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The role of fatty acids in oocyte and early embryo development

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Abstract. Growing evidence suggests that endogenous and exogenous fatty acids play diverse roles in developing mammalian oocytes and early embryos. In this review, we describe some of the regulatory roles of fatty acids in early development, in addition to their metabolic functions. We focus initially on the provision of individual fatty acids, and then discuss how these might affect metabolism, oxidative stress, membrane composition, cell signalling events and gene expression. We propose that ongoing research should focus on physiologically relevant ratios and combinations of fatty acids, rather than isolated individual fatty acids, as their combined roles are both subtle and complex. Changing the ratio of specific fatty acids in the diet of animal models, and in vitro culture medium can cause significant dysregulation of cellular processes and development, an issue that extends to human fertility.

Introduction

The study of metabolic activity of early embryos has traditionally focussed on the depletion and appearance of individual metabolites from the culture environment (Gardner and Leese 1986; Leese *et al.* 1986). From the early reports of Ralph Brinster, researchers have sought to discover how the early embryo modifies its environment *in vitro*, in the hope that improved understanding of individual embryo metabolism might yield biomarkers of viability to be used to select embryos for transfer in clinical IVF (Brinster 1968*a*, 1968*b*, 1969, 1971). This approach has been a modest success, with evidence to suggest that the pattern of consumption of any one of glucose (Rieger *et al.* 2002; Gardner *et al.* 2011), oxygen (Houghton *et al.* 1996; Thompson *et al.* 1996; Shiku *et al.* 2001; Scott *et al.* 2008; Lopes *et al.* 2010), pyruvate (Conaghan *et al.* 1993), amino acids (Brison *et al.* 2004), the appearance of lactate (Conaghan *et al.* 1993), or a combined measure of metabolism, known as metabolomics (Seli *et al.* 2008), might be predictive of a 'healthy embryo' in a variety of species. As well as generating possible markers of viability, data such as these have been fundamental in defining our current understanding of embryo metabolism. However, in many of the studies on embryo metabolism, the role of fatty acids has been overlooked. Recently we have seen the emergence of a body of literature that is beginning to consider the roles of fatty acids in periconceptual events. In this review, we will consider some of the most recent studies on the key effects and roles of fatty acids in oocyte and embryo development drawing on data from a variety of species. We will initially consider the supply and origin of fatty acids in the oocyte and early embryo, before discussing some of the effects of fatty acids on early embryo development. We will then briefly review the metabolic role of fatty acids, and conclude by considering some of the non-metabolic actions of these compounds.

The origin of fatty acids in the oocyte and early embryo

Fatty acids are typically stored in the cell cytoplasm in the form of triacylglycerol (TG), uncharged esters of glycerol subsequently arranged as neutral lipid droplets that are virtually anhydrous and thus a highly concentrated store of metabolic energy. Whilst the major site of lipid droplet storage in adult mammals is the adipocyte, a specialised cell that possesses a cytoplasm composed almost entirely of a single large globule of TG, it is of interest to note that the oocytes and embryos of mammalian species such as the porcine, bovine and ovine also contain very high quantities of lipid in the ooplasm. This endogenous content of lipid is so high that these oocytes appear dark when visualised under the light microscope (Coull *et al.* 1998; Ferguson and Leese 1999; McEvoy *et al.* 2000), especially when produced *in vitro* (Barceló-Fimbres and Seidel 2011). Cat oocytes are also high in lipid (Guraya 1965) and it has been claimed that fatty acid levels of oocytes can be increased by a carnivorous diet (Spindler *et al.* 2000). It has been suggested that, on the basis of oxygen consumption, there is sufficient lipid to support the metabolic requirements of bovine and porcine preimplantation development as a sole energy source (Sturmey *et al.* 2009*b*). However, this feature of high levels of endogenous fat is by no means universal; the oocytes and embryos of mice are pale, and contain a low level of endogenous lipid (Loewenstein and Cohen 1964), with human oocytes containing an intermediate level (Matorras *et al.* 1998). Investigation of the

cytoplasmic ultrastructure has demonstrated that there are significant numbers of lipid droplets in the cytoplasm of bovine, porcine and ovine oocytes and embryos (Kruip *et al.* 1983; Cran 1985; Abe *et al.* 2002; Leroy *et al.* 2005*a*; Sturmey *et al.* 2006; Aardema *et al.* 2011). However, the developmental period when oocytes accumulate lipid is unclear.

Oocyte development is a multistage process involving periods of growth as well as nuclear and cytoplasmic development. During the progression from primordial follicle to mature oocyte in metaphase II, there are several developmental timeframes when the oocyte might accumulate lipid. If one considers the *in vitro* oocyte, it would seem that the final stages of maturation are most susceptible to modification of cytoplasmic lipid content. For example, Aardema *et al.* (2011) have recently shown that non-esterified fatty acids (NEFA) are retained by bovine oocytes before and during meiotic maturation, and Shehab-El-Deen *et al.* (2009) found that modifying the fatty acid composition of bovine IVM medium led to significant changes in the tolerance of the resulting embryos to cryopreservation. This is an important practical consideration, as the lipid compartment of oocytes and embryos has a significant impact on their ability to survive freeze–thaw (Nagashima *et al.* 1995). Moreover, Sata *et al.* (1999) described how the presence or absence of serum in the culture medium, itself the prime source of fatty acids in culture, can influence the morphology and fatty acid composition of the bovine oocyte and embryo, a finding confirmed using a quantitative approach by Ferguson and Leese (1999) for the bovine. Such studies indicate that the lipid profile of the oocyte is dynamic and largely dictated by the environment in which it develops. It is therefore likely that oocyte development *in vivo* is also sensitive to the environment within the follicle. For example, whole-body metabolic stress has major effects on the fatty acid composition of blood and follicular fluid. One of the best-studied examples is the phenomenon of negative energy balance in the dairy cow (Leroy *et al.* 2008*a*, 2008*b*) in which fertility is compromised and associated with a modified fatty acid profile in the follicle (Leroy *et al.* 2005*b*, 2010; Vanholder *et al.* 2005). Such a situation is not unique to the bovine however; elegant work carried out by Dunning *et al.* (2010) and Jungheim *et al.* (2011) indicates that maternal overweight and obesity in women impacts the composition of follicular fluid and is associated with suboptimal fertility. The focus of much of this type of research has been on the follicular compartment and there are no reliable data on the fatty acid profile of the oviduct and uterine lumen, presumably due to the technical challenge associated with the collection of fresh samples from these environments (Iritani *et al.* 1969, 1971, 1974; Khandoker *et al.* 1997, 1998; Coyne *et al.* 2008; Hugentobler *et al.* 2008; Leese *et al.* 2008). In one of very few reports to examine oviduct and uterine fluid composition, Iritani *et al.* (1974) found that fatty acids were indeed present in the uterine secretions in the sow, although specific fatty acids were not identified. It has been suggested that the main role of many NEFA in oocytes and embryos is to provide energy, as rabbit oocytes can grow *in vitro* in salt-based medium supplemented with a sole NEFA including palmitate, oleate and stearate (Kane 1979). However, data from Menezo *et al.* (1982) suggest that fatty acids play an important role in embryo development. Using the bovine, they reported that total fatty acid content rose sharply between Days 11 and 13, with a further increase in arachidonic acid at Day 14, which may indicate the start of prostaglandin synthesis critical in embryo–maternal dialogue.

In the following sections, we describe some of the effects of key fatty acids on oocyte and embryo development.

Linolenic and linoleic acid

Marei *et al.* (2010) reported that the addition of the polyunsaturated fatty acid (PUFA) linolenic acid (C18:3 n-3) to bovine maturation medium *in vitro* increased the number of oocytes that reached metaphase II stage and that such oocytes exhibited more active mitogen-activated protein kinase (MAPK) signalling pathways. The resulting embryos were of better quality, with higher proportions at the cleavage and blastocyst stages compared with controls. However, it was interesting to note that when supplied at a supraphysiological concentration, linolenic acid induced a fall in the number of oocytes reaching metaphase II, along with a reduction in cumulus expansion. *In vivo*, the concentration of linolenic acid does not change significantly in the expanding follicle, while the concentration of linoleic acid falls (Homa and Brown 1992). Linoleic acid (18:2 n-6), the major fatty acid in bovine follicular fluid, has an inhibitory effect on oocyte maturation *in vitro* (Homa and Brown 1992; Marei *et al.* 2010), although the effect is reversible. Embryo cleavage and blastocyst development were reduced following exposure to linoleate, and MAPK 1 and 3 signalling was reduced, in stark contrast to the effect of linolenate (Marei *et al.* 2009, 2010). Linoleic acid has a similar inhibitory effect on murine embryos (Nonogaki *et al.* 1994).

In a recent report, Hughes *et al.* (2011) described the expression in ovine blastocysts of elongation of very long chain fatty acids (ELOVL5) and fatty acid desaturase 2 (FADS2), key enzymes involved in the conversion of linoleic and linolenic acid to other fatty acids. The expression profile and ratio of n-6 : n-3 PUFA were sensitive to serum supplementation. Intriguingly, most female ELOVL5 null mice are infertile (Moon *et al.* 2009), leading to speculation on the importance of fatty acid metabolism in the mouse early embryo, especially in light of the work by Dunning *et al.* (2010) who has postulated that fatty acid metabolism is active in early mouse embryo development (discussed below in further detail).

Taken together, these data suggest that the ratio of linolenic and linoleic acid is critical to mammalian reproductive success, a viewpoint we revisit later.

Oleic acid

Oleic acid (18:1) is a monounsaturated fatty acid found in high concentrations in bovine oocytes and follicular, oviducal and uterine fluids (Tsujii *et al.* 2001) and pig oocytes (Homa *et al.* 1986). In 1982, Quinn and Whittingham (1982) reported that exogenous oleic and palmitic acids inhibited fertilisation of mouse embryos but promoted blastocyst formation and hatching when added to 8-cell embryos. Aardema *et al.* (2011) showed that oleic acid had a positive effect on lipid storage, oocyte maturation and subsequent embryo development in the cow, overcoming the adverse effects of the other major saturated fatty

acids palmitic and stearic. These data further suggest that the ratio of saturated : unsaturated fatty acids is critical for oocyte maturation.

Arachidonic acid

Arachidonic acid (20:4 n-6) is a key PUFA because it is a precursor for synthesis of prostaglandins, which are vital for embryo–maternal dialogue. It becomes an 'essential' fatty acid in the absence of linolenic acid in humans and in mammals, which lack the ability to convert linolenic to arachidonic acid. Prostaglandin E_2 synthesis occurs in the bovine oocyte and increases if linolenic acid is added to the maturation medium (Marei *et al.* 2010). Menezo *et al.* (1982) reported that bovine embryos removed from the mother at Day 14 had increased levels of arachidonic acid, which was suggested might indicate the start of prostaglandin synthesis in the embryo (Menezo *et al.* 1982). Arachidonic acid is toxic to developing oocytes (Kane 1979).

Eicosapentaenoic acid and docosahexanoic acid

The n-3 PUFA eicosapentaenoic acid (C22:5 n-3) and docosahexanoic acid (C22:6 n-3), known as EPA and DHA respectively, are found in low concentrations in mammalian embryos (McEvoy *et al.* 2000). n-3 PUFA-enriched serum and albumin increased the percentage and absolute mass of EPA and DHA in Day-7 ovine blastocysts *in vitro* but also reduced quality and increased superoxide dismutase 1 (SOD1) expression at the mRNA level (Hughes *et al.* 2011). The presence of these fatty acids in embryos has been reported in most (Matorras *et al.* 1998; McEvoy *et al.* 2000; Hughes *et al.* 2011), but not all (Zeron *et al.* 2002), studies.

Palmitic acid

Palmitic acid (16:0) is the most abundant fatty acid in human follicular fluid (Jungheim *et al.* 2011) and the second most abundant in bovine follicular fluid (Leroy *et al.* 2005*b*). It has been reported that saturated fatty acids are readily taken up by bovine oocytes (Adamiak *et al.* 2006); however, bovine oocytes mature more slowly in the presence of palmitic and stearic acids and have significantly reduced fertilisation, cleavage and blastocyst rates (Leroy *et al.* 2005*a*). The mechanism of such lipotoxicity is unclear but the effects do not appear to arise through an alteration of lipid content (Leroy *et al.* 2005*b*). Interestingly, palmitate appears to be vital to cat oocyte maturation and its metabolism is significantly increased in *in vivo* compared with *in vitro* maturated oocytes (Spindler *et al.* 2000).

In the case of embryos, Jungheim *et al.* (2011) reported that mouse embryos cultured in medium rich in palmitic acid (200 μM) upregulated expression of the embryonic insulin-like growth factor 1 (IGF1) receptor and of glutamic pyruvate transaminase, a downstream target of insulin-like growth factor receptor 1 (IFG1R). Moreover, the number of inner cell mass (ICM) cells in mouse blastocysts was reduced, apoptosis of trophoblast stem cells increased, and proliferation decreased (Jungheim *et al.* 2011) following addition of palmitate. Interestingly, fetuses developing from palmitic acid-treated blastocysts were growth restricted but the offspring eventually outgrew the offspring from control blastocysts, a phenomenon with similarities to adult-onset type 2 diabetes mellitus in man (Chakravarthy *et al.* 2008). Results such as these suggest that exposure of early embryos to high concentrations of palmitic acid leads to aberrant metabolism, increased apoptosis (most likely due to damage by reactive oxygen species (ROS)), and long-term metabolic perturbation leaving the organism predisposed to diabetes and obesity. The mechanism of such a non-genomic hereditary condition has not yet been defined, although epigenetic modification is a likely candidate (reviewed by Chason *et al.* 2011). However, data such as these serve to illustrate the importance of studies on the periconceptual origin of metabolic disease (Leese *et al.* 2008), which are of critical importance in understanding the early origins of human overweight, obesity and diabetes, especially in children.

Stearic acid

Stearic acid (18:0) is a saturated fatty acid that can be converted to the monounsaturated oleic acid. It is the third most prevalent fatty acid in bovine follicular fluid, but comprises a smaller proportion in the oocyte (Tsujii *et al.* 2001). As mentioned above, stearic acid slows early embryo development (Leroy *et al.* 2005*b*). Recent studies reported similar results in somatic cells; stearic acid inhibits aortic cell growth and is cytotoxic at high concentrations and leads to a corresponding increase in intracellular adhesion molecule 1 (ICAM-1) expression and nuclear factor- kB (NF-kB) phosphorylation, but these effects are reversed by oleic acid (Harvey *et al.* 2010*a*, 2010*b*). NF-kB is expressed in the murine oocyte and zygotes through to the blastocyst stage, and is required for development from the 1- to 2-cell stage (Nishikimi *et al.* 1999), providing one possible mechanism of the action of stearic acid on early embryos.

These, and similar data, provide a wealth of information about the potential effects of fatty acids on the early embryo. However, it is important to consider the interactions between fatty acids in combination and at physiological concentrations. For example, detailed analysis of the fatty acid composition of bovine, ovine and porcine oocytes performed by McEvoy *et al.* (2000) showed that palmitic acid, oleic acid and stearic acid were the most abundant in the three species, accounting for around 70% of all fatty acids present. Linoleic acid was the fourth most prevalent at 5–8% total; an interesting observation in light of the potential toxicity of this fatty acid. The largest fractions were, in decreasing order, saturated, monounsaturated and polyunsaturated fatty acids, of which n-6 were the most common. Interestingly, good-quality oocytes tend to contain more oleic, linoleic and arachidonic acids. Haggarty *et al.* (2006) measured the fatty acid composition of supernumary human preimplantation embryos and found that those embryos that survived beyond the 4-cell stage tended to have a higher ratio of unsaturated to saturated fatty acids. These data are supported by previous findings in oocytes that failed to fertilise (Matorras *et al.* 1998). Uptake of linolenic acid increased significantly from the 2-cell to blastocyst stage, whereas uptake of the saturated palmitic acid increased by a smaller degree and the concentration actually decreased between the morula and blastocyst stages.

Of course, fatty acids are usually presented to the oocyte and embryo ultimately as a function of diet. It is beyond the scope of this article to review this area fully; however, to give a recent example of a study that fed n-3 or n-6 PUFA-enriched diets to ewes it was found that regardless of the treatment, all blastocysts had a high unsaturated fatty acid composition, particularly of linoleic acid (Wonnacott *et al.* 2010). In a mouse model, Wakefield *et al.* (2008) fed a diet rich in n-3 PUFA and assessed embryo quality following *in vivo* fertilisation. Blastocyst numbers and overall embryo quality were reduced, with mitochondrial distribution disrupted and ROS levels increased (Wakefield *et al.* 2008). These results were in stark contrast to those for oocytes removed from mice treated with an identical diet but fertilised *in vitro* and cultured to the blastocyst stage (Wakefield *et al.* 2008). How these effects might occur in the oocyte and embryo is unknown, but the ratio of presentation of fatty acids is of clear importance. For example, in recent work, Van Hoeck *et al.* (2011) have demonstrated that exposing bovine oocytes to physiologically relevant, elevated levels of palmitic acid, oleic acid and stearic acid causes significant dysregulation of metabolism and gene expression in the resulting blastocysts whereas such effects are not apparent when these fatty acids are supplemented singly. These findings highlight the importance of the changing ratio of fatty acids on successful embryonic development.

Fatty acids and metabolism

Fatty acids are widely accepted as potential metabolic substrates for the oocyte and early embryo, a topic reviewed in Sturmey *et al.* (2009*b*). For example, Sturmey and Leese (2003) and Ferguson and Leese (2006) demonstrated that inhibition of β-oxidation during oocyte maturation led to a fall in subsequent embryo viability in the pig and cow respectively. The oocytes and embryos from both of these species are rich in lipid; however, it is now becoming increasingly apparent that fatty acids may be essential for certain stages in development, even in species with low lipid reserves, such as the mouse (Downs *et al.* 2009). Using radiolabelled palmitic acid, Flynn and Hillman (1980) established that exogenous fatty acids are incorporated into murine embryos notably between the 8-cell and late blastocyst stages, in preparation for the most metabolically active part of embryo development: blastocyst expansion. Dunning *et al.* (2010) have recently suggested that β-oxidation plays a vital role in the development of mouse oocytes. The rate limiting step in β-oxidation of fatty acids is catalysed by carnitine palmitoyl transferase 1 (CPT1B), expression of which is detectable in mouse blastocysts, though not in zygotes, 2-cell or 8-cell stages (Dunning *et al.* 2010). However, treatment of zygote and 2-cell embryos with Etomoxir (Sigma-Aldrich, Castle Hill, NSW, Australia), an inhibitor of CPTB1, decreases survival rates. In the same study, enhancement of β-oxidation by supplementation with L-carnitine at the oocyte stage led to an improvement of subsequent embryo development in terms of numbers of hatching blasts and ICM : trophectoderm (TE). Other work has also found that inhibition of CPTB1 with Etomoxir or malonyl CoA arrests oocyte maturation, which may be rescued by treatment with palmitic acid or carnitine (Downs *et al.* 2009; Downs 2010). A mechanism for these responses could involve 5′ adenosine monophosphate-activated protein kinase (AMPK), a major regulator of cellular energy status, Downs and co-workers proposed inhibits malonyl CoA formation and relieves the inhibition of β-oxidation.

In a seemingly unrelated article, Miyamoto *et al.* (2010) found that oral administration of L-carnitine to mice undergoing repeated superovulation protected the oocytes against oxidative stress. It is tempting to speculate that one effect of the L-carnitine in that study was to enhance β-oxidation by the mechanism described by Dunning *et al.* (2010), which would lead to a reduction in the amount of intracellular lipid available for damage by oxidative stress. Further possible evidence of such a relationship comes from the observation that L-carnitine enrichment of porcine *in vitro* maturation medium improved oocyte maturation, reduced intracellular ROS levels (see below) and increased the concentration of glutathione, an antioxidant, in porcine oocytes (Wu *et al.* 2011). The effects of oxidative damage to the lipid compartment of the oocyte and early embryo has received relatively little attention.

Fatty acids and ROS

Production and propagation of ROS is a feature of aerobic respiration and causes damage to cellular macromolecules including DNA, protein and lipid (Stadtman and Levine 2000; Takahashi *et al.* 2000). Types of ROS include superoxide, peroxide and hydroxyl radicals. β-Oxidation of fatty acids produces ROS, which can damage mitochondrial DNA and protein, leading to compromised mitochondrial function and a predisposition to further ROS generation (Duvnjak *et al.* 2007). ROS have been shown to cause DNA damage in bovine embryos (Takahashi *et al.* 2000; Sturmey *et al.* 2009*a*) and to induce fragmentation and apoptosis in human embryos (Yang *et al.* 1998). Oxidative stress is also a major cause of developmental arrest at the 2-cell stage in mice (Johnson and Nasresfahani 1994; Favetta *et al.* 2007). At the same time, it is important to note that at low concentrations, ROS act as physiological signalling agents with well-defined functions, both in embryos and in somatic cells (Hancock *et al.* 2001).

Supplementing culture medium with serum is known to increase the fatty acid content of ovine blastocysts and to be associated with an increase in superoxide dismutase expression and a decrease in embryo quality, possibly due to an increase in superoxide production following increased peroxidation (Lequarré *et al.* 2001). Lipid peroxidation has been implicated as a cause of reduced embryo quality and arrested development in several studies (Reis *et al.* 2003). The interaction between ROS and intracytoplasmic lipid content may be species specific, however. For example in mouse oocytes and early embryos, with typically low fatty acid content, β-oxidation of lipid may protect against ROS-induced lipid peroxidation. By contrast, the oocytes and embryos of the larger domestic species contain such large amounts of lipid that β-oxidation will only deplete a small proportion, leaving the remaining lipid prone to peroxidation. Thus, the regulation of fatty acid metabolism is clearly important at the periconceptual period but the extent of the contribution of lipid may be species specific. We are seeking to address

the question of the degree to which the lipid compartment of the oocyte and early embryo *in vitro* is susceptible to oxidative stress.

In the remainder of this review, we consider some non-metabolic roles of fatty acids in early embryo development.

Incorporation into phospholipids

Homa *et al.* (1986) reported that immature pig oocyte phospholipids and TG were rich in omega-6 PUFA linolenic and arachidonic acids while Tsujii *et al.* (2001) reported that both palmitic and stearic acids are incorporated into TG and phospholipids in cultured mouse embryos following single fatty acid supplementation. Recently, a sensitive method for measuring the phospholipid content of oocytes and embryos of bovine, human and other species, as well as TG and sphingomyelin levels, was reported by Ferreira *et al.* (2010). Bovine oocytes typically contain only 10–15 pmol phospholipid, so this is an exquisitely sensitive technique that could be applied to further our understanding of the role of phospholipid in early embryos. Renaville *et al.* (2010) found reduced stearic acid but increased oleic, arachidonic and docosahexaenoic acid in the phospholipid composition of inactive bovine follicular fluid. It will be intriguing to discover if the changing concentrations of these fatty acids as follicles mature are reflected in oocyte phospholipids.

Fatty acids as cell signalling agents

Many of the extensive range of molecules required for cellular signalling have fatty acid precursors. For example, arachidonic acid is initially stored in an esterified form as part of the phospholipid bilayer of the plasma membrane. G-protein coupled receptor activation stimulates phospholipase enzymes to catalyse the hydrolysis of these phospholipids, releasing arachidonate or arachidonate-containing products. Further metabolism produces eicosanoid signalling molecules such as prostaglandins, with far-reaching effects (Piomelli 1993). These molecules have a range of effects at the cellular, tissue and whole-organism level.

Fatty acids also bind nuclear receptors, including peroxisome-proliferator activated receptors (PPAR) as well as transcription factors such as sterol-regulatory element binding protein (SREBP) and NFkB (Sampath and Ntambi 2005) and thus exert important regulatory control over the activity of such receptors and hence on gene expression (Bordoni *et al.* 2006).

PPAR

There are three known isoforms of PPAR, of which PPARα is the most widely studied (Sampath and Ntambi 2005). PPARα and PPARγ are expressed in post-implantation rat embryo development when the embryo is separating into defined regions, whereas PPARβ is considered to be expressed ubiquitously (Braissant and Wahli 1998). Moreover, PPARγ is expressed in granulosa cells surrounding the oocyte, such that PPARγ knockout in mouse oocytes and granulosa cells reduces fertility (Cui *et al.* 2002; Rees *et al.* 2008). In order to elicit their action, the PPAR dimerise with retinoid X receptor (RXR) and bind PPAR response elements to regulate expression of genes involved in lipid and carbohydrate metabolism (Desvergne and Wahli 1999). Expression of PPAR-γ mRNA, along with two of its heterodimeric partners, RXR-α and RXR-β, has been detected in bovine oocytes and zygotes through to the blastocyst stage, while PPAR-γ and RXR-β protein have been detected in trophectoderm and ICM by Mohan *et al.* (2002) who provided evidence that the proteins co-localised and could therefore form heterodimers required for signal transduction from a PUFA signal in the embryo.

All n-3 and n-6 PUFA seem to activate the PPAR isoforms, but with different affinities. For example, linoleic, linolenic and arachidonic acids activate PPARα (Lehmann *et al.* 1997), while EPA is a more potent activator than arachidonic acid (Ren *et al.* 1997). Ren *et al.* (1997) reported that mice supplemented with fish oil, a rich source of n-3 PUFA, exhibited upregulation of β-oxidation genes, a result that was absent in PPARα null mice. Lipogenic genes, however, were suppressed in both strains of mice. It has been proposed that PPARα is responsible for PUFA induction of fatty acid β-oxidation transcription, but not of PUFA-controlled repression of lipogenic genes, so a SREBP (see below) may have the latter function (Ntambi and Bené 2001).

SREBP

It has been proposed that PUFA repress lipogenic gene transcription acting through SREBP (Brown and Goldstein 1997). These transcription factors bind sterol response elements (SRE), including those encoding elements of fatty acid, triglycerides and cholesterol synthesis (Korn *et al.* 1998). *In vitro*, PUFA inhibit the proteolytic maturation of SREBP, thereby reducing the availability of mature SREBP to bind and allow transcription of SRE-controlled genes (Tabor *et al.* 1998). However, there appears to be an additional mechanism for the repression of SRE-controlled genes by PUFA. Using transfected HepG2 cells with a mature form of SREBP-1a and cholesterol to block maturation of any endogenous SREBP, it has been demonstrated that fatty acids including arachidonic acid, eicosapentanoic acid and docosahexanoic acid repressed stearoyl-CoA desaturase 1 (scd1) promoter activity in a dose-dependent manner, even though a plentiful supply of mature SREBP-1a was available (Ntambi and Bené 2001). A recent study quantified the expression in bovine embryos of nine genes typically involved with fatty acid and lipid metabolism in adipocytes. It was found that mRNA levels of SREBP1, SCD1 and FADS2, key genes involved in fatty acid signalling, decreased in bovine embryos on treatment with the PUFA docosahexanoic acid, linoleic acid and linolenic acid (Al Darwich *et al.* 2010). Thus, it is tempting to speculate that expression and regulation of SREBP might form a component of the mechanism by which the proportions of saturated and unsaturated fatty acids in the embryo are balanced.

Concluding remarks

In this article, we have considered some of the many possible roles of fatty acids in the development of early embryos, an area that is attracting increasing attention. A key feature that has emerged is the hierarchical nature of fatty acid abundance and interactions: that different reproductive organs and cells have different proportions of the major saturated, unsaturated and

polyunsaturated fatty acids, and that maintenance of these ratios is critical for proper development. As technology and understanding continues to improve, researchers will continue to uncover the intricate effects of fatty acids and the subtleties of fatty acid ratios on development. These exciting developments are timely, given the changes in human diets and effects on fertility and possible long-term health of the offspring, and represent a new frontier in fertility research.

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