feeding. Also, these data suggest that fertility responses to fat feeding is altered according to the type of FA supplemented in the diet. Feeding n-3 FA from oilseeds has improved pregnancy risk in some, but not all studies; however feeding n-3 FA as Ca-LCFA containing fish oils does not seem to influence risk of pregnancy. On the

other hand, feeding Ca-LCFA rich in n-6 and trans-octadecenoic FA improved pregnancy in lactating dairy cows. Although feeding n-3 FA has not consistently improved pregnancy risk, it has reduced pregnancy losses in dairy cows.

#### Source of selenium and reproduction

During the immediate postpartum period, the cow's immune system is challenged severely,<sup>54</sup> and the innate and humoral defense systems are reduced. The incidence of diseases and disorders can be high during this time period and have a negative impact on reproductive performance. For example the *risk* of pregnancy (odds ratio) was reduced if cows had RP or lost one BCS unit.<sup>12,19</sup> Reduction in adaptive and innate immunity at parturition increases the risk of health disorders such as RP, metritis, and mastitis.

Selenium has long been associated with immunity. Cattle supplemented with Se-yeast had an 18% increase of Se in plasma in comparison to sodium selenite in some studies.<sup>55</sup> Some regions of the US are deficient in Se, particularly the Southeast; whereas other states, such as California, are mostly adequate in Se.

We have conducted an experiment to evaluate a supplemental source of organic selenium on reproductive and immune responses by dairy cows in Florida and California.<sup>10,56,57</sup> Objectives were to evaluate effects of organic Se on health and reproductive performance of dairy cows. Cows were assigned prepartum at approximately 25 days prior to expected day of calving to one of two sources of Se, organic Se (Se-yeast [SY]; Sel-Plex®, Alltech, Nicholasville, KY) or inorganic sodium Se (sodium selenite, SS) fed at 0.3 ppm (DM basis) until 80 days posptartum. In both sites, cows followed the same study protocol and health was monitored daily throughout the study. Rectal temperature was recorded each morning for 10 days postpartum. In Florida, vaginoscopic evaluation of the reproductive tract was performed at five and 10 days postpartum. Cows were evaluated for incidence of RP, metritis, puerperal metritis, subclinical endometritis by uterine cytology, ketosis, displacement of abomasum, and mastitis. Cows had their ovulation synchronized for first postpartum AI.

Plasma Se concentrations increased with days postpartum, but source of Se did not influence Se concentrations in cows in California. However, in Florida, feeding SY improved plasma Se concentrations  $(0.087 \text{ vs}. 0.069 \pm 0.004 \mu \text{g/ml}; \text{P} < 0.01)$ . Incidence of postpartum diseases did not differ between treatments in both sites, but cows fed SY had smaller incidence of purulent vaginal discharge than those fed SS in Florida. Diet altered frequency of multiparous cows detected with > 1 event of fever (rectal temperature > 39.5°C; SY, 13.3% [25/188] vs 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%). Vaginoscopy discharge scores at five and 10 days postpartum were better for the SY group; namely, 47.1 (217/460) vs 35.0% (153/437) clear, 43.4 [200/460] vs 47.8% [209/437]) mucopurulent, and 9.3 (43/460) vs 17.1% (75/437) purulent for SY and SS groups, respectively (P < 0.05). Feeding organic Se (SY) improved uterine health and second service PR during summer.

Diet failed to alter first service pregnancy rates in California and Florida, and second service pregnancy rate in California. However, second service pregnancy rate in Florida was greater for cows fed SY than SS [SY, 17% (34/199) vs SS, 11.3% (24/211); P < 0.05]. The benefit of SY on second service pregnancy rate is intriguing. We hypothesize that cows of the SY group were better able to re-establish an embryo-trophic environment at second service following either early or late embryonic losses.

Measures of innate and humoral immune responses were unaltered by source of Se in California, but cows fed SY in Florida had improved neutrophil function and serum titers against ovalbumin. Our findings indicated that feeding SY improved measures of humoral and cellular immunity, uterine health, and second service pregnancy rate in cows in Florida which is known as a Se deficient state. However, in California source of Se had no impact on health, measures of immune response, or reproductive performance.

#### Consumption of gossypol from cotton byproducts and reproduction

Gossypol was first discovered by Chinese scientists after noticing that no children were born for more than a decade in a village where people cooked food with cottonseed oil. Since then, innumerable

reports in the literature have confirmed the anti-fertility effect of gossypol in mammals. Gossypol disrupts cell membrane metabolism, affects glycolysis, influences mitochondrial and energy metabolism in the cell, and increases fragility of cell membranes, such as in red blood cells. In fact, erythrocyte fragility has been one of the indicators of potential gossypol toxicosis.

Risco et al were among the first to show that gossypol can be toxic and even kill growing cattle.<sup>58</sup> They fed rations with 200, 400 or 800 mg/kg of free gossypol (FG) to bull calves for 120 days. The diets with 400 and 800 mg/kg of FG were considered to be toxic and could potentially cause the death of growing ruminants. Baby calves have little ability to detoxify gossypol; therefore toxicity can be easily induced by feeding cotton products.

The negative effects of gossypol on fertility of ruminants are clear in males. Studies at University of Florida and Kansas State University have shown that as little as 8 g/d of FG fed to young bulls reduced sperm quality and sexual activity.<sup>59,60</sup> However, the female ruminant seems to be relatively insensitive to the anti-fertility effect of gossypol because of rumen detoxification; but *in vitro* data indicate some inhibition of embryonic development and ovarian steroidogenesis.<sup>61</sup>

More recently, a series of experiments by our group demonstrated that consumption of up to 40 mg of FG/kg of bodyweight did not influence follicle and luteal development in dairy heifers, but feeding a diet with 40 mg of FG/kg of body weight reduced embryo quality and development *in vivo* and *in vitro*.<sup>62-64</sup> These effects likely explain the reduced risk of pregnancy in dairy cows with high plasma gossypol concentrations,<sup>65</sup> and compromised embryo survival after transfer.<sup>66</sup> Therefore, it is prudent to feed lactating dairy cows amounts of cottonseed that result in low plasma gossypol concentrations.

### Conclusions

Inadequate intake of nutrients and inadequate body reserves during early lactation are the major factors affecting reproductive performance of dairy cows. Improving energy balance by increasing caloric intake through additional non-fiber carbohydrates or supplemental fat in the diet reduces days to first ovulation and improves conception postpartum. Strong evidence suggests that management of cows during the prepartum period affects uterine health. Inadequate intake of nutrients prepartum and altered feeding behavior increases the risk of metritis in dairy cows. Supplementation with unsaturated FA of the n-3 and n-6 families usually improves fertility, as long as it does not interfere with rumen microbial metabolism. It is critical that improved methods to protect these unsaturated FA are required if precise calculations of the supply of unsaturated lipids are to be utilized in dairy cows, but response seems to be dependent upon the background Se concentrations in dietary ingredients. Lastly, although lactating dairy cows can consume substantial amounts of gossypol with no detrimental effects on health and lactation, when plasma gossypol concentrations exceed 5  $\mu$ g/ml, embryo development and establishment and maintenance of pregnancy are compromised.

#### References

- 1. Vasconcelos JLM, Sangsritavong S, Tsai SJ, et al: Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology 2003;60:795-807.
- 2. Villa-Godoy A, Hughes TL. Emery RS, et al: Association between energy balance and luteal function in lactating dairy cows. J Dairy Sci 1988;71:1063-1072.
- 3. Butler WR: Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 2003;83:211-218.
- 4. Bonczeck RR, Young CW, Wheaton JE, et al: Responses of somatotropin, insulin, prolactin, and thyroxine to selection for milk yield in Holsteins. J Dairy Sci 1988;71:2470-2478.
- Nebel RL, McGilliard ML: Interactions of high milk yield and reproductive performance in dairy cows. J Dairy Sci 1993;76:3257-3268.
- 6. Jordan ER, Fourdraine RH: Characterization of the management practices of the top milk producing herds in the country. J Dairy Sci 1993;76:3247-3256.
- 7. Curtis CR, Erb HN, Sniffen CJ, et al: Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. J Am Vet Med Assoc 1983;183:559-561.
- 8. Whiteford LC, Sheldon IM: Association between clinical hypocalcaemia and postpartum endometritis. Vet Rec 2005;157:202-203.

- 9. Markusfeld O: Relationship between overfeeding, metritis and ketosis in high yielding dairy cows. Vet Rec 1985;116:489-491.
- 10 Rutigliano HM, Lima FS, Cerri RLA, et al: Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. J Dairy Sci 2008;91:3323–3336.
- 11. NAHMS: Dairy 1996 Part III: reference of 1996 dairy health and health management. Online. Available: <u>http://nahms.aphis.usda.gov/dairy/dairy96/DR96Pt3.pdf</u>.
- 12. Goshen T, Shpigel NY: Evaluation of intrauterine antibiotic treatment of clinical metritis and retained fetal membranes in dairy cows. Theriogenology 2006;66:2210-2218.
- 13. Hammon DS, Evjen IM, Dhiman TR, et al: Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immun Immunopathol 2006;113: 21-29.
- 14. Huzzey JM, Veira DM, Weary DM, et al: Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J Dairy Sci 2007;90:3220-3233.
- 15. Urton G, von Keyserlingk MA, Weary DM: Feeding behavior identifies dairy cows at risk for metritis. J Dairy Sci 2005;88:2843-2849.
- Kimura K, Goff JP, Kehrli ME Jr, et al: Decreased neutrophil function as a cause of retained placenta in dairy cattle. J Dairy Sci 2002;85:544-550.
- 17. Wade GN, Jones JJ: Neuroendocrinology of nutritional infertility. Am J Regul Integr Comp Physiol 2004;287:1277-1296.
- 18. Hileman SM, Lubbers LS, Jansen HT, et al: Changes in hypothalamic estrogen receptor-containing cell numbers in response to feed restriction in the female lamb. Neuroendocrinology 1999;69:430-437.
- 19. Santos JEP, Bilby TR, Thatcher WW, et al: Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod Domest Anim 2008;43(Suppl 2):23-30.
- 20. Santos JEP, Thatcher WW, Chebel RC, et al: The effect of embryonic death rates in cattle on the efficacy of estrous synchronization programs. Anim Reprod Sci 2004;82-83:513-535.
- 21. Santos JEP, Juchem SO, Cerri RLA, et al: Effect of bST and reproductive management on reproductive and lactational performance of Holstein dairy cows. J Dairy Sci 2004;87:68-881.
- 22. Butler WR: Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci 1998;81:2533-2539.
- 23. Ocon OM, Hansen PJ: Disruption of bovine oocytes and preimplantation embryos by urea and acidic pH. J Dairy Sci 2003;86:1194-1200.
- 24. Rhoads ML, Rhoads RP, Gilbert RO, et al: Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Anim Reprod Sci 2006;91:1-10.
- 25. Noftsger S, St-Pierre NR: Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J Dairy Sci 2003;86:958-969.
- Santos JEP, Bisinotto RS, Ribeiro ES, et al: Applying nutrition and physiology to improve reproduction in dairy cattle. In: Lucy MC, Pate JL, Smith MF, et al, editors. Reproduction in domestic ruminants VII. Nottingham: Nottingham University Press; 2011. p. 387-404.
- 27. Kendrick KW, Bailey TL, Garst AS, et al: Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. J Dairy Sci 1999;82:1731-1740.
- 28. Grant RJ, Albright JL: Feeding behavior and management factors during the transition period in dairy cattle. J Anim Sci 1995;73:2791-2803.
- 29. Radcliff RP, McCormack BL, Crooker BA, et al: Plasma hormones and expression of growth hormone receptor and insulin-like growth factor-I mRNA in hepatic tissue of periparturient dairy cows. J Dairy Sci 2003;86: 920-3926.
- 30. Rhoads RP, Kim JW, Leury BJ, et al: Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. J Nutr 2004;134:1020-1027.
- 31. Butler ST, Marr AL, Pelton SH, et al: Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. J Endocrinol 2003;176:205-217.
- 32. Gong JG, Lee WJ, Garnsworthy PC, et al: Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 2003;123:419-427.
- 33. Santos, J.E.P., H.M. Rutigliano, and M.F. Sá Filho: Risk factors for resumption of postpartum cyclicity and embryonic survival in lactating dairy cows. Anim Reprod Sci 2009;110:207-221.
- 34. Sklan D, Kaim M, Moallem U, et al: Effect of dietary calcium soaps on milk yield, body weight, reproductive hormones, and fertility in first parity and older cows. J Dairy Sci 1994;77:1652-1660.
- 35. Ferguson JD, Sklan D, Chalupa WV, et al: Effects of hard fats on in vitro and in vivo rumen fermentation, milk production, and reproduction in dairy cows. J Dairy Sci 1990;73:2864-2879.
- 36. McNamara S, Butler T, Ryan DP, et al: Effect of offering rumen-protected fat supplements on fertility and performance in spring-calving Holstein–Friesian cows. Anim Reprod Sci 2003;79:45-56.
- 37. Schneider P, Sklan D, Chalupa W, et al: Feeding calcium salts of fatty acids to lactating cows. J Dairy Sci1988;71:2143-2150.
- 38. Scott TA, Shaver RD,. Zepeda L, et al: Effects of rumen-inert fat on lactation, reproduction, and health of high producing Holstein herds. J Dairy Sci 1995;78:2435-2451.

- 39. Sklan D, Moallem U, Folman Y: Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. J Dairy Sci 1991;74:510-517.
- 40. Schingoethe DJ, Casper DP: Total lactational response to added fat during early lactation. J Dairy Sci 1991;74:2617-2622.
- 41. Staples CR, Burke JM, Thatcher WW: Influence of supplemental fats on reproductive tissues and performance of lactating cows. J Dairy Sci 1998;81:856-871.
- 42. Staples CR, Thatcher WW: Effects of fatty acids on reproduction of dairy cows. In: Garnsworthy PC, Wiseman J, editors. Recent advances in animal nutrition. Nottingham: Nottingham University Press; 2005. p. 229-256.
- 43. Juchem SO: Lipid digestion and metabolism in dairy cows: effects on production, reproduction and health [dissertation]. Davis(CA): University of California; 2007.
- 44. Ambrose DK, Kastelic JP, Corbett R, et al: Lower pregnancy losses in lactating dairy cows fed a diet enriched in αlinolenic acid. J Dairy Sci 2006;89:3066-3074.
- 45. Fuentes MC, Calsamiglia SS, Sánchez C, et al: Effect of extruded linseed on productive and reproductive performance of lactating dairy cows. Livest Sci 2008;113:144-154.
- 46. Petit HV, Twagiramungu H: Conception rate and reproductive function of dairy cows fed different fat sources. Theriogenology 2006;66:1316-1324.
- 47. Silvestre FT, Carvalho TS, Francisco N, et al: Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: I. Uterine and metabolic responses, reproduction, and lactation. J Dairy Sci 2011;94:189-204.
- 48. Juchem SO, Cerri RLA, Villaseñor M, et al: Supplementation with calcium salts of linoleic and trans-octadecenoic acids improves fertility of lactating dairy cows. Reprod Domest Anim 2010;45:55-62.
- 49. Cerri RLA, Juchem SO, Chebel RC, et al: Effect of fat source differing in fatty acid profile on metabolic parameters, fertilization and embryo quality in high-producing dairy cows. J Dairy Sci 2009;92:1520-1531.
- 50. Mattos R, Staples CR, Williams J, et al: Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. J Dairy Sci 2002; 85:755-764.
- 51. Mattos R, Guzeloglu A, Badinga L, et al: Polyunsaturated fatty acids and bovine interferon-τ modify phorbol esterinduced secretion of prostaglandinF2α and expression of prostaglandin endoperoxide synthase-2 and phospholipase-A2 in bovine endometrial cells. Biol Reprod 2003;69:780-787.
- 52. Mattos R, Staples CR, Arteche A, et al: The effects of feeding fish oil on uterine secretion of  $PGF_{2\alpha}$ , milk composition, and metabolic status of periparturient Holstein cows. J Dairy Sci 2004;87:921-932.
- 53. Mattos R, Staples CR, Thatcher WW: Effects of dietary fatty acids on reproduction in ruminants. Rev Reprod 2000;5:38-45.
- 54. Goff JP: Transition cow nutrition: effects on immune function and postpartum health. Proc Annu Meet Conv, Dairy Cattle Reproduction Council; 2006. p. 1-8.
- 55. Weiss WP: Selenium nutrition of dairy cows: comparing responses to organic and inorganic selenium forms. In: Lyons TP, Jacques KA, editors. Alltech's nineteenth annual symposium, nutritional biotechnology in the feed and food industries. Nottingham: Nottingham University Press; 2003. p. 333-343.
- 56. Silvestre FT, Silvestre DT, Crawford C, et al: Effect of selenium (Se) source on innate and adaptive immunity of periparturient dairy cows. Biol Reprod Annual Meeting; 2006. Special Issue. p. 132.
- 57. Silvestre FT, Silvestre DT, Santos JEP, et al: Effects of selenium (Se) sources on dairy cows. J Anim Sci 2006;89(Suppl 1):52.
- 58. Risco CA, Holmberg CA, Kutches A: Effect of graded concentrations of gossypol on calf performance: toxicological and pathological considerations. J Dairy Sci 1992;75:2787-2798.
- 59. Chenoweth PJ, Chase CC Jr, Risco CA, et al: Characterization of gossypol-induced sperm abnormalities in bulls. Theriogenology 2000;53:1193-1203.
- 60. Velasquez-Pereira J, Chenoweth PJ, McDowell LR, et al: Reproductive effects of feeding gossypol and vitamin E to bulls. J Anim Sci 1998;76:2894-2904.
- 61. Randel RD, Chase CC Jr, Wyse SJ: Effects of gossypol and cottonseed products on reproduction of mammals. J Anim Sci 1992;70:1628-1638.
- 62. Coscioni AC, Villaseňor M, Galvão KN, et al: Effect of gossypol intake and plasma gossypol concentrations on follicle development and luteal function in dairy heifers. J Dairy Sci 2003;86(Suppl 1):240.
- 63. Coscioni AC, Villaseňor M, Galvão KN, et al: Effect of gossypol intake on plasma and uterine gossypol concentrations and on embryo quality and development in superovulated Holstein dairy heifers. J Dairy Sci 2003; 86(Suppl 1):240.
- 64. Villaseñor M, Coscioni AC, Galvão KN, et al: Gossypol disrupts embryo development in heifers. J Dairy Sci 2008;91: 3015-3024.
- 65. Santos JEP, Villaseňor M, DePeters EJ, et al: Type of cottonseed and gossypol in diets of lactating dairy cows: plasma gossypol, reproduction, and health. J Dairy Sci 2003;86:892-905.
- 66. Galvão KN, Santos JEP, Coscioni AC, et al: Embryo survival from gossypol-fed heifers after transfer to lactating cows treated with human chorionic gonadotropin. J Dairy Sci 2006;89: 2056-2064.