Role of fatty acids in energy provision during oocyte maturation and early embryo development

Sturmey, RG; Reis, A; Leese, HJ; McEvoy, TG

Published in:
Reproduction in Domestic Animals

DOI:
http://dx.doi.org/10.1111/j.1439-0531.2009.01402.x

First published: 01/01/2009

Document Version
Peer reviewed version

Reprod Dom Anim **44** (Suppl. 3), 50–58 (2009); doi: 10.1111/j.1439-0531.2009.01402.x
ISSN 0936-6768

Role of Fatty Acids in Energy Provision During Oocyte Maturation and Early Embryo Development

RG Sturmey[1], A Reis[2], HJ Leese[1,3] and TG McEvoy[2]

[1]*Department of Biology (Area 3), University of York, York, YO10 5YW;* [2]*SAC, Sustainable Livestock Systems Group, West Mains Road, Edinburgh, EH9 3JG;* [3]*The Hull York Medical School, University of York, York, YO10 5DD, UK*

Contents

While much is known about the metabolism of exogenous nutrients such as glucose, lactate, pyruvate, amino acids by oocytes and pre-implantation mammalian embryos, the role of endogenous stores, particularly lipid, has been largely overlooked. The presence of lipid within oocytes and early embryos has been long known, and comparisons between species indicate that the amounts and types of lipid present vary considerably. Large amounts of intracellular lipid can compromise the success of cryopreservation and the removal of such lipid has been the subject of considerable effort. In this review, we present evidence that strongly suggests a metabolic role for lipid, specifically with regard to energy provision, in the late-stage oocyte and the pre-implantation embryo. We focus initially on oxygen consumption as a global indicator of metabolic activity, before reviewing different approaches that either have been designed to investigate directly, or have revealed indirectly the role of endogenous lipid in energy generation. These fall under five headings: (i) fatty acid oxidation; (ii) inhibition of triglyceride oxidation; (iii) culture in the absence of exogenous substrates; (iv) cytoplasmic organization; and (v) delipidation. On the basis of the data derived from these studies, we conclude that there is strong evidence for the utilization of endogenous lipid as an energy substrate by oocytes and early embryos.

Introduction

The oocyte has the largest diameter of any cell in the female mammal. The developmental consequence of having a large egg is that the zygote undergoes a series of 'cleavage' divisions in which the genetic material (chromosomal DNA) is duplicated, but the cytoplasm is not and, as a result, the ratio of nuclear to cytoplasmic volume characteristic of adult cells is established approximately at the blastocyst stage. The reasons for the oocyte's large diameter are unclear, but a number of explanations have been proposed to account for the size of the mammalian egg, they are:

(1) provision of a large target for the spermatozoa;
(2) efficiency of energy expenditure – it may be more efficient to make a large egg within the ovary, rather than the alternative scenario of beginning with an adult-sized cell and having to double its volume, weight and zona pellucida at each cell division during embryogenesis;
(3) legacy of a common ancestry with reptilia, amphibia, and monotreme mammals (echidna and platypus) which have large, yolky eggs;
(4) provision of a large endogenous energy repository available to the egg/embryo until, in mammalian species, it attaches/implants.

Whatever the explanation for the large size of the egg, energy in the form of ATP is required to power the pre-implantation stages of development for which there are two potential sources: exogenous and endogenous nutrients.

Exogenous and Endogenous Nutrients

Overwhelmingly, the focus of research and practice in the culture of early embryos has been on exogenous nutrients. Thus, it is well known that oocytes and embryos at the pre-compaction 'cleavage' stages of pre-implantation development are relatively quiescent and can derive ATP from the oxidation of exogenous nutrients such as pyruvate, lactate and amino acids (Leese 1991, 1995, 2003). At the morula stage, there is a sharp rise in metabolic activity, measured in terms of oxygen consumption, in all species studied (Fig. 1) largely because of the energy demands of sodium pumping in the formation of the blastocoel cavity (Brison and Leese 1994; Donnay and Leese 1999; Houghton et al. 2003) and of increased protein synthesis, as the embryo initiates net growth (Leese 1993). Oxygen consumption reflects overall metabolic activity but does not indicate the nature of substrate(s) being oxidized. Glucose consumption also increases at this time, much of it being converted, at least *in vitro*, to lactate via 'aerobic glycolysis', which, in addition to providing a modest amount of ATP may prime the embryo for the hypoxic environment it will encounter during implantation (Leese 1995).

In contrast to our considerable knowledge on exogenous nutrients, the potential contribution to energy homeostasis of endogenous substrates in the form of glycogen, protein and fat has received relatively little research attention. Glycogen may be important at the blastocyst stage (reviewed by Leese 1993) and endogenous protein is unlikely to be expended to generate ATP leaving fat as the most likely candidate endogenous energy substrate.

Triglyceride is the major component of intracellular lipid in the oocyte (Homa et al. 1986) and thus provides a large potential energy reserve. Tables 1–3 gives the fat or triglyceride content of oocytes of a number of species as well as details of the composition of lipid present in the oocyte.

In Table 1, the striking feature is the very low fat content of mouse eggs and the exceptionally high content in the pig; so high that porcine eggs and pre-implantation embryos appear black under the

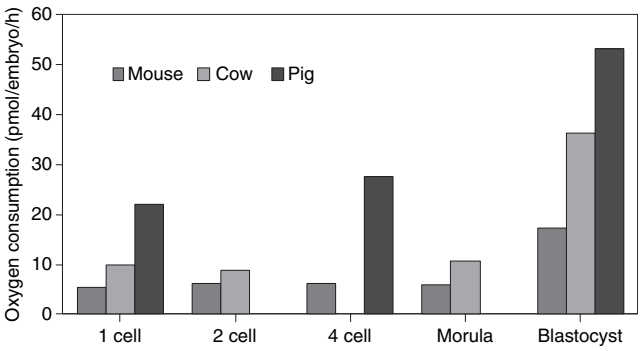

Fig. 1. Oxygen consumption rates of embryos of three mammalian species. The figure is based on the data of Sturmey and Leese (2003; pig), Houghton et al. (1996; mouse) and Thompson et al. (1996; cow)

microscope because of lipid being optically dense albeit having low buoyant density. Canine oocytes and embryos also have a high fat content and at least one study has suggested that relative scarcity of lipid at the oocyte stage is evidence of degeneracy (Durrant et al. 1998). One hypothesis for the presence of high amounts of lipid in the embryos of the domestic species is that it reflects the 'time to attachment'. Lipid-rich ruminant embryos and pig embryos remain unattached for a considerable time in the uterus *in vivo*; mouse and human eggs attach relatively speedily and may not need an 'endogenous energy reserve' to the same extent as livestock species. The lipid-rich status of the dog egg is consistent with this idea as it too is slow to implant. However, this hypothesis is speculative at this stage and requires further investigation.

Lipid Provision

The role of lipid in the peri-ovulatory (or peri-conception) phase of livestock reproduction is multifaceted and effects can be modulated at the endocrine as well as at nutrient provision level. Arachidonic acid (C20:4n-6) is a crucial pre-cursor of prostaglandins while cholesterol is needed for steroid hormone biosynthesis. Among the steroid hormones, progesterone plays a key role, through effects on endometrial activity and gene expression, in ensuring that the *in vivo* environment is conducive to embryo development. Provision of dietary fats could therefore exert effects via an endocrine or other route (Abayasekara and Wathes 1999; Kuran et al. 1999). However, in cattle and sheep, rumen-degradable dietary fats can disrupt rumen function and thereby negatively affect energy balance and well-being. A feature of dairy cattle infertility is that problems can

Table 1. Summary of lipid content of mammalian oocytes

	Stage	Cattle	Sheep	Pigs	Mice
Total lipid	Oocyte	No data	No data	No data	3.25[a]
Total FA	Oocyte	63[b]	89 ± 7[b]	161[b]	No data
Triglyceride	Oocyte	23[b]	25 ± 4[b]	74[b]–136[c]	No data
	Oocyte pre-mat	50[d*]–59[e]	No data	136[c]	No data
	Oocyte post-mat	37[d*]–46[e]	No data	123[c]	No data
	Post-fertilisation	34[e]	No data	112[c]	No data
Phospholipid	Oocyte	18[b]	25[b]	41[b]	No data

[a]Loewenstein & Cohen, (1964).
[b]McEvoy et al. (2000).
[c]Sturmey & Leese (2003).
[d]Kim et al. (2001).
[e]Ferguson & Leese, (1999).
*denotes estimated value.

Table 2. Summary of composition of mammalian oocytes

Species	Amount of TG (%)	Most abundant fatty acid	Most abundant cfatty acid in TG	Second most abundant fatty acid in TG	Report
Pig	60.0	C16:0	C16:0	C18:1	Homa et al. 1986
Pig	48.0	C16:0	C16:0	C18:0	McEvoy et al. 1997
Human	–	C18:0	–	–	Matorras et al. 1998
Pig	–	C18:1	–	–	Khandoker et al. 1997
Cow	–	C16:0	–	–	Khandoker et al. 1997
Pig	45.0	C16:0	C16:0	C18:1	McEvoy et al. 2000
Cow	36.4	C16:0	C18:1	C16:0	McEvoy et al. 2000
Sheep	28.0	C18:1	C18:1	C16:0	McEvoy et al. 2000
Cow	57.0	C16:0	–	–	Kim et al. 2001

Table 3. The amount of lipid present per unit volume

	Cattle*	Sheep*	Pig*	Mice**	Reference
Oocyte lipid mass	63	89	161	3.25	*McEvoy et al. 2000 **Loewenstein and Cohen 1964
Volume	4.20 nl	4.20 nl	4.20 nl	0.52 nl	
Lipid per nanolitre	15	21.2	38.3	6.25	

Unless otherwise stated, all values are ng.

be accentuated when energy-rich (fat-supplemented) diets are used to meet milk production requirements. Dietary lipid has been shown to influence fatty acid status of intra-ovarian follicles and the oocyte/embryo micro-environment *in vivo* but (in contrast to findings *in vitro*) we have not detected changes in lipid composition of eggs *per se*. Indeed, while the study of Zeron et al. (2002) did note effects of diets containing a polyunsaturated fatty acid (PUFA)-rich rumen-bypass dietary supplement (fed for 13 weeks) on chilling sensitivity of ovine oocytes, there were no significant differences in the abundance of key fatty acids within oocyte phospholipids and while the supplement significantly increased the abundance of some long-chain PUFAs in follicular fluid and cumulus cells, no corresponding changes were detected within the oocyte. It is therefore probable that a selective protective process has evolved to ensure that, for example, the PUFA complement of oocytes is kept to a minimum *in vivo* to minimize risks of degradation. However, this protection almost certainly does not extend to safeguarding embryos exposed to sera *in vitro* (Thompson et al. 1995; Kim et al. 2001; Reis et al. 2003, 2005). Elsewhere, there is evidence of specific, sophisticated management of fatty acid uptake, notably the avian capacity to partition docosahexaenoic acid (DHA, C22:6n-3) preferentially towards brain development (McEvoy and Speake 2001) and the mammalian data in Table 2 indicate cross-species preferences for more stable (saturated and mono-unsaturated) fatty acids. For more details of the effects of diet on reproduction, see Hess et al. (2008), Leroy et al. (2008) and Santos et al. (2008).

The situation *in vitro* is clearer. There is good evidence from a number of sources that the amount of fat in the oocyte and embryo can be altered by changing the composition of the culture medium; particularly by the provision of serum (Thompson et al. 1995; Abe et al. 1999a,b, 2002a,b; Rizos et al. 2002; Reis et al. 2003). For example, Ferguson and Leese (1999) showed a significant increase in the amount of triglyceride in cattle oocytes after culture in medium containing foetal calf serum, and using the Nile red staining method, these observations were confirmed by Genicot et al. (2005) and Leroy et al. (2005). Total lipid content is also increased *in vitro* (Kubovicova et al. 2003). Reis et al. (2005) showed that neutral lipid accounted for most of the extra material associated with *in vitro* exposure of embryos to serum, consistent with the data of Ferguson and Leese (1999).

In this review, we focus on evidence for a metabolic role for or a tendency towards triglyceride utilization during oocyte maturation and early embryo development. The evidence is derived from direct studies or indirect observations and falls under five headings: (i) Fatty acid oxidation; (ii) Inhibition of triglyceride oxidation; (iii) Culture in the absence of exogenous substrates; (iv) Cytoplasmic organization; and (v) Delipidation.

Fatty acid oxidation

Oocytes

There is indirect evidence for lipid oxidation in cow (Ferguson and Leese 1999; Kim et al. 2001) and pig (Sturmey and Leese 2003) oocytes. *In vitro*, the amount of triglyceride present in the oocyte decreases during maturation, and, at least in the pig, there is a corresponding fall in oxygen consumption sufficient to account for this depletion (Sturmey and Leese 2003). More compelling evidence comes from the use of metabolic inhibitors (see next section). In addition, Cetica et al. (2002) reported a significant rise in lipase activity in bovine oocytes during maturation, suggesting that fatty acid oxidation is occurring.

In contrast to evidence of lipid utilization, some studies indicate that lipid content does not change during *in vitro* maturation. For example, Aardema et al. (2008) recently reported that lipid droplet number or neutral lipid abundance were similar, prior to and following maturation of bovine oocytes *in vitro*. However, this invasive method of measurement precluded direct analysis of the same eggs pre- and post-maturation. Furthermore, there is considerable variation in the amount of lipid present in oocytes and early embryos (Ferguson and Leese 1999; McEvoy et al. 2000; Sturmey and Leese 2003; Genicot et al. 2005). Indeed, Aardema et al. 2008 also reported great variation in lipid droplet number among oocytes from the same ovary. This suggests that the oocyte tends to accumulate a generous surplus of lipid during its pre-maturation development. In such circumstances, the total complement of lipid could mask minor changes because of utilization for energy provision. It should also be noted that uptake of lipid from serum added to the culture medium (cattle: Ferguson and Leese 1999; sheep: Reis et al. 2005) could mask changes in endogenous reserves. Furthermore, the energy-dense nature of lipid means that only minimal amounts need to be oxidized to produce significant amounts of energy. For example, the complete oxidation of 1 mole of glucose produces between 27 and 31 moles of ATP, depending on specific transporters used and P/O ratios [that is, the number of ATP formed from ADP/Pi per molecule of oxygen; typically 2.5, (Brand 1994)]. By contrast, one mole of palmitate yields approximately 104 moles of ATP. In the case of the oocyte, the amount of lipid that would need to be consumed would be extremely small, and the differences/depletion arising from metabolic usage may be below the limits of detection using imaging methods such as those described by Genicot et al. (2005), Leroy et al. (2005), and Aardema et al. (2008).

Embryos

Waterman and Wall (1988) reported that rabbit zygotes were able to take up fatty acids from the culture environment. Incubation with [^{14}C] palmitic acid and subsequent measurement of $^{14}CO_2$ production showed that rabbit embryos can oxidize palmitic acid even at the single-cell stage, with subsequent increases, particularly from the four-cell stage onwards (Khandoker and Tsujii 1998). In that study, no other energy substrates were present in the culture medium, indicating that the fatty acid supplied was unquestionably used as a source of energy. Similar to the rabbit embryo, Hillman and Flynn (1980) found in mouse embryos that the rate of lipid oxidation was relatively constant from the unfertilized egg to the eight-cell stage (80 fmol CO_2 produced per embryo per hour), followed by a fourfold increase

between the eight-cell and blastocyst stages (up to 400 fmol CO_2 produced per embryo per hour). In tammar wallaby blastocysts, palmitate oxidation also increased from day 0 (0.3 pmol per embryo per hour) after reactivation because of removal of pouch young, to 3.8 pmol per embryo per hour by day 4 after re-activation, and increased even further by day 10 (Spindler et al. 1999).

In addition, bovine embryos cultured in the presence of acetoacetate and/or beta-hydroxybutyrate were able to reach the blastocyst stage (Gomez et al. 2001, 2002), indicating that bovine embryos can use ketone bodies as a primary energy source. This evidence that embryos can utilize lipid-breakdown products indirectly supports the suggestion that pre-implantation embryos can use intracellular lipids as an energy source.

Inhibition of triglyceride oxidation

Oocytes

In both cow and pig oocytes, inhibition of triglyceride metabolism using methyl palmoxirate during maturation *in vitro* leads to a fall in viability, reflected in reduced ability to generate a blastocyst post-fertilization (Sturmey and Leese 2003; Ferguson and Leese 2006). Methyl palmoxirate is a site-directed inactivator of Carnitine Palmitoyl Transferase 1 (Kiorpes et al. 1984) and thus blocks the entry of fatty acids into the mitochondria; the rate limiting step in β-oxidation.

Embryos

Further evidence that mammalian oocytes and embryos are capable of oxidizing fatty acids comes from studies in which mouse embryos were cultured in the presence of methyl palmoxirate (Hewitson et al. 1996). Embryo development was reduced in its presence, along with lower cell proliferation rates. In addition, on a per cell basis, glucose uptake remained constant, but a decrease in lactate production led the authors to suggest that the embryo was compensating for the inhibition of fatty acid oxidation, and that glucose was being completely oxidized to CO_2, thus maximizing ATP production and reducing lactate production. By contrast, porcine embryo development is not reduced in the presence of methyl palmoxirate in culture *in vitro*. However, in the pig, glucose metabolism is significantly increased when embryos are cultured in the presence of methyl palmoxirate (Sturmey and Leese 2008). This suggests that when denied the opportunity to metabolize lipid, the pig embryo up-regulates alternative energy generating pathways. A similar finding was observed for cattle embryos (Ferguson and Leese 2006), although the experimental details were different. In this case, when cow embryos were cultured in the presence of methyl palmoxirate in the absence of exogenous substrates, development to the blastocyst was inhibited.

Culture in the absence of exogenous substrates

Oocytes

The ability of oocytes to develop in the absence of exogenous energy substrates also supports the proposition that intracellular energy stores are utilized (Ferguson and Leese 2006). The mouse oocyte, which has only a very low triglyceride complement (see Table 3) arrests within 15 h of culture in medium lacking exogenous substrates (Downs and Hudson 2000). The cow oocyte is able to complete maturation successfully *in vitro*, as assessed by the ability to generate blastocysts post-fertilization, in the complete absence of exogenous sources of energy (Ferguson 1999). However, when triglyceride metabolism is inhibited and the culture medium lacks an energy source, the oocytes arrest (Ferguson and Leese 2006); this is the most compelling evidence for the idea that intracellular lipid plays a role in oocyte maturation *in vitro*.

Embryos

Kane (1987) showed that rabbit zygotes could complete three cleavage divisions in culture in the complete absence of nutrients added to the culture medium (i.e. with PVA replacing BSA, as the latter can be taken up by early embryos and broken down to amino acids) providing strong evidence for a role for endogenous substrates in this species. Ferguson and Leese (1999) reported that 45% of *in vitro*-produced cattle zygotes cleaved at least once in the complete absence of exogenous nutrients. To put this into context, when BSA was used as the macromolecular constituent instead of PVA, 65% underwent first cleavage and 45% developed to morulae. Furthermore, when cultured in the absence of exogenous energy sources, oxygen consumption by cattle five- to eight-cell embryos was unchanged (Ferguson and Leese 2006) indicating continued substrate oxidation; implicitly from internal stores. Inhibition of lipid metabolism reduced developmental competence and oxygen consumption. By contrast, Manser and Leese (cited in Ferguson and Leese 2006) reported that mouse zygotes deprived of exogenous nutrients degenerated within 10 h, consistent with the data in Table 2, indicating a very low fat content in the early mouse embryo.

Cytoplasmic organization

Oocytes

The final maturation of the bovine oocyte occurs in the ovulatory follicle following stimulation by the LH surge. After the LH peak, mitochondria tend to arrange themselves approximately at the lipid droplets, and by 15 h post-LH peak, these conglomerates have attained an even distribution throughout the cytoplasm (Hyttel et al. 1997). Bavister and Squirrell (2000) reported active translocation of mitochondria during oocyte maturation. Our own data are consistent with this general pattern as we found that mitochondria were homogeneously distributed in the immature oocyte, with the exception of its very inner cortex of the immature oocyte whereas they tended to be distributed more peripherally in the *in vitro*-matured oocyte (Sturmey et al. 2006). This pattern of mitochondrial relocation during oocyte maturation has been reported by others; in pig (Sun et al. 2001; Brevini et al. 2007), cattle (de Paz et al.

2001) and mouse (Van Blerkom et al. 2002) oocytes. Yet, Stojkovic et al. (2001) reported a very different pattern of mitochondrial distribution, particularly after *in vitro* maturation perhaps reflecting differences in oocyte culture media.

The distribution of lipid droplets within the cytoplasm of porcine oocytes follows a similar pattern; a homogeneous distribution throughout the cytoplasm with the exception of the very inner cortex of the immature oocyte. This shifts slightly during maturation *in vitro*, when a more pronounced peripheral distribution is apparent (Sturmey et al. 2006). Earlier studies indicated similar patterns for hamster (Guraya 1969, 1975) and mouse oocytes (Ozdzenski and Czoowska 1980; Trávník 1981). By contrast, the lipid droplets in cat and dog oocytes (Guraya 1965; Szabo 1967) tend to be located in the centre of the cytoplasm.

One possible explanation for the distribution of lipid droplets and mitochondria relates to intracellular oxygen availability. In order to generate energy from the oxidation of lipid (or exogenous substrates) oxygen must obviously be available to the mitochondria to act as a terminal electron acceptor. There are conflicting reports on the extent to which oxygen is available at the centre of the oocyte (Byatt-Smith et al. 1991; Clark et al. 2006) but it does seem plausible that lipid mitochondrial metabolic units locate to the periphery of the oocyte where oxygen is most readily available. For a more detailed description of bovine oocyte growth and maturation, refer to the review of Hyttel et al. (1997).

Fleming and Saacke (1972) reported a close spatial association between endoplasmic reticulum and mitochondria in bovine oocytes, as well as their proximity to lipid droplets, thus effectively forming 'metabolic units' (Kruip et al. 1983). We confirmed these observations using advanced imaging methods, which showed that lipid droplets and mitochondria co-localize to within 100 Å; a distance approximately equivalent to the thickness of a cell membrane, in live porcine oocytes *in vitro* (Sturmey et al. 2006). Results from live cell imaging suggested that the mitochondrial: lipid associations were maintained and were not short-term transient events. It is appropriate metabolically that mitochondria and lipid droplets be closely associated. The free fatty acids utilized to generate ATP are cleaved from triacylglycerol molecules within the lipid droplet and transported across the mitochondrial membrane by Carnitine palmitoyl transferase-1.

As noted earlier, canine oocytes have a conspicuous abundance of lipid, even higher than pig or cat (Reynaud et al. 2006) and the process of maturation *in vivo* contrasts markedly with that of other species – e.g. the retention of cumulus cells is prolonged in the oviduct (Viaris De Lesegno et al. 2008). Furthermore, this group noted the presence of lipid droplets surrounded by smooth endoplasmic reticulum, which could reflect metabolism of lipid reserves or lipoprotein synthesis. Either way, the developmental peculiarities of the canine oocyte/embryo (e.g. blastocyst stage not being reached until 14 days after the LH surge in Labrador Retrievers; Abe et al. 2008) probably require distinctly different energy provision towards which lipids are key contributors.

Embryos

In contrast to the situation with the oocyte, there is a paucity of data on the organization of lipid droplet: mitochondrial metabolic units in the early embryo. While there are reports of mitochondrial abundance and patterns of distribution (Van Blerkom 2008), and the presence of lipid droplets has been investigated by many groups (Abe et al. 1999a,b, 2002a,b; Crosier et al. 2000, 2001; Rizos et al. 2002) the lipid-mitochondrial interrelationship and scale of lipid utilization remain largely un-investigated.

Delipidation

Further evidence for the presence of an apparently overabundant supply of lipid to naturally derived oocytes and embryos of at least some mammalian species is indicated in studies where delipidation has been found not to compromise subsequent development. Among these, Nagashima et al. (1994, 1995) reported no significant difference in development rates of intact and delipidated *in vivo*-fertilized porcine embryos. Interestingly, however, in a recent study of parthenogenetic embryos produced following *in vitro*-maturation and activation, Nagashima et al. (2007) reported that delipidation on day 2 reduced blastocyst formation rates and that timing of delipidation did exert a marginal influence on subsequent development in terms of cell proliferation but not blastocyst yields (late day 2 better than early day 2 in the case of the former). The authors did not indicate whether this reflected time-related differences in tolerance of the process or the fact that at one stage, lipid removal was too excessive. Yoneda et al. (2004) also reported that development was reduced in porcine embryos delipidated at an early stage and they speculated that the decreased cell numbers at the blastocyst stage might indicate that cytoplasmic lipids are important molecules for cell division or energy metabolism. They also cited Brown (2001) who has emphasized how little is so far known about lipid droplets and their adherents, notably perilipin proteins. These proteins may modulate the extent of lipid metabolism in the cytosol by influencing access to lipid in a manner sensitive to metabolic status (Brasaemle 2007).

While delipidation has been useful in improving cryopreservation – or, more specifically, survival post-cryopreservation – of embryos that are lipid-rich, the technical process compromises biosecurity by requiring zona pellucida puncture to aspirate the excess lipid previously displaced via centrifugation (Diez et al. 2001; Karja et al. 2006; Nagashima et al. 2007; Sanchez-Osorio et al. 2008). Such removal also denies the embryo any adherents caught up in the displaced lipid (Cran 1987; Brown 2001). Consequently, some authors have investigated cryosurvival following displacement but not removal of the excess lipid. For example, Esaki et al. (2004) found this to be an effective means of facilitating vitrification of porcine embryos and further noted that while embryos that had been delipidated non-invasively subsequently (post-vitrification) emulated controls in terms of cell numbers at the blastocyst stage, those that had been delipidated invasively could

not. Previously, Tominaga et al. (2000) reported effects of stage at polarization of lipid (i.e. centrifugation without removal) on cryo-survival of bovine embryos and noted that centrifugation of zygotes was more detrimental to subsequent development than centrifugation at the two-cell stage. Notably, yet, in the absence of cryopreservation, Diez et al. (2001) reported the birth of calves (47% success vs 45% for controls) following transfer of blastocysts derived from embryos delipidated (via centrifugation plus removal) at the one-cell stage. This outcome is consistent with the view that lipid provision is more than sufficient to support development of bovine embryos when stressful circumstances are avoided.

More evidence relevant to the present review comes from studies in which, with a view to biosecurity, stimulating eggs/embryos to utilize lipid and thereby oxidize excess fat and obviating the risk of physical damage might be an alternative to invasive delipidation. Thus, for example Men et al. (2006) found that 10 μM forskolin significantly increased triglyceride lipolysis in day 6 porcine blastocysts (as determined by glycerol release). Equally notable for our own purposes is the finding that untreated controls (i.e. day 6 blastocysts not exposed to forskolin) also released glycerol during incubation, the yield being approximately 2 μM after 3 h and approaching 6 μM after 6 h. In their discussion, the authors emphasized that the glycerol measured in their assay was of endogenous origin and the result of hydrolysis of endogenous lipids. Additionally, Barcelo-Fimbres et al. (2009) recently reported that provision of phenazine ethosulphate, which reduces lipid content in cattle embryos, has no effect on live-birth parameters in cattle. An alternative approach to stimulate lipid metabolism involves the provision of oxaloacetate in place of glucose in the embryo culture medium (Sturmey and Leese 2008). Whilst the oxaloacetate can break down into pyruvate, it cannot generate energy *per se*, although its presence is required to 'prime' the TCA cycle by ensuring a steady supply of oxaloacetate. When porcine embryos were cultured in the presence of oxaloacetate, the resulting blastocysts had a significantly reduced content of intracellular triglyceride (Sturmey and Leese 2008). Whether this effect arose from oxaloacetate priming the TCA cycle to facilitate β-oxidation or through some other mechanism related to the formation of pyruvate is unclear.

Whilst the approach of encouraging embryos to deplete their intracellular lipid metabolically in order to increase cryotolerance might be of interest for freezing embryos, the physiological effects of up-regulating metabolism are largely unknown. This is particularly relevant given our concept of the *Quiet Embryo*, whereby the most viable embryos are metabolically 'quiet' (for a detailed description, see Leese 2002; Baumann et al. 2007 and Leese et al. 2008). We therefore suggest that it is probably unwise to up-regulate metabolic activity artificially, as this phenotype is often related to sub-optimal viability. Yet, the recent report by Barcelo-Fimbres et al. (2009), which demonstrates that reduction of lipid ion cattle embryos has no negative impact on the viability of calves, illustrates that this area requires further research.

Concluding Remarks

Our review of a variety of studies, each using a different experimental approach, has provided strong evidence that oocytes and early embryos are able to utilize fatty acids as energy substrates. This conclusion applies to fatty acids present endogenously in the form of triglycerides, and to those added to the culture medium but a key developmental question is to what extent, both *in vivo* and *in vitro*, embryos can selectively avoid or benignly adapt to changes in fatty acid provision and abundance in their environment. As noted by Diez et al. (2001) *the effects of lipid modification on cellular physiology and metabolic regulation are very complex* and further research is needed.

Acknowledgements

RGS is funded by a Wellcome VIP Fellowship administered by the University of York. Original research at SAC, carried out by TGM, AR and colleagues and referred to in this review, was funded by the Scottish Government and the UK Department for Environment, Food and Rural Affairs.

Author contributions

All authors contributed equally to the preparation of the manuscript.

Conflicts of interest

The authors have declared no conflicts of interest.

References

Aardema H, Knijn HM, Oei CHY, Vos PLAM, Gadella BM, 2008: Lipid droplet dynamics during in vitro maturation of bovine oocytes. Reprod Domest Anim **43**(Suppl.5)**,** 76 (Abstract).

Abayasekara DRE, Wathes DC, 1999: Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. Prostaglandins Leukot Essent Fatty Acids **61,** 275–287.

Abe H, Otoi T, Tachikawa S, Yamashita S, Satoh T, Hoshi H, 1999a: Fine structure of bovine morulae and blastocysts *in vivo* and *in vitro*. Anat Embryol (Berl) **199,** 519–527.

Abe H, Yamashita S, Itoh T, Satoh T, Hoshi H, 1999b: Ultrastructure of bovine embryos developed from *in vitro*-matured and -fertilized oocytes: comparative morphological evaluation of embryos cultured either in serum-free medium or in serum-supplemented medium. Mol Reprod Dev **53,** 325–335.

Abe H, Yamashita S, Satoh T, Hoshi H, 2002a: Accumulation of cytoplasmic lipid droplets in bovine embryos and cryotolerance of embryos developed in different culture systems using serum-free or serum-containing media. Mol Reprod Dev **61,** 57–66.

Abe H, Matsuzaki S, Hoshi H, 2002b: Ultrastuctural differences in bovine morulae classified as high and low qualities by morphological evaluation. Theriogenology **57,** 1273–1283.

Abe Y, Suwa Y, Yanagimoto-Ueta Y, Suzuki H, 2008: Preimplantation development of embryos in Labrador Retrievers. J Reprod Dev **54,** 135–137.

Barcelo-Fimbres M, Brink Z, Seidel GE Jr, 2009: Effects of phenazine ethosulfate during culture of bovine embryos on pregnancy rate, prenatal and postnatal development. Theriogenology **71,** 355–368.

Baumann CG, Morris DG, Sreenan JM, Leese HJ, 2007: The quiet embryo hypothesis: molecular characteristics favoring viability. Mol Reprod Dev **74,** 1345–1353.

Bavister BD, Squirrell JM, 2000: Mitochondrial distribution and function in oocytes and early embryos. Hum Reprod **15**(Suppl. 2)**,** 189–198.

Brand MD, 1994: The stoichiometry of proton pumping and ATP sythesis in mitochondria. Biochemist **16,** 20–24.

Brasaemle DL, 2007: The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. J Lipid Res **48,** 2547–2559.

Brevini TAL, Cillo F, Antonini S, Gandolfi F, 2007: Cytoplasmic remodelling and the acquisition of developmental competence in pig oocytes. Anim Reprod Sci **98,** 23–38.

Brison DR, Leese HJ, 1994: The role of exogenous energy substrates in blastocoele fluid accumulation in the rat. Zygote **2,** 69–77.

Brown DA, 2001: Lipid droplets: proteins floating on a pool of fat. Curr Biol **11,** R446–R449.

Byatt-Smith JG, Leese HJ, Gosden RG, 1991: An investigation by mathematical modelling of whether mouse and human preimplantation embryos in static culture can satisfy their demands for oxygen by diffusion. Hum Reprod **6,** 52–57.

Cetica P, Pintos L, Dalvit G, Beconi M, 2002: Activity of key enzymes involved in glucose and triglyceride catabolism during bovine oocyte maturation in vitro. Reproduction **124,** 675–681.

Clark AR, Stokes YM, Lane M, Thompson JG, 2006: Mathematical modelling of oxygen consumption in bovine and murine cumulus-oocyte complexes. Reproduction **131,** 999–1006.

Cran DG, 1987: The distribution of organelles in mammalian oocytes following centrifugation prior to injection of foreign DNA. Gamete Res **18,** 67–76.

Crosier AE, Farin PW, Dykstra MJ, Alexander JE, Farin CE, 2000: Ultrastructural morphometry of bovine compact morulae produced in vivo or in vitro. Biol Reprod **62,** 1459–1465.

Crosier AE, Farin PW, Dykstra MJ, Alexander JE, Farin CE, 2001: Ultrastructural morphometry of bovine blastocysts produced in vivo or in vitro. Biol Reprod **64,** 1375–1385.

Diez C, Heyman Y, Le Bourhis D, Guyader-Joly C, Degrouard J, Renard JP, 2001: Delipidating in vitro-produced bovine zygotes: effect on further development and consequences for freezability. Theriogenology **55,** 923–936.

Donnay I, Leese HJ, 1999: Energy metabolism during the expansion of the bovine blastocyst. Mol Reprod Dev **53,** 171–178.

Downs SM, Hudson ED, 2000: Energy substrates and the completion of spontaneous meiotic maturation. Zygote **8,** 339–351.

Durrant BS, Pratt NC, Russ KD, Bolamba D, 1998: Isolation and characterization of canine advanced preantral and early antral follicles. Theriogenology **49,** 917–932.

Esaki R, Ueda H, Kurome M, Hirakawa K, Tomii R, Yoshioka H, Ushijima H, Kuwayama M, Nagashima H, 2004: Cryopreservation of porcine embryos derived from in vitro-matured oocytes. Biol Reprod **71,** 432–437.

Ferguson EM, 1999: Endogenous Energy Stores in the Bovine Oocyte and Early Embryo. D.Phil Thesis, University of York, UK.

Ferguson EM, Leese HJ, 1999: Triglyceride content of bovine oocytes and early embryos. J Reprod Fertil **116,** 373–378.

Ferguson EM, Leese HJ, 2006: A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. Mol Reprod Dev **73,** 1195–1201.

Fleming WN, Saacke RG, 1972: Fine structure of the bovine oocyte from the mature graafian follicle. J Reprod Fertil **29,** 203–213.

Genicot G, Leroy JL, van Soom A, Donnay I, 2005: The use of a fluorescent dye, Nile red, to evaluate the lipid content of single mammalian oocytes. Theriogenology **63,** 1181–1194.

Gomez E, Duque P, Díaz E, Díez C, 2001: Effects of acetoacetate on in vitro development of bovine embryos in medium containing citrate and myo-inositol. Reprod Domest Anim **36,** 189–194.

Gomez E, Duque P, Díaz E, Facal N, Antolín I, Hidalgo C, Díez C, 2002: Effects of acetoacetate and D-beta-hydroxybutyrate on bovine in vitro embryo development in serum-free medium. Theriogenology **57,** 1551–1562.

Guraya SS, 1965: A histochemical analysis of lipid yolk deposition in the oocytes of cat and dog. J Exp Zool **160,** 123–136.

Guraya S, 1969: Histochemical study of lipids in the developing ovarian oocyte of the golden hamster (*Mesocricetus aurautus*). Acta Anat **74,** 65–75.

Guraya S, 1975: Histochemical observations on the juxtanuclear complex of organelles in the hamster oocyte. Acta Anat **93,** 335–343.

Hess BW, Moss GE, Rule DC, 2008: A decade of developments in the area of fat supplementation research with beef cattle and sheep. J Anim Sci **86**(E Suppl.)**,** E188–E204.

Hewitson LC, Martin KL, Leese HJ, 1996: Effects of metabolic inhibitors on mouse preimplantation embryo development and the energy metabolism of isolated inner cell masses. Mol Reprod Dev **43,** 323–330.

Hillman N, Flynn TJ, 1980: The metabolism of exogenous fatty acids by pre-implantation mouse embryos developing in vitro. J Embryol Exp Morphol **56,** 157–168.

Homa ST, Racowsky C, McGaughey RW, 1986: Lipid analysis of immature pig oocytes. J Reprod Fertil **77,** 425–434.

Houghton FD, Thompson JG, Kennedy CJ, Leese HJ, 1996: Oxygen consumption and energy metabolism of the early mouse embryo. Mol Reprod Dev **44,** 476–485.

Houghton FD, Humpherson PG, Hawkhead JA, Hall CJ, Leese HJ, 2003: Na+, K+, ATPase activity in the human and bovine preimplantation embryo. Dev Biol **263,** 360–366.

Hyttel P, Fair T, Callesen H, Greve T, 1997: Oocyte growth, capacitation and final maturation in cattle. Theriogenology **47,** 23–32.

Kane MT, 1987: Minimal nutrient requirements for culture of one-cell rabbit embryos. Biol Reprod **37,** 775–778.

Karja NWK, Otoi T, Wongsrikeao P, Murakami M, Agung B, Fahrudin M, Nagai T, 2006: In vitro development and post-thaw survival of blastocysts derived from delipidated zygotes from domestic cats. Theriogenology **65,** 415–423.

Khandoker MAMY, Tsujii H, 1998: Metabolism of exogenous fatty acids by preimplantation rabbit embryos. Jpn J Fertil Steril **43,** 195–201.

Khandoker MAMY, Tsujii H, Karasawa D, 1997: Fatty acid compositions of oocytes, follicular, oviductal and uterine fluids of pig and cow. Asian-Australas J Anim Sci **10,** 523–527.

Kim JY, Kinoshita M, Ohnishi M, Fukui Y, 2001: Lipid and fatty acid analysis of fresh and frozen-thawed immature an in vitro matured bovine oocytes. Reproduction **122,** 131–138.

Kiorpes T, Hoerr D, Ho W, Weaner LE, Inman M, Tutwiler G, 1984: Identification of 2-tetradecylglycidyl coenzyme-A as the active form of methyl 2-tetradecylglycidate (methyl palmoxirate) and its characterization as an irreversible, active site-directed inhibitor of carnitine palmitoyltransferase-A in isolated rat-liver mitochondria. J Biol Chem **15,** 9750–9755.

Kruip TAM, Cran DG, van Beneden TH, Dieleman SJ, 1983: Structural changes in bovine oocytes during final maturation in vivo. Gamete Res **8,** 29–47.

Kubovicova E, Pivko J, Landa V, Supova A, Grafenau P, Riha L, Zibrin M, Stastny P, 2003: Micromanipulation of gametes. In: J Pivko et al. (eds), Regulation and Evaluation of Ovarian Function and Embryogenesis in Normal and Transgenic Animals In Vitro and In Vivo. Publisher is Research Institute of Animal Production, Nitra, Slovak Republic, Publications of RIAP Nitra No. 8; Chapter 5, pp. 93–113.

Kuran M, Onal AG, Robinson JJ, Mackie K, Speake BK, McEvoy TG, 1999: A dietary supplement of calcium soaps of fatty acids enhances luteal function in sheep. Anim Sci **69,** 385–393.

Leese HJ, 1991: Metabolism of the preimplantation mammalian embryo. Oxf Rev Reprod Biol **13,** 35–72.

Leese HJ, 1993: Energy metabolism in preimplantation development. In: Bavister BD (ed.), Preimplantation Embryo Development. Springer-Verlag, New York, pp. 73–82.

Leese HJ, 1995: Metabolic control during preimplantation mammalian development. Hum Reprod Update **1,** 63–72.

Leese HJ, 2002: Quiet please, do not disturb: a hypothesis of embryo metabolism and viability. Bioessays **24,** 845–849.

Leese HJ, 2003: What does an embryo need? Hum Fertil **6,** 180–185.

Leese HJ, Baumann CG, Brison DR, McEvoy TG, Sturmey RG, 2008: Metabolism of the viable mammalian embryo: quietness revisited. Mol Hum Reprod **14,** 667–672.

Leroy JL, Genicot G, Donnay I, Van Soom A, 2005: Evaluation of the lipid content in bovine oocytes and embryos with nile red: a practical approach. Reprod Domest Anim **40,** 76–78.

Leroy JL, Van Soom A, Opsomer G, Goovaerts IG, Bols PE, 2008: Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. Reprod Domest Anim **43,** 623–632.

Loewenstein JE, Cohen AI, 1964: Dry mass, lipid content and protein content of the intact and zona-free mouse ovum. J Embryol Exp Morphol **12,** 113–121.

Matorras R, Ruiz JI, Mendoza R, Ruiz N, Sanjurjo P, Rodriguez-Escudero FJ, 1998: Fatty acid composition of fertilization-failed human oocytes. Hum Reprod **13,** 2227–2230.

McEvoy TG, Speake BK, 2001: Nutrition and the Egg: Safe-Guarding Reproduction in Birds and Reptiles. Proc 1st International Symposium on Assisted Reproductive Technology for the Conservation and Genetic Management of Wildlife. Henry Doorly Zoo, Omaha, Nebraska. pp. 197–200.

McEvoy TG, Coull GD, Speake BK, Staines ME, Broadbent PJ, 1997: Estimation of lipid and fatty acid composition of zona-intact pig oocytes. J Reprod Fertil Abstr Ser **20,** 10.

McEvoy TG, Coull GD, Broadbent PJ, Hutchinson JSM, Speake BK, 2000: Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. J Reprod Fertil **118,** 163–170.

Men H, Agca Y, Riley LK, Critser JK, 2006: Improved survival of vitrified porcine embryos after partial delipation through chemically stimulated lipolysis and inhibition of apoptosis. Theriogenology **66,** 2008–2016.

Nagashima H, Kashiwazaki N, Ashman RJ, Grupen CG, Seamark RF, Nottle MB, 1994: Removal of cytoplasmic lipid enhances the tolerance of porcine embryos to chilling. Biol Reprod **51,** 618–622.

Nagashima H, Kashiwazaki N, Ashman RJ, Grupen CG, Nottle MB, 1995: Cryopreservation of porcine embryos. Nature **374,** 416.

Nagashima H, Hiruma K, Saito H, Tomii R, Ueno S, Nakayama N, Matsurani H, Kurome M, 2007: Production of live piglets following cryopreservation of embryos derived from in vitro-matured oocytes. Biol Reprod **76,** 900–905.

Ozdzenski W, Czoowska R, 1980: Lipid inclusions in oocytes and preimplantational embryos of different strains of mice. Folia Morphol (Warsz) **39,** 497–502.

de Paz P, Sanchez AJ, De la Fuente J, Chamorro CA, Alvarez M, Anel E, Anel L, 2001: Ultrastructural and cytochemical comparisons between cow and calf oocytes. Theriogenology **55,** 1107–1116.

Reis A, Rooke JA, McCallum GJ, Staines ME, Lomax MA, McEvoy TG, 2003: Consequences of exposure to serum, with or without vitamin E supplementation, in terms of the fatty acid content and viability of bovine blastocysts produced in vitro. Reprod Fertil Dev **15,** 275–284.

Reis A, McCallum GJ, McEvoy TG, 2005: Accumulation and distribution of neutral lipid droplets is non-uniform in ovine blastocysts produced in vitro in either the presence or absence of serum. Reprod Fertil Dev **17,** 815–823.

Reynaud K, Fontbonne A, Marseloo N, Viaris De Lesegno C, Saint-Dizier M, Chastant-Maillard S, 2006: In vivo canine oocyte maturation, fertilization and early embryogenesis: a review. Theriogenology **66,** 1685–1693.

Rizos D, Fair T, Papadopoulos S, Boland MP, Lonergan P, 2002: Developmental, qualitative and ultrastructural differences between ovine and bovine embryos produced *in vivo* or *in vitro*. Mol Reprod Dev **62,** 320–327.

Sanchez-Osorio J, Cuello C, Gil MA, Alminana C, Parrilla I, Caballero I, Garcia EM, Vazquez JM, Roca J, Martinez EA, 2008: Factors affecting the success rate of porcine embryo vitrification by the Open Pulled Straw method. Anim Reprod Sci **108,** 334–344.

Santos JE, Bilby TR, Thatcher WW, Staples CR, Silvestre FT, 2008: Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod Domest Anim **43**(Suppl. 2)**,** 23–30.

Spindler RE, Renfree MB, Shaw G, Gardner DK, 1999: Reactivating tammar wallaby blastocysts oxidise fatty acids and amino acids. J Reprod Fertil **115,** 79–86.

Stojkovic M, Machado SA, Stojkovic P, Zakhartchenko V, Hutzler P, Gonçalves PB, Wolf E, 2001: Mitochondrial distribution and adenosine triphosphate content of bovine oocytes before and after in vitro maturation: correlation with morphological criteria and developmental capacity after in vitro fertilization and culture. Biol Reprod **64,** 904–909.

Sturmey RG, Leese HJ, 2003: Energy metabolism in pig oocytes and early embryos. Reproduction **126,** 197–204.

Sturmey RG, Leese HJ, 2008: Role of glucose and fatty acid metabolism in porcine early embryo development. Reprod Fertil Dev **20,** 149.

Sturmey RG, O'Toole PJ, Leese HJ, 2006: Fluorescence resonance energy transfer analysis of mitochondrial: lipid association in the porcine oocyte. Reproduction **132,** 829–837.

Sun QY, Wu GM, Lai L, Park KW, Cabot R, Cheong HT, Day BN, Prather RS, Schatten H, 2001: Translocation of active mitochondria during pig oocyte maturation, fertilization and early embryo development in vitro. Reproduction **122,** 155–163.

Szabo PL, 1967: Ultrastructure of the developing dog oocyte. Anat Rec **157,** 330.

Thompson JG, Gardner DK, Pugh A, McMillan WH, Tervit HR, 1995: Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. Biol Reprod **53,** 1385–1391.

Thompson JG, Partridge RJ, Houghton FD, Cox CI, Leese HJ, 1996: Oxygen uptake and carbohydrate metabolism by

in vitro derived bovine embryos. J Reprod Fertil **106,** 299–306.

Tominaga K, Shimizu M, Ooyama S, Izaike Y, 2000: Effect of lipid polarization by centrifugation at different developmental stages on post-thaw survival of bovine in vitro produced 16-cell embryos. Theriogenology **53,** 1669–1680.

Trávník P, 1981: Incidence and localization of lipids in the mouse oocyte and cleaving ovum. Folia Morphol (Praha) **29,** 292–296.

Van Blerkom J, 2008: Mitochondria in early mammalian development. Semin Cell Dev Biol [Epub ahead of print] doi:10.1016/j.semcdb.2008.12.005.

Van Blerkom J, Davis P, Mathwig V, Alexander S, 2002: Domains of high-polarized and low-polarized mitochondria may occur in mouse and human oocytes and early embryos. Hum Reprod **17,** 393–406.

Viaris De Lesegno C, Reynaud K, Pechoux C, Thoumire S, Chastant-Maillard S, 2008: Ultrastructure of canine oocytes during in vivo maturation. Mol Reprod Dev **75,** 115–125.

Waterman RA, Wall RJ, 1988: Lipid interactions with in vitro development of mammalian zygotes. Gamete Res **21,** 243–254.

Yoneda A, Suzuki K, Mori T, Ueda J, Watanabe T, 2004: Effects of delipidation and oxygen concentration on in vitro development of porcine embryos. J Reprod Dev **50,** 287–295.

Zeron Y, Sklan D, Arav A, 2002: Effect of polyunsaturated fatty acid supplementation on biophysical parameters and chilling sensitivity of ewe oocytes. Mol Reprod Dev **61,** 271–278.

Submitted: 31 January 2009

Author's address (for correspondence): RG Sturmey, Department of Biology (Area 3), University of York, York, YO10 5YW, UK. E-mail: r.g.sturmey@gmail.com