*Review*

# Antioxidant and Potential Non-antioxidant Roles of Vitamin E in Reproduction of Male Domestic Animals

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**Abstract:** Vitamin E facilitates testicular development and semen quality in farm animal. And most research tends to investigate antioxidant roles of Vitamin E in reproductive health; some research also find potential non-antioxidant roles of Vitamin E. To further uncover the mechanism by which Vitamin E modulates reproductive health, the paper reviews specific function of Vitamin E and candidate genes involved in male reproductive performance promoted by Vitamin E. The review found that cell proliferation (PDPN, BMP, Myc, AMPKa, GSK3β, PPARγ, CDK4, CDK6, Ki67, PCNA, Cyclin A2, Cyclin B1, Cyclin B3, BLM, REL, KLHL25)-, cell apoptosis (BCL-2, Caspase 9, Bax)-, spermatogenesis (CatSper1, CatSper2, NDRG1, CYP26B1, FSCN3, FLNA, SPCS3, YBX3, RARS)-, hormone receptor (PGR, FSHR, AR)-related genes are the underlying key points. And two miRNAs, miR-107 and miR-493-3p, which mediate cell cycle process, are also identified in response to Vitamin E treatment. The paper paves the way for a comprehensive understanding about effect of Vitamin E on testis development and semen quality in domestic animals.

**Keywords:** Vitamin E; testis; sperm; antioxidant; reproduction

## 1. Introduction

Vitamin E is a lipophilic antioxidant known to be essential for mammalian testicular development and spermatogenesis. It is firstly found in 1922; then the role of Vitamin E in reproduction was also verified (Evans & Bishop, 1922). Vitamin E deficiency caused abnormal spermatogenesis, abnormal epididymis epithelial cell differentiation (Bensoussan, Morales, & Hermo, 1998), seminiferous tubules with exfoliation, a reduction in number of spermatozoa and an increase in apoptotic germ cells and sperm with abnormal morphology was also found in response to Vitamin E deficiency (Saito, Hara, Kitajima, & Tanemura, 2020). Much effort has been devoted to effect of Vitamin E on male reproduction. Although species used to carry out investigations are distinct, yet most of research found that Vitamin E was capable to improve mammalian and avian semen quality (Lin, Chang, Yang, Lee, & Hsu, 2005; Umesiobi, 2012; Yousef, Abdallah, & Kamel, 2003; Yue, Yan, Luo, Xu, & Jin, 2010; Zanini et al., 2003). In addition to semen quality facilitated by Vitamin E, substantial studies also demonstrated that Vitamin E was also able to promote testicular development (Hong, Hailing, Hui, & Guijie, 2009; Momeni, Oryan, & Eskandari, 2012). Whereas the mechanism underlying that Vitamin E modulated male reproductive health mainly focused on its antioxidant property. The investigation of potential non-antioxidant mechanism was relatively rare, which still needs to be further studied.

The research on antioxidant tended to explore activity of antioxidant enzyme, antioxidant capacity of sperm and testis, or antioxidant gene expression (Hong et al., 2010; C. Xu et al., 2016). However, the research on non-antioxidant concentrated on expression about cell cycle and cell division-related genes (Amevor et al., 2022; Gao, Lu, Jian,

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Machaty, & Luo, 2022), miRNAs and target genes associated with cell cycle (Gao et al., 2020), spermatogenesis-related genes (Qu et al., 2019), cell apoptosis-related genes (Amanpour, Khodarahmi, & Salehipour, 2020; Amevor et al., 2022), hormone receptor- or kinase- related genes (Amevor et al., 2022; Zhang et al., 2021) or activity of energy metabolism enzyme (Yan, Yue, Luo, Jin, & Xu, 2010).

This review intends to provide a comprehensive summary about the mechanism that Vitamin E improves testis development and spermatogenesis in farm animals from antioxidant and potential non-antioxidant perspectives.

## 2. Testicular development

### *2.1. Roles of Vitamin E in testicular development*

Most research verified that Vitamin E promoted testicular development as Table 1 showed. Diameter of seminiferous tubule and epididymis ductile, density of spermatogenic cell and epididymis weight were increased by supplementation of Vitamin E in buck (Hong et al., 2009). Increased diameter of seminiferous tubules in response to Vitamin E treatment was also verified in rats (Momeni et al., 2012). And diet following supplementation of Vitamin E also increased testis weight in Japanese quails and Nile tilapia (Abedi, Tabatabaei Vakili, Mamouei, & Aghaei, 2017; Zhang et al., 2021). However, not all of research got the consistent conclusions. Some studies also found that Vitamin E did not enhance testicular testis weight and plasma testosterone level in quail (Biswas, Mohan, Sastry, & Tyagi, 2007). In fact, effect of Vitamin E on testis weight is not entirely dose-dependent. Adult male quails receiving moderate supplemental Vitamin E (75 and 150 IU/kg) had a higher testicular weight and plasma testosterone than quails fed on either Vitamin E-deficient or more highly supplemented diets (225 and 300 IU/kg) (Hooda et al., 2007), which suggests high level of Vitamin E weakened testicular development. And the weights of testis in the bucks supplemented with 80 IU or 320 IU Vitamin E were much more heavier than those in the bucks supplemented with 0 IU or 880 IU (Hong et al., 2009). Another research also revealed high level of Vitamin E-induced adverse reproductive performance. With supplementation of 0, 100, 1000, 10000, 20000 IU Vitamin E, weights of testis were decreased in roosters (Danikowski, Sallmann, Halle, & Flachowsky, 2002).

### *2.2. The mechanism underlying that Vitamin E promotes testicular development*

The mechanism that Vitamin E improved testicular development was overwhelmingly attributed to the antioxidant property of Vitamin E. Most of research focused on gene expression associated with antioxidant or activity of antioxidant enzymes in testis. Vitamin E could increase SOD (Superoxide dismutase) activity and decrease MDA (Malonaldehyde) level in goats, sheep and rabbits (Aydilek, Aksakal, & Karakilcik, 2004; Hong et al., 2010; Yue et al., 2010). TBARS (Thiobarbituric acid-reactive substances) was decreased by Vitamin E in roosters (Danikowski et al., 2002). However, exposure to Vitamin E increased activity of GPX (Glutathione peroxidase) (Yue et al., 2010), testicular mitochondrial ATPase (Adenosine triphosphatase), LDH (Lactate dehydrogenase), SDH (Sorbitol dehydrogenase) and ALP (Alkaline phosphatase) (Yan et al., 2010). And integrated analysis of transcriptome, qRT-PCR (Real-Time Quantitative Reverse Transcription PCR) and Western blot found dietary Vitamin E treatment altered expression of antioxidant enzyme-related genes, GPX3 (Glutathione peroxidase 3) and GSTA1 (Glutathione S-transferase alpha 1) in sheep (C. Xu et al., 2016). The expression of PGR (Progesterone receptor) and FSHR (Follicle-stimulating hormone receptor) in testis was up-regulated with 160 mg/kg Vitamin E in diet (Zhang et al., 2021).

In addition, cell proliferation and cell apoptosis was also the potential target by which Vitamin E promoted testis development. Vitamin E could enhance testis cell proliferation, and decreased the proportion of cells in the G1 phase and increased those in the S and G2/M phases in response to vitamin E supplementation. And Vitamin E was responsible for protein expression of Ki67 (Antigen identified by monoclonal antibody Ki 67), PCNA (Proliferating cell nuclear antigen), Cyclin B1 and Cyclin B3, which were

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associated with cell proliferation and cell cycle. Integrated miRNAome and mRNAome analysis found some miRNA and target gene pairs, miR-107-BLM (BLM RecQ like helicase), miR-107-REL (REL proto-oncogene) and miR-493-3p-KLHL25 (Kelch like family member 25) were key point for testis development induced by Vitamin E (Gao et al., 2020). Further, another research also found Vitamin E also facilitated Sertoli cells proliferation in sheep by regulation of genes associated with cell division and the cell cycle (PDPN, BMP6, Myc, AMPKa, GSK3β, PPARγ) (Gao et al., 2022), which were probably non-antioxidant implications that Vitamin E promoted testicular development. Although the testis weights in Vitamin E group was numerically higher than the testis weights in control group, Vitamin E was found to up-regulate gene expression of BCL-2 (BCL2 apoptosis regulator), Mfn1 (Mitofusin 1) and Mfn2 (Mitofusin 2), and down-regulate Caspase 9 gene expression (Amanpour et al., 2020).

**Table 1.** Effect of Vitamin E on testicular development.

| Doses | Ways of addition | Species | Testicular parameters | References |
|---|---|---|---|---|
| 80 IU | diet | goats | diameter of convoluted seminiferous tubule and epididymis ductules, numeric density of spermatogenic cell and epididymis weight | (Hong et al., 2009) |
| 75 and 150 IU/kg | diet | quails | increased testicular weight and plasma testosterone | (Hooda et al., 2007) |
| 150 and 300 IU kg | diet | quails | no effect on testis weight and plasma testosterone | (Biswas et al., 2007) |
| 60, 240 mg/kg | diet | quails | Increased left testis weight | (Abedi et al., 2017) |
| 100, 1000, 10000, 20000 IU/kg | diet | roosters | weights of testis were decreased | (Danikowski et al., 2002) |
| 0, 40, 80, 120, 160 mg/kg | diet | Nile tilapia | increased gonad weight (120 and 160 mg/kg) | (Zhang et al., 2021) |

## 3. Spermatogenesis

### *3.1. Roles of Vitamin E in spermatogenesis*

Most of references have confirmed that Vitamin E could facilitate spermatogenesis and improve semen quality in mammals, avian and fishes as Table 2 displayed. Vitamin E improved ejaculate volume, sperm concentration, total sperm output, sperm motility as well as decreased percentage of dead sperm and abnormal sperm in rabbits (Hashem, El-Hady, & Hassan, 2013; Yousef, 2010; Yousef et al., 2003). Percentage of progressively motile sperm and sperm viability, sperm motility, sperm count, morphology rate, plasma membrane integrity and libido were also improved in rats, roosters, turbot, boar and sheep (Lin et al., 2005; Moumeni, Soleymani, Abnousi, & Mahmoudi, 2009; Ozer Kaya et al., 2020; Umesiobi, 2012; H. Xu, Huang, Liang, Zheng, & Wang, 2015; Yue et al., 2010). A meta-analysis also concluded that dietary supplementation level of Vitamin E significantly affected sperm concentration, motility, viability, sperm fertility and the number of sperm cell deaths in chicken (Hayanti et al., 2022). And semen quality and the blood plasma testosterone were improved in the 4 dogs with poor semen quality after the 50 mg Vitamin E treatment (Kawakami, Kobayashi, Hori, & Kaneda, 2016). Ejaculate volume increased significantly from 4th week after Vitamin E treatment to the end of the experiment. The percentage of dead and abnormal sperm was reduced significantly in Vitamin E group compared with control group after the 3rd week of treatment and this effect continued until the end of the experiment. And the number of services was increased in response to Vitamin E treatment (Talib Ali, Bomboi, & Floris, 2016). The diets in roosters supplemented with Vitamin E increased sperm concentration, sperm viability, sperm motility and membrane integrity (Lotfi, Fakhraei, & Mansoori Yarahmadi, 2021). Dietary Vitamin E could increase sperm count, sperm motility, serum level of LH and FSH in aged male chicken (Amevor et al., 2022). Beside domestic animals, the ameliorative effect of Vitamin E on semen quality was also discovered in the research concerning model animal. Rate of sperm with normal morphology was also increased in mice injected with 200 mg Vitamin E (Doostabadi, Hassanzadeh-Taheri, Asgharzadeh, & Mohammadzadeh, 2021).

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Some researchers found integration of Vitamin E and selenium by diet or injection displayed better effect. Boars exposed to Vitamin E and selenium showed significantly higher sperm concentration and total sperm count, significantly lower percentage of spermatozoa with major or minor morphological changes, elevated percentage of spermatozoa with normal acrosome, and significantly higher osmotic resistance test values (Kołodziej & Jacyno, 2005). Injections of combination of Vitamin E and selenium improved during the breeding season semen quality, quantity and libido (Mahmoud, Abdel-Raheem, & Hussein, 2013). Combination of Vitamin E and selenium in diet have a higher sperm motility and blood testosterone concentration during humid hot summer in Holstein Friesian bulls (Butt, Shahid, Bhatti, & Khalique, 2019).

Not all of research got consistent conclusions. Supplementation of 220 IU Vitamin E/kg diet did not affect testicular sperm reserves, population of Sertoli cells and germ cells and structural abnormalities in the spermatozoa in boars (Marin-Guzman, Mahan, & Pate, 2000; Marin-Guzman, Mahan, & Whitmoyer, 2000). And 150 IU or 300 IU Vitamin E did not affect semen volume, sperm motility and sperm concentration (Biswas et al., 2007). The reason why is probably due to insufficient or excessive dose of Vitamin E. Indeed, some research had found that high level of Vitamin E jeopardized semen quality. With supplementation of 0 IU, 100 IU, 1000 IU, 10000 IU, 20000 IU Vitamin E, the percentage of morphologically deformed spermatozoa was increased, and semen density was decreased in roosters (Danikowski et al., 2002). Supplementation of 150 IU Vitamin E decreased the percentage of dead sperm in quail. And 300 IU Vitamin E could increase the percentage of abnormal sperm and dead sperm (Biswas et al., 2007). 200 IU or 2000 IU Vitamin E increased number of germ cells, including spermatocytes (200 IU) and spermatozoa (200 IU or 2000 IU) in sheep, however, the positive effect was reduced by 2000 IU Vitamin E (Qu et al., 2019), which suggests that it is not entirely dose-dependent and high dose of Vitamin E decreased male reproductive performance.

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**Table 2.** Effect of Vitamin E on semen quality.

| Doses | Ways of addition | Species | Semen quality | References |
|---|---|---|---|---|
| 1 g/L<br>2 mg/kg | drinking water | rabbits | increased ejaculate volume, sperm concentration, total sperm output, sperm motility, decreased percentage of dead sperm and abnormal sperm | (Yousef et al., 2003)<br>(Yousef, 2010) |
| 150 mg/kg | diet | rabbits | increased sperm concentration, decreased dead sperm number | (Hashem et al., 2013) |
| 50 mg | oral administration | dogs | improved semen quality and the blood plasma testosterone | (Kawakami et al., 2016) |
| 70 IU | diet | boars | improved libido and ejaculate volume, sperm motility, live sperm, sperm concentration and total sperm count | (Umesiobi, 2012) |
| 200 IU | diet | sheep | increased ejaculate volume, sperm concentration and sperm output | (Yue et al., 2010) |
| 175 mg | injection | sheep | increased ejaculate volume and number of services; reduced percentage of dead and abnormal sperm | (Talib Ali et al., 2016) |
| 200 or 2000 IU | diet | sheep | increased number of spermatocytes (200 IU) and spermatozoa (200 IU or 2000 IU) | (Qu et al., 2019) |
| 300 mg | intramuscular injection | sheep | decreased abnormal sperm rate, increased semen volume, semen mass activity, sperm motility and concentration | (Ozer Kaya et al., 2020) |
| 40 to 160 mg/kg | diet | cockerels | improved sperm viability and motility | (Lin et al., 2005) |
| 0.2 g/kg | diet | chicken | increase sperm count and sperm motility | (Amevor et al., 2022) |
| 244.60 and 721.6 mg/kg | diet | turbots | increased sperm concentration and integrity of plasma membrane | (H. Xu et al., 2015) |
| 150 IU or 300 IU | diet | quails | decreased the percentage of dead sperm, increase the percentage of abnormal sperm and dead sperm | (Biswas et al., 2007) |
| 100, 1000, 10000, 20000 IU/kg | diet | roosters | increased the percentage of morphologically deformed spermatozoa, and decreased semen density | (Danikowski et al., 2002) |
| 200 mg/kg | diet | roosters | increased sperm concentration, sperm viability, sperm motility and membrane integrity | (Lotfi et al., 2021) |
| 220 IU/kg | diet | boars | no effect on testicular sperm reserves, population of Sertoli cells and germ cells and structural abnormalities in the spermatozoa | (Marin-Guzman, Mahan, & Pate, 2000; Marin-Guzman, Mahan, & Whitmoyer, 2000) |

*3.2. The mechanism that Vitamin E promotes semen quality*

Just like research on testis development induced by Vitamin E, plenty of effort has been devoted to antioxidant role of Vitamin E. TBARS or MDA was decreased and superoxide dismutase activity in seminal plasma was increased by Vitamin E (Amevor et al., 2022; Kawakami et al., 2016; Lotfi et al., 2021; Yousef et al., 2003). The mRNA expression of CatSper1 (Cation Channel Sperm Associated 1) and CatSper2 (Cation Channel Sperm Associated 2), of which proteins is located in sperm, was up-regulated by Vitamin E treatment (Mohammadi et al., 2013). However, part of works found a potential non-antioxidant role of Vitamin E in spermatogenesis. The combination of qRT - PCR and transcriptome analysis verified that dietary Vitamin E increased number of germ cells via regulation of NDRG1 (N-myc downstream regulated 1), CYP26B1 (Cytochrome P450 family 26 subfamily B member 1), FSCN3 (Fascin actin-bundling protein 3) and COL4A1 (Collagen type IV, alpha1) (Qu et al., 2019). And integrated proteomics and PRM (Parallel reaction monitoring) analysis also confirmed Vitamin E improved spermatogenesis by regulating the expression of FLNA (Filamin A), SPCS3 (Signal peptidase complex subunit 3), YBX3 (Y-box binding protein 3) and RARS (Arginyl-tRNA synthetase) proteins that were associated with the plasma membranes and protamine biosynthesis of the spermatozoa (Gao et al., 2021). Dietary Vitamin E could up-regulate AR (Androgen receptor), Pgk2 (Phosphoglycerate kinase) and Cyclin A2 in testis, and down-regulate Bax (BCL2 associated X) gene expression in liver and testis (Amevor et al., 2022).

In the prior research on elucidation of mechanism by which Vitamin E promoted testis development, the GO (Gene Ontology) enrichment analysis in microbiome found

some terms, including ECM (Extracellular matrix) - receptor interaction, PI3K - Akt signaling pathway and Focal adhesion terms (Gao et al., 2020). Extracellular matrix term was also identified in the transcriptome research (Gao et al., 2020). The GO terms, ECM - receptor interaction, PI3K - Akt signaling pathway, Focal adhesion, were also found in the transcriptome investigation of spermatogenesis facilitated by dietary Vitamin E (Qu et al., 2019) and Focal adhesion terms were also found in the research on effect of dietary Vitamin E on testis expression profiles by transcriptome (C. Xu et al., 2016). Based on previous research, all of the genes associated with testis development and spermatogenesis enhanced by Vitamin E are displayed in Table 3. The aforementioned studies simply verified a relationship among antioxidant, non-antioxidant and semen quality, but the specific mechanisms still need to be revealed by more adequate evidences.

**Table 3.** Effect of Vitamin E on gene expression in testis .

| Gene symbol | Animal | Assay method | References |
|---|---|---|---|
| GPX3, GSTA1 | sheep | PCR, Western blot, transcriptome | (C. Xu et al., 2016) |
| PGR, FSHR | tilapia | PCR | (Zhang et al., 2021) |
| PDPN, BMP | sheep | PCR, Western blot | (Gao et al., 2022) |
| Myc, AMPKa, GSK3β, PPARγ, CDK4, CDK6 | sheep | PCR | (Gao et al., 2022) |
| Ki67, PCNA, Cyclin B1, Cyclin B3 | sheep | Western blot | (Gao et al., 2020) |
| miR-107-BLM, miR-107-REL, miR-493-3p-KLHL25 | sheep | microbiome-transcriptome | (Gao et al., 2020) |
| BCL-2, Mfn1, Mfn2, Caspase 9 | rat | PCR | (Amanpour et al., 2020) |
| CatSper1, CatSper2 | mouse | PCR | (Mohammadi et al., 2013) |
| NDRG1, CYP26B1, FSCN3, COL4A1 | sheep | PCR, transcriptome | (Qu et al., 2019) |
| FLNA, SPCS3, YBX3, RARS | sheep | Proteomics, PRM | (Gao et al., 2021) |
| AR, Pgk2, Cyclin A2, Bax | rooster | PCR | (Amevor et al., 2022) |

## 4. Conclusions

The review indicates that Vitamin E probably facilitates testis development or semen quality associated with cell proliferation (PDPN, BMP, Myc, AMPKa, GSK3β, PPARγ, CDK4, CDK6, Ki67, PCNA, Cyclin A2, Cyclin B1, Cyclin B3, BLM, REL, KLHL25) -, cell apoptosis (BCL-2, Caspase 9, Bax) -, spermatogenesis (CatSper1, CatSper2, NDRG1, CYP26B1, FSCN3, FLNA, SPCS3, YBX3, RARS) -, hormone receptor (PGR, FSHR, AR) - related genes or cell cycle-related non-coding RNAs, miR-107 and miR-493-3p. The mechanism still needs to be studied.

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