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Ahmed AJ Al-Mhanawi
Department of Environmental Engineering, College of Civil Engineering, University of Technology, Iraq

Asaad KM Al-Baidhani
Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq

HA Al-Mashhadani
Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq

Corresponding Author:
Ahmed AJ Al-Mhanawi
Department of Environmental Engineering, College of Civil Engineering, University of Technology, Iraq

Role of nano-dietary supplements in improving semen quality and fertility in aged roosters: physiological and molecular mechanisms, nutritional applications, and future perspectives: A review

Ahmed AJ Al-Mhanawi, Asaad KM Al-Baidhani and HA Al-Mashhadani

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Abstract
Aging reduces semen quality, fertilizing capacity, and subsequent reproductive function, primarily due to oxidative stress and related mitochondrial dysfunction, lipid peroxidation, and endocrine imbalance. Testicular degeneration, chronic inflammation, and impaired spermatogenesis ultimately lead to reduced sperm concentration, motility, viability, and membrane integrity. In the past few years, nano-enabled dietary supplements have gained much attention as a potentially effective nutritional approach to ameliorate these problems in breeder roosters since their special physicochemical properties significantly improve intestinal absorption, cellular uptake, tissue distribution, bioavailability, and biological activity compared to their conventional counterparts. This review paper presents, in a critical manner, the scientific knowledge available at this time on the use of nano-dietary supplements to enhance reproductive performance in senescent roosters. Special attention is paid to selenium nanoparticles (SeNPs), nano-curcumin, and niosomal curcumin, as these are currently considered the most researched nano-formulations in poultry reproduction. Available information suggests that these nano supplements efficiently improve antioxidant defense systems, maintain mitochondrial function, decrease lipid peroxidation, enhance testicular morphology, stimulate steroidogenesis, and protect sperm plasma membranes and DNA integrity. In most cases, therefore, they have led to significantly improved sperm concentration, motility, viability, semen quality, fertility, and reproductive performance compared with conventional dietary supplements. Selenium nanoparticles generally show superior biological efficacy over inorganic selenium and, in some reproductive traits, organic selenium as well, while nano-curcumin formulations consistently outperform conventional curcumins because of their much-improved bioavailability**.** Although these findings are encouraging, evidence is still limited due to the relatively small number of experimental studies, variations in characteristics of nanoparticles and supplementation protocols, and inadequate information on long-term safety, optimal dosage, tissue accumulation, and commercial field application. Future research that will be able to establish and develop standardized, safe, and economically viable nano-nutritional strategies to extend reproductive lifespan and fertility of breeder roosters will be that which integrates molecular biology, reproductive physiology, nanotechnology, and precision nutrition for poultry.

Keywords: Aged roosters, Nano-dietary supplements, Selenium nanoparticles, Nano-curcumin, Semen quality, Fertility, Oxidative stress, Reproductive aging

1. Introduction
The reproductive performance of breeder roosters determines flock fertility and hatchability, which are indicators of the overall efficiency of commercial poultry production. Advancements in genetics, nutrition, and flock management have increased the productive lifespan of laying hens; however, male reproductive competence during extended production cycles remains a challenge. As breeder roosters get older, there is a gradual decline in semen quality, fertilizing capacity, and reproductive consistency. In this paper we review how such changes affect flock fertility as indicated by reduced hatchability and increased production costs with substantial economic losses for the poultry industry [1, 8]. Reproductive aging in roosters represents a multifactorial biological process implicating oxidative stress, endocrine dysfunction, mitochondrial impairment, chronic inflammation, and structural degeneration of the tests.

Age-related increases in reactive oxygen species and lipid peroxidation, accompanied by decreased endogenous antioxidant defense, alter the integrity of sperm membranes, as well as their mitochondrial activity and DNA stability, and steroidogenic function. These changes occur concomitantly with those affecting Leydig cell activity and testosterone biosynthesis and the hypothalamic-pituitary-gonadal signaling and spermatogenesis; they all accelerate the deterioration of semen quality, thereby reducing sperm concentration, motility, viability, and fertility potential. These physiological changes are most often aggravated by nutritional imbalances, metabolic disorders, and environmental stressors related to prolonged breeder production cycles [1, 12].

Rooster sperm is very sensitive to attack by reactive oxygen species because its plasma membrane has very high concentrations of polyunsaturated fatty acids. Physiological levels of ROS are required for sperm maturation, capacitation-like events and fertilization; excessive oxidative stress promotes lipid peroxidation of membranes, mitochondrial dysfunction, DNA fragmentation with apoptotic signaling, and ultimately, poor reproductive performance. Therefore, current nutritional strategies are aimed at not only providing antioxidant compounds but also increasing their bioavailability, cellular uptake, and biological efficacy while maintaining the physiological redox balance necessary for normal sperm function [13, 18].

Nanotechnology is a new science that deals with particles less than 100 nm in size. It has lately come up as one of the most promising technologies in animal nutrition to compensate for the shortcomings of traditional dietary supplements. Unique physicochemical properties make nano-formulations present larger surface area, better solubility, improved absorption in the gastrointestinal tract, better distribution in tissues, release triggered and higher biological activity compared to conventional formulations. Such properties may eventually lead to more efficient delivery of bioactive compounds at the target tissues, possibly reducing losses of nutrients and improving therapeutic efficacy [19, 24]. At present, selenium nanoparticles (SeNPs), nano-curcumin, and niosomal curcumin are the three most commonly used nanotechnology-based compounds for improving poultry reproduction in breeder males. As more and more studies are proving their efficacy in enhancing antioxidant status, testicular function, semen quality, and fertility, these compounds may be considered the most effective nano-nutritional interventions against reproductive senescence in breeder roosters [25, 35].

The above gaps in knowledge have motivated the present review. A critical and comprehensive compilation of information at the physiological, molecular, nutritional, and mechanistic levels regarding nano-dietary supplements for improving reproductive performance in aged roosters is not available in the literature. Besides, issues related to nanoparticle bioavailability, molecular mechanisms of action, optimal supplementation strategies, long-term safety, tissue accumulation, and commercial practicality were insufficiently addressed by the very few relevant studies existing so far [36, 45].

Thus, this review paper will cover a summary of the present knowledge regarding nano-dietary supplements in the improvement of semen quality and fertility of aged roosters. This includes special attention to physiological and molecular mechanisms of reproductive aging, biological activities of selenium nanoparticles and nano-curcumin-based formulations, new nano-nutritional approaches, possible safety issues, research gaps, and future directions for precision reproductive nutrition in breeder males.

2. Why semen quality declines in aged roosters

Semen quality can be considered as a complex biological process because it reflects the cumulative interactions of endocrine, cellular, metabolic, and environmental factors, rather than an individual pathological alteration. This progressive impairment of testicular structure and spermatogenesis, endocrine regulation, and antioxidant defense systems finally culminate in low sperm functional integrity, rendering the breeder rooster incapable of fertilizing hens and, hence, reduced reproductive efficiency [1, 11, 15].

One of the first signs of reproductive aging is the structural degeneration of the tests. Seminiferous tubules undergo gradual atrophy, the populations of Sertoli and Leydig cells diminish, germinal epithelium degenerates, and spermatogenic activity is impaired. Morphological changes such as these reduce daily sperm production and do not allow the maturation of functional spermatozoa [16, 19].

Endocrine dysfunction exacerbates reproductive senescence in old cockerels. Senescence is frequently linked to decreased testosterone synthesis, reduced steroidogenic activity of Leydig cells, and altered responsiveness of the hypothalamic-pituitary-gonadal axis to luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Downregulation of critical steroidogenic enzymes like steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme (CYP11A1), and 3β-hydroxysteroid dehydrogenase (3β-HSD) contributes to impaired androgen production and reduced spermatogenic efficiency [20, 24].

Oxidative stress is considered to be one of the major mechanisms that underlie reproductive aging in poultry. This is because an increased level of reactive oxygen species (ROS) is accompanied by decreased activities of endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) which further enhance levels of lipid peroxidation, mitochondrial dysfunction, protein oxidation, DNA damage, and apoptotic signaling in testicular tissue and sperm cells [25, 31]. The reason for this vulnerability in rooster sperm plasma membranes is that they contain high levels of polyunsaturated fatty acids, making them more susceptible to oxidative damage, resulting in decreased membrane fluidity, reduced mitochondrial ATP production, and decreased motility, as well as low viability and fertility for sperm cells [14, 25, 32].

Chronic low-grade inflammation and mitochondrial dysfunction further aggravate reproductive senescence in addition to oxidative damage. Inflammatory signaling pathways are activated, pro-inflammatory cytokines are overproduced, and the mitochondrial membrane potential is disrupted which collectively impair cellular energy metabolism and accelerate germ-cell apoptosis hence compromising semen quality and reproductive longevity. All these factors play a role in worsening reproductive aging [28, 33].

In addition to this, management-related factors also have a bigger role in fertility decline with age. Overweight, nutrient

partitioning changes, mating activity reduction, environmental stress, and nutritional imbalances which are common during long breeder production cycles could exacerbate endocrine disruptions and oxidative stress, thereby reducing reproductive performance even further [2, 8, 31, 34].

These changes at the physiological and molecular levels in an interrelated manner therefore give a strong biological basis for dietary interventions aimed at the balance of oxidation, mitochondrial function, steroid hormone biosynthesis, and integrity of the tests. Thus, nano-supplements are relatively new but very optimistic contenders since their improved bioavailability, enhanced cellular delivery, and higher biological activity may enable better protection against the multifactorial processes responsible for reproductive aging as compared to that by conventional nutritional formulations [35, 40].

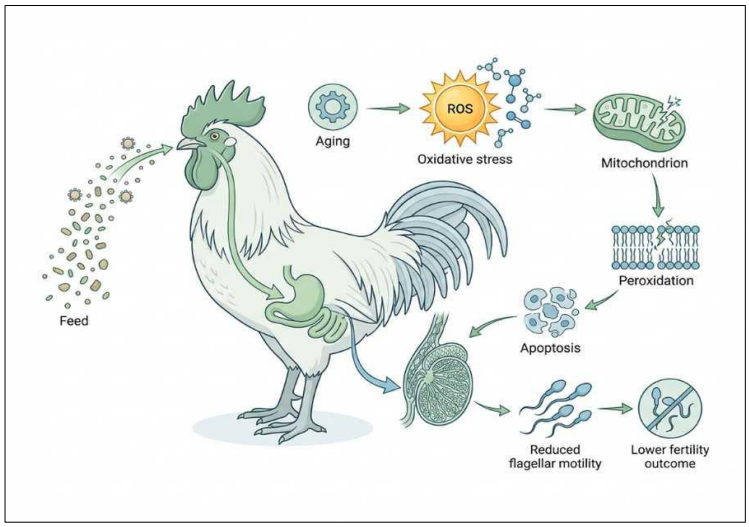

Fig 1: Conceptual illustration of the physiological and molecular mechanisms linking reproductive aging with oxidative stress, mitochondrial dysfunction, lipid peroxidation, apoptosis, impaired sperm function, and reduced fertility in aged roosters. The schematic summarizes the current understanding of age-associated reproductive deterioration and highlights the major biological pathways that may be targeted by nano-dietary interventions. Created by the authors based on the current literature [1, 2, 13, 14, 15].

3. Why nano-dietary additives may offer an advantage

The biological efficacy of dietary supplements is determined by not only the intrinsic activity of the bioactive compound but also its physicochemical properties, gastrointestinal absorption, metabolic stability, tissue distribution, and cellular availability. Many conventional antioxidants, trace minerals, and phytochemicals have relatively low bioavailability because of poor water solubility, limited absorption in the intestines, instability in a free form, rapid metabolism, or extensive excretion before they reach the target tissues. In this regard, only a fraction of the administered dose can exert biological activity within the reproductive system, as discussed elsewhere [13, 35, 38].

Nanotechnology has emerged as a smart nutritional approach to overcome these limitations. Typically, due to their reduced particle size and increased surface area to volume ratio, nano-formulations exhibit improved aqueous dispersibility, enhanced intestinal permeability, greater cellular uptake, prolonged biological retention, and better tissue delivery as compared to their conventional formulations. These attributes may enhance the bioavailability of bioactive compounds while decreasing nutrient losses and improving physiological responses at lower supplementation levels [35, 40].

Selenium nanoparticles (SeNPs) are among Nano nutritional interventions which have been widely studied. This is because selenium is a basic component of many selenoproteins which participate in the antioxidant defense, redox regulation, metabolism of thyroid hormones, and male reproductive function. The higher biological activity of SeNPs compares with inorganic sources of selenium, better absorption in the gastrointestinal tract, lower urinary excretion, and more effective tissue distribution. Experimental evidence has also indicated that SeNPs enhance the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) while they lower malondialdehyde (MDA) levels and other indices of oxidative stress. In this way, they protect testicular tissue and spermatozoa from oxidative injury [35, 39].

The reproductive benefits of nano-selenium are not limited to antioxidant defense. Some studies have also reported enhanced testicular histoarchitecture, organization of seminiferous tubules, steroidogenic activity, sperm production, semen volume, sperm concentration, motility, viability, and general fertility in breeder males on SeNP supplementation. These findings demonstrate that the nano-formulations can improve the delivery of nutrients and optimize physiological responses related to male reproductive performance at the same time [36, 40].

Another typical example is curcumin to illustrate the benefits of nano-enabled nutrition. Conventional curcumin has very good antioxidants, anti-inflammatory, and anti-apoptotic properties. But practical application is limited because of poor aqueous solubility, low absorption in the gastrointestinal tract, fast metabolism, and low systemic bioavailability. Nano-curcumin and niosomal curcumin were designed specifically to overcome these pharmacokinetic limitations by improving encapsulation, controlling release, and enhancing cellular delivery. In experimental studies on aged roosters, nano-curcumin formulations consistently outperformed equivalent doses of conventional curcumin in improving antioxidant capacity, testicular function, semen quality, and fertility all by much greater margins [41, 45].

Together, the present results indicate that the supremacy of nano-dietary additives cannot be ascribed only to the biological activity of the active ingredient by itself but rather to its optimization of delivery, increased bioavailability, improved cellular uptake, and sustained biological activity within reproductive tissues. These features would make nano-formulations very attractive nutritional tools for countering the multifactorial mechanisms responsible for reproductive aging in breeder roosters [35, 45].

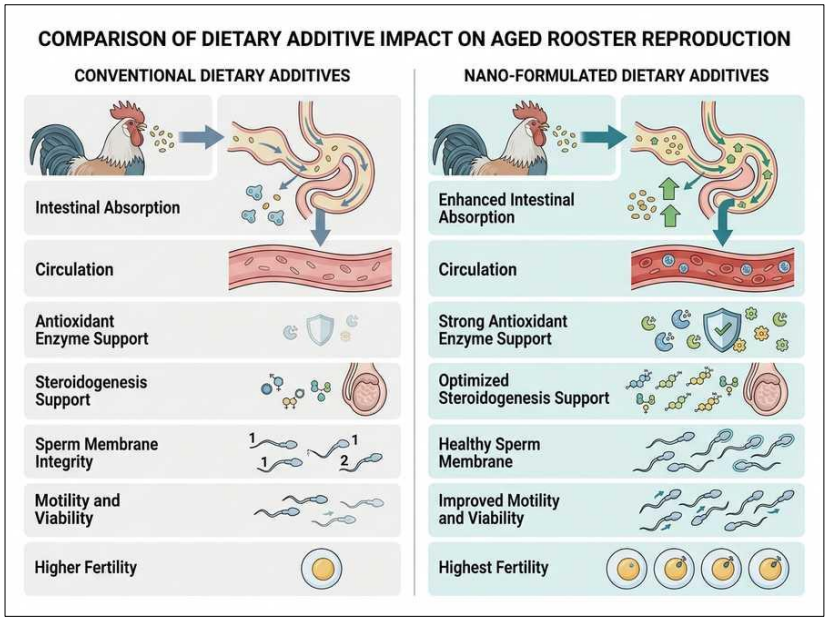

Fig 2: Schematic of the potential benefits of nano-dietary supplements over regular dietary additives in aged roosters. It shows increased gastrointestinal absorption, systemic bioavailability, tissue distribution, antioxidant defense, steroidogenic activity, testicular protection, sperm membrane integrity, mitochondrial function, sperm motility, viability, and overall fertility associated with nano-formulations. Original figure created by the authors based on published evidence [35, 45].

4. Evidence from selenium nanoparticles in aged roosters

4.1 Comparative effects of selenium nanoparticles and sodium selenite

Of the available nutritional interventions, selenium nanoparticles (SeNPs) provide the strongest direct evidence for improving reproductive performance in aged breeder roosters. Selenium is an essential trace element. Its functions are related to the synthesis of several selenoproteins that regulate antioxidant defense, redox homeostasis, mitochondrial function, and steroidogenesis. Therefore, improving selenium bioavailability has become an important strategy for delaying age-associated reproductive decline [35, 40].

A controlled feeding experiment was conducted using 50-week-old broiler breeder roosters to directly compare dietary sodium selenite with different concentrations of SeNPs under identical experimental conditions. Birds received a basal diet or diets supplemented with sodium selenite (0.30 mg Se/kg) or selenium nanoparticles at 0.15, 0.30, or 0.60 mg Se/kg. SeNP supplementation significantly improved sperm motility, sperm viability, sperm concentration, and several indices of testicular histological activity the tubular differentiation index (TDI), spermiogenesis index (SPI), Sertoli cell index (SCI), and mitotic index (MI) compared with both the control and inorganic selenium treatments. Among the evaluated treatments, dietary supplementation with 0.30 mg Se/kg as selenium nanoparticles proved to be the most consistent in improving reproductive performance.

The biological relevance of this study goes beyond the noted enhancements in semen quality. First, it evaluated aged breeder males directly, the population in which reproductive decline poses a major commercial challenge. Second, the results clearly show a dose-dependent response, indicating that the reproductive benefits of SeNPs are not related in a linear manner to increased dietary concentration. Instead, excessive supplementation did not confer additional advantages, thus underscoring the importance of optimizing dosage for maximal efficacy with minimum risk of selenium excess [41].

4.2 Comparative bioavailability of selenium sources

Selenium nanoparticles recently proved superior in a study of comparative bioavailability conducted on breeder roosters aged 47 weeks. Three sources of selenium, namely, sodium selenite, selenium-enriched yeast (Sel-Plex), and selenium nanoparticles were tested at dietary concentrations of 0.15, 0.30, and 0.45 mg Se/kg over a 40-day feeding period to determine if the earlier reproductive results could be sustained over a longer feeding trial. The study also measured oxidative stress biomarkers and antioxidant responses in parallel, to support the relative biological efficacy of each selenium source.

In comparison with inorganic selenium, supplementation of SeNPs showed much higher improvements in semen volume, sperm concentration, normal morphology, and total antioxidant capacity while markedly lowering malondialdehyde (MDA), a well-established marker of lipid peroxidation and oxidative injury [42]. Further analysis of relative bioavailability showed that selenium nanoparticles were more efficient biologically in multiple reproductive and antioxidant parameters, which indicated that the improved physicochemical properties of nano-formulations considerably enhanced selenium utilization in aged breeder males.

However, response surface optimization reported that 0.45 mg Se/kg, supplied as selenium nanoparticles, is the dietary selenium level resulting in the optimal integrated improvement of semen quality and the antioxidant status while limiting oxidative stress and sperm abnormalities [42]. Consequently, the selenium response to dietary supplementation seems to depend not only on the concentration of selenium in the diet but also on the form of selenium administered. Thus, the nanoparticles seem capable of exerting higher reproductive benefits than the conventional inorganic selenium source owing to the superior absorption, delivery, cellular uptake, and biological utilization of these compounds.

In summary, the results of this study strongly indicate that selenium nanoparticles are much better than sodium selenite for most reproductive and antioxidant parameters studied in aged breeder roosters. The superiority of SeNPs is due not only to selenium supplementation per se but also to enhanced bioavailability, better antioxidant protection, preservation of testicular function, and more efficient maintenance of structural and functional integrity of sperm [35, 42].

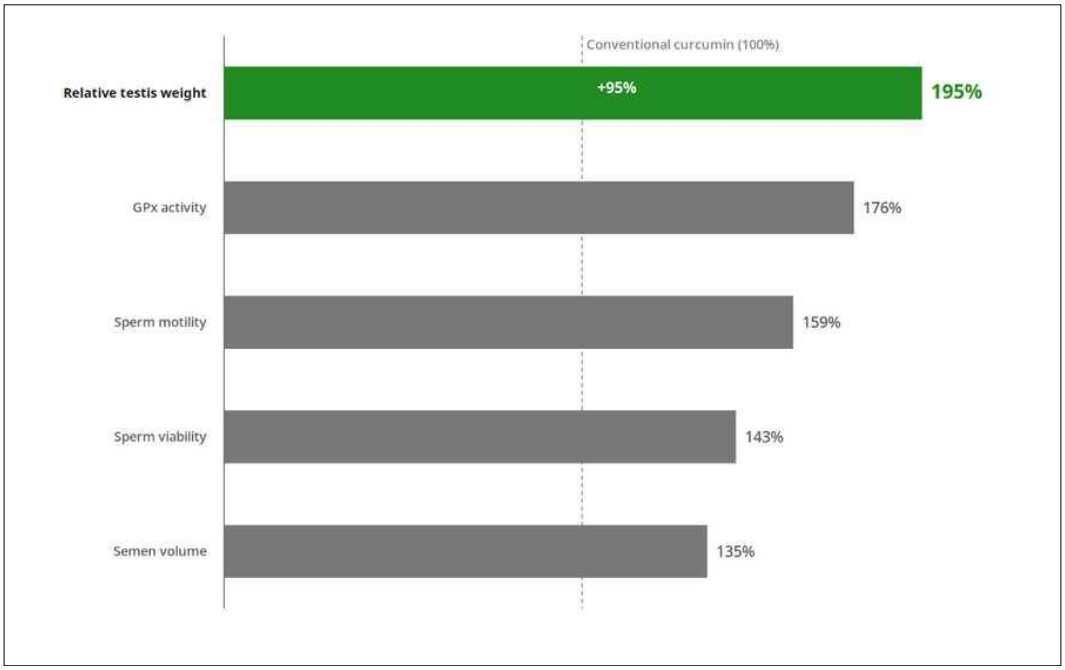

Fig 3: Relative bioavailability of nano-curcumin formulations compared with conventional curcumin in aged breeder roosters. Values are expressed relative to conventional curcumin (100%) and were calculated and graphically reconstructed from the experimental data reported by Behboodi *et al.* [43].

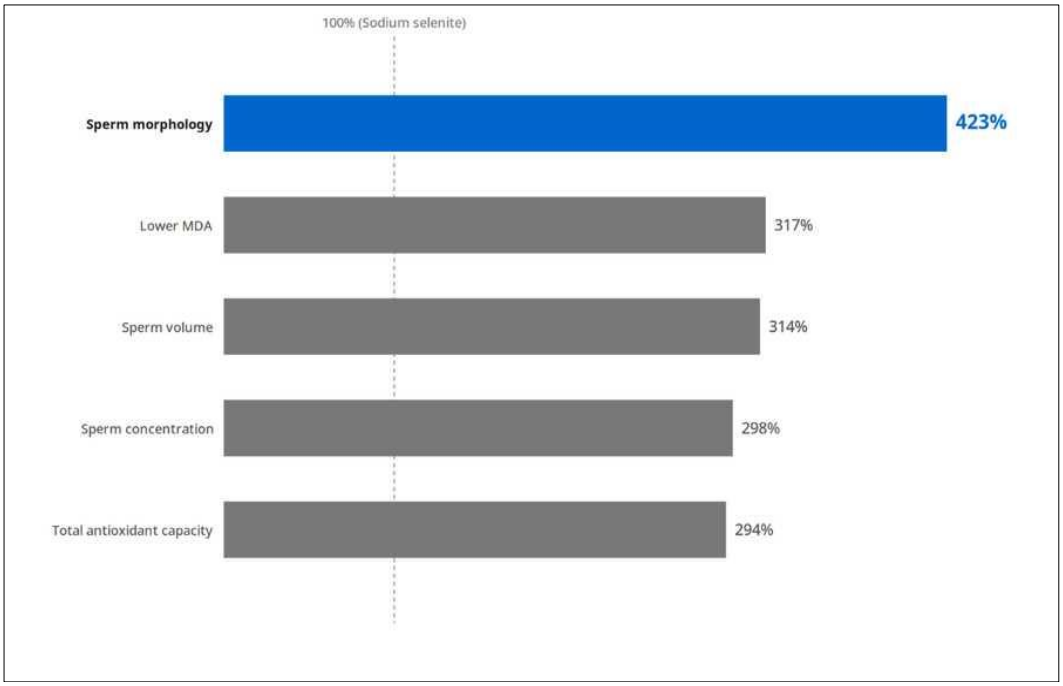

Fig 4: Comparative relative bioavailability of selenium nanoparticles and other dietary selenium sources in aged breeder roosters. Values of relative bioavailability were calculated from the experimental data reported by Asghari-Moghadam *et al.* [42] using sodium selenite as a reference (100%). For malondialdehyde (MDA), higher relative values indicate greater efficacy in reducing lipid peroxidation and oxidative stress. The graph was prepared by the authors for comparative visualization

5. Evidence from nano-curcumin in aged roosters

5.1 Nano-curcumin versus conventional curcumin

Curcumin is a polyphenolic compound that occurs naturally and is known for its strong antioxidants, anti-inflammatory, and anti-apoptotic activities. However, practical use in animal nutrition has been restricted due to its poor aqueous solubility, rapid metabolism, low absorption in the gastrointestinal tract, and consequently low systemic bioavailability. Nano-formulation has been proposed as a good approach to overcome these pharmacokinetic constraints and improve the biological activity of curcumin within target tissues [43, 45].

Direct evidence supporting this approach was provided by a controlled feeding study conducted in aged breeder roosters between 54 and 64 weeks of age [43]. Birds were given either conventional curcumin or nano-curcumin at dietary concentrations of 15 or 30 mg/kg for a 10-week experimental period. In comparison with conventional curcumin, nano-curcumin consistently showed better biological efficacy in all the reproductive and antioxidant parameters evaluated.

Relative bioavailability studies have indicated that nano-curcumin elicits much higher biological responses than conventional curcumin, with reported relative bioavailability values of 135% for semen volume, 143% for sperm viability, 159% for sperm motility, 176% for glutathione peroxidase (GPx) activity, and 195% for relative testicular weight [43]. This proves that the nano-formulation substantially improves the physiological utilization of curcumin, allowing much higher reproductive responses even at similar levels of dietary inclusion.

Apart from the conventional semen characteristics, better parameters in the treated groups were observed to include antioxidant defense capacity, testicular development, and reproductive function. All these parameters were significantly enhanced by nano-curcumin supplementation, indicating that its beneficial effects involve multiple physiological pathways rather than a single antioxidant mechanism. Improved GPx activity would mean more efficient scavenging of reactive oxygen species, which would limit lipid peroxidation and preserve membrane integrity within both testicular tissue and spermatozoa. The accompanying increase in relative testicular weight further suggests improved maintenance of testicular structure and spermatogenic activity during reproductive ageing [43].

These findings together indicate that the transformation of curcumin to a nanoscale delivery system enhances its bioavailability and biological efficacy in aged breeder roosters. The superiority of nano-curcumin may be due to increased antioxidant activity and better tissue delivery, greater cellular uptake, and more effective protection of the reproductive system against the multifactorial processes associated with age-related reproductive decline [43, 45].

5.2 Niosomal curcumin and molecular regulation of reproductive function

Better semen parameters offer direct proof of better reproductive performance, while underlying molecular mechanisms explain biological superiority of nano-formulated curcumin. Niosomal curcumin is a more recent and advanced nano-delivery system, where the main objective is to enhance stability, controlled release, cellular uptake, and bioavailability of curcumin so that its physiological activity within reproductive tissues can be maximized [43, 45].

In a recent feeding trial, 54-week-old Ross 308 breeder roosters were used to assess the reproductive effects of conventional curcumin and niosomal curcumin at dietary levels of 15 and 30 mg/kg over a nine-week experimental period [44]. The most significant enhancement in reproductive performance was noted for the 30 mg/kg niosomal curcumin, which raised sperm motility, viability, and plasma membrane integrity by nearly 19% over the untreated control group. Semen concentration was also highest in birds that received niosomal curcumin followed by those supplemented with the higher level of conventional curcumin [44].

This study contributes primarily to the molecular characterization of the reproductive response. Upregulation of the expression of genes for steroidogenic acute regulatory protein (StAR) and cytochrome P450 side-chain cleavage enzyme (*P450scc/CYP11A1*) was observed in roosters supplemented with niosomal curcumin, indicating increased capacity of steroidogenesis and higher biosynthesis of testosterone. Expression of the anti-apoptotic gene BCL-2 was also significantly elevated, showing enhanced survival cellularity and protection of germ cells against age-associated apoptotic degeneration [44].

Apart from the effects on reproductive tissues, niosomal curcumin also improved metabolic responses. Birds that received 30 mg/kg of either conventional or niosomal curcumin showed significantly higher levels of serum testosterone and high-density lipoprotein cholesterol (HDL-C) and lower levels of triglycerides, total cholesterol, very-low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol (LDL-C) [44]. These metabolic improvements may indirectly contribute to reproductive performance by optimizing endocrine functions, reducing systemic oxidative stress, and improving lipid homeostasis within the tests.

The above data indicate that curcumin in niosomal for reproduction has more benefits than its antioxidant properties. Regulation of steroidogenesis, apoptosis, oxidative balance, and systemic metabolism shows the many ways nano-curcumin works against reproductive aging, thereby probably resulting in good semen quality. This further supports the potential of niosomal curcumin as a next-generation nutritional strategy for extending the reproductive lifespan of breeder roosters since it can help molecular pathways and also improve phenotypic reproductive traits [43, 45].

Table 1 provides a summary of the available experimental studies on nano-dietary additives for improving reproductive performance in old breeder roosters. This comprises details of experimental models, nano-formulations, dietary inclusion levels, principal reproductive outcomes, and major study limitations. Comments on study limitations where the original publications did not explicitly report them are based on observable aspects of the experimental design only, such as trial duration, dosage range, experimental model, and comparator selection.

Table 1: Summary of experimental studies evaluating nano-dietary additives for improving reproductive performance in aged breeder roosters

Study	Bird model	Nano-additive (Dose)	Principal findings	Major limitation
Alavi *et al*. [41]	50-week-old broiler breeder roosters	SeNPs (0.15, 0.30, 0.60 mg/kg) vs. sodium selenite (0.30 mg/kg)	0.30 mg/kg SeNPs significantly improved sperm motility, viability, concentration, and testicular histomorphometric indices (TDI, SPI, SCI, MI).	Short-term study; only one inorganic selenium comparator.
Asghari-Moghadam *et al*. [42]	47-week-old breeder roosters (40 days)	SeNPs (0.15-0.45 mg/kg) vs. sodium selenite and selenium yeast	SeNPs showed greater relative bioavailability, improved semen quality, antioxidant capacity, and reduced MDA. 0.45 mg/kg was identified as the optimal level.	Short experimental period; selenium-specific comparison only.
Behboodi *et al*. [43]	54-64-week-old aged roosters (10 weeks)	Nano-curcumin (15, 30 mg/kg) vs. conventional curcumin	Nano-curcumin exhibited superior bioavailability, improving semen volume, motility, viability, GPx activity, and relative testicular weight.	Single experiment with only two dose levels.
Behboodi *et al*. [44]	54-week-old Ross 308 breeder roosters (9 weeks)	Niosomal curcumin (15, 30 mg/kg) vs. conventional curcumin	30 mg/kg niosomal curcumin improved semen quality, membrane integrity, testosterone, HDL-C, expression of StAR, CYP11A1 (P450scc), and BCL-2, while reducing serum lipid fractions.	One breeder genotype; single nano-carrier evaluated.

6. Selenium versus nano-curcumin: a comparative perspective

Selenium nanoparticles (SeNPs) and nano-curcumin are recent nutritional interventions applied in combating age-related reproductive degeneration in breeder cocks. Both nano-additives ameliorate semen quality and fertility by reducing oxidative stress. However, it appears that they possess different biological targets and mechanistic pathways. Recent studies indicate that SeNPs primarily enhance systems' antioxidant defense to ensure spermatogenic function while nano-curcumin appears to have broader regulatory effects on antioxidant protection, endocrine function, steroidogenesis, apoptosis, and cellular metabolism [35, 45].

6.1 Antioxidant defense and oxidative stress

Oxidative stress is one of the major mechanisms that explain the reproductive aging of breeder males. Overaccumulation of reactive oxygen species disrupts the functioning of mitochondria, initiates lipid peroxidation, and damages sperm plasma membranes, leading to reduced sperm motility and fertilizing capacity [35, 38].

Selenium nanoparticles have the best-documented evidence among all nano-additives in ameliorating classical biomarkers of oxidative stress in breeder roosters. Comparative studies have consistently demonstrated the superior biological efficacy of SeNPs over sodium selenite in reducing malondialdehyde (MDA) concentration while simultaneously enhancing total antioxidant capacity (TAC) and improving multiple semen quality parameters [41, 42]. These results are in line with the known physiological role of selenium as a component of glutathione peroxidases and other selenoproteins that participate in the maintenance of intracellular redox homeostasis [35, 38].

Nano-curcumin also offers significant antioxidant protection although available evidence highlights improvement in endogenous antioxidant enzyme activity rather than full characterization of oxidative stress biomarkers. In aged breeder roosters, nano-curcumin increased glutathione peroxidase (GPx) activity significantly with marked improvements in semen volume, sperm motility, viability, and relative testicular weight [43]. These results suggest that both nano-additives effectively alleviate oxidative stress, although selenium nanoparticles currently have a more comprehensive base of evidence on systemic oxidative stress indicators.

6.2 Steroidogenic and molecular regulation

The major difference between the two nano-additives lies in the depth of molecular evidence available at the moment. While published studies on selenium nanoparticles mainly focus on parameters related to reproductive performance, antioxidant status, and testicular morphology, those on nano-curcumin have gone further to the transcriptional regulation of genes directly involved in steroidogenesis and apoptosis.

Niosomal curcumin showed significant upregulation in the expression of steroidogenic acute regulatory protein (StAR), cytochrome P450 side-chain cleavage enzyme (CYP11A1/P450scc), and the anti-apoptotic gene BCL-2, accompanied by the major increases observed in circulating testosterone concentrations [44]. These coordinated molecular responses indicate that nano-curcumin supports reproductive function through the stimulation of testosterone biosynthesis and, at the same time, enhances germ-cell survival during reproductive aging.

To date, similar gene-expression evidence has not been reported in the available feeding studies in which selenium nanoparticles were administered to aged breeder roosters [41, 42]. Therefore, while it is evident that selenium improves reproductive performance as an antioxidant protector, the molecular mechanisms through which selenium supplementation regulates steroidogenesis are relatively less defined in this experimental model.

6.3 Reproductive performance

Semen quality was consistently improved by both selenium nanoparticles and nano-curcumin in aged breeder roosters, although their predominant physiological effects differed.

Sperm motility, viability, concentration, sperm morphology, testicular histomorphometry, and oxidative stress biomarkers showed progressive enhancement with selenium nanoparticles, which proved to be distinctly better than sodium selenite [41, 42]. This justifies the efficacy of these nanoparticles in sustaining spermatogenesis and structural integrity of the male reproductive system during senescence.

Nano-curcumin also improved sperm motility, viability, semen volume, antioxidant enzyme activity, and relative testicular weight [43]. More importantly, niosomal curcumin improved sperm membrane integrity, semen concentration, testosterone secretion, and favorable serum lipid profiles while simultaneously regulating genes associated with steroidogenesis and apoptosis [44]. This observation proves that nano-curcumin provides reproductive benefits which are not confined to the reduction of oxidative stress.

6.4 Integrated interpretation

The available evidence supports the idea that selenium nanoparticles and nano-curcumin should not be seen as rival nutritional interventions but rather complementary strategies that work through partially overlapping biological pathways.

Selenium nanoparticles seem quite effective in the enhancement of antioxidant defenses and maintenance of spermatogenic function via redox mechanisms dependent on selenium. On the other hand, nano-curcumin combines antioxidant protection with endocrine modulation regulating steroidogenic and apoptosis-related genes, as well as other metabolic pathways.

At present, available evidence does not warrant declaring nano-additive universally superior across all reproductive outcomes because no study has directly compared selenium nanoparticles and nano-curcumin under identical experimental conditions. Future research with standardized experimental designs, comprehensive molecular analyses, and long-term fertility evaluations will be needed to determine the relative efficacy of these two nano-formulations and to explore the potential synergistic benefits of combining them in breeder nutrition programmers [35, 45].

7. Visual Summary of nano-additives, mechanisms, and reproductive outcomes

Figure 5 shows an integrated schematic summarizing the major nano-dietary additives studied in aged breeder roosters along with their proposed biological mechanisms and reproductive outcomes. It also presents a summary of the current evidence on selenium nanoparticles and nano-curcumin, showing how improved bioavailability, antioxidant defense, endocrine regulation, steroidogenesis, anti-apoptotic signaling, and cellular function contribute to better semen quality, testicular function, and fertility during reproductive aging as a whole [35, 45].

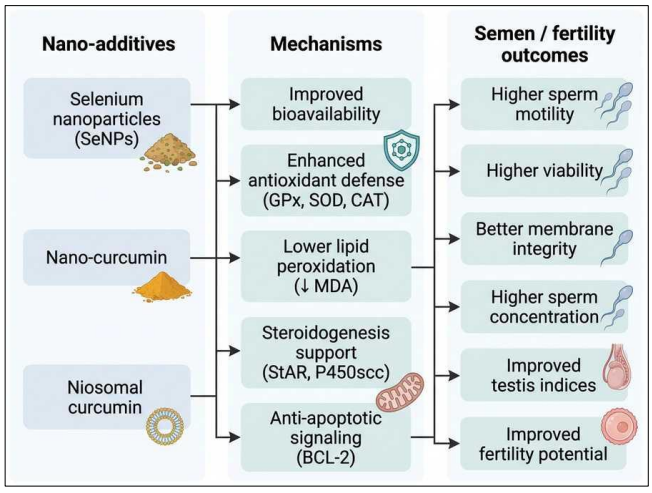

Fig 5: Visual summary infographic that explains the proposed biological mechanisms of how selenium nanoparticles, nano-curcumin, and niosomal curcumin improve reproductive performance in aged breeder roosters. This depicts the available evidence that links increased bioavailability, antioxidant activity, endocrine regulation, steroidogenesis, anti-apoptotic signaling, and metabolic modulation to the improvement of semen quality, testicular function, and fertility. Figure designed by the authors, with the structure based on information synthesized from the published literature [35, 45].

8. Practical interpretation

The data summarized in this paper prove that nano-feed supplements are a promising nutritional approach to reduce reproductive aging in breeder roosters through multiple, interconnected biological mechanisms rather than a single physiological pathway [35, 45]. Selenium nanoparticles and nano-curcumin differ in their predominant modes of action. However, both ultimately contribute to improved reproductive performance by enhancing cellular resilience against oxidative damage that accumulates with advancing age. Although selenium nanoparticles and nano-curcumin differ in their predominant modes of action, both ultimately contribute to improved reproductive performance by enhancing cellular resilience against oxidative damage that accumulates with advancing age.

One of the most consistently reported mechanisms is the reinforcement of antioxidant defense systems. Selenium nanoparticles primarily enhance the activity of selenium-dependent antioxidant enzymes, whereas nano-curcumin stimulates endogenous antioxidant responses, together leading to increased activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), accompanied by reduced lipid peroxidation as reflected by lower concentrations of malondialdehyde [35, 45]. These changes help maintain membrane stability and mitochondrial function, as well as sperm viability during reproductive senescence.

Besides antioxidant defense, nano-formulations help keep the structural and functional integrity of the male reproductive system. Increase in sperm motility, semen concentration, and testicular histomorphology point toward nano-additives sustaining spermatogenesis and maturation of sperm under conditions of progressive reproductive decline [41, 44]. In the case of nano-curcumin, further regulation of steroidogenic and apoptosis-related genes, along with increased testosterone production, indicates benefits that extend to endocrine and molecular pathways governing reproductive function [44].

Similarly important is that most of the reports available show that enhanced biological efficacy is associated with improved bioavailability. Nano-scale delivery systems permit greater gastrointestinal absorption, tissue distribution, and cellular uptake than conventional formulations; thus, they allow for lower dietary inclusion levels to elicit stronger physiological responses [42, 44]. This feature appears to be the primary advantage shared by selenium nanoparticles and nano-curcumin.

From the standpoint of management in commercial breeder operations, nano-dietary additives cannot be viewed as a magic bullet for age-related fertility decline. All these factors strongly affect the reproductive performance of aging breeder males: control of body weight, nutritional balance, male-specific feeding strategies, flock health, environmental management, and general oxidative status [46, 48]. It is therefore more likely that the best practical benefit will be obtained when nano-additives are used as part of a broad reproductive management program rather than as individual nutritional interventions.

In general, current evidence supports the use of nano-dietary supplements in precision breeder nutrition programs aimed at increasing the reproductive lifespan of roosters. However, more long-term field studies must be conducted to test fertility, hatchability, economic return, and potential interactions among different nano-formulations before universal commercial recommendations can be set up [35, 48].

9. Experimental design considerations for future nano-additive studies in aged breeder roosters

Current evidence indicates that nano-dietary additives may be used to enhance reproductive performance in aged breeder roosters. However, it is difficult to directly compare the results of published studies since there is a wide variation in experimental design, bird age, dietary inclusion levels, study duration, endpoints measured, and statistical approaches [35, 48]. Standardizing the methodological approaches would enhance reproducibility and make it easier to compare results from different studies, thereby improving the translation of research findings to commercial breeder nutrition programs.

Future studies should use clearly defined experimental models, including breeder genotype, precise age at the beginning and end of the trial, housing conditions, feeding management, and body-weight monitoring. Since reproductive ageing progresses gradually, it is important to report the exact age of the experimental birds to interpret treatment responses and for comparison with other studies [41, 44].

Dose selection should be based on dose-response principles and not on single-dose comparisons. Both selenium nanoparticles and nano-curcumin have shown biological responses, which depend on the dose. This underscores the need to evaluate multiple dietary inclusion levels together with appropriate conventional comparators. Also, details of nano-formulations in full characterization, including particle size, carrier system, stability, and delivery platform, should be given whenever available to enhance experimental reproducibility [41, 44].

A full reproductive evaluation should include not only conventional semen parameters like volume, sperm concentration, motility, viability, membrane integrity, and morphology but also mechanistic endpoints reflecting antioxidant status, endocrine regulation, and molecular responses. These are GPx, SOD, CAT, MDA, TAC, serum testosterone, steroidogenic gene expression (e.g., StAR and *CYP11A1/P450scc*), apoptosis-related markers (e.g., BCL-2), and testicular histomorphometry [35, 45].

Naturally, any improvement in semen quality should be validated with downstream reproductive outcomes whenever possible. The fertility rate, hatchability, insemination success, and repeated measurements throughout the aging period provide much more meaningful indicators of commercial reproductive performance than semen characteristics alone [46, 48].

The statistical analyses should also include appropriate techniques for data with defined experimental units, random allocation, and repeated-measures analyses where applicable, and dose-response modeling. Clear descriptions of how variances were estimated and what software was used should also be provided. Table 2 presents the major recommendations for the future design of nano-additive studies in aged breeder roosters.

Table 2: Recommended checklist for experimental design and reporting of nano-dietary additive studies in aged breeder roosters

Category	Recommended components
Experimental model	Breeder strain, age, body weight, housing, feeding management, mating system
Experimental treatments	Negative control, conventional comparator, ≥2-3 nano-additive doses
Nano-formulation	Particle size, carrier type, stability, formulation method, bioavailability characteristics
Study duration	Minimum period covering one complete spermatogenic response (approximately 6-10 weeks)
Semen evaluation	Volume, concentration, motility, viability, membrane integrity, morphology
Oxidative stress markers	GPx, SOD, CAT, MDA, TAC
Endocrine and molecular markers	Testosterone, *StAR*, *CYP11A1 (P450scc)*, *BCL-2*, testicular histomorphometry
Reproductive performance	Fertility, hatchability, repeated fertility assessment, insemination protocol
Statistical analysis	Randomization, experimental unit, repeated measures, dose-response analysis, post hoc testing, variance estimates
Reporting standards	Complete description of formulation, analytical methods, experimental timeline, and unavailable formulation details clearly stated

10. Limitations and research gaps

The available evidence strongly supports the potential of nano-dietary additives to improve reproductive performance in aged breeder roosters. However, several important limitations should be considered when interpreting the current literature. Major among them is that the studies cited were conducted under different environmental, dietary, and management conditions and with birds of different genetic backgrounds; they also vary in the type and dosage of nano-dietary additives used. Factors that could have influenced the outcomes of the studies include the physiological state of the birds and variations in the procedures used for sample collection and laboratory analyses.

First, the existing evidence base remains relatively limited. Most direct experimental studies have focused on selenium nanoparticles and curcumin-based nano-formulations, whereas numerous other nano-nutritional compounds with potential reproductive applications, including nano-zinc, nano-vitamin E, nano-coenzyme Q 9, nano-resveratrol, and nano-omega-3 formulations, have received little or no investigation in aged breeder males. So, current conclusions cannot be generalized to all nano-dietary interventions [35, 48].

Second, almost all studies available so far have been done over relatively short experimental periods, typically between about 40 days and 10 weeks [41, 44]. These durations, though adequate to detect changes in semen characteristics and antioxidant responses, may not be enough to reflect the extended reproductive lifespan of commercial breeder roosters. Therefore, long-term studies covering different production phases are needed to determine the persistence of treatment effects and long-term safety on continuous nano-additive supplementation.

Another important limitation is related to dose optimization. The reviewed studies consistently showed dose-dependent biological responses, meaning that greater dietary inclusion does not necessarily lead to superior reproductive performance [41, 44]. Excessive antioxidant supplementation may disrupt physiological redox signaling, an important part of healthy sperm function, capacitation, and fertilization. Therefore, establishing optimal supplementation ranges

remains a critical priority for future nutritional recommendations [45, 48].

This study therefore proves that nano-formulation attributes are as significant as the active compound. Selenium nanoparticles consistently outperformed conventional sodium selenite. The same was true for nano-curcumin and niosomal curcumin over their conventional forms of curcumin [42, 44]. These results stress that reproductive responses cannot be generalized across different nano-carriers, particle sizes, encapsulation systems, or manufacturing technologies. All these can substantially influence bioavailability and biological activity.

In addition, very few studies have included molecular, endocrine, and histological assessments along with conventional semen analyses. More studies on transcriptomics, proteomics, metabolomics, mitochondrial function, inflammatory biomarkers, and testicular histopathology would substantially enhance our knowledge of the mechanisms by which nano-dietary additives delay reproductive aging.

Finally, the commercial applicability of the present findings is yet to be fully validated. Future studies should go beyond laboratory-scale experiments to cover large-scale breeder operations, assessing fertility persistence, hatchability, progeny performance, economic return, feed cost-benefit relationship, and reproducibility under commercial management conditions. Another important priority for future research is comparative studies that directly evaluate different nano-additives within the same experimental design as well as synergistic combinations.

Overall, while the available evidence is highly encouraging, broader, longer-term, and more mechanistically integrated studies are required before universal recommendations regarding nano-dietary supplementation for aged breeder roosters can be established [35, 48].

11. Conclusion

Nano-dietary additives have recently been proposed as a new and promising approach to nutrition to reduce reproductive aging and improve semen quality in breeder roosters. Current evidence shows that selenium nanoparticles and nano-curcumin formulations consistently improve sperm motility, viability, concentration, membrane integrity, and antioxidant status with good reproductive performance by enhancing bioavailability and having effects on multiple physiological pathways including antioxidant defense, steroidogenesis, mitochondrial function, and cellular survival.

The available nano-formulations include selenium nanoparticles, which seem particularly effective in strengthening the selenium-dependent antioxidant systems and preserving spermatogenesis, and nano-curcumin, which has broader biological activity through the integration of antioxidant protection with endocrine regulation, steroidogenic gene activation, anti-apoptotic signaling, and metabolic modulation. In contrast to representing competing nutritional strategies, these nano-additives should be perceived as complementary approaches that target different aspects of reproductive aging.

These promising results notwithstanding, current evidence is still constrained by the relatively small number of long-term studies, diversity in experimental designs, and lack of commercial-scale validation. Future research should give priority to standardized experimental protocols, comprehensive molecular characterization, long-term fertility and hatchability assessments, economic evaluation, and direct comparative studies between different nano-formulations to establish evidence-based recommendations for breeder nutrition programs.

In general, nano-dietary supplementation is a very promising part of precision nutrition strategies to increase the reproductive lifespan of commercial breeder roosters. Further improvements in nano-formulation technology along with proper experimental validation would be anticipated to accelerate the development of more effective, targeted, and commercially applicable nutritional interventions for sustaining male fertility during reproductive aging.

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