

Lipid Peroxidation and its Impact on Infertility

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Abstract

By the age of 40, 33% of couples will face clinical infertility [1]. Furthermore, only approximately half of the remaining 66% of couples will have successful live births because of reproductive problems [2]. This evidence of infertility and its reproductive health risks raise questions about the possible mechanism of these issues. In addition to age-related effects on reproductive functioning, the incidence of infertility in both men and women is also associated with obesity, dyslipidemia, and reproductive illnesses, all which increase lipid peroxide levels in gametes. Previous and current literature suggest that excessively modified lipids increase radical oxidative stress through lipid peroxidation. This process then damages the integrity and functioning of both sperm and oocytes. The focus of this review is the molecular and applied clinical aspects of lipid peroxidation-mediated infertility. It includes the investigation of the lipid peroxidation process, the mechanisms of sperm and oocyte damage, and the clinical effects of obesity and dyslipidemia on male and female infertility.

Keywords: Lipid peroxidation, Male Infertility, Female Infertility, Obesity, Dyslipidemia

Methodology

In order to assess the relationship between lipid peroxidation and infertility, we performed a literature search of relevant publications from the 2011 to 2016 via an advanced PubMed search. Relevant search terms included "lipid peroxidation," "male infertility," "female infertility," "reactive oxidative species," "reactive oxidative species and infertility," "DNA damage," and "dyslipidemia and infertility." Pertinent and relevant articles were carefully considered, and additional older relevant articles were selected from within these papers.

Introduction

Medical infertility, or the inability to conceive after one year of unprotected intercourse under natural conditions, affects 1 in 3 couples by the age of 40 [1]. Moreover, by the age of 40, only 44% of these women have live births after conception because of the risks of spontaneous abortion and other reproductive problems [2]. These realities do reduce the incidence of successful reproductive outcomes, suggesting that infertility and its causes can pose a significant healthcare concern.

However, for many couples, the cause of this secondary infertility is largely unclear. For example, approximately 22% of infertile couples face unexplained infertility [2]. The infertility itself can be mediated through maternal, paternal, or combined parental factors [2]. Previous literature largely associates this type of secondary infertility with oxidative stress in both men and women [2]. In humans, the utilization of oxygen through the electron transport chain for energy generation allows for the creation of radical oxidative species (ROS) [3]. Under normal conditions, the body maintains a balance between oxidative and anti-oxidative species; however, an imbalance in oxidative state facilitates the increased concentration and detrimental effects of ROS. These oxidative stress mediators impair intracellular processes and DNA integrity, allowing for infertility and adverse reproductive outcomes.

One of the main causes of ROS generation includes lipid peroxidation. Lipid peroxidation is an oxidative process that modifies lipids and fatty acids in both sperm and oocyte membranes. These modified byproducts greatly affect the viability and overall quality of reproductive cells, increasing the likelihood of infertility. Therefore, a deep and complete understanding of lipid peroxidation and its causes are required

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to improve prevention and treatment of infertility. This article reviews current and previous literature on lipid peroxidation-related reproductive dysfunction and discusses its relevant risk factors, including genetic differences and lifestyles choices, on the molecular functioning of male and female gametes.

Creation of Oxidative Damage

Humans rely heavily on aerobic respiration, which utilizes oxygen to create energy molecules for daily metabolic functioning. Ninety-eight percent of the inspired oxygen is reduced during lipolysis, while the remaining 2% is used to create ROS [4]. Chemically, free radical species are molecules with one or more unpaired electrons [5]. The unpaired electrons create a state of extreme reactivity, causing these free radicals to form bonds with nucleic acids, proteins, carbohydrates, and lipids for stabilization [4]. At normal physiological levels, ROS function as messengers in vital signaling transduction in reproductive pathways, and low levels of ROS have been linked to cellular dysfunction [5]. For example, normal amounts of ROS are vital for proper capacitation, acrosomal reactions, and motility of male germ cells, which utilize redox-regulated, ROS-mediated signaling pathways [5].

Mechanisms of DNA Damage

However, high levels of ROS can also be detrimental. When excess ROS are produced, they induce oxidative stress, negatively affecting cellular homeostasis in gametes and germ cells. Oxidative stress is categorized as an imbalance between oxidant creation and anti-oxidant neutralization. The radical-dependent binding to surrounding macromolecules induces a chain of reactions that can cause mitochondrial damage, caspase activation, and apoptosis. This cellular damage affects the physical structure of DNA in oocytes and sperm, leading to DNA adducts, base oxidation, deamination, and sites of decay [6].

The main types of oxidation-mediated DNA damage (Figure 1) are due to radiation exposure, environmental causes, such as heavy metals and smoking, and intrinsic oxidative pathways [6]. This type of DNA damage stimulates DNA repair pathways, such as direct reversal of damage (DDR), single-strand damage repair (SSR), base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), double strand breaks (DSB), non-homologous end joining (NHEJ), and homologous recombination (HR) [6]. Following DNA damage, the DNA can either be repaired through cellular DNA repair mechanisms, accumulate new mutations, or trigger apoptosis of the cell [6]. While cells do have the ability to restore some cellular integrity, increased levels of damage in gametes are thought to affect reproductive outcomes. In addition to the abovementioned DNA repair pathways that are present in most cell types, gametes have specialized repair mechanisms for oxidative damage. For example, oocytes are equipped with intrinsic protective mechanisms. They utilize antioxidant enzymes, reduce glutathione, and are supported by antioxidant molecules in their surrounding environment [6]. For example, tubular epithelial cells contain hypotaurine, taurine, catalase, superoxide dismutase, and glutathione synthase to offer additional protection [6]. Similarly, sperm also neutralizes ROS through the glutathione peroxidase-mediated pathway [7].

Oxidative Stress in Infertility

To assess ROS-mediated infertility, Agarwal et al compared

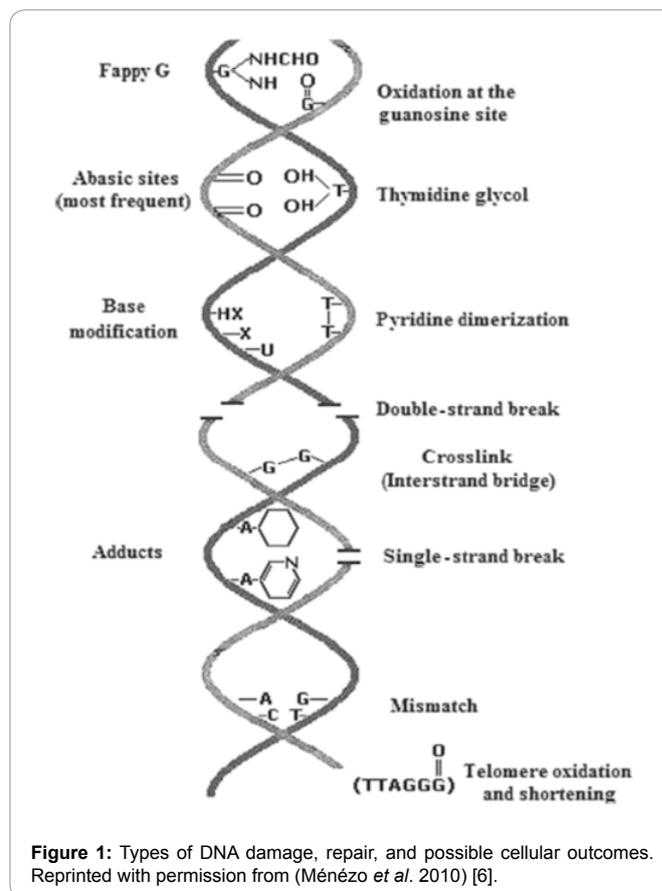


Figure 1: Types of DNA damage, repair, and possible cellular outcomes. Reprinted with permission from (Ménézo et al. 2010) [6].

ROS levels in proven donors (men who were able to initiate a pregnancy) and patients with teratozoospermia [4]. In this study, the patients with teratozoospermia had a history of difficulty in initiating pregnancy. In these teratozoospermic patients, poor sperm morphology was associated with higher ROS concentrations ($p < 0.05$) [4]. This result implies that the presence of ROS in sperm could be responsible for the subfertility and abnormal gamete functioning in the teratozoospermia group, leading to reduced live birth and pregnancy outcomes. In a 2012 study, sperm damage and live-birth rates after in-vitro fertilization (IVF) were assessed to determine an association [8]. In this study, sperm damage was determined by the percentage of sperm DNA fragmentation, a known consequence of ROS-mediated damage. The 203 patients undergoing IVF were divided into three categories based on degree of sperm DNA damage: 0-24%, 25-50%, and >50% DNA fragmentation [8]. Based on this design, patients with <25% of sperm DNA fragmentation had the highest percent of pregnancies. In this group, 39.4% of couples became pregnant, and moreover, 33.3% of these pregnancies resulted in live births. The number of pregnancies and subsequent live births decreased comparatively in both 25-50% and 50% sperm DNA damage groups, suggesting the sperm fragmentation may negatively impact pregnancy rates and outcomes [8]. In 25-50% sperm DNA damage, 29.6% of couples became pregnant, and of those couples, 23.9% resulted in live births [8]. Finally, patients with <50% of DNA damage had 16.2% rate of pregnancies with a 13.1% live birth rate [8]. Moreover, Simon et al determined that patients with less than 50% sperm DNA fragmentation

were able to conceive with IVF [8]. However, patients >50% DNA fragmentation required intracytoplasmic sperm injection (ICSI), reiterating the negative impact of DNA damage on conception [8].

As in men, oxidative stress can detrimentally impact female fertility. A 2012 comprehensive review on oxidative stress in female reproduction suggested that reproductive diseases, such as endometriosis and polycystic ovarian disease, along with obesity and other sources of radical production can impair reproductive success [9]. Karuputhula et al studied the oxidative status of granulosa cells of the oocyte in infertile IVF patients. The cells were collected from three groups of patients: 28 with endometriosis (Group A), 26 with polycystic ovary syndrome (Group B), and 32 with tubal factor infertility (Group C) [10]. ROS production was measured by mean fluorescent intensity. Both endometriosis and polycystic ovary syndrome are characterized by reproductive dysfunction secondary to oxidative stress; whereas, tubal infertility is not associated with oxidative damage. In this study, ROS production was greater in Group A and B than in Group C, associating oxidative damage with reproductive dysfunction [10].

Effect of Lipids in Reactive Oxidative Species Generation

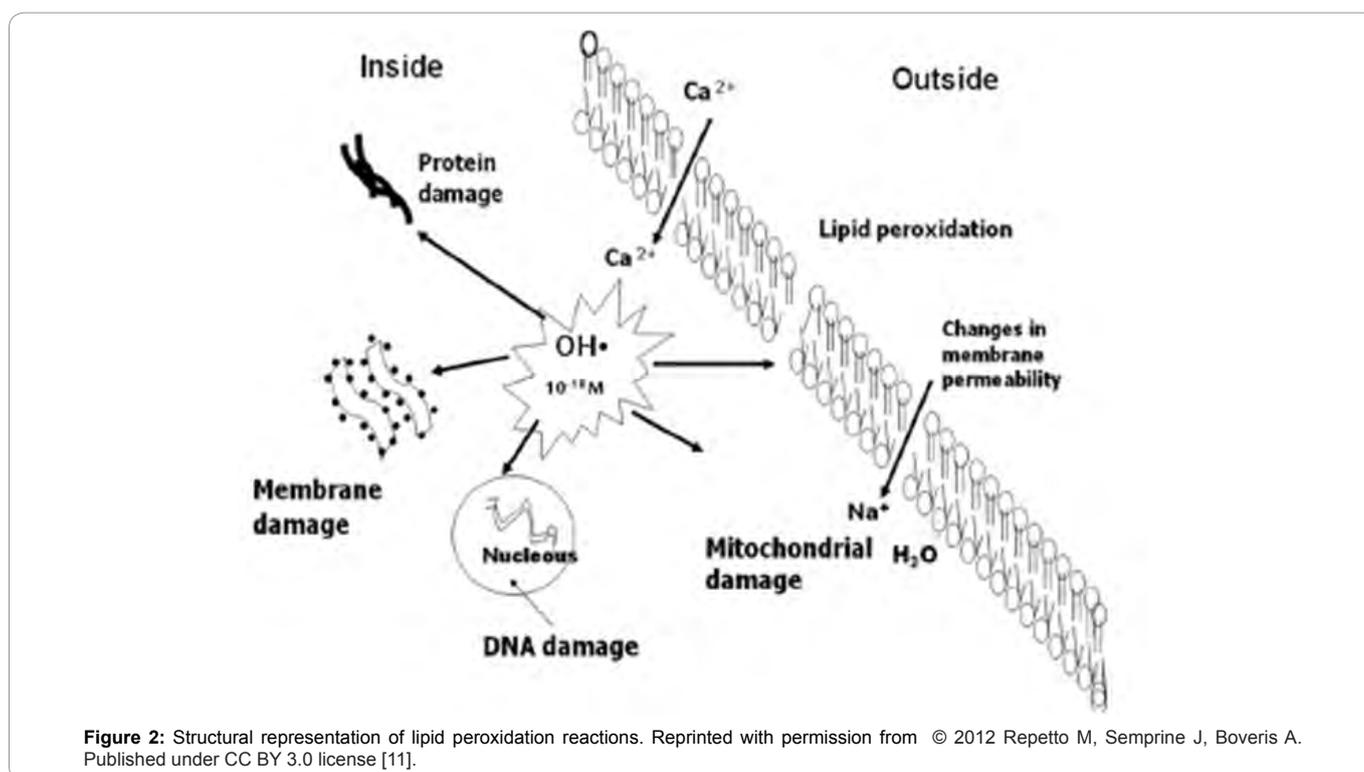
On a molecular level, ROS affect gamete integrity primarily through lipid peroxidation. Lipid peroxidation is a radical amplification process that creates oxidative stress and damage [11]. In lipid peroxidation, generated free radicals form bioactive molecules when interacting with polyunsaturated lipids, a key structural component of the phospholipid bilayers of membranes [11]. Polyunsaturated lipids contain at least two or more carbon-carbon double bonds, which are used in the initiation step of lipid peroxidation [11]. The oxidative deterioration continues with molecular rearrangement of the lipid molecules into a

radical [11]. After the creation of a carbon radical, this modified lipid interacts with oxygen to promote more lipid peroxidative radical formation in the propagation step [11]. These free radicals then attack lipids, creating damage and altering lipid structures (Figure 2) [11]. Additionally, these reactive molecules can interact with copper and iron to create additional radicals [12]. The generation of these reactive molecules induce oxidative damage to membrane proteins and cholesterol, a key membrane component [11]. These downstream effects can also affect DNA, targeting cells for apoptosis as previously mentioned [6], [11].

While mitochondria-independent oxidative reactions can form reactive species, the center of most ROS production is the mitochondria. In most cells, lipid peroxidation is mediated through improper physiological mitochondria-dependent reactions. In the mitochondria, electron transport is coupled to oxidative phosphorylation, allowing the generation of vital ATP [13]. However, uncoupling or dysregulation in the electron transport mechanism allows free radical production and in turn, creates high levels of reactive products, such as malondialdehyde [13]. In lipid-rich cells, such as sperm, lipid peroxidation overwhelms established anti-oxidative defenses, allowing for a state of oxidative stress [13]. This theory has been studied using malondialdehyde production as an index of lipid peroxidation, especially in defective sperm [14]. Aitken et al. utilized this assay to determine that lipid peroxidation levels were increased in defective sperm with high levels of ROS and found similar findings in sperm stimulated to produce oxidative radicals [14]. This type of oxidative damage, especially in the gametes, impacts fertility and reproductive outcomes.

The Effect of Lipotoxicity

Cellular damage and resulting infertility could be the result



of lipotoxicity. The concept of lipotoxicity was investigated in a 2015 study on the effect of nutrient overload in isolated rat liver beta cells [15]. In this study, cells were incubated in increasing glucose concentrations with and without palmitic acid [15]. The effects of the high glucose and palmitic acid on beta cell viability were analyzed [15].

Both previous and current research suggest that high beta cell glucose levels cause membrane phospholipid remodeling, which increases insulin secretion to maintain proper glycemic levels [15]. In this study, the glucose caused isolated insulin secretion, which is a desirable effect in a state of hyperglycemia [15]. However, after exposing the beta cells to both palmitic acid and glucose, the palmitic acid incorporation into cellular membranes in the presence of high glucose levels caused significant cell death due to stress in the endoplasmic reticulum and elevated abnormal ROS production, which was a consequence of lipid peroxidation [15].

While the previous study is focused on the lipid peroxidative effects in specifically liver cells, its controlled experimental design offers some insight on the molecular effects of excess lipid levels on cell viability. These excessive lipid levels appear to be directly related to poor cellular functioning and viability. This theory of lipotoxicity and consequential reactive oxidative species production could be related to gamete quality and infertility. With current rising trends in obesity, this phenomenon could be in effect in patients with poor diets and dyslipidemia, leading to infertility seen in both men and women.

Molecular Evidence of Lipid Peroxidation-Mediated Gamete Damage

Coupled with mitochondrial ROS production, lipotoxicity has been suggested as the biomolecular mechanism through which lipid peroxidation damages gamete viability [16]. This damage increases the likelihood of unsuccessful fertilization and reduces the rate of clinical pregnancy. As explained in previous and current literature, both sperm and oocytes are especially vulnerable to cellular changes, DNA damage, and apoptosis.

Lipid Peroxidation and Sperm

With its high lipid and mitochondrial content, sperm are especially susceptible to lipid peroxidation-mediated damage. Sperm are comprised of an acrosome cap, a middle segment, and a flagellum. The head contains densely coiled chromatin, the mid-piece has a mitochondria-filled core, and the tail functions as a

mode of transportation. This concentrated mitochondrial density with sensitive lipid membranes can increase the susceptibility of sperm to oxidative stress. Sperm have been considered especially at risk due to their exposure to oxidation by intrinsically produced ROS and those produced by leukocytes [5]. Interestingly, leukocytes can produce up to 1000 times more ROS than sperm, especially due to leukocyte activation and inflammation [5]. This process itself has been associated with infertility secondary to lipid peroxidation [5].

The ROS damage especially targets the sperm lipid membrane, modifying the fatty acids in the membrane phospholipids. These lipids function to maintain membrane fluidity. Therefore, damage to the membrane's integrity can have significant effects on sperm function. The sperm membrane itself is comprised of various monosaturated, polyunsaturated, and unsaturated fatty acids. Moreover, the elevated amount of polyunsaturated fatty acids in the sperm membrane are primarily affected by lipid peroxidation [4]. Table 1 summarizes the relevant fatty acids and their functions in the sperm.

As previously described in Table 1, the functions of these fatty acids are important in maintaining normal sperm function. Without proper sperm function, the likelihood of poor reproductive outcomes rises. Studies on the impact of lipid peroxidation on these specific fatty acids propose that modifications in these lipids are associated with male infertility. According to previous studies, asthenozoospermia, oligozoospermia, and azoospermia were associated with changes to lipids and phospholipids in the sperm membrane [17]. Previous literature also suggests that increases in linolenic, arachidonic, palmitic, and steric acid are associated with infertility [17], [18], [20]-[22]. Whereas, decreased levels of linoleic, docosahexaenoic, and eicosapentaenoic acid are found in infertile subjects [20], [22].

To further investigate the fatty acid composition and its basis in infertility, Gulaya et al analyzed semen samples of five infertile men and three healthy controls [17]. Although the sample size was small, this study offered interesting insight into the changes in fatty acid composition. Infertile men exhibited increased palmitic acid, linolenic acid, and docosahexaenoic acid ($P < 0.05$) [17]. Moreover, they also had significantly decreased eicosapentaenoic and steric acid concentrations [17]. In the discussion, Gulaya et al attributed these changes to lipid peroxidative processes of the relevant polyunsaturated fatty acids [17]. However, the decrease in steric acid levels found in the infertile group of this study is not consistent with previously established notions, but the small

Table 1: Summary of Relevant Fatty Acids and their Functions.

Fatty Acid	Classification	Chemical Formula	Function	Reference
Linolenic acid	Polyunsaturated fatty acid	18:2n-6	Presence is negatively correlated with sperm motility	Gulaya et al, 2001 [17]
Arachidonic acid	Polyunsaturated fatty acid	20:4n-6	Causes a pro-inflammatory response Has important roles in sperm capitation and the acrosome reaction	Oborna et al, 2010 [18]
Alpha-linoleic acid	Polyunsaturated fatty acid	18:3n-3	Important for maintain the properties of the lipid bilayer	Gulaya et al, 2001 [17] Lenzi et al, 1996 [19] Safarinjad et al, 2009 [20]
Palmitic acid	Saturated fatty acid	16:0	Most abundant in sperm bilayer	Tavilani et al, 2007 [21]
Steric acid	Saturated fatty acid	18:0	Second most abundant saturated fatty acid in human semen	Tavilani et al, 2007 [21]
Eicosapentaenoic acid	Polyunsaturated fatty acid	20:5n-3	Important for maintaining the properties of the lipid bilayer	Safarinjad et al, 2009 [20]
Docosahexaenoic acid	Polyunsaturated fatty acid	22:6n-3	Major polyunsaturated fatty acid in human sperm Functions in regulating membrane fluidity Related to normal sperm structure, production, and function	Stramova et al, 2014 [22]

sample size could explain this discrepancy. In a similarly sized study, Tavilani et al found that decreases in linoleic acid ($P < 0.05$), and statistically significant increases in palmitic, steric and docosahexaenoic acids in infertile patients [21].

However, both studies of Guyala et al and Tavilani et al included small sample sizes; therefore, a larger, more comprehensive study

is required for better understanding of the fatty acid composition differences [17], [21]. In 2010, Safarinejad et al assessed sperm fatty acid compositions in 82 infertile men and 74 fertile men [20]. As summarized in Table 2, there were significant decreases in eicosopentaenoic, alpha-linoleic, and docosahexaenoic acids in infertile men [20]. Additionally, levels of palmitic and steric

Table 2: Fatty Acid Composition Changes due to Lipid Peroxidation.

Study	Study Question	Subject Groups	Assay Specifics	Model	Select Phospholipid	Changes in infertility	Detected Value (p value)
Gulaya et al, 2001 [17]	Relationship between fatty acid composition and male fertility	Control group; healthy men n=3 (mean \pm SE) Experimental group; infertile men n=5 (mean \pm SE)	Gas-liquid chromatography "Chrom-5"	Human sperm	Linoleic acid ($\mu\text{g Pi}/10\text{ mL}$)	↑	$P > 0.05$
					Arachidonic acid ($\mu\text{g Pi}/10\text{ mL}$)	↑	$P > 0.05$
					Linolenic acid ($\mu\text{g Pi}/10\text{ mL}$)	↑	$P < 0.05$
					Palmitic acid ($\mu\text{g Pi}/10\text{ mL}$)	↑	$P < 0.05$
					Steric acid ($\mu\text{g Pi}/10\text{ mL}$)	↓	$P < 0.05$
					Oleic acid ($\mu\text{g Pi}/10\text{ mL}$)	↓	$P < 0.05$
					Eicosapentaenoic acid ($\mu\text{g Pi}/10\text{ mL}$)	↑	$P > 0.05$
					Docosahexaenoic acid ($\mu\text{g Pi}/10\text{ mL}$)	↓	$P < 0.05$
Tavilani et al, 2007 [21]	Differences in fatty acid composition of sperm in men with normozoospermia and those with asthenozoospermia	Control group; normozoospermic males n=8 (mean \pm SE) Experimental group; asthenozoospermic males n=15 (mean \pm SE)	Gas chromatography with flame ionization detector	Human sperm	Linoleic acid ($\text{nmol}/10^8\text{ spermatozoa}$)	↓	$P < 0.05$
					Arachidonic acid ($\text{nmol}/10^8\text{ spermatozoa}$)	↓	$P > 0.05$
					Linolenic acid ($\text{nmol}/10^8\text{ spermatozoa}$)	↓	$P > 0.05$
					Palmitic acid ($\text{nmol}/10^8\text{ spermatozoa}$)	↓	$P > 0.05$
					Stearic acid ($\text{nmol}/10^8\text{ spermatozoa}$)	↑	$P < 0.01$
					Docosahexaenoic acid ($\text{nmol}/10^8\text{ spermatozoa}$)	↑	$P < 0.001$
Safarinejad et al, 2010 [20]	Comparison of semen lipid composition in fertile and infertile men	Control group; fertile men n=78 (mean% \pm SD) Experimental group; infertile men n=82 (mean% \pm SD)	Capillary gas chromatography	Human sperm	Linoleic acid (% of total fatty acids)	↑	$P = 0.002$
					Arachidonic acid (% of total fatty acids)	↑	$P = 0.002$
					Alpha-Linolenic acid (% of total fatty acids)	↑	$P = 0.002$
					Eicosapentaenoic acid (% of total fatty acids)	↓	$P = 0.002$
					Docosahexaenoic acid (% of total fatty acids)	↓	$P = 0.003$
					↓	$P = 0.001$	

acids were both increased [20]. This study also reiterates the presence of specific fatty acid changes that could be linked to male infertility.

While the previously mentioned articles were focused on the presence of certain fatty acid acids in infertile men, Witczak et al analyzed 129 semen samples to determine if specific concentrations (weight%/total fatty acids) of these fatty acids are related to normal sperm morphology [23]. Of all the relevant fatty acids mentioned earlier, the presence of docosahexaenoic acid was positively correlated with normal sperm morphology. Therefore, lower levels of docosahexaenoic acid in infertile men may be responsible for sperm dysfunction. These studies show that lipid peroxidation could have a direct influence on overall sperm health and function. Moreover, alterations to sperm fatty acid composition appear to be associated with infertility.

Lipid Peroxidation and Oocytes

Like sperm, oocytes contain a high lipid content, which can be affected by lipid peroxidation. In a 2015 murine study, Lord et al determined that aging oocytes contained 4-hydroxynonenal, malondialdehyde, and acrolein, byproducts of lipid peroxidation [24]. While this mouse study implies that lipid peroxidation may affect oocyte function, little analysis has been done to correlate oocyte dysfunction with the fatty acid composition. In an early study on fatty acid composition of oocytes, Matorras et al studied 150 "failed fertilization" oocytes from 43 IVF patients [25]. Matorras et al compared oocyte lipid concentrations with adipose tissue, which also is used as an energy source in the body [25]. The goal of this study was to better understand the lipid composition of oocyte membranes. Based on the results, stearic and palmitic acids were most abundant of all fatty acids in oocytes [25]. Oocytes also have oleic, linoleic, eicosapentaenoic, and docosahexaenoic acids [25]. Additionally, the ratio of eicosapentaenoic acid to docosahexaenoic acid was approximately five [25]. Because this result was not seen in adipose tissue, Matorras et al concluded that this ratio may be unique to oocytes [25]. While they did not further discuss the importance of this finding, these lipids were concluded to be major sources of energy for the oocyte. However, this study design did not compare fatty acid compositions in both "failed fertilization" and unfertilized oocytes. The authors acknowledged that comparing oocytes before fertilization and unfertilized oocytes would have been scientifically important, yet ethically inappropriate [25]. Therefore, no consensus on the impact of fatty acid composition on fertility could be made. However, the fatty acid profile of oocyte membranes appears to somewhat similar to that of sperm membranes. Given this knowledge, the possibility of lipid peroxidation-mediated membrane changes in oocytes, much like in sperm, has been introduced. For example, it has been suggested that oxidatively damaged oocytes may have low levels of polyunsaturated fatty acids, which could prevent fertilization [25]. Yet, additional research in the field is still needed to assess this theory.

Effects of Dyslipidemia and Obesity on Infertility

Lipid Peroxidation and Dyslipidemia

The properties of lipid peroxidation and related lipotoxicity raise questions about the impact of overlying obesity and dyslipidemia on reproductive function. Increased lipid

peroxidation and damaging lipid membrane changes are often secondary to systemic and local accumulations of lipids. By increasing the amount of circulating lipids, there may be a higher likelihood of increased lipid peroxidation. The rising trends of obesity and associated dyslipidemia could be one possible factor in the widespread problem of infertility in the US. According to the National Health and Nutrition Examination from 2009-2010, more than 2 in 3 adults in the US are considered overweight while 1 in 3 are clinically obese [26]. While this phenomenon is not completely understood, hyperlipidemia, especially due to lifestyle choices, has been correlated to increased lipid peroxidation [27].

Effect of Dyslipidemia in Men

The direct consequence of increased dietary fatty acids may be seen in a 2015 case-control study on the effect of dietary fatty acid intake on sperm function. Eslamian et al assessed differences in fatty acid levels in normospermic and asthenozoospermic patients [28]. Classically, consumption of saturated fatty acids is associated with obesity and cardiovascular disease [28]. Eslamian et al conducted to assess the differences in dietary fat intake in asthenozoospermic men (n=107) and normozoospermic controls (n=235) [28]. They determined increased dietary intake of total saturated fatty acids, total fatty acid intake, palmitic acid, and steric acid was associated with asthenozoospermia [28].

While this study focuses on dietary intake, other literature considers the impact of systemic disease, such as dyslipidemia, hypercholesteremia, and hypertriglyceremia, on fertility. Several studies found no significant effect of dyslipidemia on infertility. Bobjer et al investigated the lipid profile changes in eugonadal and hypogonadal infertile men with non-obstructive azoospermia [29]. This study included 39 eugonadal infertile men and 26 hypogonadal infertile men [29]. Total plasma cholesterol, plasma HDL cholesterol, plasma LDL cholesterol, plasma triglycerides, and plasma LDL/HDL ratio levels were studied [29]. However, none of these variables were statistically different between the eugonadal and hypogonadal groups [29]. In several studies, low testosterone levels were used as a proxy indicator of infertility. Naifer et al found men with normal testosterone levels (n=13) and abnormal testosterone levels (n=8) had no statistically significant difference in triglyceride levels [30]. However, other studies demonstrated a link between dyslipidemia and infertility. However, Hagiuda et al found statistically significant differences in testosterone levels in 101 men with triglyceride levels of less than 150 mg/dl (control group) and 66 men with levels higher than 150 mg/dl (experimental group) [31]. Likewise, Garcia-Cruz et al found that dyslipidemic men (n=305) have significantly lower testosterone levels compared to non-dyslipidemic controls (n=78) (P=0.013) [32].

Metabolic syndrome also appears to be associated with low testosterone levels and subsequent reproductive issues, as summarized in Table 3. Metabolic syndrome is defined as three or more of the following criteria: elevated triglycerides, abdominal obesity, low HDL cholesterol, high blood pressure, and high fasting glucose. In a 2016 study, Ventimiglia et al found statistically different testosterone levels in 128 men with metabolic syndrome and 1209 men without metabolic syndrome [33]. Likewise, in a small study, Naifer et al found a similar association between men with metabolic syndrome (n=31) and without metabolic syndrome (n=9) [30].

Table 3: The Role of Dyslipidemia or Metabolic Syndrome in Male Infertility.

Study	Study Question	Subject Groups	Assay Specifics	Model	Study Variable	Detected Value (p value)
Bobjer <i>et al</i> , 2012 [29]	Lipid profile changes in eugonadal and hypogonadal infertile men with non-obstructive azoospermia undergoing testicular sperm extraction (TESE)	Control group; eugonadal infertile men with history of non-obstructive azoospermia n=39 (mean (SD)) Experimental group; hypogonadal infertile men with history of non-obstructive azoospermia n=26 (mean (SD))	Lipid concentrations determined by standard enzymatic methods (intra-assay CV: 3-5%)	Human sperm	Total plasma cholesterol (mmol/L) Plasma HDL-cholesterol (mmol/L) Plasma LDL-cholesterol (mmol/L) Plasma Triglycerides (mmol/L) Plasma LDL/HDL ratio	P=0.19 P=0.78 P=0.22 P=0.34 P=0.07
Hagiuda <i>et al</i> , 2012 [31]	Effect of elevated serum triglycerides (TG) and dyslipidemia on testosterone levels in males	Control group; standard-TG <150 mg dl ⁻¹ n=101 (mean ± SD) Experimental group; hypertriglyceridemia- TG >150 mg dl ⁻¹ n=66 (mean ± SD)	Not given	Human semen	Testosterone (ng ml ⁻¹)	P=0.00335
Ventimiglia <i>et al</i> , 2016 [33]	Effect of metabolic syndrome on testosterone levels in European men	Control group; men without metabolic syndrome n=1209 (median –IQR) Experimental group; men with metabolic syndrome n=128 (median – IQR)	Direct chemiluminescence immunometric assay on Immulite 2000 (Medical Systems SpA, Genoa, Italy)	Human serum	Total testosterone (ng/mL)	P<0.001
Garcia-Cruz, <i>et al</i> , 2012 [32]	Relationship between dyslipidemia and testosterone levels	Control group; men without dyslipidemia n=305 (mean ± SD) Experimental group; men with dyslipidemia n=78 (mean ± SD)	Automated immunometric chemiluminescent method (Cobas Roche, West Sussex, UK)	Human serum	Testosterone (ng ml ⁻¹)	P=0.013
Naifar <i>et al</i> , 2014 [30]	Relationship between hypertriglyceridemia and hypotestosteronism	Control group; patients with normal testosterone levels n=13 (mean (SD)) Experimental group; patients with abnormal testosterone levels n=8 (mean (SD))	Not given	Human serum	Hypertriglyceridemia	P=0.544
Naifar <i>et al</i> , 2014 [30]	Relationship between metabolic syndrome and hypotestosteronism	Control group; patients without metabolic syndrome n=31 (mean (SD)) Experimental group; patients with metabolic syndrome n=9 (mean (SD))	Not given	Human serum	Testosterone (ng ml ⁻¹)	P=0.004

Effect of Dyslipidemia in Women

The phenomenon of reproductive dysfunction secondary to dyslipidemia is also evident in oocytes. For example, female mice on a high-fat diet exhibited signs of oocyte dysfunction, such as heightened apoptosis and reduced mitochondrial activity [16]. Similar evidence of reproductive problems can be seen in patients with polycystic ovarian syndrome (PCOS). Along with

endometriosis, PCOS is one of the leading causes of infertility in women. Women with PCOS also face insulin resistance, obesity, dyslipidemia, and metabolic syndrome. Like endometriosis, infertility in PCOS is associated with oxidative damage. When comparing ROS levels of women with endometriosis and those with PCOS, women with PCOS were found to have higher ROS levels [10]. This suggests PCOS, which is associated

Table 4: The Role of Dyslipidemia, Obesity, and PCOS-related Changes in Female Infertility.

Study	Study Question	Subject Groups	Assay Specifics	Model	Study Variable	Detected Value (p value)
Kudesia <i>et al</i> , 2016 [34]	Relationship between abnormal lipid profiles and PCOS patients	Control group; non-PCOS infertile patients n=7 (mean \pm SD) Experimental group; PCOS patients n=10 (mean \pm SD)	Not given	Human serum	Lipid profile \approx 1 abnormality	P=0.64
Tsouma <i>et al</i> , 2014 [35]	Lipid profile differences in normal weight and over-weight/obese patients	Control group; non-PCOS patients with BMI <25 kg/m ² n= (mean \pm SD) Experimental group: non-PCOS patients with BMI >25 kg/m ² n= (mean \pm SD)	Abbott Architect c800 autoanalyser (Abbott Laboratories, North Chicago, IL, USA)	Human serum	TG (mg/dl) HDL-cholesterol (mg/dl) LDL-cholesterol (mg/dl)	P<0.001 P<0.001 P<0.001
Tsouma <i>et al</i> , 2014 [35]	Lipid profile differences in patients with PCOS	Control group; PCOS patients with BMI <25 kg/m ² (mean \pm SD) Experimental group; PCOS patients with BMI >25 kg/m ² (mean \pm SD)	Abbott Architect c800 autoanalyser (Abbott Laboratories, North Chicago, IL, USA)	Human serum	TG (mg/dl) HDL-cholesterol (mg/dl) LDL-cholesterol (mg/dl)	P<0.001 P<0.001 P<0.001

with significant dyslipidemia, may have more of an effect on reproductive function.

However, clear understanding of these mechanisms has not been elucidated. Previous and current literature have focused on finding the connection between the obesity and dyslipidemia and the PCOS-related infertility. As seen in Table 4, some studies have found no significant lipid profile abnormality in PCOS and non-PCOS patients [34]. Contrastingly, comparison of obese and non-obese PCOS patients (BMI >25 or <25 respectively) revealed significant higher lipid profile abnormalities in serum triglycerides, HDL, and LDL cholesterol in obese PCOS patients (P <0.001). This study suggests that obesity, not necessarily the presence of PCOS, may be responsible for infertility [35].

Further analysis of sexual function and obesity reveals contradictory results. In 2012, Ferraresi *et al* assessed sexual function using the Female Sexual Function Index (FSFI) questionnaire in obese and non-obese patients; however, the results were insignificant [36]. However, this paper suggested that the <26.55 FSFI scores seen from obese patients may indicate risk of sexual dysfunction [36]. This study however was based on qualitative data from patient-completed surveys; therefore, additional investigation is required to understand the relationship between obesity and sexual function. Using a more quantitative approach, Tsouma *et al* compared triglyceride, HDL, and LDL levels of non-PCOS patients with BMI >25 kg/m² and <25 kg/m² [35]. The results were statistically significant (P<0.001), suggesting again that higher BMIs lead to lipid profile abnormalities [35]. Patients with higher BMIs had higher triglyceride and LDL levels; whereas, they had lower HDL cholesterol values [35]. In the same study, the triglyceride, HDL, and LDL levels of PCOS patients with and without BMIs of <25 kg/m² were analyzed [35]. Similar results of high triglycerides, high LDL, and low HDL were appreciated in the high BMI PCOS group, showing that elevated BMI may play some role in PCOS-mediated infertility, as well as in non-PCOS patients.

Other Lifestyle Factors and Lipid Peroxidation-Mediated Infertility

Both men and women with dyslipidemia appear to face infertility. While the mechanism for this infertility is unclear, lipid peroxidation due to the excessive lipid content may be related. In men, dyslipidemia appears to be related to low levels of testosterone. Decreased testosterone is negatively correlated with fertility and sperm function. Likewise, women may also face infertility secondary to dyslipidemia as seen in PCOS-related infertility.

While obesity may be an important cause of lipid peroxidation, environmental factors may mediate this process. Shafiq-ur-Rehman *et al* observed the effects of lead on lipid peroxidation in human red blood cells [37]. Using malondialdehyde (MDA) as a marker for lipid peroxidation, the erythrocytes were exposed to increasing doses of lead [37]. As the amount of lead increased, the more MDA formed/30 minutes was appreciated [37]. Though this study was conducted in erythrocytes, a future study in gametes would reveal any association between lead exposure and infertility. In addition to lead, Eslamian *et al* demonstrated that smoking may be associated with sperm dysfunction [28]. This case-control study analyzed the differences between the normozoospermic (n=250) men and asthenozoospermic patients (n=115) [28]. The amount of smoking was significantly different in asthenozoospermic patients [28]. This group had fewer people who never smoked and more people who have smoked for \geq 20 years [28]. However, Stramova *et al* found no significant correlations among sperm function (motility and morphology) and smoking [22]. However, in this study, lower levels of malondialdehyde were correlated with better sperm motility in all patients (smoking and non-smoking) [22]. The Eslamian *et al* study utilized a case-control design, which accounted for other cofounders [28]. Similarly, Stramova *et al* tested semen samples from smokers and non-smokers; however, this study did not account for other cofounders in a case-control design. However,

because these results are contradictory, future research must be conducted to analyze the impact of factors, such as smoking, that can cause lipid peroxidation in gametes.

Discussion

With rising trends in obesity and related dyslipidemia, the impact of these metabolic abnormalities on reproductive function has been investigated. Both men and women with systemic dyslipidemia appear to be at risk for infertility. However, the mechanisms related to this infertility have yet to be completely elucidated. Previous and current research suggests that oxidative stress may be a major cause of dyslipidemia-induced reproductive dysfunction. This oxidative stress could be secondary to lipid peroxidation, a process that produces volatile, damaging radicals. Lipid peroxidation not only alters fatty acid composition of sperm and oocytes, but also could be the underlying principle that connects obesity with infertility in both sexes. The focus of the review is to understand and present the current evidence for this possible connection. However, future and further targeted research in both men and women is needed to better understand the role of lipid peroxidation in infertility. Despite this clear need for more evidence, this review promotes understanding of the damaging impact of obesity in reproductive health in order to better counsel patients.

Declaration of Interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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