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Understanding the role of long-chain polyunsaturated fatty acid and antioxidants in enhancing rooster semen quality: a comprehensive review

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A significant amount of long-chain polyunsaturated fatty acids, such as arachidonic acid (ARA) and docosatetraenoic acid (DHA) (C20:4n-6 and C22:4n-6, respectively), are present in rooster semen. The ARA is a form of omega-6 fatty acid with a vital part in several biological procedures such as cell membrane structure and sperm cell signaling pathways. The DHA is a major polyunsaturated, crucial for the rooster semen quality, thus linked to the male reproductive efficiency. Despite the DHA link to rooster semen quality and fertility, it has been found to undergo a serious decrease as roosters age. Moreover, the frozen-thawed rooster semen survival is still low, spurring innovative strategies to improve frozen-thawed rooster semen, sperm cell damage leading to the negative impact on motility, viability, and membrane integrity. These challenges sparked interest in using long-chain polyunsaturated fatty acids, particularly omega n-3 and omega n-6, to increase spermatozoa quality and reproductive efficiency. Noteworthy, chickens cannot synthesize omega n-3 and omega-n-6 *de novo*, necessitating their dietary supplementation. In rooster sperm, omega-6 polyunsaturated fatty acids are natural components that are vital for achieving an optimal fertility rate. However, the dietary supplementation of these long-chain polyunsaturated fatty acids alone has been reported to result in lipid peroxidation and sperm susceptibility to reactive oxygen species, necessitating the addition of a natural antioxidants. Although previous studies have shown that both fresh and preserved semen have improved semen parameters and a good fertility rate when antioxidants are supplemented to the diet, there have been conflicting results after adding antioxidants and long-chain polyunsaturated fatty acids (LCPUFAs) to the diet. Therefore, this review's goal is to postulate the understanding of the role of LCPUFA precursors as antioxidants, their challenges, and perspectives on the improvement of rooster semen quality. Enhancing rooster semen quality supports better fertility and hatchability in poultry, contributing to sustainable food production systems and ensuring affordable protein sources for communities, thereby addressing Sustainable Development Goals (SDGs) particularly on zero hunger and food security.

KEYWORDS

omega n-3, omega n-6, reactive oxygen species, fertility, sperm, SDGs

1 Introduction

Agenda 2063 of the African Union and the Sustainable Development Goals (SDGs) of the United Nations aim to address food security and end poverty in all its forms (Gumede, 2021; Sithole et al., 2025). In addition to being essential for farmers' survival in terms of food production and income generation, chickens provide an affordable means of obtaining vital protein sources, like eggs and meat for household consumption in order to maintain a balanced diet (Sithole et al., 2025). Moreover, smallholder farmers favor chickens because they are comparatively cheap to raise, need small space, and require fewer inputs. This alone can address the SDGs 1 and 2, which emphasize poverty elimination in all its forms (including securing balanced nutrition) and zero hunger (Street, 2023). Practically, achieving and eliminating poverty in all its forms remain the greatest challenge facing humankind despite significant interventions (Gumede, 2021). The ongoing population growth projected to reach 9 billion in 2050 will result in stagnation, inability to end poverty, and demand a doubling of food production (Adeleke and Babalola, 2020). This happens at a time when the world is highly affected by climate change, causing heat stress to animals, thus jeopardizing the production of animal products (Ngcobo et al., 2025).

The global temperature has been increasing by 0.2 to 0.3°C a decade since 1975 (Ngcobo et al., 2025). This global temperature rise has had a negative effect on the agriculture sector, thus jeopardizing its growth (Oke et al., 2024) and threatening food production. Provided that the poultry industry has been recognized as one of the crucial sectors in the livestock industry with the quickest growth rate, it is promising to improve nutrition and food security through its cheap products, such as eggs and meat, which are consumed by the majority worldwide (Oke et al., 2024). However, there are challenges in this sector, such as the lower male-to-female ratio in the flock, where males appear to be more crucial for flock fertility than females, highlighting the importance of improving male fertility (Ommati et al., 2013). Despite the recent interest in the application of long-chain polyunsaturated fatty acids (LCPUFAs) in rooster diets to improve their fertility (Alagawany et al., 2019; Cartoni Mancinelli et al., 2022), this area is still not well understood with regards to rooster semen quality. There is still an undescribed challenge of semen quality decline post-preservation (Silyukova et al., 2022), lowering the hatching rate (Maapola et al., 2023).

Poor post-thawed semen quality and hatching rate sparked an interest in the use of LCPUFAs, specifically omega n-3 (ω3) and omega n-6 (ω6), to advance reproductive efficiency through improving frozen-thawed spermatozoa. The LCPUFAs, particularly ω3 and ω6 precursors such as alpha-linolenic acid (ALA) and linoleic acid (LA), respectively, have been utilized to advance semen quality and reproductive efficiency of mammals (Ngcobo et al., 2021). The main distinction between ω3 and ω6 LCPUFAs is that ω3 has a double bond between the third and fourth carbons in its carbon chain, while the ω6 has a double bond between the 6th carbon atom (Balić et al., 2020). The presence of these LCPUFAs in the cellular membrane is vital for maintaining the soundness of the lipid bilayer (Sithole et al., 2025). Therefore, supplementing roosters' diets with ω3 polyunsaturated sources is thought to improve semen quality and fertility, especially from 39 to 47 weeks of age, as these fatty acids are minor natural constituents of chicken sperm (Alagawany et al., 2019). On the other hand, it is known that peroxidative damage is the primary cause of motility loss during rooster semen storage because it affects sperm morphology and decreases sperm motility (Long and Kramer, 2003), necessitating the enrichment of antioxidants to stabilize the production of reactive oxygen species (ROS) (Qamar et al., 2023). Sperms typically have a low antioxidant capacity; thus, enzymatic and non-enzymatic antioxidants in the seminal plasma shields the sperm by hunting ROS (Ommati et al., 2013). Furthermore, the sperm's antioxidant system functions to primarily protect the sperm's integrity and function by inhibiting apoptosis and the production of ROS (Qamar et al., 2023). This strikes a balance between the production of antioxidants and the development required to ensure that cells are functioning properly. Antioxidants are required and crucial for protecting sperm membranes from peroxidative damage (Partyka and Niżański, 2021). Despite some research done with a focus on the use of LCPUFAs on improving semen quality, very few studies are related to the cryopreservation of rooster semen. Moreover, there are mixed outcomes after the enhancement of diets with LCPUFAs, with some studies reporting negative effect of feeding LCPUFAs on semen quality and fertility. Subsequently, this review aims to postulate the understanding of the role of LCPUFA precursors and antioxidants, their challenges, and perspectives on the improvement of rooster semen quality.

2 An overview of chicken physiology

Roosters have a special reproductive system with their testicles located inside, below the dorsal abdomen and shaped in an oval or bean shape (Mfoundou et al., 2022). Their sperm remain viable inside the body and at the body temperature, unlike mammalian spermatozoa (Rutllant and Khamas, 2024). They are characterized by having semen that is milky white and highly concentrated in comparison to mammalian semen (Mfoundou et al., 2022; Rutllant and Khamas, 2024). Nevertheless, it is well known that improved roosters' reproductive success depends on early testicular development (Du et al., 2021). Therefore, the development and maturing of Sertoli and Leydig cells from 2 to 15 weeks is the crucial stage in early development of rooster testicles (Mucksová et al., 2009). By aiding pluripotent primordial germ cells in developing into spermatogonia, these cells sustain the germinal cells throughout the chickens' lives (Feng et al., 2015) hence, the time frame from 10 to 15 weeks is the fundamental stage for later testicular development with the help from steroids hormones (Feng et al., 2015).

Serum levels of hormones, including testosterone (T), follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), and luteinizing hormone (LH) on day 35 significantly increased in chickens (Feng et al., 2015). This is attributed to the fact that in the rooster testes, the FSH, LH, and testosterone determine both spermatogenesis and steroidogenesis (Vizcarra et al., 2010). Follicle-stimulating hormone and LH control spermatogenesis through cyclic adenosine 3, 5'-monophosphate (cAMP) (Huang et al., 2023) while LH further binds receptors in the membrane of Leydig cells and triggers the secretion of testosterone. Generally, the mechanism of hormones in chickens is that FSH activates spermatogenesis by binding to its receptors on the Sertoli cells' membranes (Santi et al., 2020; Shah et al., 2021). As a result, in rooster breeders, testicular function is linked to FSH concentrations, which then triggers a strong correlation between FSH and testis weight (Feng et al., 2015). On the other hand, the maturation of spermatozoa and the initiation of spermatogenesis depend on FSH (Oduwole et al., 2018). The testosterone levels, on the other hand, determine the development of testicles and how roosters behave (Feng et al., 2015) while acting on the Sertoli and peritubular cells of the seminiferous tubules and stimulating spermatogenesis.

2.1 Significance of the unique physiology of chickens and fertilization

The reproductive physiology of chickens is developed to an extent that they have one ovary that is working, as compared to mammals with two working ovaries (Rutllant and Khamas, 2024; Sithole et al., 2025). Additionally, the right ovary halts developing when the female hatches, but the left one keeps maturing, leaving the female with just the left ovary for the remainder of her reproductive life. Noteworthy, in poultry, fertilization happens internally, with the sperm meeting the egg within the chicken's reproductive tract, while with mammals, fertilization can be either internal or external depending on the species (Gwatkin, 2012). This makes it impossible to preserve chicken embryos *in vitro*. Hence currently, cryopreservation of semen (despite its challenges) is the sole feasible and less costly *in vitro* method for preserving chicken germplasm *ex situ* (Maapola et al., 2023; Sithole et al., 2025).

3 An overview of the long-chain polyunsaturated fatty acids composition

The LCPUFAs include the ω6 and ω3 fatty acid family and contain about 20- and 22-carbon chains within their carbon molecule (Abedi and Sahari, 2014). As shown in Figure 1, the primary precursor for ω6 LCPUFA is linoleic acid (LA), while ω3 is synthesized by the alpha-linolenic acid (ALA) (Miles et al., 2021). Owing to the absence of Δ-12 and Δ-15 desaturase enzymes, which are necessary for forming carbon-carbon double bonds outside the Δ-12 and Δ-15 carbons, these precursors cannot be produced *de novo* (Figure 1) in livestock, such as chickens, hence, they should be supplemented in the diet (Ngcobo et al., 2021). Among many sources of ω3 and ω6, additives such as seeds, nuts, vegetable oils and vegetable oil-based spreads are the richest source, however, eicosapentaenoic acid (EPA) and DHA ω3 can be found in seafood, particularly fatty or oily fish, and supplements like fish oils, while ARA ω6 can be located in meat and eggs (Miles et al., 2021). Despite all these knowledge, there are documented drawbacks to fish oil use, which includes the presence of heavy metal contamination and antibiotics in fish sources (Sidhu, 2003), as well as smells and odors that are undesirable, issues with stability, high cost, and challenges in purifying because of the small levels of DHA and EPA fatty acids (Abedi and Sahari, 2014). Moreover, there is a reported competition between humans and animals for fish, and human fishing activities often remove fish at unsustainable rates, which may lead to the extinction of fish due to overfishing activities (Department of Forestry, Fisheries and the Environment (DFFE) et al., 2023).

3.1 Sources of long-chain polyunsaturated fatty acids

3.1.1 Microbial sources

Microorganisms such as bacteria, fungi, algae, mosses, and protozoa can manufacture diverse polyunsaturated fatty acids (PUFAs) through aerobic and anaerobic pathways (Abedi and Sahari, 2014). Microorganisms provide superior substitutes for the production of PUFAs (Huang et al., 2023). Most notably, microalgae and oleaginous fungi, such as *Schizochytrium sp*, *Yarrowia lipolytica*, and *Mortierella alpina*, have been successfully utilized as primary sources of DHA, EPA, and ARA, respectively.

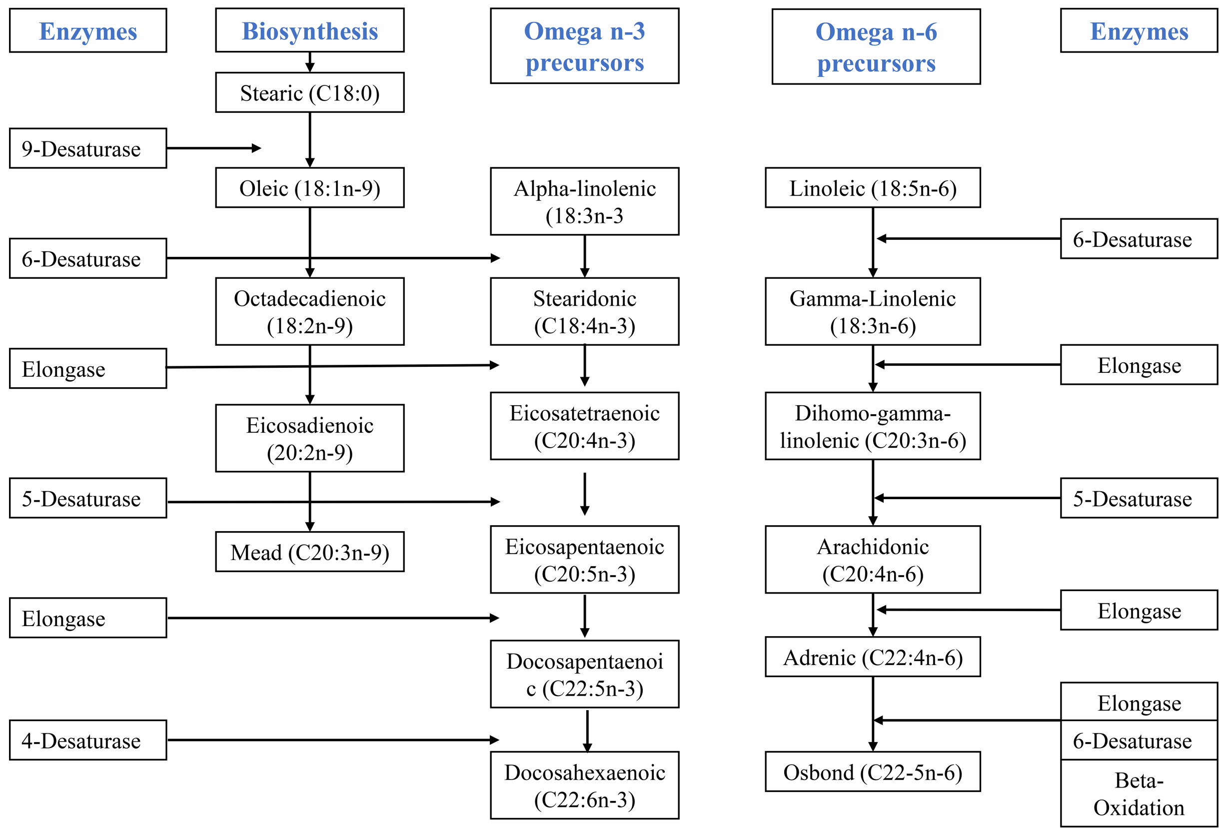

FIGURE 1
Mechanism of the biosynthesis of long-chain polyunsaturated fatty acids from precursors of critical fatty acids. Adapted from (Ngcobo et al., 2021) and modified.

The *Moritella marina* bacteria, *Thraustochytrium* spp, and *Entomophthora* spp *fungi*, and other species are from the *Thraustochytriales*, including *Thraustochytrium aureum*, *Thraustochytrium roseum*, and *Thraustochytrium* sp. *ATCC 20892*, which is a family that is known to be microorganisms that renders extreme levels of DHA (Wu et al., 2005). The microorganisms known as microalgae, on the other hand, are powered by light and generate useful metabolites including PUFAs, antioxidants, and antimicrobials (Guedes et al., 2011; Abedi and Sahari, 2014). As a substitute for more expensive plant and animal sources, they have been viewed as vectors for the commercial production of oils and fats; their benefits over fish oils include their easy refining, low danger of chemical contamination, and lack of unpleasant odor (Guedes et al., 2011).

3.1.2 Aquatic sources

The key aquatic species that have been proven to have LCPUFAs include fishes, shrimps, prawns, crabs, shellfish, and algae (Abedi and Sahari, 2014). The ω3 LCPUFAs can be acquired from fatty fish, such as salmon, tuna, mackerel, anchovies, capelin, Atlantic cod, Atlantic herring, Atlantic mackerel, Atlantic menhaden, salmonids, sardines, shark (liver), herring, sardines, and fish oil (Hoppenbrouwers et al., 2019). These sources are particularly rich in long-chain ω3 PUFAs, including EPA and DHA, which are not readily converted from plant-based ALA in the body, and are often a good source of other essential nutrients like iodine and selenium, which are not as readily available in many plant-based foods (Marsol-Vall et al., 2022).

3.1.3 Animal sources

The majority of essential fatty acids originates from milk, dairy products, lamb, beef, poultry, eggs, and pork (Woods and Fearon, 2009). These are mostly guided by the composition of the diets, the digestive system of the animal, and by the biosynthesis mechanisms within the animal (Woods and Fearon, 2009; Abedi and Sahari, 2014). Animal sources offer several benefits (higher bioavailability of EPA and DHA, and the presence of fat-soluble vitamins) over plant sources when it comes to PUFAs, especially ω3s. They provide pre-formed EPA and DHA, which are readily usable by the body, unlike plant sources, which primarily provide ALA, which requires conversion to EPA and DHA (Mahaffey et al., 2011). Nevertheless, a lot of research has reported negative and positive effects of animal sources on fertility and other factors. For example, milk with a high concentration of unsaturated fatty acids, particularly PUFAs, was found to be more prone to oxidation and the development of off-flavors (Woods and Fearon, 2009). On the other hand, egg yolk serves as a great source of PUFAs, especially DHA, which contains 0.1% EPA, 0.7% DHA, and 0.8% ALA (Abedi and Sahari, 2014).

3.1.4 Plant sources

Plant-based sources of LCPUFAs for chickens include various seeds, such as oilseeds, nuts, seeds, and certain algae (Nguyen et al., 2018). Moreover, most of these seeds are acknowledged to be rich in ALA, a precursor for the ω3 LCPUFAs EPA and DHA, which are beneficial for animal health and further utilized for the withdrawal of oils rich in ω3 PUFAs (Rizzo et al., 2023). Amongst these sources, flaxseed has been outstanding, consisting of high ALA concentrations with a total fatty acid percentage ranging from 39-60% (Goyal et al., 2014; Zamani Ghaleshahi et al., 2020). It is considered as a good source of ALA, which can be converted to other beneficial fatty acids in chickens (Kartikasari et al., 2012). Moreover, incorporating flaxseed in chickens' diet helps in supporting their immune systems, making them more resistant to common chicken diseases since flaxseed oil contains anti-inflammatory properties of ω3 fatty acids help in reducing the risks associated with inflammation (Lee et al., 2021). Black raspberry seed oil is another source of LCPUFAs, comprising 35% ALA of the overall fats and 98-99% of unsaturated fatty acids; however, with slightly lower ratios of ω6 to ω3 at 1:6:1 (Parry and Yu, 2004). Boysenberry seed oil is another plant-based source comprising a high percentage (19.5%) of ALA and a ratio of ω6 to ω3 fatty acid of 2:8:1. It has a very high PUFA concentration of 73.3% and more than 91% of the seed oil made up of all unsaturated fatty acids (Yu et al., 2020).

4 Role of long-chain polyunsaturated fatty acids in improving fertility rates and semen quality of roosters

Dietary supplementation with LCPUFAs, particularly ω3 such as DHA and EPA, can influence rooster semen quality (Yuan et al., 2023) and has many advantages on semen quality as shown in Figure 2. Moreover, adding ω3 to the diet is reported to influence chicken's immunity, resulting in the production of chicken products that are good for consumers' health (Mousa et al., 2017). This has triggered numerous studies in chickens to focus on the functional action of various LCPUFA varieties and their dietary levels on the mechanism of lipids. The use of LCPUFAs in chicken diets has been confirmed to significantly decrease cholesterol and overall lipid content in the blood (Alagawany et al., 2019). Fatty acids have several effects, such as improving sperm cell quality through improving fertility, quantity, and overall semen quality (Ngcobo et al., 2024), hence, several studies reported good results on the use of PUFAs (Al-Daraji et al., 2010; Eslami et al., 2016; Badwy et al., 2024). Moreover, a study by (Bongalhardo et al., 2009) reported that adding animal sources such as fish oil to rooster diets influenced fertility through lowering fatty acid ratio (ω3:ω6) in sperm membranes, which may modify the physical properties of the membrane or induce resistance to peroxidative

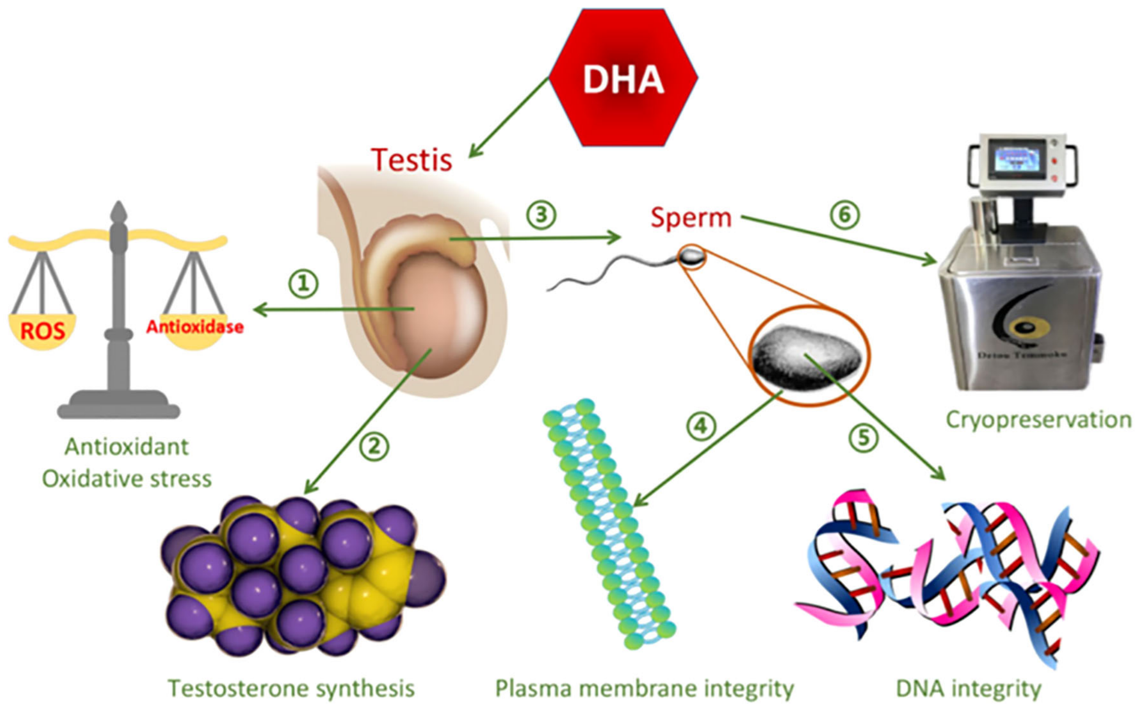

FIGURE 2
The functions of DHA on semen quality: 1. Improves antioxidant capacity and decrease oxidative stress 2. Enhance testosterone synthesis 3. Enhance sperm maturation 4. Improves plasma membrane integrity of the sperm 5. Improves integrity of the DNA 6. Prevent sperms from damage caused by cryopreservation (Yuan et al., 2023).

damage. Furthermore, a diet enriched with a medium ratio of ω3: ω6 fatty acids influenced DHA and ω-3 PUFAs while reducing DHA and ARA in rooster sperm (Al-Daraji et al., 2010). However, some studies have indicated that high doses of unsaturated fatty acids, which are examples of high doses of LCPUFAs, are harmful to health, raise the amount of oxidative stress, and reduce the production of testosterone crucial enzymes, all of which will have a negative impact on semen quality (Yuan et al., 2023).

Lipids are an essential element of the sperm which aids not only in the energy metabolism of sperm but also in various roles linked with fertility (Zaniboni et al., 2006). Rooster sperm contain high levels of LCPUFAs, in which the primary LCPUFAs are AA and DHA (C20:4n-6 and C22:4n-6, respectively, which are positively correlated with fertility rate (Abbaspour et al., 2020). Their multiple double bonds enhance membrane flexibility and support signal transduction pathways crucial for sperm motility and viability. However, this same structural feature makes LCPUFAs highly vulnerable to reactive oxygen species (ROS), predisposing spermatozoa to lipid peroxidation, DNA fragmentation, and reduced functionality during storage and cryopreservation (Wang et al., 2025). Reactive oxygen species are by-products of normal cellular metabolism and play dual roles in sperm physiology (Chandimali et al., 2025). At physiological levels, ROS are necessary for capacitation, hyperactivation, and acrosome reaction (Dutta et al., 2019). It has been proven that the leading fatty acids in sperm of roosters are ω6. Many studies (see Table 1) have investigated the effect of dietary enrichment of LCPUFAs from diverse sources on semen quality and fertility of roosters. For instance (Zanussi et al., 2019), reported that supplementing with flaxseed at 2% improved overall semen fertility (control-85.4% and flax- 91.67%) by enhancing semen performance and fertility potential. According to (Badwy et al., 2024), 1% PSO improved semen variables such as volume, sperm quality, sperm morphology, and velocities. Comparable results were observed by (Qi et al., 2019) where the inclusion of 2% flaxseed was used, and the conclusion was that the 2% group had an improved semen quality (control- 70.83% and 2%flaxseed- 82.29%) compared to the other treatment group. These benefits are attributed to enrichment of DHA and eicosapentaenoic acid (EPA) in sperm membranes, which optimize membrane stability and protect against premature acrosome reaction. However (Abbaspour et al., 2020), reported that whole flaxseed supplementation at 2% was unable to modify the fatty acid composition of the sperm or enhance the quality of the sperm from aged broiler breeder roosters. A documented belief about the effect of dietary ω3 fatty acids on sperm is that they can alter the quantity and composition of PUFA in the head and tail of sperm (Esmaeili et al., 2015). Furthermore, it is clear that ω3 PUFAs have a role in many sperm physiological processes, and that dietary ω3 fatty acids enhance certain sperm traits, such as sperm motility (Feng et al., 2015; Abbaspour et al., 2020). Notably, a study by (Feng et al., 2015) used ω3 and ω6 additives (soya bean oil and flaxseed oil, respectively) and reported an increase in the spermatogonia development and germ cell layers of roosters.

Despite these promising results, some studies have reported negative results such as that high levels of LCPUFA supplementation increase oxidative stress, elevate malondialdehyde (MDA) production (a marker of lipid peroxidation), and even reduce testosterone synthesis (Table 1). These detrimental effects appear to be dose-dependent and are more pronounced when antioxidant supplementation is absent. For instance (Cerolini et al., 2006) reported that dietary supplementation

TABLE 1 Effect of long-chain polyunsaturated fatty acids on the reproductive performance of cockerels fed different supplementation at different levels.

Breed	Source of LCPUFAS	Concentration used	Findings	References
Ross 308 broiler	Whole flaxseed	2% flaxseed	Did not improve the sperm quality of aged broiler cockerels	(Abbaspour et al., 2020)
			Exhibited no effect on the fatty acid profile of the sperms	
Cobb broiler	Fish oil	1% fish oil	Decreased non-progressive motion significantly	(Cerolini et al., 2006)
			Significant increase in MDA production	
Ross 308 breeder	Fish oil	15g/kg	Improved the quality of frozen-thawed semen	(Gandeshmin et al., 2020)
Ross 308 broiler	Oleate	0.125 and 0.25 mm	Both levels increased forward progressive motility at 24 hours .	(Eslami et al., 2016)
Japanese quail	Flax oil	3%	Improved reproductive performance of Japanese quail	(Al-Daraji et al., 2010)
Hy-line Silver-Brown	Fish oil	30g/kg	Decreased sperm viability of cockerels	(Olubowale et al., 2014)
Ross broiler breeder	Fish oil	15 g per kg	Increased total motility, progressive motility, and velocities	(Pourazadi et al., 2020)
Ross 308 breeder	Flaxseed oil	2%	Improved the semen performance and fertility potential	(Zanussi et al., 2019)
Sinai local strain	Pumpkin seed oil	1% PSO	Improved semen volume, concentration, sperm motility, abnormality, and velocity parameters	(Badwy et al., 2024)
Jing Hong breeder roosters	Soya bean oil and flaxseed oil	0.5% SO/1.5% FO	Developed 5–6 layers of germ cells on day 21	(Feng et al., 2015)
			Serum levels of GnRH were increased on day 21 and 35	

with 1% fish oil significantly increased MDA production (lipid peroxidation) (0.002 mg/10^9). The ability of LCPUFAs to shield the sperm from chemical (oxidative) and physical (cryopreservation) harm has been outlined in earlier research (Hudson and Wilson, 2003). Moreover, the contradiction in most research outcomes vary with the source of LCPUFAs, the age and physiological status of the chickens, and genetic background (Kartikasari et al., 2012). For instance, younger roosters respond more positively to supplementation compared to older breeders with semen quality that is already declining (Authaida et al., 2023). Furthermore, experimental design factors such as duration of feeding, type of extender used for semen storage, and housing conditions also influence results, contributing to the apparent inconsistencies across the literature (Arif et al., 2025; Halawa et al., 2025).

Noteworthy, the incorporation of LCPUFAs into sperm membranes modifies membrane lipid composition and biophysical properties (Catala, 2015). Higher levels of DHA have been associated with increased membrane fluidity, improved mitochondrial activity, and enhanced motility. In addition, LCPUFAs modulate hormonal signaling pathways and regulate the expression of genes linked to steroidogenesis and spermatogenesis (Mora et al., 2023). Nevertheless, according to (Sithole et al., 2025) if delivered in high concentrations, the membrane's polyunsaturated fatty acids make the sperm to be very vulnerable to reactive oxygen species (ROS), resulting in lipid peroxidation (see Figure 3). This then necessitates the addition of antioxidants such as vitamin E and ascorbic acid to maintain a stability between the generation of ROS and the readily available defensive antioxidants (Qamar et al., 2023).

The principal element of the sperm's antioxidant system is responsible for protecting the membrane from ROS and preventing apoptosis so to preserve the integrity and function of the sperm. Provided that the survival of sperm cell post the freeze-thaw procedure is reported to be still poor, cryopreservation of rooster semen remains a tough challenge (Maapola et al., 2023). Thus, using frozen-thawed semen for AI leads to decreased fertilization rate. An attempt to research the impact of a diet enhanced with LCPUFAs on the improvement of the sperm subsequent to cryopreservation has been made by minor studies in roosters. For instance (Gandeshmin et al., 2020), reported that enriching the diet of roosters using 15 g fish oil per kg diet increased the quality of frozen-thawed semen.

The evidence provided indicates that LCPUFAs are indispensable for optimal sperm function, however, their benefits are conditional. Supplementation improves semen quality when provided in moderate doses and in combination with sufficient antioxidant support. Conversely, excessive or unbalanced supplementation increases susceptibility to oxidative damage, counteracting potential gains (Chandimali et al., 2025). Thus, the key challenge is not whether LCPUFAs are beneficial, but rather how to optimize their levels, sources, and combinations with antioxidants for reliable improvements in fertility and semen preservation.

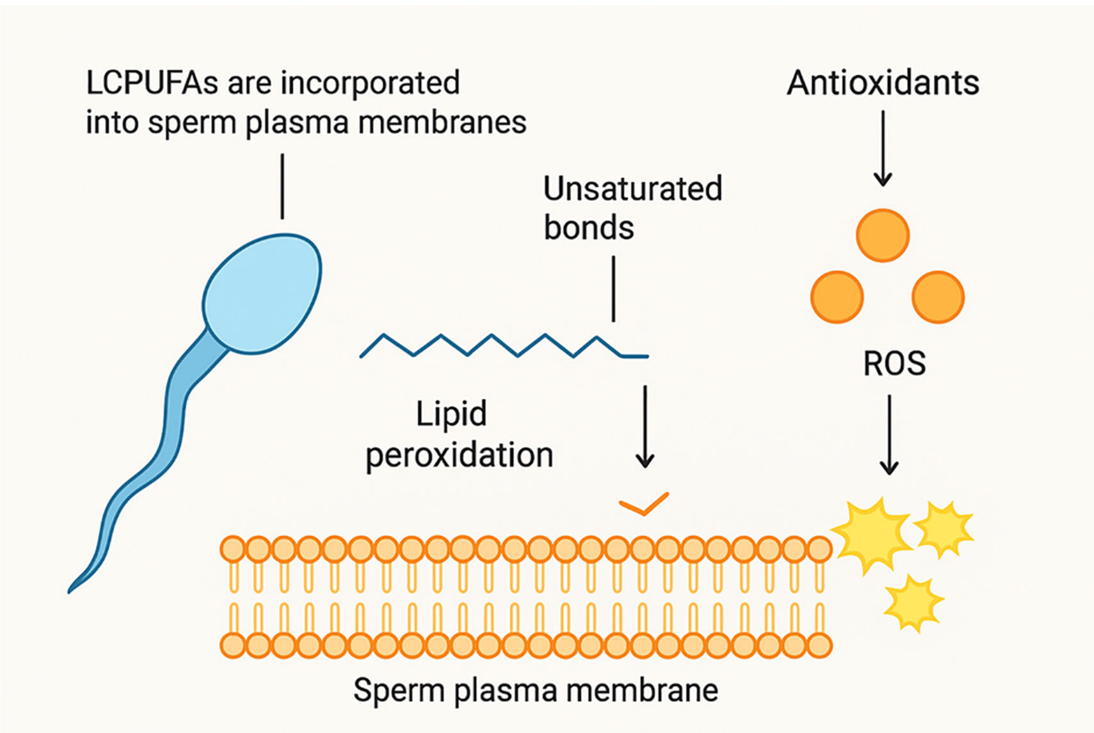

FIGURE 3
Mechanisms by which LCPUFAs and antioxidants affect the quality of cockerel semen (Wang et al., 2025).

5 Antioxidants role on the fertility preservation of sperm in roosters

Antioxidants are substances or enzymes that can eliminate, hunt, or defuse ROS and their effects on sperm quality (Jena et al., 2023). Antioxidants are there to preserve the structure and function of cells by defending the plasma membrane from ROS (Jena et al., 2023).They prevent deoxyribonucleic acid (DNA) fragmentation and early sperm maturation, lessen cryodamage, enhance sperm quality, and protect sperm from ROS generated by abnormal sperm or leukocytes. Additionally, they protect the integrity of the acrosome by stopping the premature acrosome response while operating by disrupting the oxidative chain reaction, which lowers oxidative stress (Qamar et al., 2023). Reactive oxygen species may be delivered physiologically and at low amounts to help with sperm maturation, capacitation, and acrosome response (Wang et al., 2025). They further help in striking a balance between ROS production on the physiological and pathological levels (Figure 4). Ironically, elevated ROS levels harm nuclear DNA and sperm membrane lipids, resulting in infertility and subfertility (Qamar et al., 2023). This is where the significance of a tightly regulated balance between ROS production and antioxidant defenses becomes evident. A variety of antioxidants can be used to treat cases of infertility and subfertility in order to alter the level of balance.

The antioxidant capacity of the sperm is lowered throughout the process of spermatogenesis as it loses the majority of its cytoplasmic contents; therefore, the antioxidant capacity of the seminal plasma is what determines how well the sperm is protected against ROS (Qamar et al., 2023; Antinozzi et al., 2025). Seminal plasma serves as the primary line of defense against extracellular ROS because it is composed of a variety of enzymatic and non-enzymatic antioxidant molecules, such as carotenoids (vitamin A), catalase (CAT), GSH, coenzyme Q 10 (CoQ10), glutathione reductase (GSH) glutathione peroxidase (GPx), pyruvate, superoxide dismutase (SOD), vitamin C, vitamin E, taurine, hypotaurine, and uric acid (Saleh and Hcld, 2002). The antioxidant system of the body is influenced by the dietary intake of antioxidants, minerals, and vitamins (Agarwal et al., 2005). The use of antioxidants to lessen the impact of ROS overproduction, whether right into the semen extenders or through dietary supplements, has been thoroughly studied and documented in the literature (Qamar et al., 2023). Dietary antioxidants often need treatment strategies that are more consistent and last longer in order to improve male fertility. Therefore, the dosage determines the impact of each antioxidant.

Table 2 below depicts the effect of antioxidants on the reproductive performance of roosters fed different supplementations, investigated by different studies. The positive effects of antioxidants inclusion to

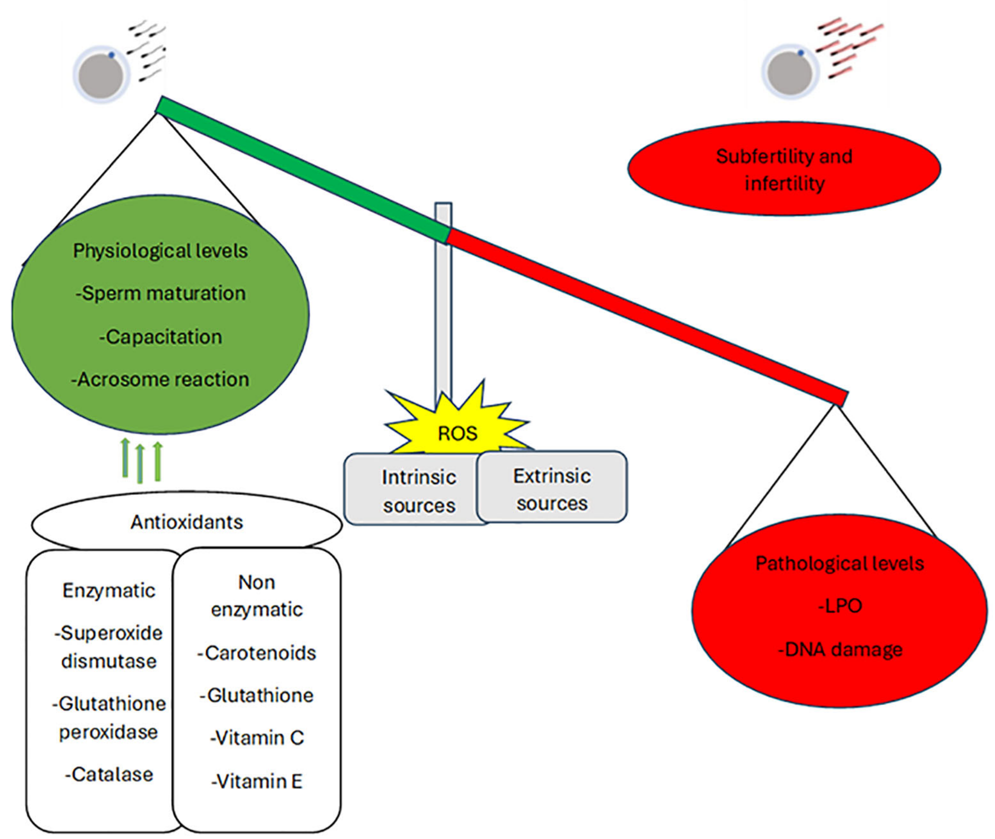

FIGURE 4
The impacts and balance of ROS on sperm fertilizing ability (Qamar et al., 2023).

TABLE 2 Effect of antioxidants on the reproductive performance of cockerels fed different supplementations.

Breed	Antioxidants	Level used	Findings	References
Arbor Acres	Vitamin E	150mg vE/kg	Advanced semen volume, amount of sperm cells, percentage live sperm, and reduced sperm abnormality	(Abioja et al., 2022)
Cobb broiler	Vitamin E	300 mg vE/kg and 100 mg vE/kg	Both levels improved the total sperm output and progressive motility	(Cerolini et al., 2006)
Ross broiler	Vitamin E	0.5%, 1%, 2% and 3%	Increased progressive sperm motility and viability rates for treatments enhanced with vitamin E	(Tabatabaei and Aghaei, 2012)
		0.5%, 1%, 2% and 3%	Decreased the rate of abnormal sperms in all the treatments groups with different levels of vitamin E	(Tabatabaei and Aghaei, 2012)
Noi crossbred roosters	Vitamin E	E75/kg	Increased the percentage of sperm abnormalities	(Khang et al., 2022)
Ross 308 breeder	Vitamin E	200 mg/kg VE	Improved the semen performance and fertility potential	(Zanussi et al., 2019)
Taiwan native	Vitamin E	160 mg/kg of VE	Improved maximum length of fertility at 49 weeks age	(Lin et al., 2005)
Inshas strain	Selenomethionine	0.30 and 0.45 mg/kg	Improved semen quality, fertility, hatchability and oxidative status of roosters	(Shamiah et al., 2017)
Rhode Island Red	Vitamin E	20, and 200 mg/kg	Led to increased levels of α-tocopherol in semen, testes, and liver	(Surai et al., 1998)
	Selenium	0. 3 mg Se/kg	The GSH-Px activities were stimulated in the seminal plasma, spermatozoa, testes, and liver	(Surai et al., 1998)
Egyptian local cross	Organic selenium	0.3 mg/kg	Enhanced the seminal plasma GSH-Px activity, enhanced total antioxidant capacity significantly, enhanced semen quality	(Ebeid, 2009)
Sinai local strain	Vitamin E	100 mg Vit. E/kg	Improved semen volume and concentration, sperm motility, abnormality, and velocity parameters	(Badwy et al., 2024)
Inshas cockerels	Glutathione	0.2 mM	Improved semen characteristics and fertility rates to extender of cockerel semen stored at 5 °C for up to 72 h	(Shamiah et al., 2017)
Lveyang black-boned breeder	VC +VE	300 mg/kg VC and 200 mg/kg VE	Boosted serum testosterone and sperm motility significantly Alleviated the oxidative stress made by dexamethasone	(Min et al., 2016)
Mandarah		200 mg/kg ascorbic acid or 150 mg/kg VE	Improved the quality of semen, seminal plasma, fertility and blood biochemistry and hematology of heat-stressed roosters	(Attia et al., 2020)

improve semen quality are evident from the above results. For instance, vitamin E inclusion at 150mg vE/kg increased semen volume (0.71% to 0.94%), number of sperm cells (1.42% to 2.52%), and percentage live sperm (86.8% to 96.9%) and reduced sperm abnormality (from 11.0% to 5.6%); decreased morphological defect rates of chicken spermatozoa; and improved the semen performance and fertility potential. When roosters were exposed to heat stress, the characteristics of semen quality improved; increased sperm count and motility, decreased the percentage of dead sperm, and improved the antioxidative status of seminal plasma. In contrast, the addition of antioxidants like selenium, selenomethionine, and glutathione increased the stimulating effect on GSH-Px activity in seminal plasma. Moreover, 300 mg/kg VC (84.0 ng/dL) and 200 mg/kg VE (91.2 ng/dL) were reported to enhance serum testosterone and sperm motility remarkably while also contributing to alleviating the oxidative stress caused by dexamethasone (Min et al., 2016). A study by (Attia et al., 2020) reported that 200 mg/kg ascorbic acid improved semen quality, fertility, seminal plasma, and blood biochemistry and hematology of heat-stressed roosters.

Contrary to the strong evidence for beneficial effects, in some studies, high doses of antioxidants such as excessive vitamin E or cysteamine have reduced motility and impaired sperm function. This paradox may be explained by the "antioxidant paradox," where excessive scavenging of ROS disrupts their physiological role in capacitation and acrosome reaction (Dutta et al., 2022). Moreover, variations in experimental design such as antioxidant form, administration procedure, treatment duration, environmental stressors such as temperature, housing, and genetic background of the chickens contribute to divergent results. Another factor is the interaction between antioxidants and LCPUFAs. While antioxidants are essential for preventing peroxidation of PUFA-enriched membranes, inadequate antioxidant support during LCPUFA supplementation amplifies oxidative stress (Kodali et al., 2020). Thus, contradictory findings often arise when LCPUFAs are fed without appropriate antioxidant balancing. Antioxidants act at multiple levels to protect spermatozoa, this may include membrane protection (vitamin E, being lipid-soluble, localizes within sperm membranes and prevents peroxidative chain reactions initiated by ROS); enzymatic defense (enzymes such as SOD catalyze the dismutation of superoxide anions into hydrogen peroxide, which is subsequently neutralized by CAT and GPx); DNA protection (water-soluble antioxidants like vitamin C scavenge ROS in the seminal plasma, reducing oxidative DNA damage); mitochondrial function (by limiting ROS accumulation, antioxidants help maintain mitochondrial activity, thereby sustaining ATP production and motility) (Kowalczyk, 2022; Qamar et al., 2023).

5.1 Antioxidants in semen cryopreservation

Though rooster semen is considered to have high activity of antioxidants, this cannot be enough to completely scavenge the detrimental effect of peroxidative sperm injury during semen cryopreservation (Partyka et al., 2012). There is no doubt that cryopreservation of semen enhances lipid peroxidation, leading to susceptibility of rooster semen to lipid peroxidation as the antioxidant activity decreases (Partyka et al., 2012; Fleming and Thomson, 2025). Numerous studies have shown that antioxidant-supplemented diluents offer significant defense against ROS and the development of lipid peroxidation in the semen (Baumber et al., 2000). However, minor percentage of these studies were addressing cryopreservation of rooster semen. Noteworthy previous studies addressed the impact of supplementing antioxidants in the diet on the quality of rooster semen (Bréque et al., 2003). Numerous studies have also used non-enzyme and enzyme antioxidants to improve semen quality before cryopreservation. For instance, in Table 3, antioxidants at different levels improved semen quality parameters like mitochondrial activity, sperm motility, and enhanced the functionality of seminal plasma, decreased MDA levels; however, 0.001- and 0.004-mM levels of cysteamine decreased sperm motility. That could be attributed to the fact that cysteamine leads to hydrogen peroxide production and oxidative stress, which declines glutathione peroxidase activity at high concentrations (Salimi et al., 2024).

Collectively, antioxidants are indispensable for protecting spermatozoa against oxidative stress and ensuring functional competence, particularly during storage and cryopreservation (Kaltsas, 2023). However, their effects are dose- and context-dependent, for instance, moderate supplementation enhances fertility, while excessive levels may disrupt normal physiological ROS signaling (Manful et al., 2025). Importantly, antioxidants should not be considered in isolation but as part of an integrated nutritional strategy with LCPUFAs. Optimizing their ratios, delivery methods, and timing of administration is likely to yield the most reliable improvements in semen quality and fertility.

6 Future prospects and or research directions into improving rooster semen quality

6.1 Optimizing LCPUFA supplementation for enhancing reproductive performance in roosters

The balance between ω3 and ω6 fatty acids in the diet is vital (Jeong et al., 2024). Therefore, future research is needed to focus and continue to explore optimal ratios for enhancing reproductive performance in roosters, as an imbalance can negatively impact semen quality (Clément et al., 2012). Moreover, adding antioxidants to semen extenders is one of the strategies currently practiced, and thus, future studies should explore the novel and more effective

TABLE 3 Effects of antioxidants on cryopreserved semen quality of cockerels.

Type of antioxidants	Level used	Findings	References
N-acetyl-L-cysteine	5 mM	Improved activities in the mitochondria, motility of the sperm; improved plasma membrane integrity of sperm, and protects against cell death	(Partyka et al., 2012)
Catalase (CAT)	200µg/mL	Improved sperm motility and viability,	(Amini et al., 2015)
Catalase (CAT)	100µg/mL	Decreased MDA level	(Amini et al., 2015)
L-carnitine (LC)	1mM	Protected against LPO, DNA fragmentation, apoptosis, and increases mitochondrial potential	(Partyka et al., 2017)
Superoxide dismutase	200 U/ml	Reduced lipid peroxidation in the membrane of the sperm, protects sperm against cell death, boosted sperm motility, and plasma membrane integrity	(Partyka et al., 2012)
Melatonin	10^{-3}M and 10^{-6}M	Both levels increased membrane integrity, mitochondrial activity, sperm motility, cell death, gave protection from the fragmentation of DNA, decreased LPO	(Mehaisen et al., 2020)
Cysteamine	0.001 and 0.004mM	Both levels decreased sperm motility	(Thananurak et al., 2020)
Taurine	1mM	Protected against apoptosis and DNA fragmentation, reduces LPO, and protected plasma membrane integrity	(Partyka et al., 2017)

natural and synthetic antioxidants to further alleviate oxidative damage during storage and improve post-storage semen quality.

6.2 Synergistic effects of LCPUFAs with other nutrients to improve protection and fertility

While there is evidence out there supporting a beneficial interaction between LCPUFAs and antioxidants, particularly in reducing oxidative stress and inflammation, the synergistic effect of these two is still evolving and not fully established across all scenarios (Ngcobo et al., 2021; Mishra et al., 2023). Prospects research should focus on ideal combinations and dosage of LCPUFAs and several antioxidants to improve protection and fertility (Mishra et al., 2023). Future studies should again focus on determining the utmost effective and practical methods for administering dietary versus extender supplementation for ideal results (Bailey et al., 2019). Overall, a focus on modifying diets with precise LCPUFAs and antioxidant profiles for different rooster breeds, ages, and production systems should be tested (Cartoni Mancinelli et al., 2022). This should stress on combining nutritional strategies with ARTs and cryopreservation to maximize their efficacy. Moreover, deep insights into understanding the molecular pathways by which LCPUFAs and antioxidants influence spermatogenesis, sperm function, and fertility will lead to more targeted and effective options (Sengupta et al., 2024; Wang et al., 2025).

6.3 Targeted delivery of antioxidants via nanotechnology

Nanotechnology offers a promising approach for the targeted delivery of antioxidants, while enhancing their efficacy and reducing potential side effects (Falchi et al., 2018; Saadeldin et al., 2020). Thus, targeted delivery of antioxidants using nanotechnology in rooster semen can significantly improve semen quality by giving protection to the sperm from oxidative damage, especially during cryopreservation (Falchi et al., 2018). For instance, inclusion of Nano-Se to semen extender enhanced the post-thawing quality and oxidative variables of rooster semen (Safa et al., 2016). Thus, prospect studies should be geared towards using nanoparticles to engineer and transport antioxidants such as vitamin E, selenium, or quercetin directly to sperm cells or their mitochondria to enhance their effectiveness on protection compared to traditional methods (Tiwari et al., 2022).

6.4 Mycotoxin challenges

Mycotoxins are defined as secondary metabolites that are produced by molds that contaminate a wide variety of plants and feed (Olariu et al., 2025). Their control in fields such as agriculture is considered one of the challenges because of their several effects on chickens (Murugesan et al., 2015). Hence, future studies aiming at addressing mycotoxins and rooster reproduction should pay focus on emerging innovative diagnostic tools for early detection, discovering novel strategies to mitigate mycotoxin contamination in feed, and investigating the long-term effects of mycotoxin exposure on rooster reproductive performance (Olariu et al., 2025). This can be tailored in a way of understanding the epigenetic changes induced by mycotoxins and their transgenerational impact on fertility and reproductive success (Bilska et al., 2018). Moreover, future research can contribute to focusing on the role of LCPUFAs and antioxidants in reducing the detrimental effects of mycotoxins on rooster reproduction, improving flock health, and fostering sustainability of the poultry industry (Gómez-Osorio et al., 2024).

6.5 Use of omics technologies to improve rooster reproductive performance

By offering greater view of the molecular mechanism underpinning reproduction, the application of omics technologies such as transcriptomics, proteomics, metabolomics, and genomics offers significant opportunities to improve chicken reproductive efficiency (Zhou et al., 2025). In addition to helping in identifying the metabolic pathways essential for fertility, these tools can be used to identify genes associated with reproductive parameters such as fertility, comprehend the patterns of gene expression at numerous stages of reproduction, and offer insight into the locating proteins involved in reproductive processes (Panner Selvam et al., 2019; Wadood, 2024). Furthermore, researchers' understanding of the critical processes governing fertility, hatchability, and inclusive reproductive efficiency in chicken, could be greatly enhanced by these technologies (Wadood, 2024). These technologies can then be employed to gain a complete view of the molecular changes in rooster semen in response to LCPUFA and antioxidant supplementation. These may further assist in identifying novel biomarkers for semen quality and fertility.

6.6 Influence of gut microbiome on rooster reproductive performance

Microbiome is defined as the sum of microorganisms related to an organism and its interaction (Moszak et al., 2020). The gut microbiome in chickens plays a significant role in reproductive performance, with evidence suggesting that manipulating the gut microbiota using probiotics, prebiotics, and other interventions can improve overall reproductive health (Aruwa et al., 2021; Naeem and Bourassa, 2025). Thus, understanding the intricate relation between the gut microbiome and chicken reproduction will assist researchers and poultry producers to develop strategies to improve poultry health and enhance productivity (Naeem and Bourassa, 2025). Therefore, upcoming prospects should focus on exploring the possible relationship between LCPUFAs and antioxidants, the gut microbiome of roosters, and their subsequent impact on nutrient absorption and overall reproductive health (Yue et al., 2024).

7 Conclusion

This review highlights the dual role of LCPUFAs and antioxidants in shaping rooster semen quality. LCPUFAs are essential components of sperm membranes, enhancing fluidity, signaling, and fertilization potential, yet their susceptibility to oxidative damage makes them a double-edged sword. Antioxidants, both dietary and extender-based, provide critical protection against ROS and lipid peroxidation, but their effectiveness depends on precise dosing and physiological context. A critical synthesis of the literature reveals that contradictory findings in semen quality outcomes largely stem from imbalances: excessive LCPUFA supplementation without sufficient antioxidants leads to oxidative stress, while overly high antioxidant doses can suppress the physiological ROS signaling required for capacitation and acrosome reaction. The most consistent improvements occur when LCPUFAs and antioxidants are provided together in optimized ratios, underscoring their interdependence. Despite encouraging evidence, several gaps remain. Few studies have systematically tested optimal LCPUFA-to-antioxidant ratios, considered genetic variation among breeds, or explored advanced delivery systems such as nanotechnology. Moreover, most existing data are limited to short-term experiments, leaving long-term reproductive outcomes and implications for genetic resource conservation underexplored. The effective use of LCPUFAs and antioxidants should be viewed not as isolated strategies but as integrated nutritional interventions. Optimizing their synergy has the potential to improve semen quality, enhance fertility, and increase the success of cryopreservation in poultry breeding programs. Such advances will strengthen genetic conservation efforts and contribute to global food security by supporting more sustainable poultry production systems.

Author contributions

SS: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. KN: Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – review & editing. MM: Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – review & editing. JN: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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