Can Feeding Fats Improve Reproductive Performance in Dairy Cows?

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Take Home Messages

- Growing evidence indicates that feeding fat to dairy cows improves their reproductive performance.
- Polyunsaturated fatty acids (PUFA) have important beneficial effects on several reproductive functions.
- Feeding omega-3 PUFA sources such as flaxseed and fish oil reduced pregnancy losses in dairy cows in several studies.
- New research indicates that exposure to omega-3 PUFA enhances early embryo development through differential activation of genes regulating cellular function and proliferation.
- Many questions still remain on the optimization and economics of dietary fats and fatty acids to improve dairy cow reproductive function.

Introduction

Poor reproductive efficiency of dairy cows continues to be a challenge for the dairy industry (Ambrose and Colazo, 2007; Leblanc, 2005; Lucy, 2001). Embryonic losses are significant contributors to poor reproductive efficiency in dairy cows, with post-fertilization losses estimated to be up to 60% (Santos et al., 2004).

Nutritional management is one of the strategies available to improve reproduction in dairy cows. There is growing evidence that supplementing fats in dairy rations has beneficial effects on reproductive function and performance of lactating dairy cows. A recent report (Rodney et al., 2015), adopting the meta-analysis approach, screened over 5000 research papers on the subject of feeding fats and reproductive function in dairy cattle but found only 17 studies with 26 dietary comparisons that were suitable for inclusion in the meta-analysis. Based on their meta-analysis, the authors concluded that feeding fats during the transition period has a positive effect on fertility, with a 27% increase in the probability of pregnancy to service. In addition, cows fed fats during the transition period had a reduction in the interval from calving to pregnancy (9 of 11 comparisons), and a tendency to increase milk production (16 of 23 comparisons).

How dietary fats improve reproductive performance in dairy cattle is not fully understood, but several hypotheses exist (Staples et al., 1998). Improved fertility may result from (a) improvement in energy status, shortening the interval from calving to first postpartum estrus, (b) increased production of steroid hormones, e.g., progesterone, which is essential for pregnancy maintenance, (c) alterations in serum insulin concentrations, which could improve ovarian follicular development, and (d) reduced synthesis and release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the uterus by specific long-chain fatty acids, creating a conducive environment for early embryo survival and pregnancy establishment. Polyunsaturated fatty acids (PUFA) such as linoleic acid, α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acids have garnered much attention lately for their positive effects on reproductive function in cattle, and several excellent reviews have been written on this topic (Mattos et al., 2000; Santos et al., 2008; Wathes et al., 2013). Diets enriched in n-3 PUFA can have positive effects on the development of the early embryo, potentially through the differential activation of genes involved in embryonic cellular growth and proliferation (Salehi et al., 2016).

▪ Fats and Fatty Acids

One of the reasons for feeding fats to dairy cows is to increase energy intake efficiently, because of the higher caloric content packed in fats compared to other feed ingredients. Although both plant (e.g. oilseeds) and animal fats (e.g. tallow, fishmeal) have been used to supplement dairy cow rations primarily to improve energy, many studies have shown beneficial effects of dietary fat on reproductive function (Rodney et al., 2015). Such benefits could be independent of improved energy status, through the action of certain long chain fatty acids on the reproductive axis (Staples et al., 1998). Animals cannot synthesize essential fatty acids such as linoleic and α-linolenic acids; hence, these fatty acids must be made available through diets. Specifically, linoleic (C18:2, n-6) and α-linolenic (C18:3, n-3) acids get converted to very long chain fatty acids through processes of desaturation and elongation. For example, linoleic acid gets converted to arachidonic acid (C20:4, n-6), a precursor for prostaglandins of 2-series, such as $PGF_{2\alpha}$, which plays a major role in the regulation of the estrous cycle, regression of corpus luteum and termination of pregnancy in cattle. Alpha-linolenic acid gets converted to eicosapentaenoic (C20:5, n-3) acid and docosahexaenoic (C22:6, n-3) acids, precursors for prostaglandins of 3-series (e.g. PGE_3), which can alter PGE:PGF ratios, potentially diminishing the luteolytic effects of $PGF_{2\alpha}$. Moreover, eicosapentaenoic and docosahexaenoic acids can directly act at the level of the uterus (site of $PGF_{2\alpha}$ production) to suppress $PGF_{2\alpha}$ release by reducing the availability of arachidonic acid. Therefore, supplying dietary n-3 PUFA that alter $PGF_{2\alpha}$ release could be a strategy to improve embryo survival in cattle.

Understanding the influence of diets enriched in omega-3 (n-3) and omega-6 (n-6) PUFA on reproductive function in dairy cows has been of interest to us for many years. In this paper, we present research findings on the effects of dietary PUFA, mainly from work done in our laboratory, drawing parallels from related studies done elsewhere, where relevant.

▪ Effects of Dietary Fat (n-3 PUFA) Inclusion During Postpartum Period on Conception Rates and Pregnancy Losses

Flaxseed is one of the richest sources of α-linolenic acid, an n-3 PUFA. In recent years, we have conducted 3 studies investigating the effects of adding flaxseed as an n-3 PUFA supplement in postpartum dairy cow rations on conception rates and pregnancy losses.

Study 1. Alberta

In early work (Ambrose et al., 2006), we compared the effects of type of PUFA on ovarian function, early embryonic survival, conception rates and pregnancy losses in dairy cows receiving a diet supplemented with rolled flax seed (high in α-linolenic acid, n-3 PUFA) vs. sunflower seed (high in linoleic acid, n-6 PUFA). Cows in the study were housed in tiestalls, fed individually and milked twice daily. We randomly assigned 121 lactating dairy cows to 1 of 2 diets starting at approximately 55 days after calving. Diets were isocaloric and contained ~9% rolled oilseeds (either flax or sunflower) on a dry matter basis, providing approximately 750 g of oil per cow per day. The experimental diets were fed for a minimum period of 8 weeks. In a subset of 16 cows (8 per diet), ovarian follicular dynamics were monitored every other day from the day of insemination (d 0) until day 21. To remove the variations associated with estrus detection, timed artificial insemination (TAI) was performed on all cows following a Presynch/Ovsynch protocol. Presumptive conception rate at day 24 was assessed based on progesterone measured in blood (plasma) at 0, 21 and 24 days after TAI. Cows were presumed conceived on day 24 if progesterone concentration had been lower than 1 ng/mL on day 0 (i.e. at

TAI) and greater than or equal to 1 ng/mL on day 21 and 24. Pregnancy diagnosis was performed by ultrasonography 32 days after TAI. Cows confirmed pregnant at 32 days after TAI stopped receiving the experimental rations, whereas non-pregnant cows were placed on the Ovsynch protocol for a second time and rebred by TAI 10 days later. In these cows, the experimental diets continued until pregnancy diagnosis, 32 days after second TAI.

The numbers of small, medium and large ovarian follicles were not affected by diets but the ovulatory follicle before first TAI was larger (16.9 vs. 14.1 mm) in cows fed flaxseed compared to those fed sunflower seed. Presumptive conception rate 24 days after TAI was higher in cows fed flax than in those fed sunflower seed (72.9 vs. 47.5%; $P<0.01$).

Conception rate: Conception rate to first TAI, confirmed by ultrasound 32 days after TAI, tended to be higher in cows fed flaxseed than in cows fed sunflower seed (48.4 vs. 32.2%; $P<0.07$). Conception rates to the second TAI and cumulative conception rates (combined for both TAI) were not different between diets.

Pregnancy loss: Cumulative pregnancy loss (from 32 days until calving) in cows fed the flaxseed diet was significantly lower than in cows fed the sunflower seed diet (9.8 vs. 27.3%). In other words, 90.2% of pregnant cows calved in the flax group compared to only 72.7% in the sunflower group.

To determine if the above findings of increased conception rates and reduced pregnancy losses in flaxseed-supplemented rations were repeatable, we conducted 2 independent studies in Oregon and British Columbia. In both studies, lactating Holstein cows were assigned to receive a total mixed ration (TMR) containing 6% fat on a dry matter basis. Each cow received ~2.2 kg of flaxseed per day (flax) or a no-flaxseed supplement (control).

Study 2. Oregon

This work was done in collaboration with the Oregon State University in a large (1300 cow), high-producing commercial dairy herd, with a rolling herd average of 11,435 kg. We randomly assigned 303 early postpartum cows to receive a TMR supplemented with (n=156) or without (n=147) rolled flaxseed. The flax ration was formulated to supply 750 grams of oil per cow per day from rolled flaxseed. The control ration supplied an equal amount of fat from a combination of corn dried distillers grain and solubles, High Fat Product (Archer Daniels Midland Co) and Megalac®. Cows were fed freshly mixed TMR twice daily and milked 3 times daily. Diets began approximately 32 days after calving and continued for 31 days after TAI, which occurred following a Presynch/Ovsynch protocol. Cows were subjected to TAI by 1 of 2 technicians, with TAI occurring ≥28 days after initiation of experimental diets. Pregnancy diagnosis was performed by ultrasonography 31 days after TAI. Cows diagnosed pregnant were rechecked at 94 days to assess pregnancy losses between 31 and 94 days of gestation.

Conception rate: The conception rate was numerically lower in cows fed flaxseed compared to those fed the control diet, both at 31 days (28.2 versus 42.9 %; P= 0.13) and 94 days (25.6% versus 36.7%; P=0.31). Pregnancy rate at 31 days was influenced (P<0.02) by AI technician (24.4 versus 40.5% for the two technicians) but there was no diet-by-technician interaction.

Pregnancy loss: Overall, 12.2% of the pregnancies were lost between 31 and 94 days of gestation. Though not statistically different, the proportion of pregnancy loss was 37% lower (P= 0.20) in cows fed flaxseed (9.0%; 4 of 44) than in cows fed the control diet (14.3%; 9 of 63).

Study 3. British Columbia

The next study was conducted in collaboration with the University of British Columbia at the Dairy Education and Research Centre in Agassiz, BC. We randomly assigned 266 lactating dairy cows to receive either a TMR supplemented with rolled flaxseed (n=141) or a control ration with no-flaxseed (n=125). As in the previous studies, the flaxseed ration was formulated to provide 750 g of oil per cow per day from rolled flax seed. The control diet was formulated to provide 750 g of fat supplied from tallow and Megalac®. Diets began a minimum of 28 days before TAI and continued until pregnancy diagnosis at 35 days after TAI. Reproductive management procedures were similar to that of the previous study, except that pregnancy was confirmed by ultrasonography at 35 days after TAI, and reconfirmed by rectal palpation at 90 days of gestation. Data on calving were also collected from this location. Early pregnancy loss between 35 and 90 days of gestation, and cumulative pregnancy loss between 35 days and term (calving) were determined.

Conception rate: Diets did not affect the conception rate at 35 days, with flax and no-flaxseed cows averaging 43.3 and 41.6%, respectively. Conception rates at 90 days for the 2 dietary treatments were 40.4 and 38.4%, respectively.

Pregnancy loss: Pregnancy losses between 35 days of gestation and calving were numerically lower (P=0.20) in cows fed flaxseed (8.3%) than in cows fed a control ration (16.3%).

Other Related Studies with Postpartum n-3 PUFA Diets on Conception Rate and Pregnancy Loss

In a Quebec study, Petit and Twagiramungu (2006) assigned 3 groups of 46 cows each to 1 of 3 isonitrogenous, isoenergetic, and isolipidic supplements based on either whole flaxseed (flax), Megalac® or micronized soybeans (soybeans). Rations contained ~10.6% whole flaxseed and ~7.3% total fat on a dry matter basis. The experimental diets were fed from calving until 50 days of gestation in cows that conceived to first AI, or until 120 days for those found not pregnant after the first AI.

Conception rate and pregnancy loss: Conception rates did not differ among treatments, with first service conception rates of 44.4, 55.9 and 40.0%, respectively, for flax, Megalac® and soybean diets. Total embryo mortality was lower (P=0.07) for cows fed flax (0%) compared to those fed either Megalac (15.4%) or soybean (8.0%). Progesterone concentrations were higher from day 17 to 21 of an estrous cycle in cows fed flax. The authors concluded that pregnancy losses could be reduced by feeding whole flaxseed through possible modulations in concentration of progesterone and size of the corpus luteum.

In a large study at the University of Florida, Silvestre et al. (2011) assigned 1380 Holstein cows to diets containing calcium salts of either palm oil (high in saturated fatty acids) or safflower oil (high in linoleic acid) from 30 days before calving until 30 days after calving, and then to receive either calcium salts of palm oil or fish oil (high in eicosapentaenoic and docosahexaenoic acids) from 30 to 160 days postpartum. The experimental diet combinations fed during the transition (-30 to +30 d) and breeding periods (30 to 160 days postpartum), respectively, were palm oil + palm oil; palm oil + fish oil, safflower oil + palm oil, and safflower oil + fish oil. Total dietary fat (dry matter basis) in the breeding period was about 5%, with 1.5% being supplemental fat from calcium salts of either palm oil or fish oil.

Conception rate and pregnancy loss: Although pregnancy per AI at 32 and 60 days after first AI was not affected by diets, pregnancy loss was significantly (P<0.01) reduced in cows fed fish oil versus palm oil (6.0 vs. 11.8%) during the breeding period, regardless of whether the cows were fed diets supplemented with palm or safflower oils during the transition period.

Summary of findings: When n-3 PUFA of either flaxseed or fish oil origin was included in the postpartum rations, conception rate (pregnancy per AI) did not consistently increase. In 2 of the 5 studies, conception rate tended to increase in cows fed n-3 PUFA, whereas in the other 3 studies, conception rate was not affected by diet; however, pregnancy loss was consistently lower in cows fed diets enriched in n-3 PUFA in all 5 studies (Figure 1). Mean pregnancy losses in the n-3 PUFA dietary group were significantly lower or tended to be lower in 3 of the 5 studies, and numerically lower in 2 studies. These findings strongly suggest that PUFA diets of flaxseed or fish oil origin (predominantly n-3 fatty acids) can reduce pregnancy losses in dairy cows.

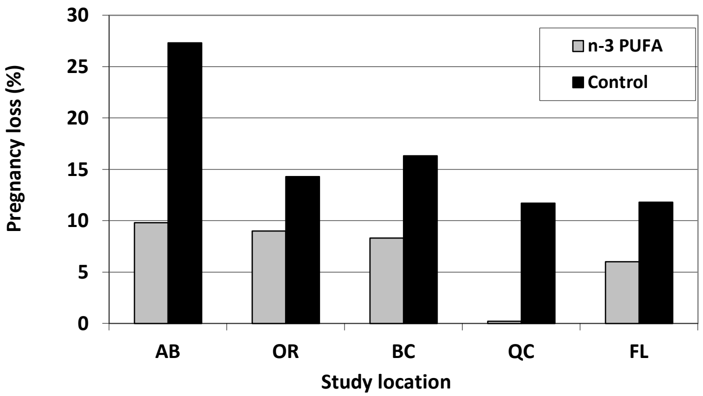

Figure 1. Pregnancy loss in 5 different studies in which lactating dairy cows were fed a diet enriched in n-3 PUFA of either flaxseed or fish oil origin vs. a control diet containing low or no n-3 fatty acids. Study locations were Alberta (AB), Oregon (OR), British Columbia (BC), Quebec (QC) and Florida (FL). The total number of cows in each study and probability of difference for pregnancy losses in AB, OR, BC, QC and FL were 121 ($P<0.05$), 303 ($P>0.10$), 263 ($P>0.10$), 138 ($P=0.07$), 1380 ($P<0.01$).

▪ Effects of Dietary Fat Inclusion During the Prepartum Period on Postpartum Reproductive Performance

Study 1. (Colazo et al., 2009)

This study was designed to determine the effects of feed restriction and source of dietary fatty acids during the close-up dry period on postpartum reproductive performance of dairy cows. We hypothesized that (1) restricted feed intake during the prepartum period will improve postpartum intake and reduce negative energy balance, thereby contributing to improved reproductive performance, and (2) that inclusion of oilseeds with different fatty acid profiles in prepartum diets will have a differential influence on resumption of cyclicity and carryover beneficial effects on fertility.

We assigned 72 cows to receive 1 of 6 diets starting 34 days before expected calving date in a 2 x 3 factorial arrangement. Dietary treatments were 2 levels of feed intake (ad libitum; AL or 24% feed restriction; FR), and 3 types of rolled oilseed supplements: canola, linola or flax at 8% of dry matter, to enrich the diets with oleic, linoleic or α-linolenic acids, respectively. A common lactation diet containing no oilseeds was fed to all cows after calving. Reproductive tracts were examined by ultrasonography twice weekly from 7 days after calving until confirmation of first ovulation. After an elective waiting period of 65 days, 66 of the 72 cows were subjected to Ovsynch/TAI and pregnancy was diagnosed 32 days after TAI.

The mean dry matter intake during the prepartum period of cows fed AL (11.9 kg/d) was higher than that of FR cows (9.0 kg/d). Prepartum energy balance was higher in AL than in FR cows (3.6 vs. -0.4 Mcal/d; $P<0.01$) but fatty acid source (i.e., oilseed) did not affect energy balance. Milk production was not affected by FR but affected by the source of dietary fatty acids with canola, linola and flax fed cows yielding 32.9, 36.4 and 34.6 kg, respectively. The birth weight of calves was not affected by level of intake or fatty acid source. A significantly higher proportion of cows in the AL group suffered uterine infections relative to FR (27 vs. 6%; $P<0.01$) but FR cows tended to have a higher incidence of ovarian cysts (20 vs. 5%; $P=0.09$). Feed restriction during the close-up dry period had a negative impact on conception to first TAI (19 vs. 47%; $P=0.02$) compared to AL, without affecting the interval from calving to uterine involution or ovarian function. However, the mean interval from calving to first ovulation was longer ($P=0.02$) in cows fed canola seed (35 d) compared to those fed either linola (24 d) or flax seed (21 d). Pregnancy to first AI, cumulative pregnancy from 75 to 280 days postpartum, and days open were not affected by oilseed type (Table 1) indicating that feeding fats in the prepartum period has little carryover beneficial effects in terms of reproductive outcomes, although feeding oilseeds high in PUFA (e.g. linola, flax) shortened the interval from calving to first ovulation.

Table 1. Postpartum reproductive performance indices in cows fed canola, linola or flaxseed during the prepartum period (Colazo et al., 2009)

	Prepartum diet			
Index	Canola	Linola	Flax	*P*
Cows (n)	24	24	24	
Calving to 1st ovulation[1] (d)	34.7 ± 3.1	23.7 ± 3.2	21.0 ± 3.1	0.02
Pregnancy to 1st TAI (%)	26.3	43.5	29.2	0.43
Cumulative pregnancy[2] (%)	54.2	70.8	62.5	0.49
Days open	186 ± 10.9	167 ± 14.3	166 ± 14.5	0.24

[1]Canola > linola and flax

[2]Pregnancies from 75 to 280 d postpartum

Study 2. Salehi et al (2016)

The finding in our previous study (Colazo et al., 2009) that the interval from calving to first ovulation was shorter in cows fed diets supplemented with linola (high in linoleic acid) or flax seed (high in linolenic acid) than in those fed diets supplemented with canola seed (high in oleic acid) prompted us to investigate further. As canola seed is a common ingredient in dairy cattle rations, we set out to confirm our previous observations and further investigate the effect of dietary canola seed on reproductive function. In our earlier study (Colazo et al., 2009), we did not have a control diet with no supplemental fat; therefore, we also included a control (no added fat) ration in this study.

We hypothesized that cows given a prepartum diet supplemented with canola seed will have a longer interval from calving to first ovulation, as observed previously, compared to those fed diets supplemented with no-oilseed or sunflower seed. Due to difficulties in sourcing linola seed, we used sunflower seed instead because of its high linoleic acid content (73%), comparable to that of linola (72%). Our objectives were to determine the effects of supplemental fat (no-oilseed vs. oilseed) during late gestation and the source of fat (canola vs. sunflower seed) on intake, milk production and composition, calf birth weight, postpartum health disorders, ovarian function and reproductive performance in dairy cows. Pregnant Holstein cows blocked by parity and body condition were assigned to 1 of 3 diets containing rolled canola seed (high in oleic acid; n = 43) or sunflower (high in linoleic acid; n = 45) at 8% of dry matter, or no-oilseed (control; n = 43), for the last 35 days of gestation. All cows received a common lactation diet after calving. Ovarian ultrasonography was performed twice weekly to monitor follicular growth and to determine the interval from calving to first ovulation.

Prepartum oilseed supplementation, more specifically sunflower seed, increased postpartum intake in primiparous cows without affecting prepartum intake or milk yield. On the contrary, in multiparous cows, prepartum oilseed supplementation decreased intake both pre and postpartum, and milk yield during the first 2 weeks. Regardless of parity, prepartum diet containing canola seed reduced postpartum feed intake compared to those fed sunflower seed. Mean nonesterified fatty acids (NEFA) concentrations at week -3 were greater in cows given supplemental oilseed than those given no-oilseeds.

Gestation length (276 vs. 273 d) and calf birth weight (43.7 vs. 41.0 kg) were increased in cows given supplemental oilseed prepartum compared to those fed no-oilseed. Interestingly, a disproportionate increase in the birth weight of female calves was evident in cows fed oilseed (43.9 vs. 40.4 kg; P=0.02). The type of oilseed also had a differential effect on calf birth weight with female calves born of cows fed sunflower being heavier than female calves born of cows fed canola seed (45.4 vs. 42.3 kg; P=0.08). Reproductive disorders tended to be greater in cows fed supplemental oilseed than those fed no-oilseed (42 vs. 23%). Furthermore, cows fed sunflower seed had greater incidence of dystocia (35 vs. 18%) and total health disorders (52 vs. 32%) than those fed canola seed. Oilseed supplementation did not have any significant effect on ovarian function or fertility (Table 2). More detailed results of this work can be found in our recent paper in the Journal of Dairy Science (Salehi et al., 2016).

Table 2. Postpartum ovarian function and fertility in cows fed control, canola or sunflower seed during prepartum period (Salehi et al., 2016)

	Prepartum diet			
Index	Control	Canola	Sunflower	*P*
Cows (n)	31	31	33	
Calving to 1st ovulation (d)	20.7 ± 1.6	22.9 ± 1.5	20.6 ± 1.4	0.47
Cows ovulated by 35 d (%)	71.0	77.4	84.8	0.21
Pregnancy to first AI (%)	22.6	25.8	33.3	0.53
Cows pregnant by 150 d[1] (%)	41.9	51.6	42.4	0.78
Cows pregnant by 250 d[2] (%)	58.1	74.2	72.7	0.06

[1]Cumulative pregnancies up to 150 d postpartum
[2]Cumulative pregnancies up to 250 d postpartum

Summary of findings: In the first study, the mean interval from calving to first ovulation was longer in cows fed canola seed compared to those fed either linola or flax seed. However, the results were not repeatable in the second study. In the second study, prepartum oilseed supplementation at ~8% reduced intake during the entire experimental period (pre and postpartum) and decreased milk yield during early lactation in multiparous cows. Oilseed supplementation also increased calf birth weight and postpartum health disorders. However, we found no significant differences in postpartum ovarian function and reproductive performance between the 2 prepartum diets.

▪ Effects of Dietary Fat Inclusion on Embryonic Development

Several studies have shown that fats and fatty acids can affect embryonic development in cattle (Tables 3 and 4). Both in vivo and in vitro studies have investigated the role of fats and fatty acids on bovine embryo development. Three such studies conducted in our laboratory are summarized below.

Study 1. (Thangavelu et al., 2007)

Because inclusion of flaxseed in dairy cow rations increased conception rates in some studies and reduced pregnancy losses in many studies, we proposed that reduced pregnancy losses were due to enhanced embryonic development during the early days of gestation in cows fed a diet enriched in α-linolenic acid. We (Ambrose et al., 2006) previously found that lactating dairy cows fed flaxseed had significantly lower pregnancy losses than those fed sunflower seed; however, a no-PUFA control was not included in that study. Therefore, in the study by Thangavelu et al. (2007), we fed 3 diets, 2 high in unsaturated fatty acids and 1 high in saturated fatty acids.

Table 3. Effects of fats and fatty acids on embryo development (in vivo studies)

Source ref.	Type of fat	Main result
Salehi et al., 2014; Reprod Fertil Dev 26:218	-Flax seed -Sunflower seed -Canola seed	Feeding rolled flaxseed reduced the proportion of degenerated embryos.
Moallem et al., 2013; Reproduction 146:603	-Flaxseed oil -Fish oil -Saturated fatty acid	Feeding flaxseed oil enhanced the cleavage rate of in vitro fertilized oocytes and tended to improve blastocyst rate compared to a diet enriched in saturated fatty acid.
Zachut et al., 2010; J Dairy Sci 93:529	-Control -Encapsulated flax oil -Encapsulated sunflower oil	Feeding flax oil increased the cleavage rate of in vitro matured oocytes as compared with those of control cows.
Cerri et al., 2009; J Dairy Sci 92:1520	-Calcium salts of palm oil -Calcium salts of linoleic and trans-octadecenoic acids (LTFA)	Feeding LTFA improved the proportion of excellent-, good-, and fair-quality embryos, and embryos from cows fed LTFA had a greater number of blastomeres than embryos from cows fed palm oil
Childs et al., 2008; Theriogenology 70:992	-Palmitic acid -Rumen protected n-3 PUFA	Feeding n-3 PUFA reduced the proportion of degenerated embryos.

Table 4. Effects of fats and fatty acids on embryo development (in vitro studies)

Source ref.	Type of fat	Main result
Salehi et al., 2014; WCDS Adv Dairy Technol 26:379	-Serum collected from cows fed flax (vs.) -Commercial fetal calf serum	Adding serum collected from cows fed flax improved development of embryo derived from low quality oocytes.
Adrema et al., 2011; Biol Reprod 85:62	-Palmitic acid -Stearic acid -Oleic acid	Palmitic and stearic acid had detrimental effect on oocyte developmental competence, whereas oleic acid improved oocyte developmental competence and blastocyst rate.
Marei et al., 2010; Reproduction 139:979	-Control -Linoleic acid	Adding linoleic acid inhibited cumulus cell expansion, delayed development of the oocytes to the metaphase II stage and reduced cleavage and blastocyst rate.
Marei et al., 2009; Biol Reprod 81:1064	-Control -α-Linolenic acid	Adding α-linolenic acid to in vitro maturation medium enhanced oocyte maturation and subsequent embryo development.
Leroy et al., 2005; Reproduction 130:485	-Palmitic acid -Stearic acid -Oleic acid	Addition of palmitic or stearic during oocyte maturation had negative effects on maturation, fertilization, and subsequently cleavage rate and blastocyst yield.

We hypothesized that feeding flaxseed (n-3 PUFA) will enhance early embryonic development compared to sunflower seed (n-6 PUFA) or saturated fatty acids. The objective was to compare embryonic development (as determined by the number of blastomeres, i.e., cells of the early embryo) in cows fed rations supplemented with saturated fatty acids or unsaturated fatty acids (flaxseed or sunflower seed). Twenty-four cyclic lactating Holstein cows (86 ± 22 d postpartum; 3.0 ± 0.4 lactations) were randomly assigned to 1 of 3 dietary groups. Diets were isonitrogenous and estimated energy intake was similar across diets. After receiving the diets for approximately 20 days, cows were subjected to superovulatory treatments and artificially inseminated twice (0800 h and 1900 h) with frozen-thawed semen from a single young sire. Seven days after AI, embryos were collected non-surgically. Transferable (excellent and good) quality embryos from each of the 3 dietary groups were stained and blastomere nuclei counted using a microscope and automated software.

Total ova and embryos, or transferable embryos, did not differ among the diets, but the overall recovery rate, defined as embryos/ova recovered as the proportion of corpora lutea, was higher in cows of the sunflower group. Fertilization rate was also not affected by diets. Total blastomere number was affected by diet (P<0.01; Table 5). When all categories of embryos were considered, embryos collected from cows fed saturated fat had fewer blastomeres than those from cows fed flax or sunflower seed. The differences were clearly evident in the expanded blastocyst stage, where embryos of cows fed flax or sunflower seed had a greater number of blastomeres than those from cows fed saturated fats. Blastomere numbers of expanded blastocysts did not differ between flax and sunflower seed dietary groups.

Table 5. Mean (± SEM) total number of blastomere nuclei of embryos recovered from cows fed diets supplemented with saturated fatty acid (SAT), flaxseed (FLX) or sunflower seed (SUN). Embryos (n = 61) were collected, non-surgically 7 days after AI, stained, and blastomere nuclei counted under a confocal microscope.

	Dietary Groups			
Stage of embryo	SAT	FLX	SUN	*P*
Morula	64.4 ± 4.1[a]	76.3 ± 4.4[b]	65.6 ± 4.1[a]	0.09
Blastocyst	77.5 ± 6.1[a]	88.6 ± 6.5[ab]	93.7 ± 5.7[b]	0.07
Expanded blastocyst	89.3 ± 9.5[a]	115.4 ± 6.3[b]	132.3 ± 8.3[b]	0.02
All embryo stages[1]	77.1 ± 3.9[a]	93.4 ± 3.4[b]	97.1 ± 3.1[b]	0.01

[1]Includes morula, blastocyst, and expanded blastocyst
[ab]Means with different superscripts within rows differ or tend to differ

Another study (Cerri et al., 2009), conducted jointly by researchers in the universities of California-Davis and Florida, supported the above findings. Holstein cow diets were supplemented with fat (2% of dry matter), either a calcium salt of palm oil (mostly saturated fatty acids) or a calcium salt high in linoleic acid and a blend of trans-octadecenoic acid (mostly unsaturated fatty acids) from 25 days before calving until 70 days postpartum. Cows were inseminated following a Presynch/Ovsynch protocol and embryos collected 5 days after insemination. Approximately 75 cows were assigned to each dietary treatment. The cows that received the mostly-unsaturated fatty acid diet had a higher proportion of excellent, good and fair-quality embryos. In addition, embryos from cows fed the unsaturated fatty acid diet had a greater number of blastomeres than those from cows fed the mostly-saturated fatty acid diet.

Study 2. (Salehi et al., 2013)

We investigated the effects of diets enriched in oleic, linoleic or α-linolenic acid on the development and transcriptomic profile (gene expression) of embryos collected from non-lactating dairy cows. Cows received 1 of 3 diets supplemented with rolled oilseeds (8% of dry matter): flax (n=8), sunflower (n=8) or canola (n=8). After a minimum 35-day diet adaptation, cows were superovulated, artificially inseminated with semen of the same sire and embryos collected 7½ days after AI. Cows fed flax had fewer degenerated embryos compared to those fed either sunflower or canola seed. The proportion of viable embryos was also higher in cows fed flaxseed (Salehi et al. 2013). The transcriptome profile of in vivo produced embryos revealed that 175 genes were differentially expressed in embryos from cows fed flax compared to those fed either canola or sunflower seeds. The differentially expressed genes mainly had roles in cellular growth and proliferation, and lipid metabolism (data unpublished).

Study 3. (Salehi et al., unpublished)

Using a whole animal (in vivo) model, it is not possible to determine whether the effect of PUFA on embryo quality is exerted at the follicular level (on oocytes, before fertilization) or at the oviduct/uterine level (on embryos, after fertilization). Therefore, we used an in vitro model to investigate whether fatty acids specifically influenced post-fertilization development of embryos. Serum collected from cows fed 2 of the above rations (flax and sunflower) was added separately to groups of early-stage embryos produced by in vitro fertilization of oocytes harvested from slaughter-house ovaries. Serum was added to the medium used for post-fertilization culture so that embryos were exposed to either of the serum treatments for up to 7 days. Excellent and good quality embryos from each treatment group were used for gene expression studies. Adding serum collected from cows fed flaxseed compared to those fed sunflower seed increased the expression of genes responsible for cell proliferation and differentiation as well as genes involved in maternal recognition of pregnancy without affecting morphological development.

Summary of findings: Collectively, our results and those from other researchers indicate that fats and fatty acids can influence early embryonic development. When compared to n-3 and n-6 PUFA, saturated fatty acids seem to exert a detrimental effect on embryos. In contrast, n-3 PUFA have a positive effect on early embryo development, including differential expression of genes that favour cell proliferation and pregnancy recognition.

▪ Conclusions

Getting back to our original question regarding whether feeding fats can improve reproductive performance in dairy cows, although there are inconsistences among reports, data from many studies compared by meta-analysis (Rodney et al., 2015) indicate that feeding fats can improve reproductive performance. Several studies that have used dietary n-3 PUFA (flaxseed/fish oil) in postpartum rations found a consistent reduction of pregnancy losses in lactating dairy cows (Figure 1). There is also a growing body of evidence showing positive effects of PUFA, particularly n-3 PUFA, on embryo development (Tables 3 and 4). All of these findings, and many other positive effects of fats and fatty acids on reproduction reported elsewhere (Ambrose and Kastelic, 2003; Santos et al., 2008; Wathes et al., 2013), strongly suggest that feeding fats high in n-3 PUFA to dairy cows can be beneficial. One of the biggest and most common problems with studies where the measured endpoint is conception rate or pregnancy loss, is the lack of sufficient statistical power due to inadequate animal numbers. Therefore, large multi-location, multi-year field studies are essential to find a more definitive answer to our question. Finally, a major consideration to feeding supplemental fats high in n-3 PUFA is the cost factor. Where practical, selective feeding of n-3 PUFA diets to cows with a high risk of reproductive loss (e.g., multiparous cows, those in poor body condition, etc.) might be a cost-effective approach.

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