Dietary Fatty Acids and Dairy Cow Fertility

Divakar J. Ambrose¹ and John P. Kastelic²

¹Pork, Poultry and Dairy Branch, Livestock Development Division, Alberta Agriculture, Food and Rural Development, Edmonton, Alberta T6H 4P2
²Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1
E-mail: divakar.ambrose@gov.ab.ca

■ Take Home Messages

¬ Polyunsaturated fatty acids such as linoleic (C18:2n-6), α-linolenic (C18:3n-3), eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) acids can affect reproductive function and fertility
¬ Linoleic acid is found mainly in oilseeds, whereas α-linolenic acid is found predominantly in forages and in some oilseeds (e.g. flaxseed); Eicosapentaenoic and docosahexaenoic acids are high in fish oils
¬ Dairy cows fed diets high in eicosapentaenoic and docosahexaenoic acids (supplementation with menhaden fish meal) or α-linolenic acid (supplementation with flaxseed) during early pregnancy had reduced PGF₂α production and increased pregnancy rates
¬ Feeding diets high in α-linolenic acid during the dry period may increase the incidence of placental retention
¬ Dietary supplementation of select polyunsaturated fatty acids during the postpartum period has the potential to improve fertility in dairy cows, but more research is essential

■ Introduction

It has been emphasized that genetic selection of dairy cows primarily for higher milk yield, without considering important non-production traits (e.g. fertility, mastitis resistance) will cause a further decline in fertility (Lucy, 2001; Westwood et al., 2002). With average first service conception rates in dairy cows currently less than 40%, any means of improving fertility must be explored. Fortunately, dietary manipulation to improve fertility holds much promise. Several excellent reviews are available on this topic (Grummer and Carroll, 1991; Staples et al. 1998; Mattos et al. 2000; Santos, 2001). In one of these reviews, Staples et al (1998) reported that dietary fat improved dairy cow
fertility in 11 of 20 research papers. Although the mechanisms through which dietary fats enhance reproductive performance in dairy cattle are not well-understood, several hypotheses have been put forth. These hypotheses, as reviewed by Staples et al (1998), propose that supplemental dietary fat improves fertility via: 1) an amelioration of negative energy status, leading to an earlier return to estrus during the postpartum period and, therefore, improved fertility; 2) an increase in steroidogenesis (i.e. production of steroid hormones, e.g. progesterone) favorable to improved fertility; 3) manipulation of serum insulin concentrations, thereby stimulating development of ovarian follicles; and 4) altering the production and release of prostaglandin F\(_2\omega\), (PGF), which causes regression of the corpus luteum (CL).

The positive effect of dietary fat on fertility in dairy cows could be due to effects of certain dietary fatty acids on the pituitary, ovaries and uterus, rather than via improved energy status (Lucy et al., 1992; Staples et al., 1998). It is believed that only certain dietary fats can suppress PGF production because specific fatty acids (e.g. polyunsaturated fatty acids; PUFA) are known to play a key role in PGF synthesis. Therefore, by manipulating the fatty acid profile of diets, uterine synthesis of PGF during early pregnancy can potentially be suppressed, resulting in a reduction in embryonic mortality. This is an area of research that is beginning to receive attention. In the past few years, several reports have indicated that diets enriched in specific fatty acids (particularly those of the omega-3 family) improve fertility in dairy cows. This paper will summarize some of the recent research findings on this topic.

### Fats, Fatty Acids and Essential Fatty Acids

Fat, derived from either animals or plants, is an important nutrient that provides energy, fatty acids and fat-soluble vitamins. In general, fats of animal origin are high in saturated fatty acids, whereas fats of plant origin are high in unsaturated fatty acids. Fatty acids are classified as either “unsaturated” or “saturated” based on the presence or absence of “double bonds” in their chemical structure. Saturated fatty acids have no double bonds, whereas unsaturated fatty acids have double bonds in their structure. Unsaturated fatty acids may be further categorized as either “monounsaturated” (one double bond) or “polyunsaturated” (more than one double bond). Unsaturated fatty acids are also classified into different classes according to the number of carbon atoms and the number of double bonds present in their structure. Linoleic acid, for example, has 18 carbon atoms and two double bonds; thus, it is conventionally written as C18:2. Alpha-linolenic acid, on the other hand, has 18 carbon atoms and three double bonds, and therefore is written as C18:3. Depending on the location of the first double bond relative to the methyl end of the fatty acid chain, PUFA are also classified as members of either the Omega-3 (n-3) family (e.g. C18:3n-3) or the Omega-6 (n-6) family (e.g. C18:2n-6).
Animals can synthesize most fatty acids, with the notable exception of fatty acids belonging to the n-3 and n-6 families. Since animals must have a dietary source of these fatty acids, they are considered essential fatty acids. When consumed by animals, linoleic acid, found mainly in oilseeds, is desaturated (insertion of double bonds) and elongated (addition of carbon atoms) to form arachidonic acid (C20:4n-6). In contrast, \( \alpha \)-linolenic acid, found predominantly in forage (e.g. grass, legume leaves) and select oilseeds (e.g. flaxseed), is desaturated and elongated to form two fatty acids that are unique to fish oils: eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:n-6).

The essential fatty acids have three important functions (Sinclair, 1984); the most important function is structural, contributing to the maintenance and function of cellular membranes. In addition, essential fatty acids are important in the metabolism of cholesterol and the synthesis of eicosanoids (bioactive compounds such as prostaglandins, leukotrienes and thromboxanes, all derived from 20-carbon fatty acids).

### Essential Fatty Acids and Reproductive Function

Deficiency of essential fatty acids in experimental animals can cause various disorders associated with defective cell membranes, including impaired growth, skin and connective tissue abnormalities, and impaired fertility (Sinclair, 1984). Zeron et al., (2001) reported that a decline in the fertility of Holstein cows during summer months was associated with a significantly lower content of linoleic acid in the oocyte membrane. Although \( \alpha \)-linolenic acid content did not have any seasonal variation, EPA and DHA were not detectable in oocyte membranes. In contrast, these two fatty acids (EPA and DHA) are present at high concentrations in bull sperm membranes and play a major role in membrane fluidity (Parks and Lynch, 1992). It has been reported that roosters fed a diet high in n-3 PUFA had increased n-3 PUFA in sperm membranes, had improved sperm quality and fertilizing ability (Abayasekara and Wathes, 1999). It is also believed that, at the cellular level, fatty acids may have a direct effect on the transcription of genes that encode proteins essential for reproductive events (Mattos et al., 2000).

In some animals, PUFA may play an important role in many reproductive processes including ovulation, fertilization and parturition (Abayasekara and Wathes, 1999). In cattle, dietary fatty acids can influence ovarian follicular growth, CL function and progesterone production (Abayasekara and Wathes, 1999; Mattos et al., 2000). Prepartum diets high in PUFA of the n-3 family delayed parturition in sheep (Baguma-Nibasheka et al., 1999) and increased the incidence of placental retention in cattle (Barnouin and Chassagne, 1991). In contrast, increasing the dietary availability of n-3 PUFA during the postpartum period improved pregnancy rates in cattle (Armstrong et al., 1990;
Ambrose and Kastelic

Burke et al., 1997; Petit et al., 2001; Ambrose et al., 2002) likely through a decrease in uterine PGF secretion during early pregnancy (Thatcher et al., 1997). The essentiality of specific fatty acids in reproductive performance of dairy cows and possible mechanisms through which PUFA may influence reproductive function (as proposed by researchers at the University of Florida), were presented at this conference last year (Spain, 2002).

It is clear that the PUFA are important for reproduction. The practical significance to the dairy farmer is the potential ability to reduce embryonic loss by feeding PUFA-enriched diets to reduce/suppress PGF secretion in dairy cattle during the critical period of maternal recognition of pregnancy. Some of the published literature on this topic will be discussed later in this paper. But first, let's see how and why suppression of PGF will help improve fertility.

**PGF Suppression in Early Pregnancy Improves Embryo Survival**

In a normal, cycling, non-inseminated cow, the lifespan of the corpus luteum (CL) is about 16 days. Approximately 12 d after estrus, low amplitude pulses of PGF secretion begin in the uterus of both cyclic and pregnant cattle. This initial increase in PGF is followed by high amplitude pulses of PGF-release by the uterus of cyclic (non-pregnant) animals, leading to regression of the CL. In the event of a pregnancy, the high amplitude pulsatile release of PGF is suppressed, allowing the continued maintenance of CL and pregnancy establishment. The suppression of PGF depends on a timely “signal” from the developing embryo. Interferon-\(\tau\) has been recently identified as the primary embryonic signal that leads to maternal recognition of pregnancy (Thatcher et al. 1997). Embryos start secreting interferon-\(\tau\) in small quantities as early as Day 10, with increasing secretion as the conceptus elongates. However, secretion of interferon-\(\tau\) in quantities sufficient to prevent the pulsatile release of PGF secretion may not happen until the embryo acquires a filamentous form (that is, beyond Day 13). Because all embryos do not develop at the same rate, it is very likely that before slow-growing embryos get a chance to signal their presence through expression of interferon-\(\tau\) in sufficient quantities, the high amplitude pulses of PGF may set-in, leading to regression of the CL, resulting in low plasma progesterone concentrations, pregnancy loss, and a return to estrus. However, an alternate means of suppressing PGF secretion, at least temporarily, would give slow-growing embryos an increased time-window to “catch-up” and “signal” their presence, vastly increasing their chance of survival.

Because PUFA suppress PGF during early pregnancy in dairy cows, feeding diets enriched in PUFA to improve fertility is an exciting new possibility in dairy reproductive management.
### Effect of Fishmeal-Based Rations on Fertility

Unsaturated fatty acids are largely metabolized by rumen bacteria in a process known as biohydrogenation. During biohydrogenation, unsaturated fatty acids are converted to saturated fatty acids. However, two unique fatty acids, EPA and DHA (high in fish oils), are known to largely escape ruminal biohydrogenation (Ashes et al., 1992). Because these fatty acids are also known to decrease PGF secretion (Sinclair, 1984; Thatcher et al., 1997), feeding fishmeal to cattle has the potential to enhance fertility.

In an Israeli study involving 240 Holstein cows, Bruckental et al. (1989) reported that overall pregnancy rate at 16 wk postpartum was higher (P<0.05) in cows fed a fishmeal-based ration (72%) compared to cows fed a ration supplemented with soybean meal (52%) when both groups of cows received 210 g crude protein per kg dry matter (DM). An Irish group (Armstrong et al., 1990) subsequently reported that British Friesian cows (about 160 cows per year over a 2-yr period) fed fishmeal (0.8 kg/day) had improved conception rates to all services (64% vs 44% in control cows; P<0.05), with a reduction in the number of services per conception (1.62 vs 2.31, P<0.01).

More recently, Burke et al. (1997) tested this hypothesis by feeding menhaden fishmeal (MFM) to dairy cattle in two commercial herds in Florida (total of > 600 cows). Cows were assigned to either a fishmeal diet (MFM @ 0.7 kg/d) or Control diet (no fishmeal) from 24 to 109 d postpartum. All cows were synchronized for estrus at 51 d postpartum and inseminated at detected estrus. Overall pregnancy rate at 120 d was similar between diets in one herd (60.2 vs 65.4% for MFM vs Control, respectively), but in the second herd, MFM-fed cows had a higher pregnancy rate (41.3 vs 31.9%; P<0.06). It is noteworthy that a greater proportion of cows fed MFM had plasma progesterone concentrations >1 ng/mL 48 h after PGF injection than cows fed a control ration (29 vs 4%). The authors assumed that MFM delayed CL regression and suggested that EPA and DHA present in the MFM diets might have reduced uterine synthesis of PGF, leading to a slight delay in CL regression. Such a slight delay in the complete regression of CL is not considered detrimental to conception.

Although the inclusion of fishmeal in the diet of dairy cattle improved pregnancy rates, the mechanisms were not clear. The proposed hypothesis was that the PUFA in fishmeal, mainly EPA and DHA, were reducing PGF secretion in the uterus. Therefore, Coelho et al. (1997) designed an experiment to determine if feeding a diet enriched in EPA and DHA actually contributed to the reduction of uterine PGF secretion in dairy cows. Primiparous cows (approximately 83 d in milk) were randomly assigned to receive either MFM (n=7) or Control (n=8) diets. Diets were isonitrogenous (17.7% of DM) and isocaloric (1.59 Mcal/kg of DM). Dried distillers grains and part of the soybean meal in the control diet were replaced by fishmeal (5.4% of DM) in the MFM diet. Cows were fed twice
daily as a TMR ad libitum; the mean dry matter intake was 20.6 kg/d. The fat content of MFM was 8.3%. Of the total fats, 11.4% was EPA and 6.6% was DHA. It was estimated that each cow receiving the MFM diet consumed 10.8 g of EPA and 6.2 g of DHA, compared to 0 g of each, by cows in the control diet. Fifteen days after a synchronized ovulation, all cows were subjected to an experimental protocol (estradiol, 3 mg iv; followed 4 h later by 100 IU oxytocin, iv) to induce the release of PGF from the uterus. Blood samples were taken to measure the PGF metabolite (PGFM) in plasma. Cows fed MFM had significantly lower plasma PGFM (P<0.01; Figure 1), indicating a suppression of uterine PGF release. Peak concentrations of PGFM occurred at 30 min, and MFM attenuated the estradiol- and oxytocin-induced increase in PGF.

![Figure 1. Plasma concentrations of PGFM after estradiol and oxytocin challenge in cows fed fishmeal versus a control diet (Coelho et al., 1997).](image)

In a more recent study, Mattos et al. (2002) assigned 32 cycling multiparous cows to rations containing 0, 2.6, 5.2 or 7.8% MFM. The diet with 7.8% MFM also contained fish oil at 0.28% of dietary DM in order to increase the intake of EPA and DHA. Average dry matter intake was 24.9 kg/d and was unaffected by diet. Combined intakes of EPA and DHA averaged 0, 12.8, 24.1 and 54.0 g/d, from the 0, 2.6, 5.2 and 7.8% MFM diets, respectively. As in the previous study, cows were treated with estradiol and oxytocin 15 d after ovulation. In cows fed MFM, plasma concentrations of PGF metabolite were significantly lower (than in control cows) 15, 30, and 45 minutes after injection of oxytocin, confirming the findings of Coelho et al. (1997).
In another study, Thatcher et al. (2001) provided evidence that EPA, DHA and \( \alpha \)-linolenic acid can reduce PGF secretion by bovine endometrial cells (cells of the uterus) under in vitro conditions. Although all three fatty acids inhibited PGF secretion compared to an untreated control (\( P<0.05 \)), EPA and DHA were more effective than \( \alpha \)-linolenic acid in suppressing PGF secretion. Adding 20 \( \mu \text{M} \) \( \alpha \)-linolenic acid, DHA or EPA resulted in a 22\% (\( P=0.04 \)), 60\% (\( P<0.01 \)), and 61\% (\( P<0.01 \)) reduction in PGF at 6 h of culture (Figure 2), respectively, in comparison to the control without fatty acid (6203 pg/ml).

![Figure 2](image)

**Figure 2.** PGF secretion by cells of the cow uterus (bovine endometrial cells) in culture after 6 h of co-incubation with 20 \( \mu \text{M} \) \( \alpha \)-linolenic acid (ALA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) versus an untreated control (Adapted from Thatcher et al., 2001).

Certain oilseeds (e.g. flaxseed) are high in \( \alpha \)-linolenic acid, which, upon elongation and desaturation, forms EPA and DHA. Although flaxseed is a good source of fat (about 30\%) and high in \( \alpha \)-linolenic acid (55-60\% of total fats), it is not commonly fed to cattle. Can flaxseed-based rations improve fertility in dairy cows?

### Influence of Diets Enriched in \( \alpha \)-Linolenic Acid on Fertility

Feeding whole or processed flaxseed or abomasal infusion of flaxseed oil to dairy cows increased long chain fatty acids concentration in milk (Kennelly, 1995), including that of EPA (Hagemeister et al. 1991). Because flax oil is rich
in \( \alpha \)-linolenic acid, and \( \alpha \)-linolenic acid can suppress PGF secretion directly or through its desaturates (EPA and DHA), feeding flaxseed to dairy cows has the potential to increase fertility through the mechanism discussed above.

In a recent study, Petit et al., (2001) assigned 35 lactating dairy cows to receive (from 9 to 19 weeks of lactation) total mixed rations based on ryegrass silage and supplemented with either Megalac and solvent-extracted flaxseed meal or formaldehyde-treated whole flaxseed. Dry matter intake and body weight change did not differ between treatments. Milk production was higher for cows fed Megalac than for those fed flaxseed (19.8 vs 18.6 kg/day). Cows fed formaldehyde-treated flaxseed had a higher conception rate than those fed a Megalac diet (87.5 vs 50%, respectively; \( P < 0.05 \)), but there were no significant differences in the size or number of ovarian follicles.

More recently, we (Ambrose et al., 2002) presented preliminary findings of a similar study involving a larger number of cows; this study has subsequently been completed. The objectives were to determine if a diet enriched in \( \alpha \)-linolenic acid would enhance ovarian follicular growth, plasma progesterone concentrations, embryo survival, and pregnancy rate in lactating dairy cattle. Holstein cows (\( n=121 \)) were assigned to diets supplemented with either rolled flaxseed (55% of lipid content is \( \alpha \)-linolenic acid) or rolled sunflower seed (<1% of lipid content is \( \alpha \)-linolenic acid), to provide approximately 750 g oil/cow/day. [The oilseeds contained approximately 32% fat. On average, each cow was fed 2.29 kg sunflower seed or 2.41 kg flaxseed]. Diets began 4 wk before breeding when cows were about 55 d postpartum. Barley silage and barley grain-based rations were formulated to meet or exceed NRC 2001 requirements. Diets were similar in metabolizable protein and net energy of lactation. Based upon a mean dry matter intake of 24.2 kg/d, cows on the flaxseed diet consumed 410 g \( \alpha \)-linolenic acid compared to cows on the sunflower diet (~ 5 g \( \alpha \)-linolenic acid). Ovulation was synchronized with a Presynch/Ovsynch protocol and cows were bred by timed-insemination. The gonadotropin releasing hormone (GnRH) preparation used in the Ovsynch protocol was Fertiliene (Vetoquinol N-A Inc., Lavaltrie, QC) at a dose of 2 ml; the prostaglandin product was Estrumate (Schering Canada Inc., Pointe-Claire, QC), also at a 2 ml dose. Pregnancy diagnosis was conducted 32 d after AI by ultrasound. Once pregnancy was confirmed, pregnant cows were removed from the experimental diets. Nonpregnant cows were immediately placed on another Ovsynch regimen, rebred 10 d later (i.e. 42 d after the first AI), and experimental diets continued until pregnancy diagnosis, 32 d after second AI. In a subset of 16 cows (\( n=8 \) per diet) ovarian structures were monitored by transrectal ultrasonography and serum progesterone was measured on alternate days from Days 0 to 20 and 0 to 18, respectively.

Blood samples were obtained from all other cows (for progesterone assay), on Days -10, -3, 0 (day of AI), 7, 21, and 24. Pregnancy rate was estimated on Day 24 (presumptive pregnancy) based on progesterone concentrations being
<1ng/ml at AI (Day 0) and >1ng/ml 7, 21 and 24 d after AI. Presumptive pregnancy rate to the first AI was higher in cows fed flaxseed than in those fed sunflower seed. Confirmed pregnancy rate to first AI at 32 d also remained higher in cows fed flax, compared to those fed sunflower seed (see Figure 3). If anestrous cows were excluded and only the remaining cows (n=107) were considered, pregnancy rates to first AI improved slightly, but the difference between sunflower seed (37.3%) and flaxseed (53.6%) became relatively less significant (P=0.09) because of the decrease in animal numbers.

Figure 3. Presumptive pregnancy rate 24 d after AI (PP24), confirmed pregnancy rate at 32 d (Preg32) and embryo survival (ES) from 24 to 32 d post AI in cows fed a ration supplemented with either rolled sunflower seed or rolled flaxseed.

Embryo survival rate between Days 24 and 32 (post AI) did not differ between diets, but there was a higher rate of early (AI to Day 24) embryo survival in cows fed the flaxseed-based ration. Cumulative pregnancy rates (combined for both inseminations) were 67.7% (flax) and 59.3% (sunflower, P>0.10).

In the subset of 16 cows with more intensive monitoring, the preovulatory follicle (prior to the first AI) was larger in cows fed flaxseed compared to those fed sunflower seed (16.9 vs. 14.1 mm; P<0.05). Cows fed sunflower seed had a larger number of small (2-5 mm) follicles compared to flaxseed fed cows (30.2 vs 25.4; P<0.01). Mean number of larger follicles or size of CL was not affected by diet. Although mean serum progesterone concentrations were not different between diets, progesterone concentration at AI remained elevated in flax-fed cows (0.41 vs 0.15 ng/ml; P<0.05) suggesting that a flaxseed-based diet may have slightly delayed the CL regression process, likely via reduced PGF secretion. Burke et al. (1997) made similar observations in cows fed a
fishmeal-based ration. Linoleic and $\alpha$-linolenic acid content of milk increased by 74% and 187%, respectively (relative to pre-diet levels), in cows fed flaxseed, and by 121% and 21%, respectively, in cows fed sunflower seed.

In our study, inclusion of rolled flaxseed in the diets of postpartum dairy cows improved fertility, possibly through enhanced embryo survival during the early gestation period. Was this improvement in fertility through suppression of PGF? Most likely so. However, this hypothesis was not supported in at least one study (Petit et al., 2002) where the researchers failed to demonstrate a difference in mean plasma PGFM among cows fed formaldehyde-treated whole flaxseed, flaxseed oil, or a Megalac-based ration. In fact, cows fed a combination of flaxseed and fish oil had elevated concentrations of PGF. This contradicts previous reports of Coelho et al. (1997) and Mattos et al. (2002), who showed significant reductions in PGFM concentrations in cows fed menhaden fishmeal. It is noteworthy that plasma PGFM concentrations in the study by Petit et al. (2002) remained substantially lower than those reported by Coelho et al. (1997) and an acute increase in PGFM as a consequence to oxytocin challenge did not occur even in the control cows, unlike in the studies by Coelho et al. (1997) and Mattos et al (2002). Another difference is that Petit et al. (2002) used grass silage as a major ingredient in the TMR fed to all cows. Grass silage is a very rich source of $\alpha$-linolenic acid (63.7% of total fatty acids, in this case – as reported by the authors); therefore, a high concentration of $\alpha$-linolenic acid in all experimental diets may have contributed to an overall suppression of PGF. Even the type of fish (menhaden vs herring) used as the fatty acid source may have contributed to some difference between the studies. More studies are essential to elucidate the mechanism(s) through which diets enriched in $\alpha$-linolenic acid may be enhancing fertility in cattle.

**Summary**

Current evidence indicates that dietary supplementation of essential fatty acids, specifically those of the Omega-3 series, will enhance fertility in dairy cattle. Inclusion of menhaden fishmeal or flaxseed in the ration of lactating dairy cows has improved pregnancy rates. The mechanism(s) of action are still not completely understood. Though postpartum diets enriched in EPA, DHA or $\alpha$-linolenic acid helped improve fertility, some studies suggest that such diets, if fed during the dry period, may increase the incidence of placental retention. However, at least one report (Kemp et al., 1998) found no such evidence. This is a relatively new area of research and much more remains to be uncovered.
Acknowledgements

Our study (using diets supplemented with flaxseed or sunflower seed) was funded by Alberta Milk, and the Matching Investment Initiative of Agriculture and Agri-Food Canada. The technical assistance provided by Phyllis Day and Pavol Zalkovic, the expert advice of Rick Corbett on ration formulations, and cooperation of the staff at the dairy research unit of the Dairy Research and Technology Centre, Edmonton, are greatly appreciated. Estrumate and Fertiline used in the synchronization protocols, and sunflower seed for the diets were graciously donated by Schering Canada, Vetoquinol N-A Inc., and Pioneer Hi-Bred, respectively.

References


