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Oleic acid in the modulation of oocyte and preimplantation embryo development

Shabnam Fayezi[2], *Jo L.M.R. Leroy*[3], *Marefat Ghaffari Novin*[2] *and Masoud Darabi*
Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium; and Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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Summary

Potential reproductive effects are considered as the major aspect of biomolecules functionality in an organism. The recent identification of differential patterns of fatty acids across ovarian follicles and their association with levels of sexual maturity highlights the importance of these biomolecules. It is well known that fatty acids are highly diverse in terms of their functional properties. Oleic acid is chemically classified as an unsaturated omega-9 fatty acid. Besides serving as an important energy source, oleic acid is involved in metabolic and structural roles. Free and esterified oleic acids are compartmentalized into discrete extracellular fluids, cell organelles and found within the cytosol. This review summarizes the current knowledge on the contribution of oleic acid in regulating female fertility, particularly its involvement in female germ cell growth and development. Oleic acid has been identified as a blastomeric and post-cryopreservation survival biomarker in bovine. Several related studies have shown the critical role of oleic acid in counteracting the detrimental effects of saturated fatty acids and in paracrine support of oocyte development. Although available data are not ideally detailed, most data suggest that oleic acid can contribute to normal oocyte and preimplantation embryo development via mechanisms involving metabolic partitioning of fatty acids, change in the membrane structural organization, attenuation of oxidative stress and regulation of intracellular signalling. Thus, oleic acid may play a significant role in oocyte and early embryo development, suggesting that future studies should explore in more detail its potential effects on the physiopathology of female reproduction.

Keywords: Desaturation, Embryo, Fatty acids, Fertilization, Ovary

Introduction

It is well known that fatty acids are highly diverse in terms of their metabolic and functional properties. Oleic acid (OlAc) is chemically classified as a long-chain unsaturated omega-9 fatty acid (Fig. 1). Over the last decade large numbers of studies have suggested that the physiological role of OlAc extends beyond that of an energy source and includes the regulation of cell metabolism, inflammation, tissue development and longevity (Lee & Park, 2014; Han *et al*., 2017). A single *cis* double bond at the position Δ9 gives a high capability to OlAc to solubilize lipids (Robinson & Cistola, 2014). Indeed, OlAc is a major determinant of plasma membrane fluidity (Funari *et al*., 2003), which is obviously associated with important functions including cell–cell interaction, membrane transport and signalling events. This fatty acid is capable of donating a hydrogen cation to an acceptor, and opposes oxidation or inhibits reactions brought about by dioxygen or peroxides (Choe & Min, 2009). Moreover, OlAc can directly be attached to proteins, which is known as an important step in growth and differentiation of many cell types (Rios-Esteves & Resh, 2013).

The main lipid classes of bovine oocytes are triglycerides, phospholipids, cholesteryl esters and free fatty acids (FFAs) which accounted, respectively,

[1]All correspondence to: Masoud Darabi. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz 5165665811, Iran. Tel: +98 41 33363234. Fax +98 41 33363231. E-mail: darabim@tbzmed.ac.ir
[2]Department of Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3]Gamete Research Center, Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium.

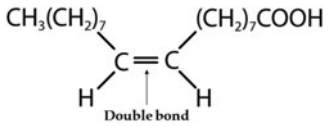

Figure 1 Oleic acid molecule. The organic structure of oleic acid has 18 carbons and a single cis configuration double bond on the ninth carbon (n-9 or ω-9) from the carboxyl (acid) end. This double bond is located at the centre of the molecule and causes a bend in the carbon chain which alters the physical properties and promotes fluidity. Derived from the Protein Data Bank ID: OLA. C, carbon; H, hydrogen; O, oxygen.

for 57.3, 16.2, 15.5 and 10.9% of the total lipid extract (Kim *et al*., 2001). Free and esterified OlAc are metabolically regulated and compartmentalized into discrete extracellular fluids, cell organelles and within the cytosol. Oleic acid is a major monounsaturated fatty acid in the lipid extracts of bovine, sheep, porcine (Homa *et al*., 1986; Prates *et al*., 2013; Dunning *et al*., 2014) and human oocytes (Matorras *et al*., 1998), with relative proportions between 10 and 25% of total fatty acids. Moreover, the phospholipid fractions from oocytes of cattle and other ruminants contained even more OlAc ranging from 20 to 26% (Fig. 2).

Potential reproductive effects are considered as the major aspect of biomolecules functionality in an organism (Ströhle & Döring, 2010). The recent identification of OlAc as a blastomeric and post-cryopreservation survival biomarker in bovine may indicate the positive effects of this fatty acid on embryo development (González-Serrano *et al*., 2013; Ferreira *et al*., 2014). Other recent studies have shown the critical role of OlAc in paracrine supporting of oocyte development and in counteracting the detrimental effect of saturated fatty acids on oocyte development. The association between fertilization and fatty acids in general has been reviewed previously (McKeegan & Sturmey, 2011; Dunning *et al*., 2014). However, to date, there has been no dedicated review of the literature dealing specifically with potential role of OlAc in reproduction.

This review will summarize current knowledge on the role of OlAc in the modulation of oocyte and preimplantation embryo development. We will focus primarily on changes in female sex hormones production, follicular development and early embryo development that are relevant to future clinical studies.

Oleic acid in endocrine-related reproduction function

Fatty acids may act more generally and indirectly via actions in the central nervous system to alter peripheral organ function. For instance, long-term control of food intake is affected by circulating OlAc in ruminants (Ingvartsen & Andersen, 2000).

Pituitary control

While dietary OlAc and linoleic acid (LnAc) caused no change in basal luteinizing hormone (LH), these long-chain fatty acids reduced gonadotropin-releasing hormone-induced LH release in dairy cows (Salehi *et al*., 2015). Despite the fact that the physiological significance of these effects on cattle ovulation remains to be investigated, these results suggest that concentration of circulating OlAc is important in the neuroendocrine control of reproduction, which appears to be independent of hypothalamic function and directly modulates gonadotropin release from the pituitary.

Steroidogenesis

The follicle cells and the corpus luteum in the ovary secrete the female sex hormones estrogen and progesterone, respectively, which regulate ovulation and control the reproductive cycle. These functions appear to be related to cellular fatty acid status. Notably, the amounts of unsaturated fatty acids including OlAc were significantly greater in minor plasma membrane phospholipids of the corpus luteum in pregnant ewes than in regressing corpus luteum of non-pregnant ewes at day 13 of the estrous cycle (Zelinski *et al*., 1988). This selective increase in the OlAc content of membrane phospholipids reflects dynamic changes in OlAc partitioning during the reproductive cycle. In addition to dietary intake, OlAc is supplied by a fine regulated *de novo* synthesis mechanism (Fig. 3). Endogenous synthesis of OlAc is primarily regulated by stearoyl-coenzyme A desaturase (SCD) in multiple cell types, including follicular cells (Feuerstein *et al*., 2007; Aardema *et al*., 2017; Fayezi *et al*., 2017). A recent study showed that prepubertal heifers versus cows had higher levels of *SCD* gene expression in granulosa cells and OlAc in follicular fluid (Warzych *et al*., 2017). Moreover, SCD activity was profoundly increased by follicle-stimulating hormone (FSH), which initiates and supports follicular development and regulates steroidogenesis in females (Moreau *et al*., 2006).

Free OlAc supplementation at an amount of 200 μM to the maturation medium of bovine cumulus–oocyte complexes (COC) significantly increased estradiol production, although no effect was found on progesterone (Maya-Soriano *et al*., 2013). Vanholder *et al.* (2005) also reported that a 2.5-fold higher free OlAc (500 μM), such as stearic acid and palmitic acid, stimulated estradiol secretion of bovine granulosa cells, while simultaneously inhibiting cell proliferation. A relatively lower concentration of OlAc (300 μM) also inhibited human granulosa cell proliferation (Mu *et al*., 2001). It is of note

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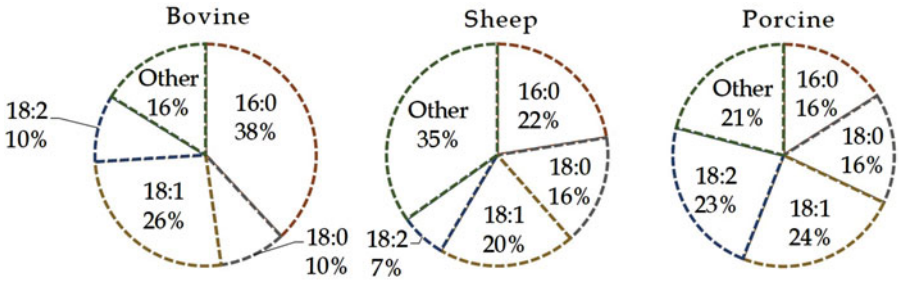

Figure 2 Fatty acid composition in phospholipids from bovine, sheep and porcine oocytes. Data of bovine are the average from Zeron *et al.* (2001) and McEvoy *et al.* (2000) studies. Other parts are based on data of Homa *et al.* (1986). 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid.

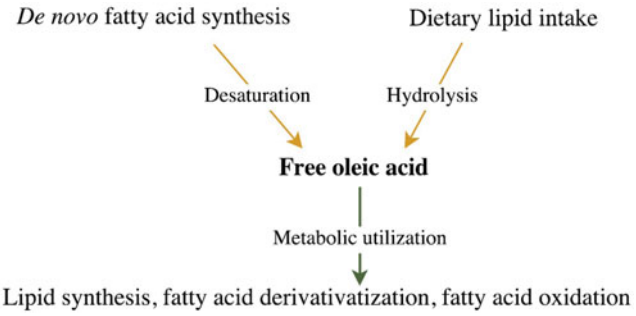

Figure 3 Diagram illustrating the metabolic pathways for oleic acid. Free oleic acid is derived from dietary lipid intake or endogenous *de novo* production. The latter is dependent to desaturation reaction, which is a rate-limiting step in the oleic acid synthesis. Oleic acid can be incorporated into cellular lipids, metabolized into bioactive derivatives or catabolized to release energy.

that all of these OlAc concentrations are 3-fold to 7.8-fold higher than the follicular fluid value of ~64 μM (Jungheim *et al.*, 2011) and are also in 1.5-fold to 2.3-fold excess of the free OlAc in plasma. In a later study, these fatty acids individually did not reproduce such effects on bovine theca cells (Vanholder *et al.*, 2006). Such cell type-specific differences may be attributed to an *in vivo* adaptation process that modulates cellular sensitivity to FFAs. This hypothesis was confirmed by a more recent study, which reported decreases in estradiol and progesterone along with the gonadotropin hormone receptors following long-term (8 day) OlAc incubation in a serum-free, immature bovine granulosa cell culture model (Yenuganti *et al.*, 2016). It has been suggested that OlAc-induced changes are due to a reduced response to gonadotropic hormones (Yenuganti *et al.*, 2016). However, because OlAc increased estradiol secretion in short-term cultures of mature bovine granulosa cells (Vanholder *et al.*, 2006), it rather appears to be due to cell-specific adaptive responses. Consistently, theca cells are more directly supplied with blood, which is rich in FFAs (Vanholder *et al.*, 2006).

Altogether, these data underscore the dynamic changes occurring in the OlAc content of follicles during the reproductive cycle, which can modulate steroidogenesis.

Oleic acid in formation of competent oocytes

A unique diversity of lipid molecular compounds has been documented for oocytes. For instance, a more complex lipid composition was recorded during the *in vitro* maturation process of porcine oocytes (Pirro *et al.*, 2014). The knowledge accumulated regarding the effect of OlAc on follicular development and measures of oocyte developmental competence, including maturation and fertilization rate, is discussed further below. Table 1 summarizes data related to cattle, as the most studied species.

Follicular fluid

The free fatty acid fraction in bovine (Leroy *et al.*, 2005) and human (Valckx *et al.*, 2014a) sera and ovarian follicular fluid is rich in palmitic acid, stearic acid and OlAc. The concentration of individual FFA in human serum was reflected in follicular fluid, but overall the relationship was weak (Jungheim *et al.*, 2011). Notably, human follicular fluid showed an increase of 3.3% in stearic acid and a reduction of −7.0% in OlAc relative to serum (Jungheim *et al.*, 2011). These data suggest that a selective transport mode determines OlAc concentration in follicular fluid. This selective transport may be accompanied by other metabolites, as evidenced in the placental transport of OlAc coupled with amino acids in primary human trophoblasts (Lager *et al.*, 2013). Hudson *et al.* (2014) have shown that in a low amino acid environment the physiological concentration of OlAc, but not palmitic acid or stearic acid, caused a reduction in fluorescence dye transfer from bovine cumulus cells to the oocyte. The expression of connexin proteins, as the main

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Table 1 Effect of oleic acid supplementation on cumulus-granulosa cell function, oocyte characteristics and embryo development in cattle

Model	Main findings	Potential mechanism	Reference
Dietary intake (IVF)			
800 g/day, OlAc-enriched high-fat versus low-fat diet	↑ IVM and ED *in vitro*	↑ Cell ingredients; ↓ Adverse effects of insulin	(Fouladi-Nashta *et al.*, 2007)
Dietary intake			
1.35–1.50%, Sunflower oil	↑ Retrieved oocytes, CR and ED	↑ Essential nutrients	(Bilby *et al.*, 2006)
COC culture			
200 µM, OlAc combined with SFA versus stearic acid	↓ IVM and CR; ↑ Reduced glutathione, LD in blastocysts and cryotolerance	↓ Oxidative stress	(Van Hoeck *et al.*, 2013; Van Hoeck *et al.*, 2015)
500 µM	↑ Steroidogenesis	↑ Membrane stability, free cholesterol	(Vanholder *et al.*, 2005)
80–200 µM	↔ Apoptosis in GC, FR, CR and blastocyst yield	–	(Leroy *et al.*, 2005; Nandi *et al.*, 2014)
200 µM	↔ Developmental competence and total blastocyst cell number	–	(Van Hoeck *et al.*, 2011)
200 µM	↑ Steroidogenesis; ↔ Progesterone and cytoplasmic maturation; ↓ IVM	–	(Maya-Soriano *et al.*, 2013)
40 and 160 µM	↔ Developmental competence and proliferation of GC	–	(Nandi *et al.*, 2014)
100 mg/ml, OlAc-enriched phospholipids	↑ CR and blastocyst formation	↓ Transition temperature	(Zeron *et al.*, 2002)
500 µM, OlAc	↑ FR, post-fertilization development	↑ Energy storage	(Leroy *et al.*, 2003; Aardema *et al.*, 2011)
1000 µM, OlAc	↓ Proliferation of GC, FR, CR and ED	↑ Membrane formation	(Jorritsma *et al.*, 2004)
GV culture			
100 µM, OlAc versus SFA and linoleic acid	↑ Breakdown rate and MII	↓ Adenylate cyclase	(Homa & Brown, 1992)
Fertilized oocytes culture			
100–1000 µM	↑ CR and ED	↓ Oxidative stress	(Karaşahiṅ & Arikan, 2015)

*COC, cumulus–oocyte complex; CR, cleavage rate; d, days; ED, embryo development; FR, fertilization rate; GC, cumulus–granulosa cells; GV, germinal vesicles; IVM, *in vitro* maturation rate; LD, lipid droplets; MII, metaphase II oocytes; OlAc, oleic acid; SFA, saturated fatty acids. ↓, significant decrease; ↑, significant increase; ↔ no significant change.

structural components of gap junctions, has been considered to be modulated by nutrients (Hudson *et al.*, 2014). These data support the hypothesis that OlAc in the follicle microenvironment can contribute to the regulation of metabolite transport through the control of gap junctional communication between cumulus cells and the developing oocyte.

Fatty acid composition and glucose content in follicular fluid appear to be generally involved in follicular development (Warzych *et al.*, 2014). Notably, OlAc in follicular fluid was related to a higher bovine oocyte quality, in terms of COC morphology and follicular diameter (Warzych *et al.*, 2014). However, the fatty acid composition of follicular fluid was not associated with bovine (Sinclair *et al.*, 2008) or human (O'Gorman *et al.*, 2013) oocyte cleavage following *in vitro* fertilization (IVF). In another study, the concentration of palmitic acid and stearic acid in human follicular fluid was associated with morphologically poor and good COCs, respectively (Sinclair *et al.*, 2008). In a clinical study, we have reported that the number of mature oocytes from women undergoing IVF cycle was inversely associated with total saturated fatty acid content of phospholipids in follicular fluid (Shaaker *et al.*, 2012). Associations between phospholipid OlAc and the number of retrieved mature oocytes showed a positive trend (Shaaker *et al.*, 2012). Moreover, a higher concentration of total saturated and a lower concentration of total unsaturated fatty acids were found in patients with cleavage failure (O'Gorman *et al.*, 2013). Intrafollicular OlAc concentration is significantly increased in phospholipid fraction while

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showing a reduction in the non-esterified fatty acids fraction as bovine follicles become dominant (Renaville *et al*., 2010). Follicular fluid of women with higher body mass index and lower IVF outcome showed increased phospholipid content (Fayezi *et al*., 2014) but reduced concentration of OlAc in the phospholipid fraction (Valckx *et al*., 2014a). These observations argued for a link between relative ratio of OlAc/other fatty acids in follicular fluid phospholipids and oocyte development.

Follicular growth

A recent study using high-resolution mass spectrometry technique demonstrated that the fatty acid patterns of porcine follicles were largely different from non-follicular ovarian tissue (Uzbekova *et al*., 2015). Moreover, porcine follicles were heterogeneous in their lipid composition, possibly reflecting changes in fatty acids over the natural course of ovarian folliculogenesis (Uzbekova *et al*., 2015). More recently, Warzych *et al.* (2017) have shown that sexual maturity in bovine alters the follicular environment with regard to the lipid droplet content within the oocyte and follicular fluid fatty acid composition. Overall, these data further support the idea that dynamic change in fatty acid content of follicular compartments contributes to regulating female gametogenesis. Several studies, which are discussed below, point to the specific effect of OlAc on the process of follicular growth.

Diet supplementation with long-chain fatty acids has been shown to increase the ovulation rate, the number of follicles and serum progesterone in ewes (El-Shahat & Abo-El Maaty, 2010). However, depending on the type of fatty acid used in the diet, the effect on oocyte development can be very different (Leroy *et al*., 2014). Consistent with this hypothesis, more oocytes were collected from dairy cows fed OlAc as compared with cows fed *trans* OlAc, LnAc or linolenic acid (Bilby *et al*., 2006).

Follicular growth and antrum formation in bovine are affected by elevated FFAs concentrations *in vitro* (Van Hoeck *et al*., 2014; Valckx *et al*., 2015). Moreover, experimental data support the idea that FFAs not only modulate final oocyte maturation, but also potentially interfere with follicular growth at the early stages of development (Leroy *et al*., 2015). The same manner as *in vivo*, however, depending on the type and ratio of fatty acids the final effect may be different (Leroy *et al*., 2015). The diameter of *in vitro*-cultured murine follicles was higher and apoptosis markers in luteinized granulosa cells were reduced with OlAc treatment (210 μM), compared with high stearic acid (Valckx *et al*., 2014b). In addition, when OlAc was used in combination with stearic acid and palmitic acid these differences were eliminated (Valckx *et al*., 2014b). Furthermore, in a rat model, the FSH/FSH receptor pathway in granulosa cells that triggers ovarian follicular growth from primordial stage involves the activation of *de novo* OlAc synthesis as a downstream consequence (Moreau *et al*., 2006). Finally, a more complete understanding of the role of OlAc in follicular growth will require developing new strategies that focus on earlier stages of gametogenesis, including primordial germ cell specification and proliferation.

Oocyte quality and fertilization

Post-fertilization events such as the formation of zygote and embryogenesis are considered as markers for developmental capacity of oocytes. It has long been suggested that the polyunsaturated fatty acid (PUFA) supplements can beneficially affect fertility (Wathes *et al*., 2007). Although PUFA-enriched diet increased dominant follicle size in dairy cows and OlAc-enriched diet increased recovery rate of oocytes, no significant additional beneficial effects were seen between OlAc- and PUFA-enriched diets with respect to oocyte quality as characterized by subsequent embryo development (Bilby *et al*., 2006). Compared with a low-fat diet, a high-fat diet containing mainly palmitic acid and OlAc resulted in significantly improved developmental potential of oocytes in lactating dairy cows, as evidenced by increased rate of blastocyst production and quality during IVF, possibly through enhanced biosynthesis of cell ingredients and neutralizing the adverse effects of insulin (Fouladi-Nashta *et al*., 2007).

Although a 100 μM dose of albumin-bound OlAc did not show a statistically significant difference compared with the control albumin alone, the breakdown rate of bovine germinal vesicles and oocyte progression to MII were higher after incubation with OlAc as compared with palmitoleic acid, stearic acid and LnAc, possibly due to a specific activation of adenylate cyclase (Homa & Brown, 1992). Moreover, LnAc significantly inhibited *in vitro* bovine cumulus cell expansion and decreased the percentage of oocytes at MII stage in a dose-dependent manner (Marei *et al*., 2010). Although maturation rate was not affected, incubation with 500 μM albumin-bound OlAc during *in vitro* maturation significantly increased the percentage of bovine oocytes that were fertilized (Aardema *et al*., 2011). However, adding both saturated fatty acids palmitic acid and stearic acid reversed these effects. Furthermore, OlAc reversed the detrimental effects of palmitic acid and stearic acid (Aardema *et al*., 2011). Free stearic acid was much more potent than the combined OlAc, palmitic acid and stearic acid to increase the expression of genes related to apoptosis and oxidative stress, including Bcl-2-associated X protein (*BAX*), glutathione peroxidase 1 (*GPX1*) and superoxide dismutase 1 (*SOD1*), in bovine oocytes

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and luteinized granulosa cell cultures (Van Hoeck *et al*., 2013; Valckx *et al*., 2014b). However, the expression level of the proliferation-related gene growth arrest and DNA-damage-inducible (*GADD45B*) was increased after treatment with 210 μM OlAc (Van Hoeck *et al*., 2015). In addition, the glutathione content of bovine oocytes and the number of lipid droplets in the resulting morulae were lower following high stearic acid treatment compared with that of combined saturated fatty acids and OlAc (Van Hoeck *et al*., 2015). These observations were attributed to the compensating action of OlAc.

A few studies have shown no significant effect at lower doses of free OlAc alone compared with vehicle control. OlAc at concentrations equivalent to that of FFAs in postpartum cows follicular fluid (200 μM) showed no effect (Leroy *et al*., 2005) on *in vitro* maturation, fertilization and cleavage rate and blastocyst yield. Despite significant increasing effect on cumulus cell steroidogenesis, exposure to a 200 μM concentration of OlAc resulted in no beneficial effects on cytoplasmic maturation, and nuclear maturation of heifer oocytes (Maya-Soriano *et al*., 2013). In contrast to palmitic and stearic acid, OlAc at concentrations between 40 and 160 μM showed no detrimental effect on bovine oocyte maturation rate and subsequent fertilization rate and embryo yield as well as on granulosa cell growth (Nandi *et al*., 2014). These data further highlight the importance of the ultimate OlAc ratio of the cellular lipids, over the entire quantity, which needs to be taken into account in future studies.

Concentration and form of OlAc supplementation in the growth medium may also determine the effect on the developmental capacity of oocytes. Albumin-bound OlAc at a high concentration equivalent to plasma FFAs in postpartum cows (1 mM) reduced the proliferation of granulosa cells, *in vitro* fertilization and subsequent cleavage and embryo development (Jorritsma *et al*., 2004). However, the study did not distinguish between supplementation alone with albumin and the control condition. Thus, besides the high concentration, these adverse effects can be caused by addition of albumin alone (Leroy *et al*., 2005, 2008). Notably, incomplete extraction of fatty acids from albumin has been postulated as a possible explanation for variations in embryo formation rate with different concentrations of albumin (Cagnone & Sirard, 2014). In contrast, a previous study showed that a high dose of albumin-bound OlAc (1.25 mM) was the most effective treatment among other unsaturated fatty acids for promoting rat embryos development to blastocyst stage (Khandoker & Tsujii, 1999). Other studies have shown that a lower amount of OlAc (500 μM) exerts a promoting effect on oocyte developmental competence post-fertilization (Leroy *et al*., 2003; Aardema *et al*., 2011), possibly by increasing lipid storage in bovine oocyte (Aardema *et al*., 2011). In addition, OlAc prevents suppression of post-fertilization development induced by saturated fatty acids in bovine (Aardema *et al*., 2011; Van Hoeck *et al*., 2011). It has been hypothesized that OlAc may mitigate the negative effects induced by saturated fatty acids. As evidenced in bovine oocytes (Aardema *et al*., 2011) and in hamster ovary cells (Listenberger *et al*., 2001), co-exposure to OlAc resulted in the metabolic channelling of palmitic and stearic acid into the non-toxic lipid droplets.

Altogether, these results suggest that the ultimate effect of free OlAc on oocyte *in vitro* maturation (IVM) is determined by the relative ratio of cellular OlAc to other fatty acids. While no consensus has been reached regarding the effect of OlAc alone on IVM, it is evident that OlAc counteracts the detrimental consequences of exposure to saturated fatty acids during IVM.

The efficient metabolic incorporation of OlAc by bovine oocyte has been demonstrated by altered lipid droplets (Aardema *et al*., 2011), energy metabolism-related gene expression, and mitochondrial function and ultrastructure (Van Hoeck *et al*., 2013) following exposure to OlAc during IVM. The physical properties and thermal responses of the plasma membrane remarkably changed during the process of oocyte maturation. A study performed using Fourier transform infrared analysis detected a large difference in membrane lipid phase transition temperature among human germinal vesicles, mature (MII) oocytes and zygotes (Ghetler *et al*., 2005). The higher phase transition temperature in zygotes may reflect differences in the fatty acids within the membrane phospholipids. Indeed, a high ratio of OlAc to saturated fatty acids reduced the transition temperatures of lipids and increased membrane fluidity in animal oocytes (Arav *et al*., 2000; Zeron *et al*., 2001). Electrofusion of bovine COCs with liposomes prepared from egg phosphatidylcholine with a high content of OlAc decreased transition temperature and increased blastocyst formation *in vitro* (Zeron *et al*., 2002). Bovine GV-stage oocytes were much more susceptible to cryoinjury than mature oocytes and embryos (Arav *et al*., 1996). Interestingly, the fusion of large membrane fragments from mature oocytes with bovine GV oocytes membrane enhanced their chilling resistance (Arav *et al*., 1996). Notably, in contrast with palmitic acid and stearic acid, no adverse effect on the embryo cryotolerance was observed with OlAc treatment during *in vitro* maturation of bovine oocytes (Shehab-El-Deen *et al*., 2009). These observations were attributed to alteration in the membrane lipid composition. Because of the large capacity of OlAc to solubilize lipids (Robinson & Cistola, 2014), such potential changes in OlAc will profoundly affect cell membrane function.

The lower bovine fertility during summer than winter is attributed to disrupted development of

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oocyte due to heat stress (Rivera & Hansen, 2001). Notably, OlAc reaches 38% of total fatty acids and became the most abundant fatty acid in membrane phospholipids of bovine germinal vesicle oocytes during winter, an increase of 28% as compared with the summer season (Zeron *et al*., 2001). This compositional change generally modifies physical properties of membranes, such as melting temperature (Zeron *et al*., 2001). Such a process may be involved in increased oocyte quality in winter. Whether there is any change in the plasma membrane fatty acids during the oocyte maturation remains to be determined.

As noted earlier, the concentrations of fatty acids, including OlAc, in follicular fluid and oocyte quality potential were intimately correlated in bovine (Warzych *et al*., 2017). According to these results, during the prepubertal period *de novo* synthesis of fatty acids, as the main energy resources in ovarian follicles, is low and insufficient to fully support oocyte development (Warzych *et al*., 2017). A study on human cumulus–granulosa cells has demonstrated that SCD activity is required for cumulus cell lipid storage and steroidogenesis (Fayezi *et al*., 2017). In addition, oocyte maturation was negatively affected by SCD inhibition in cumulus–granulosa cells, possibly due to deficient lipid-mediated paracrine support. Subsequently, these effects were rescued by supplementation of the main product of SCD OlAc, confirming the idea that its *de novo* production in cumulus cells contributes to the acquisition of oocyte meiotic and developmental competence during folliculogenesis (Fayezi *et al*., 2017). It is interesting to note that *de novo* synthesis and paracrine release of OlAc by astrocytes induced neuronal differentiation (Tabernero *et al*., 2001). This function was mediated by the activation of protein kinase C (Tabernero *et al*., 2001), which is known to promote oocyte maturation and early embryo development (Mondadori *et al*., 2008; Tepekoy *et al*., 2014). In addition, Aardema *et al.* (2017) have shown that treatment of SCD inhibitor plus stearic acid during IVM led to a decrease in the SCD activity index in cumulus cells, as well as a reduction in the number of produced blastocysts. The authors attributed these findings to the protective effect of SCD against saturated fatty acids via conversion to unsaturated fatty acids. Taken together, a sufficient accessibility of metabolic processes to OlAc and its relative amount to the cell fatty acid content are particularly important in oocyte maturation.

Oleic acid in preimplantation embryo development

Embryo cleavage and blastocyst formation or hatching are the main stages of early embryogenesis. The ratio of various fatty acids is an important metabolic feature and may strongly influence reproductive outcomes. The relative percentage of OlAc markedly increased during the developmental stages of bovine (Menezo *et al*., 1982) and rabbit (Yahia Khandoker *et al*., 1998) embryos. Consistent with these data, OlAc and LnAc were more abundant in the late preimplantation stage human embryos (Haggarty *et al*., 2006). These observations can reflect a differential pattern of fatty acid uptake during embryo development which may be conserved among species. Indeed, at later developmental stages, a trend towards increasing uptake of OlAc (Wang & Tsujii, 1999; Haggarty *et al*., 2006) and LnAc (Haggarty *et al*., 2006) over palmitic acid was seen in human and mouse embryos. In addition, during preimplantation embryo development the channelling of exogenous OlAc was preferentially directed towards phospholipids and away from intermediate energy substrates such as triglycerides (Wang & Tsujii, 1999). According to these data, active uptake and metabolic channelling of OlAc towards phospholipids can contribute to early embryo development.

The data obtained from phosphosphingolipidome analysis showed increased concentration of phosphatidylcholine classes containing OlAc (3.8-fold), LnAc (4.5-fold) and linolenic acid (3-fold) for *in vivo* produced compared with *in vitro* produced bovine embryos (Sudano *et al*., 2012). Moreover, phosphatidylethanolamines, ceramides (Ferreira *et al*., 2014) and cholesteryl ester (González-Serrano *et al*., 2013) containing primarily OlAc have been identified as blastomeric biomarkers for bovine embryo quality using ionization mass spectrometry approach. Indeed, *in vivo* produced bovine blastocysts showed higher concentrations of OlAc in phospholipids and cholesteryl-ester fractions compared with their *in vitro* derived counterparts (González-Serrano *et al*., 2013). Accordingly, unsaturated fatty acid content may be an important factor in the post-cryopreservation survival of *in vivo* (Sudano *et al*., 2012) and *in vitro* (Pereira *et al*., 2007) produced bovine embryos. Moreover, a diet fed to cows enriched in LnAc compromised the embryo cryotolerance and decreased the hatching rate of *in vivo* produced embryos (Guardieiro *et al*., 2014). Therefore, it appears that among unsaturated fatty acids, a beneficial effect is produced by OlAc on embryo cryotolerance.

Hatching rate of bovine blastocyst in a culture supplemented with 0.1% serum lipid fraction tended to be lower compared with total serum-containing medium. This lipid fraction was extracted using ether, a nonpolar solvent capable of extracting major lipid classes. Compositional analysis showed that this fraction had a fatty acid concentration comparable with total serum. However, it contained significantly reduced concentrations of OlAc and increased amounts

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of palmitic acid and stearic acid compared with total serum (Cagnone & Sirard, 2014). Nevertheless, a large-scale gene expression analysis of bovine blastocysts failed to detect differences in transcriptome following these *in vitro* culture conditions (Cagnone & Sirard, 2014). These results suggest that posttranscriptional modifications may be responsible for the changes in the embryo development upon serum lipid, particularly OlAc, supplementation.

Based on previous evidence for the association between unsaturated fatty acid content and cryopreservation success of embryos (Shehab-El-Deen *et al*., 2009; Sudano *et al*., 2012), an *in vitro* study demonstrated that supplementation of bovine embryo culture medium with OlAc at a concentration of 1.0 mM increased the rates of embryonic cleavage, development and quality (Karaşahiṅ & Arikan, 2015).

Stinshoff *et al.* (2014) have observed that conjugated LnAc significantly suppressed the expression of *SCD*, a key enzyme for *de novo* OlAc synthesis, and resulted in reduced bovine embryo development *in vitro*, a phenotype very similar to when mouse embryos (Ben-David *et al*., 2013) were exposed to selective inhibitors of SCD. The requirement for LnAc, as another abundant unsaturated fatty acid, in embryo development is controversial. Only limited positive effects on *in vitro* embryonic development and quality were observed in bovine by supplementation of LnAc (Karaşahiṅ & Arikan, 2015). Remarkably, treatment of mouse pronuclear and 2-cell stages with albumin-bound PUFAs including LnAc resulted in a significantly lower percentage of cleaved embryos and blastocysts (Nonogaki *et al*., 1994). Consistent with these results, the treatment of bovine COCs with LnAc, even at a concentration not affecting oocyte maturation (50 mM), significantly decreased subsequent cleavage and blastocyst rates (Marei *et al*., 2010). These adverse effects were accompanied by a sharp increase in the arachidonic acid-dependent pro-inflammatory pathway (Marei *et al*., 2010). In agreement to these findings, a reduced arachidonic acid content in bovine oocytes was associated with the improved quality of derived embryo without an increase in oocyte maturation or embryo production rates (Lapa *et al*., 2011).

Overall, the current data from *in vitro* models suggested that OlAc supplementation may beneficially effect embryo development that is obviously distinct from other bioactive fatty acids like LnAc and arachidonic acid.

Potential mechanism of reproductive function of oleic acid in female

A general weakness in the most studies reviewed lay in their observational nature, which did not allow mechanistic insights into the reproductive role of fatty acids, in particular OlAc. Nevertheless, current knowledge suggests that potential mechanisms underlying association of OlAc with female reproduction may include altered metabolic channelling, plasma membrane fluidity, oxidative stress and intracellular signalling events (Fig. 4).

The division from one to four blastomeres requires a large increase in membrane surface area, as much as 1.74-fold (Pratt & George, 1989). Oleic acid is a major fatty acid in membrane phospholipids (Homa *et al*., 1986; Kim *et al*., 2001; Zeron *et al*., 2001) and is essential for organization and normal function of membrane (for review see Lopez *et al*., 2014). The functional significance of OlAc in membrane formation at the earliest stages of zygote development remains to be investigated.

Oleic acid activates proliferative and maturational signalling pathways in which protein kinase C participates (Tabernero *et al*., 2001). In light of the information available on the critical role of protein kinase C in bovine oocyte maturation and early embryo development (Mondadori *et al*., 2008), OlAc may be considered as an important regulator of reproduction acting at the level of a common signalling pathway. This aspect of reproduction control by OlAc will require further study, which will help to deal with conflicting viewpoints.

Advanced lipidomics analyses point to prostaglandins and cannabinoids as markers for female fertility (Vilella *et al*., 2013a; Agirregoitia *et al*., 2015). Metabolism of OlAc and omega-6 fatty acids – also known as n-6 PUFAs – shares common enzymatic pathway steps catalyzed by desaturases and prostaglandin-endoperoxide synthases (Cheng *et al*., 2015). Therefore, OlAc can modify PUFA metabolism and, subsequently, prostaglandin and cannabinoids synthetic pathways. Indeed, OlAc significantly increased the ratio of prostaglandin E2 to prostaglandin F2α produced by endometrial cells isolated from the late pregnant ewes (Cheng *et al*., 2015). Such change in prostaglandin ratio has been associated with embryo implantation in human (Vilella *et al*., 2013b) and fetal maturation in porcine (Cao *et al*., 2005; Ziecik *et al*., 2008), which may similarly contribute to ovarian folliculogenesis or granulosa cell differentiation.

Conceiving and sustaining a pregnancy to term are the most reliable markers for oocyte quality and embryo competence. This aspect has been particularly examined in the study of dietary PUFAs. Indeed, feeding PUFAs dynamically during the postpartum period increased the occurrence of pregnancy in cattle (Silvestre *et al*., 2011; Dirandeh *et al*., 2013). Regarding dietary OlAc, this point has not been studied.

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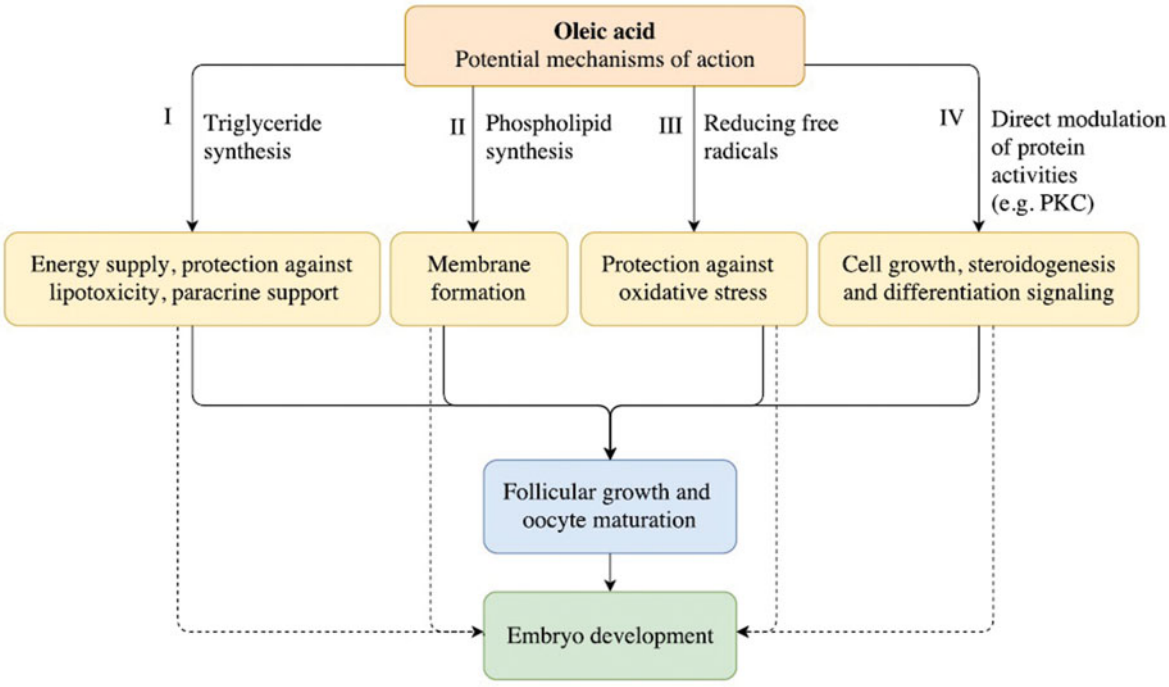

Figure 4 Potential mechanisms underlying the effects of oleic acid on ovarian function and embryo development. The additive effects on embryo development are shown in dot-dash lines. Oleic acid profoundly increases partitioning of fatty acids towards lipid droplet which in turn serves as energy supply and protects oocytes and granulosa cells against lipotoxicity, and enhances lipid-mediated paracrine support of oocytes (I). Oocyte plasma membrane fluidity and cell divisions at the early stages of embryo development can be modified by oleic acid-promoted phospholipid synthesis (II). Oleic acid can influence follicular metabolic activities, steroidogenesis and oocyte development either directly by acting as a regulator of cellular signalling via the modulation of oxidative stress (III) and the activation of protein kinase C (PKC) (IV).

Conclusions

Although available data are not ideally detailed, most studies suggest that dietary or *de novo* synthesis of OlAc contributes to the acquisition of developmental competence by oocytes and to early stages of embryo development in mammals. The beneficial effects of OlAc may involve one or more of the following possible mechanisms. Firstly, OlAc profoundly increased the partitioning of fatty acids towards lipid droplet which in turn not only protects oocytes and granulosa cells against lipotoxicity but also may promote the lipid-mediated paracrine support of oocytes by surrounding cells. Secondly, OlAc acts as a major determinant of plasma membrane structural organization in follicular cells. Thirdly, OlAc acts directly as a metabolic component or regulator of oxidative stress and cellular signalling. Therefore, this monounsaturated fatty acid may play a significant role in oocyte and early embryo development, suggesting that future studies should explore in more detail its potential effects on physiopathology of female reproduction.

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Authors' contributions

S.F. wrote the manuscript and made the figures. J.L., M.G.N. and M.D. contributed to the manuscript writing and editing. All authors read and approved the final manuscript.

Declaration of interest

The authors declare that they have no competing interests.

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