

Effects of dietary fatty acids on reproduction in ruminants

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Fats in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins. Dietary fatty acids of the n-3 family reduce ovarian and endometrial synthesis of prostaglandin $F_{2\alpha}$, decrease ovulation rate in rats and delay parturition in sheep and humans. Polyunsaturated fatty acids such as linoleic, linolenic, eicosapentaenoic and docosahexaenoic acids may inhibit prostaglandin $F_{2\alpha}$ synthesis through mechanisms such as decreased availability of its precursor arachidonic acid, an increased competition by these fatty acids with arachidonic acid for binding to prostaglandin H synthase, and inhibition of prostaglandin H synthase synthesis and activity. It is not known whether polyunsaturated fatty acids regulate expression of candidate genes such as phospholipase A_2 and prostaglandin H synthase via activation of nuclear transcription factors such as peroxisome proliferator-activated receptors. Manipulation of the fatty acid profile of the diet can be used potentially to amplify suppression of uterine synthesis of prostaglandin $F_{2\alpha}$ during early pregnancy in cattle, which may contribute to a reduction in embryonic mortality. Feeding fats and targeting of fatty acids to reproductive tissues may be a potential strategy to integrate nutrition and reproductive management to improve animal productivity.

Several studies report improved reproductive performance in lactating dairy cows fed supplemental fat. A recent review on the effects of dietary fat on fertility of dairy cows showed that 11 of 20 articles reported improvement (Staples *et al.*, 1998). Authors suggest that this effect was probably not a result of improvement of the energy status of the cow but that increased fertility could be due to effects of dietary fatty acids on the pituitary, ovaries and uterus.

Fats are classified as lipids, which are biological compounds that are soluble in organic solvents. Lipids include cholesterol and fats such as triacylglycerols and phospholipids. Phospholipids are major components of cellular membranes, and are a source of fatty acids for the synthesis of a variety of effector molecules such as the eicosanoids, a group of compounds that includes prostaglandins, thromboxanes and leukotrienes. Cholesterol is another component of the cellular membrane and is the precursor for the synthesis of steroid hormones.

The structure of fatty acids, that is, the length of the acyl chain, the number of double bonds in the chain, and the types of isomer formed by each double bond largely determines their function. Saturated fatty acids do not have double bonds in the acyl chain. Unsaturated fatty acids are classified into different families according to the position of the first double bond relative to the methyl end. For example, linoleic acid has 18 carbon atoms and two double bonds (C18:2), with its first double bond at the sixth position from the methyl end, and is therefore a member of the n-6 family. In contrast, linolenic acid (C18:3) belongs to the n-3 family because the first of its double bonds is at the third carbon position. Processing of fatty acids in one family can only generate fatty acids of the same family, i.e. a fatty acid of the n-3 family cannot be converted into a member of n-6 family and vice versa (Fig. 1).

Fatty acids present in vertebrates are either generated by *de novo* synthesis or are absorbed from the diet. Fatty acids from any source may undergo elongation and desaturation to generate fatty acids with different biochemical properties. Elongation involves the addition of two-carbon units to a carbon chain through the action of elongase. Desaturation is a process catalysed by desaturase enzymes that insert a double bond in the acyl chain (Fig. 1). Desaturase enzymes are classified according to the position of insertion of the double bond. For example, $\Delta 6$ desaturase inserts a double bond between the sixth and seventh carbon from the carboxyl end. The range of possible positions for insertion of double bonds varies according to the organism. In animals, desaturation of fatty acids does not occur at positions greater than $\Delta 9$ (Cook, 1996). This prevents animals from synthesizing fatty acids of the n-3 and n-6 families. Since animals have absolute requirements for some fatty acids of the n-3 and n-6 families, these fatty acids are considered to be essential and must be provided by the diet. For example, linoleic acid (C18:2, n-6) is an essential fatty acid required for the synthesis of arachidonic acid (C20:4, n-6), and of eicosanoids (Fig. 2) (Cook, 1996).

Reproduction in ruminants is associated closely with the availability of energy. Fats are glyceride esters of fatty acids and are important sources of energy. Dietary fats favour reproductive function by supplying energy and by actions on reproductive processes that are not related to energy. For example, increased availability of fatty acid precursors allows increased steroid and eicosanoid secretion, which can alter ovarian and uterine function and affect pregnancy rates. At the cell, fatty acids may have a direct effect on the transcription of genes that encode proteins that are essential to reproductive events. The focus of this review is to examine the reproductive

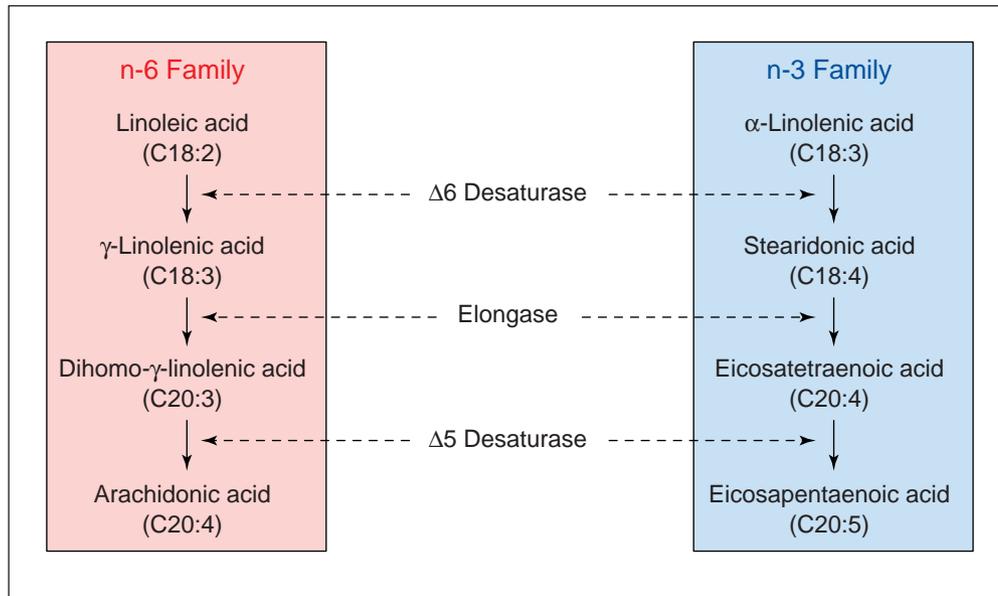


Fig. 1. Pathway of desaturation and elongation of n-3 and n-6 fatty acids. Precursor fatty acids, linoleic (C18:2, n-6) and linolenic (C18:3, n-3), are sequentially acted upon by $\Delta 6$ desaturase, elongase and $\Delta 5$ desaturase enzymes. Adapted from Bezard *et al.* (1994).

processes that are regulated by fats and the potential mechanisms of action.

Digestion and absorption of fats

Dietary fats can undergo modification in the rumen. Bacteria of the ruminal flora digest dietary triacylglycerols, phospholipids and galactolipids releasing fatty acids from their glycerol backbone. Released unsaturated fatty acids have some of their double bonds reduced, and the isomer orientation changed in a process called fatty acid biohydrogenation. Some PUFAs of the n-3 family, eicosapentaenoic acid (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3) have been shown to undergo negligible biohydrogenation (Ashes *et al.*, 1992). Eicosapentaenoic acid and docosahexaenoic acid are found typically in feed ingredients derived from fish, such as oil and meal. Fat supplements partially resistant to biohydrogenation, such as calcium salts of long-chain fatty acids (CaLCFA), have been developed to increase the amount of unsaturated fatty acids reaching the duodenum and being incorporated into adipose tissue and milk.

Effect of dietary fats on LH secretion, ovarian follicular dynamics, function of corpus luteum and steroidogenesis

Secretion of LH from the pituitary and follicular growth in cattle are regulated partially by the energy status of the animal. The energy status in lactating animals has been defined as the net energy intake of the animal minus the net energy required for maintenance and that required for milk production. Negative energy states prolong post-partum anoestrus (Randel, 1990) and reduce the frequency of pulses of LH that are necessary for growth of ovarian follicles to the preovulatory

stage in cattle and sheep (Schillo, 1992). Supplemental fat is included frequently in rations to increase energy concentration in the diet, which could result in an increase in energy intake and improve the energy status of the cow (Blum *et al.*, 1995; Harrison *et al.*, 1995; Palmquist and Weiss, 1994). Energy provided by fat supplementation increases LH secretion in animals that consume less energy than required (Hightshoe *et al.*, 1991; Sklan *et al.*, 1994). However, a mechanism independent from energy by which dietary fatty acids affect LH secretion has not yet been established in ruminants.

Several studies have shown that feeding supplemental fat alters the growth dynamics of the ovarian follicle and that this effect is somewhat independent from energy. Supplemental fat feeding in a manner that was isocaloric with the control diet without fat supplement stimulated programmed growth of a preovulatory follicle (Lucy *et al.*, 1993). Effects of supplemental fat also include increased total number of follicles (Lucy *et al.*, 1991a,b; Wehrman *et al.*, 1991; Thomas and Williams, 1996; Beam and Butler, 1997; Lammoglia, 1997) and increased size of preovulatory follicles (Lucy *et al.*, 1990, 1991a, 1993; Beam and Butler, 1997; Oldick *et al.*, 1997). Increased size of preovulatory follicles may be due in part to increased concentrations of plasma LH, which stimulates the latter stage of follicular growth. Further research is required to determine whether dietary fatty acids affect the secretion of LH and whether increased size and numbers of follicles are associated with increased pregnancy rates.

In one study, fat supplementation increased the concentration of total cholesterol in follicular fluid. This finding might be related to the fact that granulosa cells collected from follicles of supplemented cows showed increased progesterone and androstenedione secretion *in vitro* (Wehrman *et al.*, 1991). The ovulation of larger follicles, as observed in the supplemented

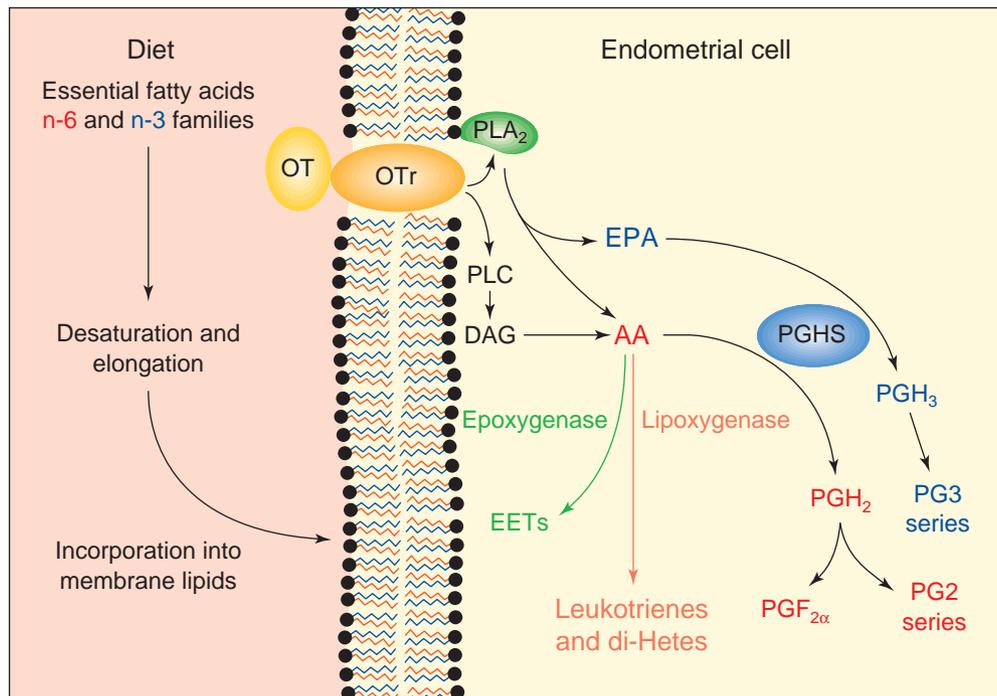


Fig. 2. Schematic representation of the metabolism of dietary n-6 and n-3 polyunsaturated fatty acids (PUFAs) and potential mechanisms for regulation of $\text{PGF}_{2\alpha}$ secretion. Absorbed PUFAs are desaturated and elongated in sites such as the mammary gland, adipose tissue, testis, brain, placenta and the liver (non-ruminants). Dietary PUFAs and their desaturation and elongation products are incorporated into phospholipids of the plasma membrane. The amount of each fatty acid incorporated depends on the amount of precursor present in the diet. External stimuli such as the binding of oxytocin (OT) to the oxytocin receptor (OTr) stimulates the activity of phospholipase A₂ (PLA₂) and phospholipase C (PLC), which cleave phospholipids from the plasma membrane and ultimately increase availability of fatty acids for processing by prostaglandin H synthetase (PGHS). Eicosapentaenoic acid (EPA; C20:5, n-3) is processed by PGHS to generate prostaglandins of the 3 series. Arachidonic acid (AA; C20:4 n-6) can be processed by PGHS, epoxygenase and lipoxygenase to generate prostaglandins of the 2 series, epoxyeicosatrienoic acids (EETs), leukotrienes and hydroxyeicosatetraenoic acids (di-HETEs), respectively. DAG, diacylglycerol.

cows, may result in the formation of larger corpora lutea with increased steroidogenic capacity. Dietary fat supplementation in cows consistently increases plasma concentrations of cholesterol (Grummer and Carroll, 1991), the precursor for the synthesis of progesterone. The fact that ruminants treated with supplemental fat often have small increases in plasma concentrations of progesterone (Staples *et al.*, 1998) indicates that greater availability of cholesterol results in increased secretion of progesterone. However, incubation of dispersed bovine luteal cells with PUFAs such as eicosapentaenoic and docosahexaenoic acid decreases the secretion of progesterone (Hinckley *et al.*, 1996). Further research is required to determine whether dietary fats affect progesterone secretion *in vivo*.

There is also evidence for a reduced rate of clearance of progesterone when animals are supplemented with fats. When the corpora lutea of cows were removed by ovariectomy, the rate of decrease of plasma concentrations of progesterone observed was smaller in cows fed a fat supplement of CaLCFA than in cows not fed supplemental fat (Hawkins *et al.*, 1995). Increased concentrations of plasma progesterone in the luteal phase before and after insemination have been associated

with higher pregnancy rates (Butler *et al.*, 1996; Maurer and Etschekamp, 1982). Since progesterone prepares the uterus for implantation of the embryo and helps maintain pregnancy by stimulating histotroph for nourishment of the conceptus, increased plasma progesterone may improve pregnancy rates in animals fed supplemental dietary fat.

Dietary fatty acids and synthesis of prostaglandins

Prostaglandins are one type of bioactive compound derived from 20 carbon fatty acids (Box 1). Prostaglandins of the 2 series (for example $\text{PGF}_{2\alpha}$) are important regulators of parturition, and, in some species, cause regression of the corpus luteum that leads to the initiation of a new oestrous cycle. Arachidonic acid for synthesis of prostanoids of the 2 series can be acquired either from the diet or synthesised *de novo* from linoleic acid. Conversion of linoleic acid into arachidonic acid involves two steps of desaturation at the 5 and 6 positions and a 2-carbon elongation catalysed by an elongase enzyme (Fig. 1). Formation of eicosanoids of the 2 series starts with the release of arachidonic acid from the phospholipids through the action

Box 1. Eicosanoids

The bioactive compounds derived from 20 carbon fatty acids include prostaglandins, thromboxanes and leukotrienes, which are grouped under the general term eicosanoids. Prostaglandins and thromboxanes are products of the prostaglandin H synthase (PGHS) pathway and are called prostanoids. The fatty acids dihomo- γ -linolenic, arachidonic acid and eicosapentaenoic acid are precursors for the synthesis of prostanoids of the 1, 2 and 3 series, respectively (Bergstrom *et al.*, 1964). The number of the series corresponds to the number of double bonds in the prostanoid, that is, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) contains 2 double bonds.

of phospholipase A_2 (PLA_2). Released arachidonic acid can be processed by the PGHS enzyme complex to produce prostaglandins and thromboxane (PGD_2 , PGE_2 , $PGF_{2\alpha}$, TXA_2 , PGI_2), by lipoxygenases to produce leukotrienes (LTA_4 , LTB_4 , LTC_4 , LTD_4) and hydroxyeicosatetraenoic acids (HETEs), and by cytochrome P_{450} epoxygenases to produce epoxyeicosatrienoic acids (EETs) (Fig. 2). Arachidonic acid is stored in the phospholipids of the cellular plasma membrane and its concentrations are relatively high in rat uterine tissues (5–10% of total uterine lipid (Howie *et al.*, 1992)). The PGHS contains both cyclooxygenase (COX) and peroxidase activity, and converts arachidonic acid into PGH_2 . This compound is processed further by the different prostaglandin synthases to generate PGD_2 , PGE_2 , $PGF_{2\alpha}$, TXA_2 and prostacyclin (PGI_2).

Essential fatty acids of the n-3 and n-6 families have been shown to inhibit the secretion of eicosanoids in several cell types cultured *in vitro* (Levine and Worth 1994; Achard *et al.*, 1997). Other studies have demonstrated reduced eicosanoid synthesis when fatty acids of the n-3 and n-6 families are fed in the diet. Supplementation of dairy cows with fish meal (5.4% of dietary dry matter) containing eicosapentaenoic acid and docosahexaenoic acid affects uterine $PGF_{2\alpha}$ secretion of lactating dairy cows (Thatcher *et al.*, 1997). Secretion of $PGF_{2\alpha}$ from the uterus was induced by sequential injections of oestradiol (0 h) and oxytocin (4 h) given on day 15 of a synchronized oestrous cycle, 34 days after the introduction of diets. Feeding of fish meal resulted in an attenuation of the induced PGFM response in peripheral plasma compared with control animals. However, the suppression was not observed in all cows fed fishmeal. Failure to suppress $PGF_{2\alpha}$ secretion also was reported in 20% of cows infused with interferon τ (IFN- τ), indicating that responsiveness of the endometrium to anti-luteolytic signals can be another factor affecting establishment of pregnancy (Thatcher *et al.*, 1995). Similar results were reported in the human endometrium. Dietary supplementation with γ -linolenic acid (C18:3, n-6) or eicosapentaenoic acid reduced the synthesis *in vitro* of PGE_2 and $PGF_{2\alpha}$ from human endometrial samples collected 6 months after initiation of dietary treatments. Endometrial explants collected before supplementation were used as controls (Graham *et al.*, 1994). Infusion of a fat source rich in linoleic acid (17%) into the abomasum of lactating dairy cows resulted in a significant attenuation in the release of PGFM, as measured in peripheral plasma, in response to an injection of oxytocin on

day 15 of a synchronized oestrous cycle (Oldick *et al.*, 1997). Collectively, these results indicate that high concentrations of PUFAs in the diet can decrease the endometrial secretion of prostaglandins.

The dynamics of bovine corpus luteum regression in response to exogenous $PGF_{2\alpha}$ also can be altered by dietary fish meal. Cows ($n = 56$) were fed fish meal at either 0 or 2.8% of diet dry matter from about 24–109 days post-partum. On day 58 post-partum all cows were injected with a luteolytic dose of $PGF_{2\alpha}$. Two days after injection of $PGF_{2\alpha}$ the proportion of cows with plasma concentrations of progesterone that were >1 ng ml^{-1} was greater when fish meal was included in the diet than when the control diet was fed (29 versus 4%) (Burke *et al.*, 1997). Thus, it is possible that fatty acids present in the fish oil reduce the sensitivity of the corpus luteum to $PGF_{2\alpha}$. Since eicosapentaenoic acid present in fish meal is the precursor for the formation of the vasodilatory PGI_3 (Needleman *et al.*, 1979), it is possible that the delay in corpus luteum regression is mediated by a partial neutralization of the vasoconstrictive action of $PGF_{2\alpha}$ that normally occurs during luteolysis. Alternatively fish meal feeding may reduce the uterine secretion of $PGF_{2\alpha}$ that delayed the completion of functional luteolysis.

Involvement of PUFAs in the suppression of $PGF_{2\alpha}$ secretion during early pregnancy in cows also has been demonstrated. When monitoring the metabolism of radiolabelled arachidonic acid by bovine microsomes, addition of endometrial cytosol from pregnant cows had a greater inhibitory activity than that from non-pregnant cows at day 17 of the oestrous cycle (Thatcher *et al.*, 1994). Purification indicated that the inhibitor was enriched in linoleic acid. The ability of linoleic acid to inhibit PGHS activity has been reported by Pace-Asciak and Wolfe (1968) and Thatcher *et al.* (1994).

Increased pregnancy rates observed when fat supplements are fed may be mediated by reduced $PGF_{2\alpha}$ secretion from the uterus and decreased sensitivity of the corpus luteum to $PGF_{2\alpha}$. Suppression of $PGF_{2\alpha}$ secretion and maintenance of the corpus luteum are obligatory steps for establishment of pregnancy in cows, and failures in this process can cause the loss of up to 40% of the pregnancies (Ayalon, 1978; Maurer and Chenault, 1983; Thatcher *et al.*, 1994). Reducing $PGF_{2\alpha}$ secretion through the feeding of PUFAs could improve fertility by reducing embryonic losses caused by defective suppression of $PGF_{2\alpha}$ secretion during early pregnancy.

Effects of prostaglandins on ovulation

Prostaglandins E_2 and $F_{2\alpha}$ are important mediators of the ovulatory process. Their concentration in follicular fluid increases sharply before ovulation. The administration of inhibitors of prostaglandin synthesis such as indomethacin blocks ovulation in rats, rabbits, pigs, sheep, monkeys and humans through the reductions of both PGE_2 and $PGF_{2\alpha}$ (Sogn *et al.*, 1987). When rats were fed diets containing high concentrations of eicosapentaenoic acid and docosahexaenoic acid, the number of corpora lutea found after the first oestrus was reduced. Since the release of GnRH in the rat has been associated with activation of PGE_2 -dependent pathways (Ojeda *et al.*, 1979; DePaolo *et al.*, 1982; Kim and Ramirez, 1986; Zhang *et al.*, 1992), eicosapentaenoic and docosahexaenoic acids may have caused a decrease in

hypothalamic synthesis of PGE₂, which prevented GnRH release and decreased the frequency of ovulations. Experiments have not been designed to examine specifically whether feeding of supplemental fats, such as fish meal or fish oil, interferes with ovulation of the preovulatory follicle in cattle. However, after injection of GnRH and PGF_{2α} given 7 days apart, oestrous detection and conception rates to the synchronized service were normal in cows that were fed fish meal (Burke *et al.*, 1997).

Effects of prostaglandins on labour

Parturition is a process that is accompanied by the massive release of prostaglandins. Alterations of fatty acids in the endometrium have been described in normal parturition, and manipulations of fatty acid content used experimentally to delay onset of parturition. Fatty acids of the n-3 family have been shown to affect uterine activity during parturition in rats and sheep, and to delay the onset of parturition in humans (Olsen *et al.*, 1992). Supplementing linolenic acid to a diet deficient in essential fatty acids resulted in an impairment of parturition in rats (Leat and Northrop, 1979). This also occurred when fish oil was given to rats as the major dietary essential fatty acid source, and an inhibition of uterine synthesis of PGE₂ was detected (Leaver *et al.*, 1986). In pre-term pregnant sheep, intravenous infusion of a 20% n-3 fatty acid emulsion resulted in a delay in the onset of induced labour and delivery compared with a control group infused with an emulsion of soybean oil containing 7% n-3 fatty acids. In animals treated with the 20% n-3 fatty acid infusion, maternal and fetal plasma concentrations of PGE₂ were lower than in control animals, suggesting a possible effect on PGHS. Infusion of betamethasone resulted in a decrease of plasma progesterone concentrations in both groups and in an increase in oestradiol concentration only in animals of the control group (Baguma-Nibasheka *et al.*, 1999). These workers suggested that n-3 fatty acids may have affected the conversion of progesterone to oestradiol by downregulating placental 17β-hydroxylase (P₄₅₀ 17β-OH). Since oestradiol upregulates PGHS, it is possible that PGE₂ did not increase in the n-3 treated group because of insufficient oestradiol support. An alternative explanation is that labelling the phospholipid pools with n-3 fatty acids altered the dynamics of prostaglandin secretion. Indeed, concentrations of eicosapentaenoic acid esterified to membrane phospholipids of the ovine endometrium normally drop significantly during the last third of gestation (Zhang *et al.*, 1995). This finding suggests that factors inhibitory to prostaglandin synthesis are decreased before parturition. Concentrations of arachidonic acid in endometrial phospholipids of sheep declined during labour, suggesting utilisation for synthesis of prostaglandins (Zhang *et al.*, 1995).

Mechanisms of inhibition of prostaglandin secretion

Dietary PUFAs can decrease PGF_{2α} synthesis by different actions, which include decreasing the availability of precursor arachidonic acid, increasing the concentration of fatty acids that compete with arachidonic acid for processing by PGHS, and inhibiting PGHS. Reduced availability of arachidonic acid in the uterine phospholipid pool for conversion to prosta-

glandins of the 2 series can occur through a reduction in the synthesis of arachidonic acid or through displacement of existing arachidonic acid from the phospholipid pool by other fatty acids (Fig. 3).

Reduced synthesis of arachidonic acid

One possible step contributing to the reduction of uterine content of arachidonic acid involves decreased synthesis of arachidonic acid in uterine tissue or in tissues that are exporters of fatty acids. Since there are no reports of arachidonic acid synthesis in the ruminant endometrium, supply from the circulation is likely the most important source. Desaturation in ruminants occurs primarily in adipose tissue as the ruminant liver has a limited capacity for exporting triacylglycerols and lipoproteins (Emery *et al.*, 1992). Synthesis of arachidonic acid in the liver can be decreased by high concentrations of PUFAs in the diet, as dietary PUFAs, particularly eicosapentaenoic acid and docosahexaenoic acid, are major inhibitors of desaturation and elongation in liver cells (Fig. 3; Bezard *et al.*, 1994).

Dietary supplementation of rats with fish oil (eicosapentaenoic acid and docosahexaenoic acid) or linseed oil (high in linolenic acid) reduced the Δ₆, Δ₅ and Δ₉ desaturation of linoleic acid and linolenic acid by liver microsomes, and the concentration of arachidonic acid in liver phospholipids (Christiansen *et al.*, 1991; Garg *et al.*, 1988). In addition, when fish oil rich in n-3 fatty acids was fed to rats, there was an increase in the incorporation of n-3 fatty acids into liver microsomal lipid relative to the n-6 fatty acid family. Incubation of tissues containing desaturase activity with PUFAs also decreases desaturation. The Δ₅ and Δ₆ desaturase activity of rat hepatoma cells was reduced when they were incubated with eicosapentaenoic acid and docosahexaenoic acid (Larsen *et al.*, 1997). In one study, elongation activity was increased and desaturation decreased when rat hepatoma cells were incubated with fatty acids of the n-3 and n-6 families (De Alaniz and De Gomez Dumm, 1990).

Competition of n-3 fatty acids for desaturase activity

Another step to account for reduced arachidonic acid synthesis involves preferential processing of n-3 fatty acids by Δ₆ desaturase at the expense of desaturation of n-6 fatty acids (Sprecher, 1981). When deuterated linoleic acid and linolenic acid were fed simultaneously to humans, the conversion of linolenic acid to eicosapentaenoic acid and docosahexaenoic acid was greater than the conversion of linoleic acid to arachidonic acid (Emken *et al.*, 1990). In this case, the presence of high concentrations of linolenic acid in the diet would compete with linoleic acid for binding with Δ₆ desaturase and reduce the conversion of linoleic acid to arachidonic acid. In human intestinal epithelial cells, the desaturation-elongation pathway was reported to have a preference for linolenic acid and to be subject to an efficient feedback regulation by eicosapentaenoic acid (Chen and Nilsson, 1993).

Altered fatty acid profile in the plasma membrane

Reduced availability of arachidonic acid for incorporation into phospholipids of the plasma membrane will result in greater incorporation of other fatty acids, which may or may

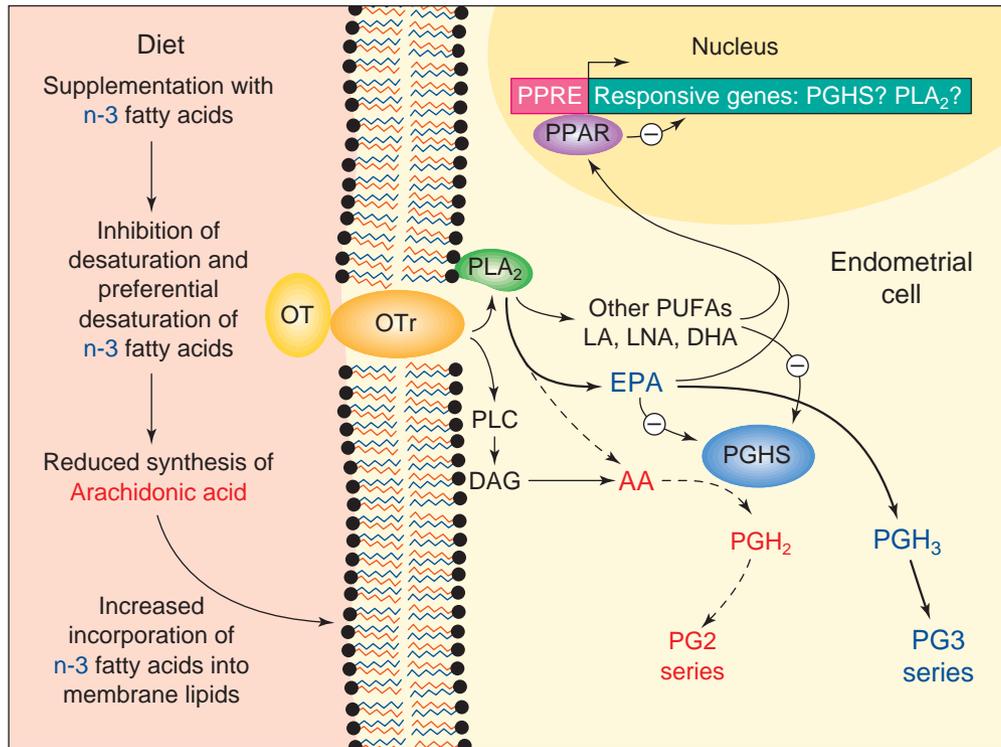


Fig 3. Proposed schematic model of the effect of a diet rich in n-3 fatty acids on the synthesis of eicosanoids. The n-3 fatty acids inhibit desaturase activity and undergo preferential desaturation at the expense of desaturating n-6 fatty acids. This results in increased incorporation of n-3 fatty acids and reduced incorporation of arachidonic acid (AA; C20:4, n-6) into plasma membrane lipids. Decreased availability of arachidonic acid and increased availability of eicosapentaenoic acid (EPA; C20:5, n-3) in the plasma membrane result in decreased synthesis of prostaglandins of the 2 series (indicated by dotted lines) and increased synthesis of eicosanoids of the 3 series. Eicosapentaenoic acid competes with arachidonic acid for processing by prostaglandin H synthetase (PGHS). Eicosapentaenoic acid and other polyunsaturated fatty acids released into the cytoplasm by phospholipase activity, such as linolenic (LNA; C18:3, n-3), linoleic (LA; C18:2, n-6) and docosahexaenoic (DHA; C22:6, n-3), can inhibit PGHS activity. The fatty acids may activate nuclear transcription factors such as the peroxisome proliferator activated receptor (PPAR) to repress transcription of genes encoding phospholipase A₂ (PLA₂) and PGHS. The PPARs bind to the peroxisome proliferator response elements (PPREs) in the regulatory region of target genes. DAG, diacylglycerol.

not be precursors of other eicosanoids (Fig. 3). Indeed, displacement of arachidonic acid from cell membrane phospholipids has been demonstrated in animals fed diets rich in n-3 fatty acids. Trujillo and Broughton (1995) reported a significant reduction in the proportion of arachidonic acid present in phospholipids extracted from liver cells from rats fed for 4 weeks with diets rich in n-3 fatty acids. The fatty acid composition of the diet was related to the content of C20 and C22 fatty acids in the rat uterus (Howie *et al.*, 1992). Feeding a diet rich in n-3 fatty acids for 3 weeks resulted in a 50% replacement of the rat uterine phospholipid pool of n-6 fatty acids with n-3 fatty acids. Another interesting finding of this study was the ability of the uterus to conserve a relatively stable concentration of arachidonic acid in its lipid pools in spite of a prolonged period of dietary deprivation of its precursors. This conservation of arachidonic acid was further supported by the fact that rats fed diets with a n-3:n-6 ratio that was 700–800 times greater than the control had an n-3:n-6 ratio in the uterus just 10 times

greater than that of rats fed the control diet. On the basis of this observation, it was proposed that the process of incorporation of fatty acids into uterine lipid pools is selective and favours the incorporation of arachidonic acid. Other studies also support this concept (Leaver and Ning, 1981; Ning *et al.*, 1983). Nevertheless, arachidonic acid pools can be replaced substantially by feeding n-3 fatty acids. Changes in uterine fatty acid composition were detected 3 weeks after the introduction of treatment diets, indicating that the metabolic turnover of the uterine phospholipid pool is high.

Competition of PUFAs for PGHS

Another possible mechanism for dietary reduction in prostaglandin synthesis is the competition of PUFAs such as dihomo- γ -linoleic (C20:3, n-6) and eicosapentaenoic with arachidonic acid for PGHS (Fig. 3). Increased amounts of eicosapentaenoic acid could result in increased synthesis

of prostaglandins of the 3 series, and reduced synthesis of $\text{PGF}_{2\alpha}$. Consequently, reproductive responses regulated by $\text{PGF}_{2\alpha}$ would be reduced. Concentrations of arachidonic acid in the rat uterus are consistently greater than those of dihomo- γ -linoleic and eicosapentaenoic acid, even when high eicosapentaenoic acid diets are fed. However, the ratio of eicosapentaenoic acid:arachidonic acid can be altered considerably through manipulation of the diet (Howie *et al.*, 1992). Indeed, feeding rats with diets rich in n-3 fatty acids resulted in increased secretion of prostaglandins of the 3 series from uterine explants cultured *in vitro* (Leaver *et al.*, 1991).

Inhibition of PGHS synthesis and activity

The PUFAs can also inhibit PGHS synthesis *in vitro* (Achard *et al.*, 1997), and competitively inhibit the conversion of arachidonic acid to prostaglandins of the 2 series (Corey *et al.*, 1983; Fig. 3). When bovine aortic endothelial cells were incubated with eicosapentaenoic acid or docosahexaenoic acid, PGHS-1 mRNA was reduced. Since the reduction in PGI_2 synthesis was greater than the reduction in PGHS-1 message, these authors suggested that the activity of PGI synthase was affected (Achard *et al.*, 1997). Incubation of rat hepatoma cells with arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, or with heineicosapentaenoic acid (C21:5, n-3), a synthetic fatty acid, caused reductions in activity of the COX enzyme, a component of PGHS. Eicosapentaenoic acid inactivated the enzyme almost completely when added 30 s before addition of arachidonic acid substrate (Larsen *et al.*, 1997). Although there are no data indicating that PGHS synthesis is inhibited *in vivo* by dietary PUFAs, findings from studies *in vitro* support this concept and may partially explain reduced secretion of eicosanoids when high concentrations of PUFAs are fed.

Effects on gene expression

Dietary fatty acids inhibit the expression of genes involved in hepatic lipogenesis (Jump *et al.*, 1996) and may repress genes involved in the synthesis of prostaglandins, such as those encoding PLA_2 and PGHS-2. The proposed mechanisms for regulation of gene expression by PUFAs involve activation of nuclear transcription factors such as peroxisome proliferator-activated receptors (PPARs). The PPARs interact with peroxisome proliferator response elements (PPREs) in the regulatory region of target genes to activate or repress transcription (Jump *et al.*, 1996; Fig. 3). A mechanism for repression of gene expression independent of PPARs has been proposed in which PUFAs bind to PUFA-binding proteins. The PUFA-PUFA binding protein complex then binds to response elements to repress gene transcription (Sessler and Ntambi, 1998). To date, it is not known whether PPARs regulate PLA_2 and PGHS-2 genes in the uterus.

Collectively, these findings suggest that increasing dietary PUFAs can alter the synthesis of prostaglandins of the 2 series by: (i) partial replacement of arachidonic acid in phospholipid pools, which limits the amount of arachidonic acid precursor available for prostaglandin synthesis; (ii) decreasing arachidonic acid biosynthesis by inhibition of $\Delta 6$ and $\Delta 5$ desaturase enzymes that are necessary for conversion of linoleic acid to

arachidonic acid; (iii) acting as a direct competitive inhibitor with arachidonic acid for PGHS; and (iv) directly decreasing gene expression of PGHS.

References

Key references are indicated by asterisks.

- *Achard D, Gilbert M, Bénistant C, Slama SB, DeWitt DL, Smith WL and Lagarde M (1997) Eicosapentaenoic and docosahexaenoic acids reduce PGH synthase 1 expression in bovine aortic endothelial cells *Biochemical and Biophysical Research Communications* **241** 513–518
- Ashes JR, Siebert BD, Gulati SK, Cuthbertson AZ and Scott TW (1992) Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants *Lipids* **27** 629–631
- Ayalon N (1978) A review of embryonic mortality in cattle *Journal of Reproduction and Fertility* **54** 483–493
- Baguma-Nibasheka M, Brenna JT and Nathanielsz PW (1999) Delay of pre-term delivery in sheep by n-3 long chain polyunsaturates *Biology of Reproduction* **60** 698–701
- Beam SW and Butler WR (1997) Energy balance and ovarian follicle development prior to the first ovulation in dairy cows receiving three levels of dietary fat *Biology of Reproduction* **56** 133–142
- Bergstrom S, Danielsson H, Klenberg D and Samuelsson (1964) The enzymatic conversion of essential fatty acids into prostaglandins *Journal of Biological Chemistry* **239** 4006–4009
- *Bezard J, Blond JP, Bernard A and Clouet P (1994) The metabolism and availability of essential fatty acids in animal and human tissues *Reproduction Nutrition Development* **34** 539–568
- Blum JW, Jans F, Moses W, Frohli D, Zemp M, Wanner M, Hart IC, Thun R and Keller U (1985) Twenty-four hour pattern of blood hormone and metabolite concentrations in high-yielding dairy cows: effects of feeding low or high amounts of starch, or crystalline fat *Zentralblatt Veterinaermedizin* **32** 401–418
- Burke JM, Staples CR, Risco CA, De la Sota RL and Thatcher WW (1997) Effect of ruminant grade menhaden fish meal on reproductive and productive performance of lactating dairy cows *Journal of Dairy Science* **80** 3386–3398
- Butler WR, Calaman JJ and Beam SW (1996) Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle *Journal of Animal Science* **74** 858–865
- Chen Q and Nilsson A (1993) Desaturation and chain elongation of n-3 and n-6 polyunsaturated fatty-acids in the human CACO-2 cell-line *Biochimica et Biophysica Acta* **1166** 123–201
- Christiansen EN, Lund JS, Rortveit T and Rusta AC (1991) Effect of dietary n-3 and n-6 fatty acids on fatty acid desaturation in rat liver *Biochimica et Biophysica Acta* **1082** 57–62
- *Cook HW (1996) Fatty acid desaturation and chain elongation in eukaryotes. In *Biochemistry of Lipids, Lipoproteins and Membranes* pp 129–152 Ed. DE Vance and J Vance. Elsevier, Amsterdam
- Corey EJ, Shih C and Cashman JR (1983) Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis *Proceedings of the National Academy of Sciences* **80** 3581–3584
- De Alaniz MJ and De Gomez Dumm (1990) Role of fatty acids of (n-3) and (n-6) series on alpha-linolenic acid desaturation and chain elongation in HTC cells *Molecular Cell Biochemistry* **93** 77–85
- DePaolo LV, Ojeda SR, Negrovilar A and McCann SM (1982) Alterations in the responsiveness of median-eminence luteinizing hormone releasing-hormone nerve-terminals to norepinephrine and prostaglandin-E2 *in vitro* during the rat estrous cycle *Endocrinology* **110** 1999–2005
- Emery RS, Liesman JS and Herdt TH (1992) Metabolism of long chain fatty acids by ruminant liver *Journal of Nutrition* **122** 832–837
- Emken EA, Adlof RO, Rakoff H, Rohwedder WK and Gulley RM (1990) Metabolism *in vivo* of deuterium-labelled linoleic acid and linoleic acids in humans *Biochemical Society Transactions* **18** 766–769
- Garg ML, Sebkova E, Thomson AB and Clandinin MT (1988) Delta 6-desaturase activity in the liver microsomes of rats fed diets enriched with cholesterol and/or n-3 fatty acids *Biochemistry Journal* **249** 351–356
- Graham J, Franks S and Bonney RC (1994) *In vivo* and *in vitro* effects of γ -linolenic acid and eicosapentaenoic acid (C20:5, n-3) on prostaglandin production and arachidonic acid (C20:4, n-6) uptake by human endometrium *Prostaglandins Leukotrienes and Essential Fatty Acids* **50** 321–329

- Grummer RR and Carroll DJ (1991) Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle *Journal of Animal Science* **69** 3838–3852
- Harrison JH, Kincaid RL, McNamara JP, Waltner S, Loney KA, Riley RE and Cronrath JD (1995) Effect of whole cottonseeds and calcium salts of long-chain fatty acids on performance of lactating dairy cows *Journal of Dairy Science* **78** 181–193
- Hawkins DE, Niswender KD, Oss GM, Moeller CL, Odde KG, Sawyer HR and Niswender GD (1995) An increase in serum lipids increases luteal lipid content and alters the disappearance rate of progesterone in cows *Journal of Animal Science* **73** 541–545
- Highshoe RB, Cochran RC, Corah LR, Kiracofe GH, Harmon DL and Perry RC (1991) Effects of calcium soaps of fatty acids on post-partum reproductive function in beef cows *Journal of Animal Science* **69** 4097–4103
- Hinckley T, Clark RM, Bushmich SL and Milvae RA (1996) Long chain polyunsaturated fatty acids and bovine luteal cell function *Biology of Reproduction* **55** 445–449
- Howie A, Leaver HA, Wilson NH, Yap PL and Aitken ID (1992) The influence of dietary essential fatty acids on uterine C20 and C22 fatty acid composition *Prostaglandins Leukotrienes and Essential Fatty Acids* **46** 111–121
- *Jump DB, Clarke SD, Thelen A, Liimatta M, Ren B and Badin M (1996) Dietary polyunsaturated fatty acid regulation of gene transcription *Progress in Lipid Research* **35** 227–241
- Kim K and Ramirez VD (1986) Effects of prostaglandin E₂, forskolin and cholera toxin on cAMP production and *in vitro* LH-RH release from the rat hypothalamus *Brain Research* **386** 258–265
- Lammoglia MA, Willard ST, Hallford DM and Randel RD (1997) Effects of dietary fat on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol 17 β , 13,14-dihydro-15-keto-prostaglandin F₂, and growth hormone in estrous cyclic Brahman cows *Journal of Animal Science* **75** 1591–1600
- Larsen LN, Hovik K, Bremer J, Holm KH, Myhren F and Borretzen B (1997) Heneicosapentaenoate (21:5n-3): its incorporation into lipids and its effects on arachidonic acid (C20:4, n-6) and eicosanoid synthesis *Lipids* **32** 707–714
- Leat WMF and Northrop CA (1979) Supplementation with linolenic acid of a diet deficient in essential fatty acids results in impaired parturition in rats *Journal of Physiology* **290** 37–?
- Leaver HA and Ning ACWS (1981) Endometrial uptake and esterification of arachidonic acid (C20:4, n-6) during the estrous cycle of the guinea pig *British Journal of Pharmacology* **733** 223–223
- Leaver HA, Lytton FDC, Dyson H, Watson ML and Mellor DJ (1986) The effect of dietary ω 3 and 6 polyunsaturated fatty acids on gestation, parturition and prostaglandins in intrauterine tissues and the kidney *Journal of Lipid Research* **25** 143–146
- Leaver HA, Howie A and Wilson NH (1991) The biosynthesis of the 3 series prostaglandins in rat uterus after alpha-linolenic acid feeding: mass spectroscopy of prostaglandin E and F produced by rat uteri in tissue culture *Prostaglandins Leukotrienes and Essential Fatty Acids* **42** 217–224
- Levine L and Worth N (1984) Eicosapentaenoic acid – its effects on arachidonic acid metabolism by cells in culture *Journal of Allergy and Clinical Immunology* **74** 430–436
- Lucy MC, Gross TS and Thatcher WW (1990) Effect of intravenous infusion of soybean oil emulsion on plasma concentrations of 15-keto-13,14-dihydro-prostaglandin F₂ and ovarian function in cycling Holstein heifers. In *International Atomic Energy Agency Livestock Reproduction in Latin America* 119–132
- Lucy MC, Staples CR, Michel FM and Thatcher WW (1991a) Effect of feeding calcium soaps to early post-partum dairy cows on plasma prostaglandin F₂, luteinizing hormone, and follicular growth *Journal of Dairy Science* **74** 483–489
- Lucy MC, Staples CR, Michel FM and Thatcher WW (1991b) Energy balance and size and number of ovarian follicles detected by ultrasonography in early post-partum cows *Journal of Dairy Science* **74** 473–482
- Lucy MC, De la Sota RL, Staples CR and Thatcher WW (1993) Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (somatotribove) or saline and fed diets differing in fat content and energy *Journal of Dairy Science* **76** 1014–1027
- Maurer RR and Chenault JR (1983) Fertilization failure and embryonic mortality in parous and nonparous beef cattle *Journal of Animal Science* **56** 1186–1189
- Maurer RR and Echternkamp SE (1982) Hormonal asynchrony and embryonic-development *Theriogenology* **17** 11–22
- Needleman P, Raz A, Minkes MS, Ferrendeli JA and Sprecher H (1979) Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties *Proceedings of the National Academy of Sciences* **76** 944–948
- Ning ACWS, Leaver HA and Poysner NL (1983) Arachidonic acid uptake into and release from guinea pig endometrium *in vitro*. *Prostaglandins Leukotrienes and Medicine* **10** 369–380
- Ojeda SR, Negrovilar A and McCann (1979) Release of prostaglandin Es by hypothalamic tissue – evidence for their involvement in catecholamine-induced luteinizing-hormone-releasing-hormone-release *Endocrinology* **104** 617–624
- Oldick BS, Staples CR, Thatcher WW and Gyawu P (1997) Abomasal infusion of glucose and fat – effects on digestion, production, and ovarian and uterine functions of cows *Journal of Dairy Science* **80** 1315–1328
- Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS and Grant A (1992) Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration *Lancet* **25** 1003–1007
- Pace-Aciac C and Wolfe LS (1968) Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids *Biochimica et Biophysica Acta* **152** 784–787
- Palmquist DL and Weiss WP (1994) Blood and hydrolyzed feather meals as sources of undegradable protein in high fat diets for cows in early lactation *Journal of Dairy Science* **77** 1630–1643
- Randel RD (1990) Nutrition and post-partum rebreeding in cattle *Journal of Animal Science* **68** 853–862
- Schillo KK (1992) Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep *Journal of Animal Science* **70** 1271–1282
- Sessler AM and Ntambi JM (1998) Polyunsaturated fatty acid regulation of gene expression *Journal of Nutrition* **128** 923–926
- Skalan D, Kaim M, Moallem U and Folman Y (1994) Effect of dietary calcium soaps on milk yield, body weight, reproductive hormones, and fertility in first parity and older cows *Journal of Dairy Science* **77** 1652–1660
- Sogn JH, Curry TE, Brannstrom M, Le Maire WJ, Koos RD, Papkoff H and Janson PO (1987) Inhibition of follicle-stimulating hormone-induced ovulation by indomethacin in the perfused rat ovary *Biology of Reproduction* **36** 536–542
- Sprecher H (1981) Biochemistry of essential fatty acids *Progress in Lipid Research* **20** 13–22
- *Staples CR, Burke JM and Thatcher WW (1998) Influence of supplemental fats on reproductive tissues and performance of lactating cows *Journal of Dairy Science* **81** 856–871
- Thatcher WW, Staples CR, Danet-Desnoyers G, Oldick B and Schmitt EP (1994) Embryo health and mortality in sheep and cattle *Journal of Animal Science* **72** Supplement 3:16
- Thatcher WW, Meyer MD and Danet-Desnoyers (1995) Maternal recognition of pregnancy *Journal of Reproduction and Fertility Supplement* **49** 15–28
- Thatcher WW, Binelli M, Burke JM, Staples CR, Ambrose JD and Coelho S (1997) Antiluteolytic signals between conceptus and endometrium *Theriogenology* **47** 131–140
- Thomas MG and Williams GL (1996) Metabolic hormone secretion and FSH-induced superovulatory responses of beef heifers fed dietary fat supplements containing predominantly saturated and polyunsaturated fatty acids *Theriogenology* **45** 451–458
- Trujillo EP and Broughton KS (1995) Ingestion of n-3 polyunsaturated fatty acids and ovulation in rats *Journal of Reproduction and Fertility* **105** 197–203
- Wehrman ME, Welsh TH, Jr and Williams GL (1991) Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics, and hastens the onset of post-partum luteal activity *Biology of Reproduction* **45** 514–522
- Zhang Z, Benson B and Logan JL (1992) Dietary fish oil delays puberty in female rats *Biology of Reproduction* **47** 998–1003
- Zhang Q, Wu WX, Nathanielsz PW and Brenna JT (1995) Distribution of arachidonic, eicosapentaenoic, docosahexaenoic and related fatty acids in ovine endometrial phospholipids in late gestation and labor *Prostaglandins Leukotrienes and Essential Fatty Acids* **53** 201–209