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Review

The effects of obesity and non-esterified fatty acids on preimplantation embryo development

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ABSTRACT

Obesity is an increasingly prevalent condition worldwide and is one of the main factors for female infertility. Obesity affects fertility by impacting oocyte and embryo quality, as well as the receptivity of the maternal environment, among many other outcomes. The effect of obesity, specifically of non-esterified fatty acids (NEFAs), on embryo development, has been extensively examined in the literature. This review discusses the relationship between non-esterified fatty acids and preimplantation embryo development, the underlying mechanism(s) that contributes to lipotoxicity in embryos, as well as outlines potential therapeutic implications of embryos exposed to NEFAs.

KEYWORDS: preimplantation embryo, non-esterified fatty acids, palmitic acid, oleic acid, obesity, infertility, lipotoxicity.

1. Introduction

As classified by the World Health Organization (WHO), individuals with a body mass index (BMI) of 25 or more are considered overweight; and individuals with a BMI of 30 or more are considered obese [1]. The WHO estimated more than half of the adults worldwide were overweight or obese in 2016 [1]. Statistics Canada also reported that 63% of Canadian adults were overweight or obese in 2018, in which 57% are females within that population [2]. Obesity is a condition with an excess accumulation of lipid that leads to inflammation, perhaps metabolic disorder, and certainly cellular stress in many parts of the body. It impairs vital bodily functions by placing stress on the cardiac system [3, 4], weakening immunity [5, 6], and disrupting endocrine functions like insulin sensitivity [7, 8]. In addition, obesity impairs reproductive functions in females, as it imparts negative effects on embryo development at the preimplantation stages [9]. In this review, the effects of exposure to an elevated fatty acid environment, specifically palmitic acid (PA) and oleic acid (OA), on preimplantation embryo development are discussed.

2. Preimplantation embryo development

Preimplantation embryo development defines the period from the fertilization of an oocyte with a sperm to the attachment of an embryo to the uterine wall [10]. Fertilization occurs when a sperm enters the oocyte and a zygote is formed, initiating preimplantation development [11]. Preimplantation embryo development can be separated into two parts – cleavage division(s) and blastocyst formation (cavitation) [10]. Cleavage division (cell division) begins approximately 24 h after fertilization in human embryos, where the

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one-cell zygote subdivides into two-, four-, eight-, then 16-cells and onwards at regular intervals from the two-cell stage to the early blastocyst stage [12]. Blastocyst formation occurs when cavitation is initiated [13]. Cavitation begins as a morula stage embryo undergoes compaction and initiates fluid transport across the outer cell layer of the embryo *via* ion channels, sodium/potassium-ATPase (Na/K-ATPase) activity, aquaporins, and tight junctions, all collaborating to form a nascent fluid-filled blastocyst cavity [13, 14]. The blastomeres polarize during compaction and express lineage-specific genes for either trophectoderm or inner cell mass development [15]. Upon embryo hatching from its surrounding zona pellucida, the trophectoderm mediates embryo implantation by interacting with the uterine wall and eventually contributes to the placenta during implantation [16]; whereas the inner cell mass consists of pluripotent epiblast cells that represent the origin of the three primary germ layers [15]. The timing from cavitation to implantation takes approximately 48 hours in the human and 24 hours in the mouse [12, 17].

3. Female infertility and assisted reproductive technologies (ARTs)

Infertility defines the inability of otherwise healthy human couples to achieve pregnancy within a year of unprotected intercourse. The World Health Organization (WHO) estimates that infertility affects 186 million individuals worldwide [18]. The global burden of disease study in 2017 identified that infertility prevalence rate significantly increased by 15% from 1990 to 2017 [19]. The study discovered that countries with high socioeconomic development, for example, Canada, had the highest increase in infertility rate in females. Roughly 16% of Canadian couples experience infertility and at least 40% of the time it is due to difficulties experienced by females [20]. Female infertility is influenced by many factors including age, genetics, as well as lifestyle-related influences. Presently, the mean maternal age of first childbirth is in the early 30s in European countries [21]. Infertility highly correlates with maternal age and can begin as early as 25 years of age [22]. Eijkemans *et al.* [22] reported that age-related infertility exponentially increases from 4.5% at age 25 to about 50% at age 40, likely due to the increasingly rapid loss of oocyte numbers and their reduced developmental competence. Genetics could also predispose women of conditions and diseases that affect fertility, for example, polycystic ovary syndrome, that results in irregular ovulation patterns [23]. Lifestyle choices like smoking, alcohol, and drug consumptions are evidently factors for infertility that impair reproductive function [21].

As the infertility rate increases, many people turn to assisted reproductive technologies (ART) for their fertility needs. In 2010, the International Federation of Fertility Societies reported that there are at least 4,400 ART clinics worldwide [24], with 37 ART clinics across Canada in 2019 [25]. According to the Canadian Assisted Reproductive Technologies Register, more than 35,000 ART treatment cycles were initiated in 2019 and resulted in approximately 17,000 clinical pregnancies [26]. Some of the ART interventions performed in 2019 were *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), intrauterine insemination (IUI), *in vitro* oocyte maturation (IVM), and frozen embryo transfer (FET), to name a few [26].

4. The effect of obesity on female reproductive health

Studies have clearly determined that obesity negatively affects reproductive health in women. These effects include menstrual abnormalities [27], ovarian dysfunction [28], diminished oocyte quality [29], reduced endometrial sensitivity for implantation [30], and overall significant reduction in pregnancy rate [9]. Overweight and obese women have twice the likelihood of experiencing irregular menstrual cycles than healthy individuals [31]. These women experience heavier menstrual bleeding, compared to normal and underweight women [32]. Additionally, obesity and high body fat percentage is significantly associated with mean menstrual cycle length of longer than 32 days [33].

Leptin is a hormone released from adipose tissue and leptin levels in the serum are positively correlated to BMI [34, 35]. Normally, leptin stimulates the hypothalamic-pituitary-gonadal (HPG)

axis, resulting in the induction of gonadotrophin-releasing hormone (GnRH) release in the hypothalamus [34]. GnRH expression induces luteinizing hormone (LH) release from the pituitary glands which directly acts on receptors located on the ovaries [34]. Under obese conditions, however, high leptin levels from excess adipose tissue continuously stimulate the HPG axis and eventually results in leptin resistance, disrupting normal GnRH and LH expression [34]. LH is a gonadotrophin hormone that regulates the ovulatory cycle and initiates ovulation of an oocyte [34]. LH levels are implicated in obesity-related effects in women. A study has found a significantly higher LH pulse frequency in obese teenage girls than girls of healthy weight [36]. Another study of obese adult women identified a lower amplitude of LH secretion than normal-weighted women, although no difference in LH pulse frequency was observed in this study [37]. Along with many others, these studies support that abnormal secretory pattern of gonadotrophin hormones like LH are present in women with high BMI levels. Changes in gonadotrophin hormones derived from effects of elevated body fat control evidently disrupts the menstrual cycle, which contributes to reduced reproductive competency of obese women.

A meta-analysis identified that overweight and obese women (BMI > 25 kg/m^2) experience significantly higher levels of miscarriage than women with normal BMI, regardless of the method of conception [38]. Elevated body fat levels disrupt ovarian functions by inducing inflammation and macrophage infiltration. Mice placed under a prolonged high fat diet displayed increased ovarian tissue inflammation and lowered pregnancy rate [39]. In women undergoing IVF treatments, monocyte chemotactic protein-1 (MCP-1) levels in serum and follicular fluid were positively correlated with BMI [40]. MCP-1 is an important factor for the recruitment and infiltration of immune cells that is normally elevated during the menstrual cycle to support ovulation; however, obesity appears to additionally increase MCP-1 expression at the ovaries during ovulation [41]. This aberrant elevation of MCP-1 results in the infiltration of additional monocytes and contributes to inflammation of the ovaries, thus impacting normal ovarian functions [40]. Studies previously suggested that elevated leptin levels in obese women may also lower endometrial receptivity for embryo implantation due to the regulatory role of leptin in endometrial cells [9, 42]. However, the role of elevated leptin in achieving pregnancy is controversial as studies are inconsistent with regards to reporting obesity-induced hyperleptinemia as a factor for miscarriage [43]. Women with high BMI levels also have higher levels of triglycerides, insulin, and lactate present in the follicular fluid compared to women with normal BMI [44]. Investigations of women undergoing IVF treatments have discovered an important correlation between high free fatty acid levels in ovarian follicles and increased numbers of poor-quality oocytes [45]. Women displaying higher follicular free fatty acids also have fewer normally fertilized oocytes compared to women with a normal BMI and they experience a 50% lower chance of achieving a clinical pregnancy after IVF treatments [46]. Nevertheless, the decline in oocyte developmental competency is a key factor in which hyperlipidemia reduces the likelihood of a successful embryo implantation.

In addition to the physiological effects on oocyte and embryo competency, obesity disrupts endocrine functioning of reproductive hormones that are vital for a successful pregnancy. Hyperandrogenism, defined as the elevated levels of androgens, such as testosterone, is common in obese women [47]. Corbould [48] identified that chronic exposure to testosterone impairs glucose metabolism in subcutaneous adipose tissue. The phosphorylation of protein kinase C zeta, downstream of the insulin receptor in adipose tissue, was inhibited, thus resulting in insulin resistance, and altered glucose uptake in adipose tissue [48]. Hyperandrogenism contributes to insulin resistance and adipose accumulation at the ovaries, which exacerbates the effects of obesity on women's reproductive health [49]. In addition to the change in testosterone levels, estrogen levels are also affected by BMI. Shah and colleagues [46] observed a significantly lower estradiol level in women with a high BMI. The lowered level of estradiol supports the occurrence of reduced fertilization rate in the high BMI group [46], as proper estrogen levels are essential to support healthy oocyte maturation.

Overall, obesity is detrimental to not only maternal reproductive health, but more specifically to oocyte and early embryonic developmental competency. Most obese women of reproductive age experience difficulties with conception [50], even with the help of ART. Women with high BMI levels have much lower ART success which substantially results from reduced oocyte developmental competence, lower *in vitro* fertilization success, and overall lower live birth rates [46]. However, the underlying mechanisms that lead to these effects of obesity on fertility largely remain to be discovered.

5. Lipid metabolism in preimplantation embryos

Lipid metabolism is an important source of energy for cell physiology. Nutrients like carbohydrates and lipids from dietary sources are taken up by intestinal cells [51] and then transported to the liver and other peripheral organs like the adipose and muscle tissues for metabolism. These nutrients provide energy for many organ systems including the reproductive system. The developmental competency of oocytes is highly dependent on surrounding cumulus cells and their ability to utilize glucose or fatty acids as an energy source [52]. Cumulus cells contribute largely, *via* gap junctions, to oocyte development by supplying most of the adenosine triphosphate (ATP) and substrates required for energy expenditure [52]. Oocytes can also generate ATP, although mitochondria are immature at that stage. Richani *et al.* [52] showed that the production of ATP occurs in denuded mouse oocytes by the adenosine salvage pathway, but ATP production was at a much lower level. Primarily, oocytes and cumulus cells utilize glucose and fatty acids in the follicular environment to produce energy by means of glycolysis and β-oxidation, respectively [53]. The inhibition of β-oxidation in mouse cumulus-oocyte complex inhibits oocyte maturation and reduces blastocyst development [54], suggesting that fatty acids are an important energy source throughout development. Fatty acids are stored as triacylglycerol (TAG) in lipid droplets in the oocytes until oxidation for energy consumption [55]. The amount and composition of lipid droplets in the oocyte changes throughout follicular development and differs between species [55]. Clearly, fatty acid metabolism is required to provide sufficient energy substrates like nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide in reduced form ($FADH_2$), guanosine triphosphate (GTP), and ATP to support oocyte maturation. The energy produced from lipid metabolism also supports development of an early embryo, especially during the earliest cleavage events. For example, oxidation of stored TAG liberates fatty acids and phospholipids supports the increase of cell membrane surface area throughout preimplantation development [55].

6. Non-esterified fatty acids (NEFA)

Obesity results from the accumulation of adipose tissue, which can be evaluated by the level of non-esterified fatty acids (NEFA) in the serum [56]. NEFAs are fatty acids released into the circulation from adipose tissues, as a result of lipolysis of TAG in the adipocytes [57]. The release of NEFA allows transport of fatty acids to other organs to produce energy. Another major source of NEFA in the circulation is dietary lipid intake. These lipid droplets are transported to the liver as TAG in chylomicrons and must be hydrolyzed into NEFA by lipoprotein lipase before taken up for long-term storage [57]. Although early literature suggested that an increase in adipose tissue mass leads to elevated levels of NEFA in the circulation [58], many studies have shown the opposite. For example, McQuaid *et al.* [59] reported that obese individuals have a systemic NEFA concentration no different from lean individuals. The rate of lipoprotein lipase activity in obese individuals was in fact lowered, which reduces NEFA released from the adipose tissue [59]. Karpe *et al.* [57] also completed a systematic review of the literature prior to 2009 and found that plasma NEFA concentration is unrelated to body fat mass. However, while the reliability of serum NEFA levels as an estimation for obesity or body fat accumulation is questioned, the composition and relative ratios of varying NEFAs in the circulation of obese individuals likely reflect the degree of negative effects related to the obese environment. In terms of NEFA levels in the reproductive system, follicular NEFA composition and ratios were found to vary across individuals.

Multiple studies reporting analyses of oocyte follicular fluids have found varying composition of fatty acids based on different diet composition and variation in tissue metabolic rate differences [55]. However, analysis of human follicular fluids identified that approximately 45% of total NEFAs are palmitic acid (PA) and oleic acid (OA) [60]. These free fatty acids are at even higher concentrations in women who are obese compared to women with a normal BMI [60]. Although little is known about the NEFA levels in human oviductal fluids, reports of NEFA existing in the follicular fluid suggest that embryos are likely exposed to fatty acid environments throughout the reproductive tract. This is supported as studies using animal models have reported the presence of NEFAs in oviducts and the uterus [61, 62]. Embryos of animal models also showed the uptake of environmental fatty acids into lipid compartments [62, 63].

NEFAs can be categorized into two main categories – saturated fatty acids and unsaturated fatty acids. Saturated fatty acids are fatty acids with no double-bonds between their carbon chain, whereas unsaturated fatty acids are fatty acids with at least one double-bond between their carbon chain. PA and OA are the most abundant NEFAs in the circulation, estimating at 23% and 31% of total NEFA, respectively of normal-weight subjects [60].

6.1. Palmitic acid (PA)

Palmitic acid (16:0; PA) is a 16-carbon saturated fatty acid. It can be synthesized in the body *de novo* through lipogenesis from other macromolecules and it can also be consumed in the diet [64]. PA is abundant in palm oil, meat, and dairy products. Under nutrient-rich conditions, PA concentration is tightly controlled by desaturation to other unsaturated fatty acids like palmitoleate (16:1) for long-term lipid storage [65]. However, with an imbalance of PA homeostasis, implications in metabolism occurs leading to metabolic syndromes like insulin resistance and even cancer [64].

6.2. Oleic acid (OA)

Oleic acid (18:1; OA) is an 18-carbon monounsaturated fatty acid. It is a major component of olive oil, representing up to 83% of olive oil's total composition [66]. Like PA, OA can be synthesized *de novo* through lipogenesis and thus dietary intake is not deemed a necessity. However, the recent increase in popularity of the Mediterranean diet places an emphasis on the beneficial effects of ingesting unsaturated fatty acids like OA. Recent research efforts have identified that the Mediterranean diet, which consists of extra virgin olive oil and plant fats as main sources of fats, reduces the risk of chronic diseases and mortality [67]. With promising results from multiple randomized clinical trials, the American Heart Association recommends lower intake of saturated fats and replacing fat intake with unsaturated fats to lower the risk of cardiovascular events [68]. A recent meta-analysis of gestational studies has also shown that close adherence to the Mediterranean diet improves clinical pregnancy and live birth rates after ART treatments, improves fetal development, as well as reduces gestational complications in pregnant mothers [69].

7. The effects of NEFA on embryo development

7.1. Palmitic acid (PA)

The effects of PA on bovine and mouse preimplantation embryo development are extensively described in the literature. PA is one of the major fatty acids making up approximately 25% of total NEFA present in bovine follicular fluid [70]. Aardema *et al.* [63] reported that denuded bovine oocytes actively take up PA into TAG for lipid storage. Though, the uptake of PA in bovine oocytes resulted in lipid droplets that are smaller in size and number when compared to control oocytes in a dose-dependent manner [63]. Additionally, bovine embryo development was greatly impaired in a dose-dependent manner after PA-exposure [63]. Leroy *et al.* [70] reported that i*n vitro* maturation of bovine oocytes in medium containing PA results in a significantly lower fertilization rate compared to controls. Oocyte maturation was also disrupted by the presence of PA in the culture media, as a significantly higher percentage of oocytes experienced delayed progression through meiosis [70]. Furthermore, the investigation by Van Hoeck *et al.* [71] on the effects of PA-treated bovine embryos found that

high PA-supplemented culture medium significantly lowered blastocyst development as well as increased apoptosis. Our group recently reported similar effects in mouse preimplantation development. After *in vitro* incubation of mouse embryos at the 2-cell stage in PA-supplemented culture medium, Yousif *et al.* [62] demonstrated impairments of mouse blastocyst development in a dose-dependent manner. We further identified that PA upregulated endoplasmic reticulum (ER) stress markers at the transcript level, providing evidence that exposure to elevated PA may induce cell stress that impairs mouse preimplantation development [62]. A similar study by Jungheim *et al.* [45] reported a reduced cell count in mouse blastocysts after *in vitro* incubation with PA for 30 hrs. The researchers also identified a significantly upregulated insulin-like growth factor type-1 receptor (IGF-1R) and glutamic-pyruvate transaminase 2 (GPT2) protein expression after PA treatment, which may contribute to abnormal insulin signaling in these embryos. When the PA-treated blastocysts were transferred into pseudo-pregnant female mice and grown to birth, those embryos developed into significantly smaller fetuses than the fetuses from control embryos [45]. Although fetuses from PA-treated embryos were possibly growth-restricted, a catch-up growth pattern was observed, and those fetuses eventually grew heavier than control fetuses by post-natal day 50 [45]. These findings imply that PA exposure alone can lead to metabolic changes in the offspring, which shows relevancy to insulin resistance in obese individuals. Figure 1 summarizes the effect of PA on oocyte and preimplantation embryo development.

7.2. Oleic acid (OA)

Among the many NEFAs, OA is another major fatty acid found in the reproductive system, including the follicular fluid. It is estimated to make up approximately 30% of total NEFA in bovine follicular fluid [70]. A study by Aardema *et al*. [63] investigated the effects of OA treatment on bovine oocyte developmental competency and lipid metabolism. OA had no negative effect on oocyte maturation but i*n vitro* exposure to OA simply increased lipid droplet sizes and numbers in the oocytes [63]. It was also indicated that OA was primarily metabolized into its subsequent products from β-oxidation or processed as TAG for long-term storage [63]. Similarly, Leroy *et al*. [70] reported that i*n vitro* incubation of bovine oocytes with OA had no significant effects on oocyte maturation nor blastocyst development after fertilization. Another study of bovine embryos with exogenous medium supplementation with OA indicated a significant acceleration in cleavage rate at the 2-cell stage as well as a significant increase in blastocyst development [72]. Recently, Yousif *et al.* [62] investigated the dose-dependent effects of OA up to 500 μM on developing mouse preimplantation embryos and observed no significant impairments in blastocyst development at any tested dose. Cell morphology at all preimplantation cell stages was unaffected as well [62]. OA-treated mouse embryos did not induce mRNA levels of ER stress markers but OA treatment significantly enhanced lipid droplet accumulation [62]. Overall, it is concluded that OA has no discernable negative impact on oocyte and early mammalian embryo developmental competency (Figure 1).

8. Lipotoxicity in preimplantation embryos

During embryogenesis, the growing embryo experiences various types of stress. These stress factors can be categorized into two main categories, local and environmental stress. The source of local stress on the embryo includes oxidative stress and inflammation among many others [73], whereas environmental stress factors include maternal hormone and toxins [73]. *In vitro* embryo manipulation and growth also poses additional stress during embryogenesis through handling and culture conditions, to name a few [74, 75]. Ultimately, the early embryo must utilize its capacity to adapt and offset exposure to stressors to achieve and maintain high developmental capacity.

One of the main factors for stress during embryogenesis is energy expenditure. Energy production is required to support the exponential growth and cell division during the early cleavage stages. One source of energy comes from fatty acid oxidation. Fatty acids are taken up by an oocyte through the surrounding cumulus cells and follicular fluid [55]. These lipids are then converted into TAG by diacylglycerol acyltransferase (DGAT) enzyme to be stored as lipid droplets in the

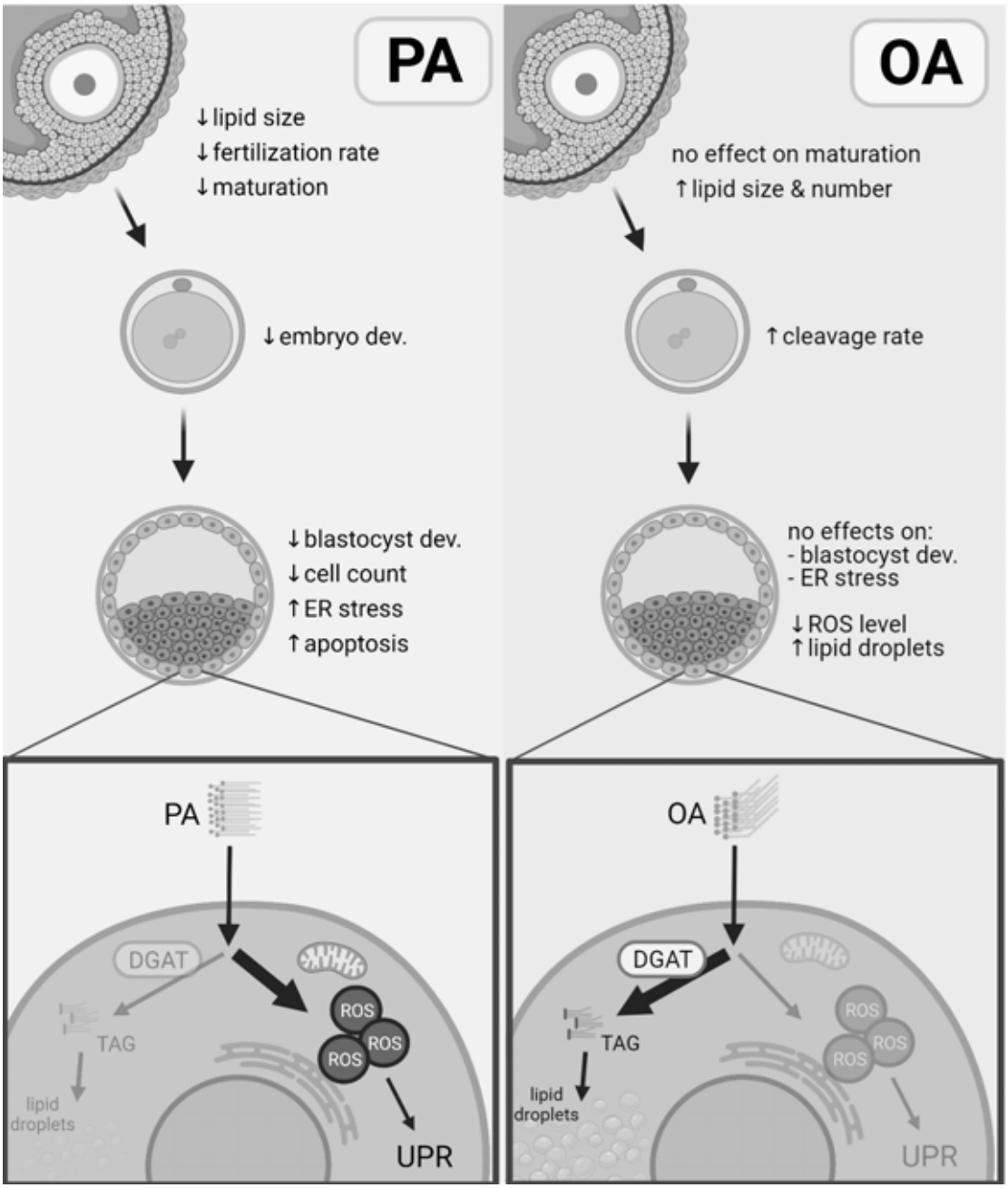

Figure 1. Model of PA and OA treatment effects on preimplantation embryo development.

Treatment with palmitic acid (PA) results in overall reduction in developmental potential in preimplantation embryos. PA impairs oocyte maturation and fertilization; whereas treatment of preimplantation embryos with PA disrupts blastocyst development, cell count, as well as ER stress markers that eventually lead to apoptosis. Evidence suggests that PA build-up in the cytoplasm results in ROS and lipid peroxide production that leads to lipotoxicity. Lipotoxicity-induced dysregulation of the ER can result in the imbalance of protein folding, thus activating the ER stress response mechanisms like the unfolded protein response (UPR) to maintain ER homeostasis. Treatment with oleic acid (OA) has no overall negative effects on oocyte maturation or preimplantation embryo development. However, it lowers ROS level, and it also increases lipid droplet accumulation. OA enters the embryo and is preferentially converted to TAG by DGAT enzymes. TAG can be easily stored in lipid droplets for long term storage without disrupting regular cellular functions. Created with BioRender.com.

cytoplasm and the ER [55]. A study of porcine preimplantation embryos reported a significant decrease in lipid droplet volume at the blastocyst and late-blastocyst stages compared to other developmental stages [76], indicating a potential utilization of fatty acids as an energy source at the blastocyst stage. Although embryos can utilize lipids as a source of energy metabolites, a high fat environment is detrimental to preimplantation embryo development. Follicular fluids of mothers with high BMI levels tend to have higher concentrations of free fatty acids and triglycerides [44]. High lipid contents taken up by the oocytes produce reactive oxidative substrates (ROS) and lipid peroxides that lead to lipotoxicity [77]. Many organelles including the mitochondria and ER experience structural remodeling and dysfunction due to lipotoxicity that can ultimately induce apoptotic cell death [78]. In fact, exposure of mouse preimplantation embryos to elevated levels of PA not only disrupts blastocyst development but it also increases the expression of ER stress transcripts, revealing that ER stress was induced in response to high fat environment [62]. Lipotoxicity-induced dysregulation of the ER can also promote an imbalance in ER protein folding, resulting in the accumulation of unfolded proteins and activation of ER stress response mechanisms. The unfolded protein response (UPR) is activated to maintain ER homeostasis by removing unfolded proteins and preventing further protein unfolding [79]. Under prolonged ER stress, cells may opt to activate autophagy [80] to maintain cellular homeostasis and to alleviate cell stress. However, apoptosis may soon be initiated when homeostasis cannot be re-established. A study of trophoblast cell lines revealed a dose-dependent effect of PA and stearic acid (18:0; SA) on increased apoptosis and caspases 3 and 7 activity [81], revealing the detrimental effects of high fatty acid environments. It is therefore evident that lipotoxicity in preimplantation embryos not only affect blastocyst development, but it also impairs ER stress pathways which ultimately induces apoptotic cell death.

9. Therapeutic implications

9.1. Palmitic acid (PA) and Oleic acid (OA) co-treatment

The protective ability of OA on embryo development has sparked interest in its effects when presented with saturated fatty acids that are generally believed to be harmful. Common consensus from the literature would suggest that a higher unsaturated-to-saturated fatty acid ratio should provide benefits to preimplantation embryo development. In bovine oocytes, Aardema *et al.* [63] investigated the effect of OA co-treatment at 1:1 ratio with saturated fatty acids, PA and SA, on oocyte developmental competency and lipid metabolism. It was reported that although PA and SA, individually, had adverse effect on oocyte maturation in a dose-dependent manner, the addition of OA with each saturated fatty acid alleviated the adverse effects and restored developmental competency [63]. Furthermore, in bovine oocytes, the combination of OA with each saturated fatty acid showed significantly greater lipid droplet accumulation (size and number) compared to PA and SA treatments individually, highlighting the change in lipid storage as a potential mechanism of the compensatory effects of OA [63]. The hypothesis that OA counteracts PA-induced effects is also supported in murine embryos. Yousif *et al.* [62] reported that incubation of mouse embryos in both PA- and OA-supplemented culture medium at 1:1 ratio restored blastocyst development to control levels, compared to the significant developmental arrest observed in the PA-alone group. The rescuing effects of OA in the combination treatment beneficially affected ER stress pathway gene expression, lipid metabolism, and mitochondrial ROS levels by reversing PA-induced effects to control levels [62]. Although OA is well-recognized for its protective effects, its abilities may be limited when exposed to a high proportion of saturated fatty acids. The study by Van Hoeck *et al.* [71] investigated the effect of PA, SA, and OA combination treatment on bovine oocyte and embryo development at a higher saturated-to-unsaturated fatty acid ratio (1.125:1 ratio). When bovine oocytes were treated with PA, SA, and OA in combination prior to fertilization, blastocyst development was significantly lower than the supplemented control [71]. Additionally, the total cell number and apoptotic cell index of those embryos were significantly lower and higher, respectively, in the combination treatment [71], revealing the limitation of OA rescue effects. These findings support that OA can, in fact, counteract the effect of PA exposure in early

embryo development; however, the protective mechanism can still be overwhelmed, or even fail, when exposed to higher proportions of saturated fatty acids than unsaturated fatty acids (Figure 2).

9.2. Antioxidants

As lipotoxicity implicates ROS level and related pathways in the embryos [77], the incorporation of antioxidants in *in vitro* embryo culture medium

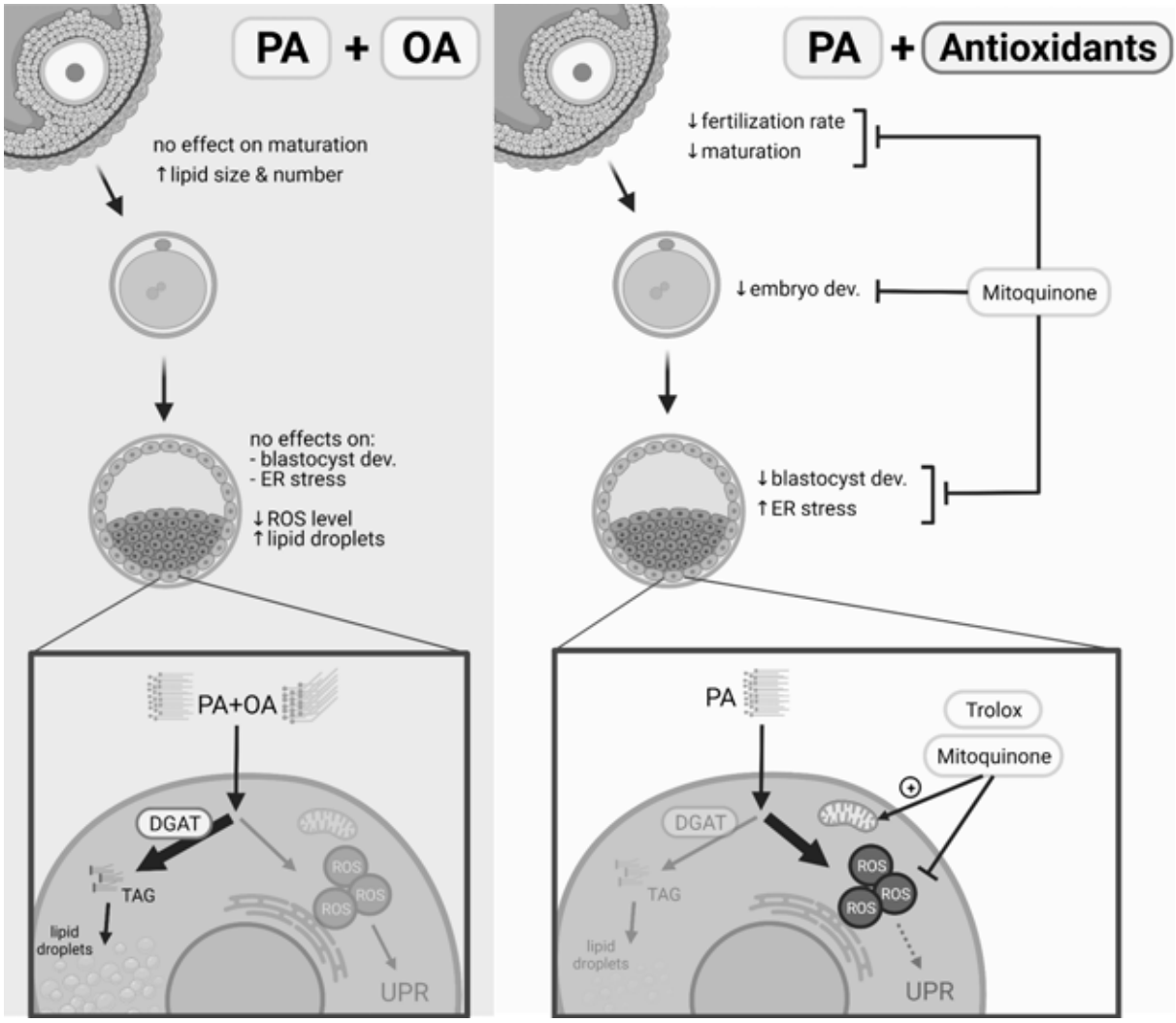

Figure 2. Model of PA and therapeutic addition effects on preimplantation embryo development.

The co-treatment of PA and OA reversed PA effects at both the oocyte and the embryo level. Oocytes co-treated with PA and OA had no implications on maturation but resulted in more and larger lipid droplets. Preimplantation embryos also had no developmental impact from the PA and OA co-treatment. Lipid droplets increased by size and number while ROS levels were reduced after co-treatment. Evidence suggests that the presence of OA in the cytoplasm redirected the metabolism of PA into lipid droplets instead of diacylglycerol (DAG) and ceramides that elevate ER stress and ROS production. Thus, OA potentially serves as an important addition to culture media to counteract PA-effects on oocyte and embryo developmental competency. Antioxidants like Trolox and Mitoquinone targets the mitochondria, where pre- or co-treatment with the antioxidants restored mitochondrial activity of PA-exposed embryos. Both Trolox and mitoquinone reduce ROS levels in the embryos; however, only mitoquinone was shown to alleviate PA effects on developmental competency of preimplantation embryos. Created with BioRender.com.

to enhance developmental success has become a highly investigated area. A study by Marei *et al.* [82] investigated the effect of PA and the addition of Mitoquinone, a mitochondrial-targeted antioxidant, on bovine oocyte maturation and blastocyst development. The researchers reported that after PA exposure, oxidative stress was significantly increased while mitochondrial functioning was disrupted in a dose-dependent manner. But placing these PA-insulted embryos into culture medium with mitoquinone relieved embryos from elevated oxidative and ER stress, restored mitochondrial functioning, while also improving blastocyst quality [82]. Another antioxidant, Trolox (droxy-2,5,7,8-tetramethylchroman-2-carboxylicacid), was employed in the study by De Bie *et al.* [83] as a protective agent against ROS effects. Trolox is a synthetic vitamin E analogue which also acts as a standard reference for evaluating antioxidant properties [84]. Bovine oocytes were placed into culture medium supplemented with Trolox during *in vitro* oocyte maturation or during embryo development. The authors revealed that pre-treatment of bovine oocytes with Trolox before PA exposure during embryo development protected embryos against PA effects on developmental competency and ROS levels [83]. Trolox pre-treatment increased mitochondrial activity that potentially enabled embryos to cope with elevating cell stress as embryos were exposed to PA, although Trolox pre-treatment could not restore blastocyst development to control levels [83]. These studies demonstrated that the addition of antioxidants in *in vitro* culture medium may potentially improve oocyte or embryo development by protecting embryos against hyperlipidemia-induced oxidative stress. Future investigations regarding the supplementation of culture medium with antioxidants are necessary to define optimal conditions for improving developmental capacity as well as ART success (Figure 2).

10. Conclusion

In this review, the association between obesity and preimplantation embryo development was discussed in various contexts. We highlighted the prevalence of obesity and the utilization of ARTs in achieving pregnancy. The outcomes of obesity on female reproductive health as well as embryo competency were explored extensively. Specifically, this review emphasized on two main NEFAs, PA and OA, and their effects, at elevated levels, on preimplantation embryo development. We proposed that the lipotoxicity effects on fatty acid metabolism and ER stress in preimplantation embryos is a potential underlying mechanism which impacts development as observed in NEFA-treated embryos. Lastly, two therapeutic strategies were outlined regarding current research efforts that aim to alleviate PA influences on developmental competency and cellular stress in preimplantation embryos. It is expected that as we gain knowledge about the underlying mechanisms of hyperlipidemia, therapeutic options will be revealed and more readily translated to assisting infertile individuals, especially those who are obese, with achieving a healthy pregnancy.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest to declare.

ABBREVIATIONS

ART	:	Assisted reproductive technologies
ATP	:	Adenosine triphosphate
BMI	:	Body mass index
DGAT	:	diacylglycerol acyltransferase
ER	:	Endoplasmic reticulum
FADH2	:	Flavin adenine dinucleotide (reduced)
FET	:	Frozen embryo transfer
GnRH	:	Gonadotrophin-releasing hormone
GPT2	:	Glutamic-pyruvate transaminase 2
GTP	:	guanosine triphosphate
HPG	:	Hypothalamic-pituitary-gonadal
ICSI	:	Intracytoplasmic sperm injection
IGF-1R	:	Insulin-like growth factor type-1 receptor
IUI	:	Intrauterine insemination
IVF	:	In vitro fertilization
IVM	:	In vitro maturation
LH	:	Luteinizing hormone
MCP-1	:	Monocyte chemotactic protein-1
NADH	:	Nicotinamide adenine dinucleotide
NEFA	:	Non-esterified fatty acids
OA	:	Oleic acid
PA	:	Palmitic acid
ROS	:	Reactive oxidative substrates

SA : Stearic acid
TAG : Triacylglycerol
UPR : Unfolded protein response
WHO : World health organization

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