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Dietary lipid supplementation on cow reproductive performance and oocyte and embryo viability: a real benefit?

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Abstract

The practice of "fat feeding" has become common in the dairy industry in a number of countries. There are several ideas as to how dietary lipids could influence reproductive performance. Highly saturated triacylglycerols (TAG), like palm oil, can increase milk yield but may aggravate negative energy balance and consequently impair fertility when fed during the first weeks postpartum. However, priming the lipid oxidation in the liver by feeding saturated lipid sources during the dry period has recently been shown to be a potentially promising strategy to mitigate fat mobilization and liver accumulation postpartum. Furthermore, polyunsaturated free fatty acids (FFA), such as omega-3 fatty acids and conjugated linoleic acids are fed to reduce the 'de novo' fatty acid synthesis in the udder and thus the milk TAG content, which may be of modest benefit for overall energy balance. Furthermore, omega-6 and -3 poly unsaturated FFA are reported to alter follicular growth, steroid synthesis and prostaglandin metabolism in the ovary and endometrium, respectively. Omega-6 FFA are believed to have proinflammatory and thus PGF2α-stimulating properties rendering them extra value as "neutraceutical" early postpartum, while omega-3 FFA can weaken this inflammatory potency, leading to a higher chance of survival of the embryo when supplemented during the periconceptual period. Unfortunately, research results rarely provide a consensus in this perspective. The consequences of these fat feeding strategies on oocyte and embryo quality remain an intriguing issue for debate. Dietary lipid supplementation may alter the microenvironment of the growing and maturing oocyte, of the early and older embryo and thus may affect reproductive outcome. We recently reported that dietary induced hyperlipidemic conditions can be harmful for embryo development and metabolism. However, to date, research results remain somewhat conflicting most probably due to differences in fat sources used in diet, and duration of supplementation and in experimental set up.

Keywords: dietary fat supplementation, energy balance, oocyte and embryo quality, reproduction.

Introduction

The dairy cow industry has changed dramatically over the past decades. Per-cow milk yields have increased dramatically as a combined result of improvements in animal management, nutrition, and genetics. A prerequisite for good lactation performance during a cow's life span is the production of offspring at regular intervals. Consequently, reproductive efficiency is fundamental for the modern dairy industry, as fertility influences average daily milk production, average days in milk, number of calves born per year, the generational interval, and ultimately the farmer's income (Leroy and de Kruif, 2006; Inchaisri *et al.*, 2011). Many studies have reported a worrisome decrease in the reproductive performance of dairy cows in recent decades, and this problem appears to affect all countries benefiting from high yielding dairy herds (for review see: Leroy and de Kruif, 2006). Reproductive failure is a major reason for rapid culling, threatening longevity of dairy cows and the sustainability of modern dairying. Furthermore, only an optimal reproduction at herd level guarantees an acceptable environmental ecological foot print of milk production (Garnsworthy, 2004). Reproductive failure in dairy cows is a multifactorial and complex problem. Calving under hygienic conditions and devoid of stress should guarantee optimal uterine involution and the absence of endometritis. Good feeding strategies (composition, quantity, palatability, availability, and the access of the feed) are also important. More and more farmers know that keeping the cows eating throughout this sensitive transition period represents their greatest challenge (Janovick and Drackley, 2010). Any drop in appetite and thus in dry matter intake increases the pressure on the cow's metabolic health. Recently, Walsh *et al.* (2011) elegantly considered all of the key steps in dairy cow reproduction and listed the pathways on how reproductive failure can originate, as well as provided known risk factors. The interactions between early postpartum negative energy balance (NEB), and the hypothalamus-pituitary-ovary-uterus axis have been particularly well studied (Ducker *et al.*, 1985; Lucy, 2001; Armstrong *et al.*, 2002; Butler, 2003). Disrupted

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endocrine signalling delays resumption of ovarian cyclicity postpartum; a relationship well-recognized as a major factor in dairy cow reproductive failure (Opsomer *et al.*, 1998; Roche, 2006; Vanholder *et al.*, 2006). However, attention has recently shifted to the widely reported fall in conception rates (Bousquet *et al.*, 2004; Roche, 2006), and a remarkably high incidence of early embryonic mortality (Mann and Lamming, 2001; Bilodeau-Goeseels and Kastelic, 2003). How sub-optimal metabolism or nutrition in the dairy cow can affect oocyte and embryo quality has been reviewed extensively (Leroy *et al.*, 2008b, c, 2011). To summarize some excellent epidemiological research (Santos *et al.*, 2009; Dubuc *et al.*, 2012), it may be concluded that compromised metabolic health of the dairy cow during the transition period is associated with impaired reproductive outcome, in terms of anovulation or embryo mortality.

A number of strategies have been proposed to tackle impaired reproductive performance through an improvement of the metabolic health status of the animal. Nutrition is one of critical importance and several concepts for feeding towards an 'optimal fertility' have been proposed (Santos *et al.*, 2010; Thatcher *et al.*, 2011). One of these so-called promising strategies is feeding of fatty acids (FFA) and sources of triacylglycerols (TAG). However, without definition, this is a broad-brush approach, which could have the very opposite effect to that intended. It is vital to consider that there are different types of FFA which can be provided in varying amounts and ratios during a number of sensitive time periods. Depending of the type of fat feeding, direct effects at the level of the uterus, corpus luteum, follicle, oocyte or embryo can be expected, as well as indirect effects mediated by changes in energy balance or immune function which will ultimately impact on reproductive physiology. Therefore, comparing studies is difficult and may explain the often conflicting results in the literature. In this review, we will consider many of the key studies in an attempt to make sense of the bewildering complexity of the relationship between dietary fat and reproductive performance in dairy cows.

Fat feeding and its effects on energy balance

Striving for an optimal metabolic health is the best strategy to safeguard normal ovarian physiology and good oocyte and embryo quality. Modern dairy rations are often supplemented with rumen protected fat to increase the energy intake in the early postpartum period and to increase fertility (Beam and Butler, 1997; Thatcher *et al.*, 2006). Dietary lipid supplementation provided to improve energy balance (DeFrain *et al.*, 2005), increases the overall dietary energy content, which stimulates milk production. An unintended downstream consequence of this increased milk production is net energy loss, ultimately resulting in elevated levels non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (β-OHB) and lower concentrations of glucose and insulin (McNamara *et al.*, 2003; van Knegsel *et al.*, 2005; Moallem *et al.*, 2007). In a recent study with isocaloric diets, Van Knegsel *et al.* (2007) found out that lipogenic diets resulted in a higher energy partitioning to milk production. In particular, saturated FFA seem to induce a state of peripheral insulin resistance, increasing the amount of glucose available for lactose synthesis and thus for milk production, which further stimulates peripheral lipid mobilization (Pires *et al.*, 2007); a self-perpetuating cycle. The reported positive effects of dietary lipid supplementation on milk production depend on the precise timing of provision, with the most positive results obtained when lipids are provided as the animal reaches positive energy balance (Grummer, 1995). Together, these data suggest that supplying dietary lipids during the early postpartum period to ameliorate the negative energy balance is of little benefit to overall reproductive outcome. Indeed, pressure on metabolic health tends to increase further. Only glucogenic diets are able to alleviate the adverse effects of negative energy balance on reproductive outcome.

Recently, there has been interest on the benefits of lipid feeding during the final weeks of the dry period, in an attempt to stimulate the fatty acid provision and metabolism in the liver. Feeding lipids to the dry cow induces a rise of NEFA prepartum, but is associated with lower NEFA and lower liver TAG after calving. Researchers claim that the liver is primed to cope with FFA when presence in excess during an episode of significant lipid mobilization. However, caution is warranted as dietary fat significantly reduces dry matter intake during the dry period, which may explain part of the observations (Douglas *et al.*, 2006; Andersen *et al.*, 2008).

Another dietary strategy to minimize negative energy balance postpartum is the induction of milk TAG content depression in order to significantly reduce the energy output, since the synthesis of TAG has a high demand for energy (Bauman *et al.*, 2008). It is currently well known that several rumen fatty acid bio-hydrogenation intermediates (such as trans mono unsaturated FFA and conjugated linoleic acids) induce a significant drop in de novo synthesized fatty acids in the mammary gland. It has been proposed that sparing fatty acid precursors (β-OHB and acetate) and NADPH (made from glucose in the pentose phosphate pathway), might be of benefit to the cow. Indeed, Odens *et al.* (2007) and Castaneda-Gutierrez *et al.* (2007) demonstrated that feeding trans 10, cis 12 CLA induced MFD which was paralleled with lower NEFA and higher IGF-I concentrations and thus an improved energetic status. However, many other studies could not find any beneficial effect of the induced MFD on energy balance. We recently showed that feeding marine algae, which is rich in long chain omega-3 (n-3) FFA, caused a drop in milk fatcontent, but no beneficial effects could

be seen on energy balance. The concomitant milk yield increase suggests that at least part of the spared energy is used to stimulate milk production (Hostens *et al.*, 2011).

In conclusion, it can be stated that feeding FFA, irrespective of the fatty acid type, is not a good strategy to improve the dairy cow's energy balance. Robust scientific evidence is lacking and study results lack any consensus. Lipid feeding during the transition period can significantly reduce dry matter (DM) intake and stimulate milk yield, further aggravating the metabolic pressure on the animal.

Lipid feeding as a strategy to stimulate ovarian activity and follicular growth and to alter the uterine environment

Wathes and co-workers provided a comprehensive review of the different pathways on how dietary FFA can effects different aspects of reproduction (Wathes *et al.*, 2007). Supplemental dietary lipids increases the size of the preovulatory follicle and its production of estradiol (Lucy *et al.*, 1991; Beam and Butler, 1997; Moallem *et al.*, 2007; Zachut *et al.*, 2008), most likely via the induction of high cholesterol concentrations in follicular fluid and plasma. This increased follicle size may have beneficial effects on both oocyte quality and corpus luteum function (Vasconcelos *et al.*, 2001). The resulting hypercholesterolemia also enhances progesterone secretion, thus, supporting early embryo developmental competence (Ryan *et al.*, 1992; Lammoglia *et al.*, 1996; McNamara *et al.*, 2003). It is generally accepted that the nutritional requirements for early resumption of ovarian activity and follicular growth are different from the nutritional conditions optimal for conception and early embryo growth. In that light, Garnsworthy *et al.* (2008) advised not to increase the lipid content over 5% of the DM to avoid a depression in circulating insulin concentrations during the first weeks postpartum. However, they deliberately added dietary lipids to attenuate insulin concentrations during breeding in order to avoid oocyte and zygote overstimulation (Garnsworthy *et al.*, 2009). Apart from rations supplemented merely with saturated or monounsaturated FFA (to increase energy intake), polyunsaturated FFA (PUFA) are becoming increasingly popular; particularly as a way to increase milk concentrations of n-3 FFA and lipids containing n-3 fatty acyl residues. Supplementation with these polyunsaturated FFA can supress prostaglandin secretion by the endometrium, and hence support the lifespan of the CL (Staples *et al*., 1998; Cheng *et al.*, 2001; Thatcher *et al.*, 2006), an effect which would be beneficial for embryo survival. The mechanism behind this observation was reported by Bilby *et al.* (2006b) who showed that diets rich in fish oil (high in n-3 poly-unsaturated) have the potential to reduce the expression of endometrial cyclooxygenase-2, an essential enzyme for prostaglandin biosynthesis (Thatcher *et al.*, 2006). In stark contrast, Hinckley *et al.* (1996), demonstrated that fish oil inhibited progesterone production by luteal cells cultured *in vitro*. This observation was confirmed *in vivo* by a study in which cows were fed a linseed rich diet (linolenic acid, C:D18:3, n-3) which lead to significantly reduced plasma progesterone concentrations (Robinson *et al.*, 2002). Mattos *et al.* (2002) were not able to corroborate this negative effect on progesterone production in synchronized cows fed either eicosapentaenoic acid (EPA, C:D 20:5, n-3) or docosahexaenoic acid (DHA, C:D 22:6, n-3). In other words, it is important to consider the exact type of the supplemented FFA (length of the carbon chain and degree of unsaturation) when estimating a specific effect on fertility. Feeding n-6 FFA to dairy cows stimulates PGF2-α synthesis improving uterine health (Petit *et al.*, 2004). A sequential and selective feeding of extra n-6 FFA around calving and of n-3 rich diets during the breeding period has therefore been proposed as an optimal reproductive management strategy in dairy cows (Silvestre *et al.*, 2011). The optimal immune response at the uterine level early postpartum should prevent endometritis while the n-3 supplementations around conception should safeguard embryo survival through sustained corpus luteum function. Clearly, a conclusive result of the effects of fat supplementation in dairy rations on the reproductive outcome, awaits further investigation.

Lipid feeding and the effects on the oocyte and embryo microenvironment

It is widely reported that changes in serum FFA are reflected in the lipid composition of the follicular environment (Childs *et al.*, 2008b; Fouladi-Nashta *et al.*, 2009). For example, PUFA content in follicular fluid is highly correlated to that of the diet (Adamiak *et al.*, 2005) and it is generally accepted that alterations in dietary fatty acid intake cause a similar shift in the fatty acid profile of the follicular fluid (Wonnacott *et al.*, 2010; Zachut *et al.*, 2010) although the ovary can, to some extent, buffer against major fluctuations in plasma n-3 and n-6 fatty acids (Fouladi-Nashta *et al.*, 2009).

One of the best-studied examples of metabolic changes in the follicle fluid is the phenomenon of NEB in high-yielding dairy cows (Leroy *et al.*, 2008a). In summary, there is good evidence that the ovary can selectively accumulate NEFA in a way that means that the concentration of FFA in plasma correlates to that measured in follicular fluid (Canfield *et al.*, 1990; Grummer *et al.*, 1995; Rabiee *et al.*, 1997; Comin *et al.*, 2002; Leroy *et al.*, 2005). Similar correlations between plasma and follicular fatty acid composition have recently been reported in humans (Robker *et al.*, 2009; Valckx *et al.*, 2012). Interestingly, palmitic acid, stearic acid, and oleic acid are the predominant NEFA in

bovine (Leroy *et al.*, 2005) and human ovarian follicle (Valckx *et al.*, 2012).

Data concerning the microenvironment within the oviduct and uterus are less well established due to technical difficulties in sampling the environment. Leese *et al.* (2008) proposed the epithelia lining the endosalphinx and endometrium as the final components in a supply line that links maternal diet at one end and embryo uptake of nutrients at the other. Also Tsujii *et al.* (2009) emphasized that serum and oviduct fluids play an important role in the development of blastocysts. The concentrations of nutrients in tubal fluid are documented to be below their plasma concentrations (Leese and Barton, 1984), which suggests that their overall transport across the tube occurs principally by diffusion (Leese and Gray, 1985); however, there are ongoing reintensified efforts to attempt to model transport of nutrients into the female reproductive tract. It is clear from the work of Childs *et al.* (2008a) that PUFA feeding affects the fatty acid composition of the genital tract.

The influence of fat feeding on the oocytes and embryonic lipid profile

Although the fatty acid composition of oocytes across a number or mammalian species has been reported (McEvoy *et al.*, 2000), little is known about the uptake of specific FFA by the follicle enclosed oocyte, how this may be altered by maternal metabolism and the consequences this might have for postfertilization development. A number of *in vitro* studies from different species and using diverse approaches have shown that the lipid profile of oocytes is dynamic and can be influenced by the external environment (Ferguson and Leese, 1999; Sata *et al.*, 1999; Kim *et al.*, 2001; Adamiak *et al.*, 2005; Aardema *et al.*, 2011). The lipids stored within the oocyte and early embryo represents an important source of energy for the early embryo (Sturmey *et al.*, 2009; McKeegan and Sturmey, 2012), however the consequences of endogenous lipids on early development have historically been overlooked. Oocytes have been shown to have increased TAG content when cultured with 'lipid enriched' follicular fluid, leading to compromised nuclear maturation (Yang *et al.*, 2012). In the broadest physiological terms, unsaturated fatty acids tends to have beneficial effects, whereas saturated fatty acids tend to have more deleterious effects. This is largely borne out in the oocyte and early embryo. For example, human embryos containing a higher ratio of unsaturated to saturated fatty acids are more likely to progress beyond the 4 cell stage (Haggarty *et al.*, 2006). What is less clear is the extent to which lipid composition of the oocyte and embryo *in vivo* can be altered in response to diet (Zeron *et al.*, 2002) and whether this impacts embryo quality. Santos *et al.* (2008) suggested the existence of a selective uptake process to ensure that the PUFA content of oocytes is kept to a minimum to minimize risks for degradation. Also Fouladi-Nashta *et al.* (2009) proposed that the ovary can buffer the oocyte against major fluctuations in plasma PUFA. In embryos, a similar protection mechanism might exist, as they found higher concentrations of saturated fatty acids than unsaturated fatty acids in rabbit embryos (Tsujii *et al.*, 2009).

Fat feeding and the effects on oocyte and embryo quality

As discussed earlier, the period of follicular development and early embryo development may represent a 'window of susceptibility' to dietary induced changes in the maternal environment (Ashworth *et al.*, 2009). In cows, supplementation with linoleic and linolenic acid, as present in sunflower and linseed oils, has little effect on *in vitro* maturation, subsequent oocyte quality, fertilization, or embryo development (Bilby *et al.*, 2006a). Feeding ewes with fish oil supplemented diet improved oocyte quality, oocyte membrane integrity, and increased the proportion of PUFA in the plasma, follicular fluid, and cumulus cells, but not in the oocyte (Zeron *et al.*, 2002). A diet with high n-3:n-6 ratio has been shown to increase linolenic acid and estradiol levels in the follicle and improve embryo cleavage rate (Zachut *et al.*, 2010), and conjugated linoleic acids, decrease embryo development rate and also suppress expression of stearoyl-CoA desaturase-1, the enzyme which converts stearic acid to oleic acid (Stinshoff *et al.*, 2013). A very recent study done in Brazil could not show any positive effects of supplementing linoleic acid to the diet of Nellore heifers on embryo production. On the contrary, embryo cryotolerance was significantly reduced in the fat supplemented group (Guardieiro *et al.*, 2013). Dietary intakes of women in the month preceding *in vitro* fertilization or intracytoplasmic sperm injection treatment showed that a high n-3 intake was associated with improved embryo morphology (Hammiche *et al.*, 2011). Confusingly however, high maternal dietary n-3 PUFA supplementation periconception reduced normal oocyte development in the mouse, perturbed mitochondrial metabolism, and adversely affected the morphological appearance of the embryo (Wakefield *et al.*, 2008). Furthermore Petit *et al.* (2008) reported that feeding flaxseed as a source of alpha-linolenic acid (ALA) did not improve embryo quality or the maintenance of gestation after embryo transfer.

Combined, the studies described in the preceding paragraph, as well as a great number of other important studies, illustrate the complexity of the relationship between nutrition and oocyte/embryo quality. For an overview of some recent studies about the effects of dietary lipid supplementation on oocyte and embryo quality, see Table 1. When designing and evaluating studies in this area, careful consideration

must be given to the precise timing and duration of dietary intervention, as well as to the amount and chemical nature of the lipid supplement. It is also important to note that there may be species-specific response to dietary lipid supplementation in terms of oocyte and embryo quality and care must be taken when extrapolating from mouse models. Furthermore, studies often identify the level (oocyte and/or embryo) where the dietary induced lipid changes impact on fertility, though they do not distinguish the specific lipid fraction and its structural composition responsible for observed effects. We have previously reported that exposure of preimplantation embryos to dietary-induced hyperlipidemic serum can result in reduced embryo development and quality, hence poorer fertility (Leroy *et al.*, 2010). The mechanistic insights for these findings are lacking so far, as the supplemented sera contained several lipid fractions that were significantly altered in response to the dietary lipid supplements, including doubled cholesterol concentrations, more than doubled total fatty acid concentrations, and increased levels of both long chain saturated and unsaturated fatty acids (Leroy *et al.*, 2010). Here, *in vitro* studies become invaluable.

Table 1. Survey of studies focusing on the effect of different types of fatty acids on oocyte and embryo quality in ruminants.

Author	Findings
Aardema *et al.*, 2011	**Oleic acid** rescues effects of palmitate and stearate and promotes maturation and development.
Adamiak *et al.*, 2006	**Altered lipid intake** is reflected in changed fatty acid composition in follicular fluid and cumulus oocyte complex.
Bilby *et al.*, 2006	Negative effects of **n-6 rich** diets on oocyte quality.
Chankitisakul *et al.*, 2013	**L-carnitine** treatment dislocates lipid droplets and improved cryopreservation of bovine oocytes.
Fouladi-Nashta *et al.*, 2007	Positive effect of 800 g **Megalac®** supplementation for 14 days on oocyte quality.
Fouladi-Nashta *et al.*, 2009	Holstein cows fed **palmitic** and **oleic, linoleic** or **linolenic acids** had altered plasma fatty acid profile, but no effect on embryo development rate.
Haggarty *et al.*, 2006	Human embryos with higher **unsaturated:saturated fatty acid ratios** are more likely to develop.
Hughes *et al.*, 2011	**EPA and DHA** may increase oxidative damage in ovine oocytes.
Jungheim *et al.*, 2011	Predominant human follicular fluid and serum NEFA were oleic, palmitic, linoleic, and stearic acid. Elevated NEFA correlated with poor COC morphology
Lapa *et al.*, 2011	Improved development and embryo quality after **trans-10 cis-12 CLA** supplementation during bovine oocyte maturation.
Marei *et al.*, 2009	Positive effect of **linolenic acid** on oocyte *in vitro* maturation.
Marei *et al.*, 2010	Negative effects of **linoleic acid** on oocyte *in vitro* maturation and developmental potential.
Oba *et al.*, 2013	High concentration of **NEFA** *in vivo* derived serum might adversely affect early cleavage stages in bovine embryos.
Ponter *et al.*, 2012	Bovine diet high in **linolenic acid** increases Prostaglandin E2 synthase-1 expression in COCs.
Yang *et al.*, 2012	**Lipid-rich human follicular fluid** decreases murine oocyte maturation rate.
Zachut *et al.*, 2010	Better cleavage rate after *in vitro* fertilization of oocytes from **linolenic acid** supplemented cows.
Zachut *et al.*, 2010	Bovine diet with a high n-3:n-6 ratio increases **linolenic acid** and estradiol concentrations in the follicle.
Zeron *et al.*, 2002	Positive effects of **fish oil** supplemented diets on oocyte quality and chilling sensitivity.

Use of *in vitro* models to understand oocyte an embryo responses to 'fat'

By using *in vitro* models, it is possible to assess the direct effects of individual and combinations of FFA on oocyte and early embryo development in a controlled way. This research has been reviewed previously (Sturmey *et al.*, 2009; McKeegan and Sturmey, 2012). Whilst care must be taken when extrapolating such studies to the whole animal, *in vitro* studies have given us a wealth of understanding on how specific lipid molecules can impact early development. For example, addition of physiological concentrations of n-3 PUFA to oocyte maturation media resulted in improved oocyte nuclear maturation rate, whereas n-6 PUFA-treated oocytes had reduced resumption of meiosis (Marei *et al.*, 2009, 2010). In bovine oocytes, n-3 PUFA may play a critical role in maintaining meiotic arrest (Homa and Brown, 1992), possibly acting through protein kinase C (Murakami *et al.*, 1986), which plays a significant role in

metabolic regulation on a cellular level, in cell growth, and differentiation (Nishizuka, 1988).

What is especially interesting are emerging data showing that the phenotype of the early embryo can be dramatically altered by exposure to FFA during the final oocyte maturation. Bovine *in vitro* maturation models have demonstrated that elevated concentrations of saturated NEFAs, such as stearic acid and palmitic acid, can reduce oocyte developmental competence (Jorritsma *et al.*, 2004; Leroy *et al.*, 2005; Aardema *et al.*, 2011). We have recently shown that exposure to elevated NEFA during oocyte maturation can lead to profound changes in metabolic regulation and gene expression in the resulting embryo (Van Hoeck *et al.*, 2011, 2013). Of particular note was the observation that oocyte exposure to elevated NEFA lead to embryos which, at the blastocyst stage, did not consume glucose. This is a startling observation, since a sharp increase in glucose consumption at the blastocyst stage is common to all species studied thus far (Smith and Sturmey, 2013). The impact of this metabolic deregulation in the early embryo is currently unclear, but does suggest that the period when follicles are developing may represent a 'window of susceptibility' to the dietary or metabolically induced differences in fatty acid availabilitty with the consequences persisting in the embryo. Too high fatty acid provision in the oocyte's microenvironment due to massive lipolysis (negative energy balance) or due to specific fat feeding strategies may lead to reduced fertility due to compromised early embryo quality. Much more research, is needed to define optimal dietary lipid supplementation strategies. And finally, enough attention should be paid to unforeseen potential negative effects of dietary lipid supplementation having indirect negative effects on reproduction. To give one example, Wullepit *et al.* (2012) recently demonstrated that PUFA feeding to dairy cows significantly increased the level of oxidative stress.

Conclusions

It can be concluded from this overview that dietary lipid supplementation has very limited additive value in alleviating the negative energy balance during the transition period. Dietary lipid supplementation can be a good strategy to stimulate follicular growth and steroid production. Depending on the source of lipids given, the effect on prostaglandin synthesis in the uterus or corpus luteum can be very different. Direct effects on oocyte and embryo quality tend to vary significantly and the results may depend on experimental set up and the animal model used. More *in vitro* studies are warranted to provide us with in depth knowledge on the pathways involved. Finally it is important to consider the more indirect effects of dietary lipid supplementation on reproduction, for example due to an altered dry matter intake, ruminal health, immunity, oxidative stress, and endocrine signalling.

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