ARCHIVES OF VETERINARY SCIENCE

March/2025

WAAVP

30th Conference of the World Association for the Advancement of Veterinary Parasitology

August 17th to 21st, Viasoft Experience

CURITIBA - 2025

Supplementation of biotin to the semen dilution medium improves sperm quality parameters in turkeys

http://dx.doi.org/10.5380/avs.v30i1.96899

ARTICLES

Archives of Veterinary Science https://www.ufpr.br/

Supplementation of biotin to the semen dilution medium improves sperm quality parameters in turkeys

Submitted: 16/09/2024
Accepted: 22/02/2025

Marziyeh Bazpour, Saleh Tabatabaei Vakili, Khalil Mirzadeh, Ali Aghaei*

Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran, ORCID: 0009-0002-1997-0616, ORCID: 0000-0003-1598-7154, ORCID: 0009-0008-7066-1214, ORCID: 0000-0003-2934-0431

Author for correspondence: Saleh Tabatabaei Vakili - tabatabaei@asnrukh.ac.ir

Abstract: Turkey's reproduction relies heavily on artificial insemination, making short-term semen preservation crucial. This study investigated the impact of biotin supplementation on turkey sperm quality during short-term cold storage. Semen was diluted with extenders containing various biotin concentrations (0, 25, 50, 100, and 200 nmol) and stored at 5°C for 24 hours. While initial sperm quality was unaffected by biotin levels, prolonged storage revealed significant differences. A biotin concentration of 25 nmol consistently outperformed other levels in maintaining sperm motility, viability, and membrane integrity, with the lowest rates of morphological abnormalities. Conversely, a 200 nmol biotin concentration negatively impacted sperm viability ($P < 0.05$). For each treatment, 25 nmol of biotin consistently preserved the best sperm quality and viability ($P < 0.05$). These findings suggest that adding 25 nmol of biotin to turkey semen extenders is an effective strategy for preserving sperm quality and extending its lifespan under cold storage conditions.
Keywords: Antioxidant, biotin, extender, sperm quality, turkey.

1. Introduction

Several factors contribute to reduced sperm quality and fertility during *in vitro* storage. One of the most significant factors is the irreversible alteration of the sperm's phospholipid membrane due to lipid peroxidation (Pardyak et al., 2024). Storing sperm at low temperatures increases the production of reactive oxygen species (ROS), which have been shown to cause harmful effects on the sperm cell structure (Gualtieri et al., 2021). During spermatogenesis, sperm lose a large portion of their cytoplasm, where most enzymes are located, resulting in limited antioxidant capacity. Additionally, the concentration of antioxidant factors in seminal plasma decreases during semen processing and dilution. Therefore, adding antioxidants to semen during storage is a practical approach to maintaining sperm quality and enhancing fertility (Qamar et al., 2023).

The sperm membrane in poultry contains unsaturated fatty acids, predominantly n-6 fatty acids, which are highly susceptible to lipid peroxidation and ROS. Peroxidation of these unsaturated fatty acids in the sperm membrane leads to cellular dysfunction and loss of membrane integrity (Amini et al., 2015; Elomda et al., 2024). Moreover, due to its lower cytoplasm content than mammalian sperm, poultry sperm receives less protective material and is consequently more vulnerable to oxidative damage (Zong et al., 2023).

Biotin, or vitamin B7, is a water-soluble vitamin from the B complex, also known as vitamin H. It acts as a coenzyme in the metabolism of fatty acids, carbohydrates, and amino acids, as well as in the synthesis of vitamins B6 and B12 (Montazeri et al., 2021). It has been shown that vitamins E and B are beneficial in testicular tissue, and play a positive role in sperm functional parameters (Hassani-Bafrani et al., 2019). Vitamins D and B are also effective in treating male infertility and protecting the nuclear DNA from damage caused by environmental stress and pollution. A deficiency in B vitamins, due to their role in DNA synthesis, development, and repair, can impair spermatogenesis. B vitamins improve semen quality and reduce sperm apoptosis by decreasing ROS (Kaltsas et al., 2023).

Protecting the plasma membrane against oxidative reactions with antioxidants improves the quality and function of poultry sperm. Considering that the long-term storage of avian sperm can be highly beneficial in the poultry industry, developing suitable extenders for avian semen preservation is essential (Khoso et al., 2024). Thus, this study aimed to evaluate the effect of adding different levels of biotin to turkey semen on sperm viability and quality parameters.

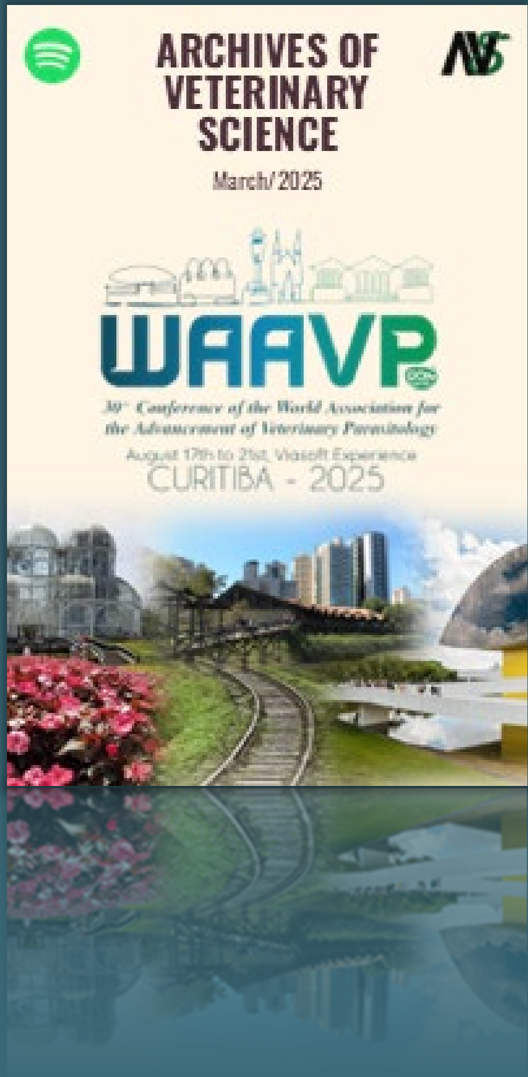
2. Materials e Methods

2.1. Study location and animals

The present study was conducted at the poultry research farm of Agricultural Sciences and Natural Resources University of Khuzestan, located in Khuzestan province, Iran. A total of 18 male turkeys, averaging 7 months old, were used for this study. The birds were kept indoors as a group and fed a diet according to the National Research Council (NRC) recommendations. The diet was based on corn (61%), barn (11%), alfalfa (3%), barley (9%), soybean (11%), oyster (1.3%), phosphate (1.2%), soda (0.1%), salt (0.25%), methionine (0.15%), vitamin supplement (0.25%), oil (1.5%); with metabolizable energy (2855 kcal/kg), crude protein (12%), calcium (0.83%), phosphorus (0.36%), chlorine (0.18%), and sodium (0.18%). The turkeys had free access to water.

2.2. Semen collection and preparation

Semen were collected by abdominal massage method from each turkey weekly for five weeks. Samples were immediately transferred to the laboratory and were pooled to reduce errors due to individual differences and diluted in Modified Ringer's solution



Authors:

Marziyeh Bazpour
Saleh Tabatabaei Vakili
Khalil Mirzadeh
Ali Aghaei

Author for correspondence:

Saleh Tabatabaei Vakili – tabatabaei@asnrukh.ac.ir

ARCHIVES OF VETERINARY SCIENCE

March/2025

To read full article visit Vol. 30 No. 1 (2025): Archives of Veterinary Science: WAAVP 2025 Special Edition at https://revistas.ufpr.br/veterinary/ and look for by this title.

publish.avs@ufpr.br
editor.avs@ufpr.br

UFPR
UNIVERSIDADE FEDERAL DO PARANÁ

50 anos Setor de Ciências Agrárias Universidade Federal do Paraná 1973 - 2023

PPGCV PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS-UFPR

ARTICLES
Archives of Veterinary Science
https://www.ufpr.br/

Supplementation of biotin to the semen dilution medium improves sperm quality parameters in turkeys

Submitted: 16/09/2024
Accepted: 22/02/2025

Marziyeh Bazpour, Saleh Tabatabaei Vakili, Khalil Mirzadeh, Ali Aghaei*

Department of Animal Science, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran, ORCID: 0009-0002-1997-0616, ORCID: 0000-0003-1598-7154, ORCID: 0009-0008-7066-1214, ORCID: 0000-0003-2934-0431

Author for correspondence: Saleh Tabatabaei Vakili - tabatabaei@asnrukh.ac.ir

Abstract: Turkey's reproduction relies heavily on artificial insemination, making short-term semen preservation crucial. This study investigated the impact of biotin supplementation on turkey sperm quality during short-term cold storage. Semen was diluted with extenders containing various biotin concentrations (0, 25, 50, 100, and 200 nmol) and stored at 5°C for 24 hours. While initial sperm quality was unaffected by biotin levels, prolonged storage revealed significant differences. A biotin concentration of 25 nmol consistently outperformed other levels in maintaining sperm motility, viability, and membrane integrity, with the lowest rates of morphological abnormalities. Conversely, a 200 nmol biotin concentration negatively impacted sperm viability ($P < 0.05$). For each treatment, 25 nmol of biotin consistently preserved the best sperm quality and viability ($P < 0.05$). These findings suggest that adding 25 nmol of biotin to turkey semen extenders is an effective strategy for preserving sperm quality and extending its lifespan under cold storage conditions.

Keywords: Antioxidant, biotin, extender, sperm quality, turkey.

1. Introduction

Several factors contribute to reduced sperm quality and fertility during *in vitro* storage. One of the most significant factors is the irreversible alteration of the sperm's phospholipid membrane due to lipid peroxidation (Pardyak et al., 2024). Storing sperm at low temperatures increases the production of reactive oxygen species (ROS), which have been shown to cause harmful effects on the sperm cell structure (Gualtieri et al., 2021). During spermatogenesis, sperm lose a large portion of their cytoplasm, where most enzymes are located, resulting in limited antioxidant capacity. Additionally, the concentration of antioxidant factors in seminal plasma decreases during semen processing and dilution. Therefore, adding antioxidants to semen during storage is a practical approach to maintaining sperm quality and enhancing fertility (Qamar et al., 2023).

The sperm membrane in poultry contains unsaturated fatty acids, predominantly n-6 fatty acids, which are highly susceptible to lipid peroxidation and ROS. Peroxidation of these unsaturated fatty acids in the sperm membrane leads to cellular dysfunction and loss of membrane integrity (Amini et al., 2015; Elomda et al., 2024). Moreover, due to its lower cytoplasm content than mammalian sperm, poultry sperm receives less protective material and is consequently more vulnerable to oxidative damage (Zong et al., 2023).

Biotin, or vitamin B7, is a water-soluble vitamin from the B complex, also known as vitamin H. It acts as a coenzyme in the metabolism of fatty acids, carbohydrates, and amino acids, as well as in the synthesis of vitamins B6 and B12 (Montazeri et al., 2021). It has been shown that vitamins E and B are beneficial in testicular tissue, and play a positive role in sperm functional parameters (Hassani-Bafrani et al., 2019). Vitamins D and B are also effective in treating male infertility and protecting the nuclear DNA from damage caused by environmental stress and pollution. A deficiency in B vitamins, due to their role in DNA synthesis, development, and repair, can impair spermatogenesis. B vitamins improve semen quality and reduce sperm apoptosis by decreasing ROS (Kaltsas et al., 2023).

Protecting the plasma membrane against oxidative reactions with antioxidants improves the quality and function of poultry sperm. Considering that the long-term storage of avian sperm can be highly beneficial in the poultry industry, developing suitable extenders for avian semen preservation is essential (Khoso et al., 2024). Thus, this study aimed to evaluate the effect of adding different levels of biotin to turkey semen on sperm viability and quality parameters.

2. Materials e Methods

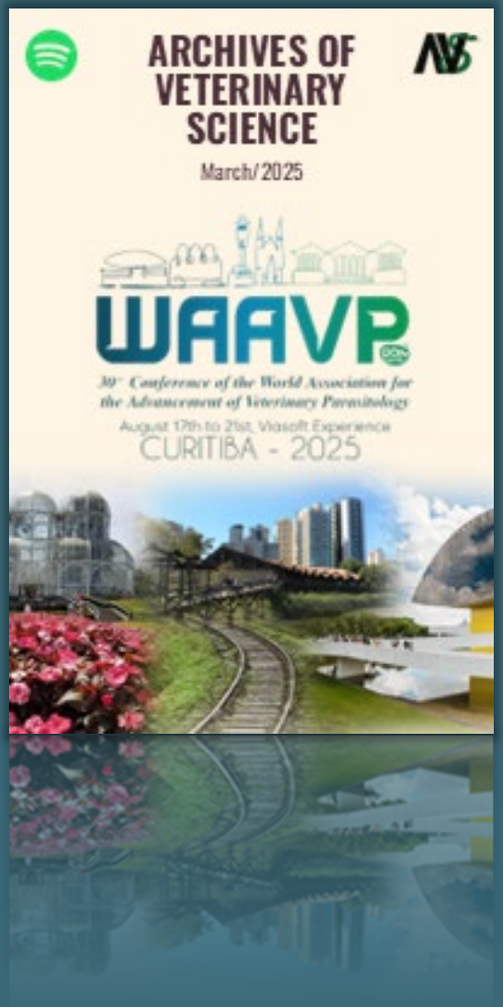
2.1. Study location and animals

The present study was conducted at the poultry research farm of Agricultural Sciences and Natural Resources University of Khuzestan, located in Khuzestan province, Iran. A total of 18 male turkeys, averaging 7 months old, were used for this study. The birds were kept indoors as a group and fed a diet according to the National Research Council (NRC) recommendations. The diet was based on corn (61%), barn (11%), alfalfa (3%), barley (9%), soybean (11%), oyster (1.3%), phosphate (1.2%), soda (0.1%), salt (0.25%), methionine (0.15%), vitamin supplement (0.25%), oil (1.5%); with metabolizable energy (2855 kcal/kg), crude protein (12%), calcium (0.83%), phosphorus (0.36%), chlorine (0.18%), and sodium (0.18%). The turkeys had free access to water.

2.2. Semen collection and preparation

Semen were collected by abdominal massage method from each turkey weekly for five weeks. Samples were immediately transferred to the laboratory and were pooled to reduce errors due to individual differences and diluted in Modified Ringer's solution

http://dx.doi.org/10.5380/avs.v30i1.96899



ARCHIVES OF VETERINARY SCIENCE

March/2025

publish.avs@ufpr.br
editor.avs@ufpr.br

UFPR UNIVERSIDADE FEDERAL DO PARANÁ
50 anos Setor de Ciências Agrárias Universidade Federal do Paraná 1973 - 2023
PPGCV PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS-UFPR

(Sodium chloride: 6.80 gr, potassium chloride: 1.73 gr, calcium chloride: 0.64 gr, magnesium sulfate: 0.25 gr, sodium bicarbonate: 2.45 gr, distilled water: 100 ml) in a ratio of 1:10 (Tabatabaei et al., 2011).

2.3. Semen processing and experimental groups

The pooled and diluted semen was divided into equal aliquots and supplemented with different concentrations of biotin (Sigma-Aldrich), including 0, 25, 50, 100, and 200 nmol.

2.4. Assessment of sperm quality parameters

The treated semen samples were stored at 5°C in a refrigerator, and sperm quality parameters were examined at 1, 6, 12, 18, and 24 hours after storage. Sperm total motility and progressive motility were estimated using computer-assisted sperm analysis (CASA, video test-sperm 2.1, St. Petersburg, Russia). Five microscopic fields were analyzed for each semen sample (Santos et al., 2019). Sperm plasma membrane integrity was evaluated using a hypotonic swelling test (HOST). A 10 µL semen sample was mixed with 100 µL hypo-osmotic solution (1.35 g fructose and 0.375 g sodium citrate dissolved in 100 ml distilled water, pH=7) and incubated at 37°C for 30 minutes. The percentage of swollen and coiled tails in 200 sperm was counted using a 400x phase contrast microscope and considered indicative of normal plasma membrane integrity (Wang et al., 2022). Sperm viability was evaluated using the eosin-nigrosin staining method. A small drop (10 µL) of semen was placed on a pre-warmed slide and mixed with a larger drop (20 µL) of the stain (10 g nigrosin, 1.6 g eosin, and 2.9 g sodium citrate dissolved in 100 ml distilled water). After drying, viability was assessed by counting 200 cells by microscope at 1000× magnification using immersion oil. Spermatozoa with unstained sperm heads were considered viable, while sperm with stained or partially stained heads were counted as dead (Amini et al., 2015). Morphological sperm abnormalities were determined by examining semen smears stained with eosin-nigrosin on a glass slide. A total of 200 spermatozoa was counted on each slide at 1000× magnification using immersion oil, and the percentage of morphologically abnormal sperms in the head, mid-piece, and tail was estimated (Wang et al., 2022).

2.5. Statistical analysis

Data were analyzed using SPSS software (version 20) with a completely randomized design. One-way analysis of variance (ANOVA) was conducted to compare sperm quality parameters among treatment groups at each storage time point. Repeated measures ANOVA was employed to assess changes in sperm parameters over time for each treatment group. Duncan's multiple range test was used for post-hoc comparisons of means.

3. Results

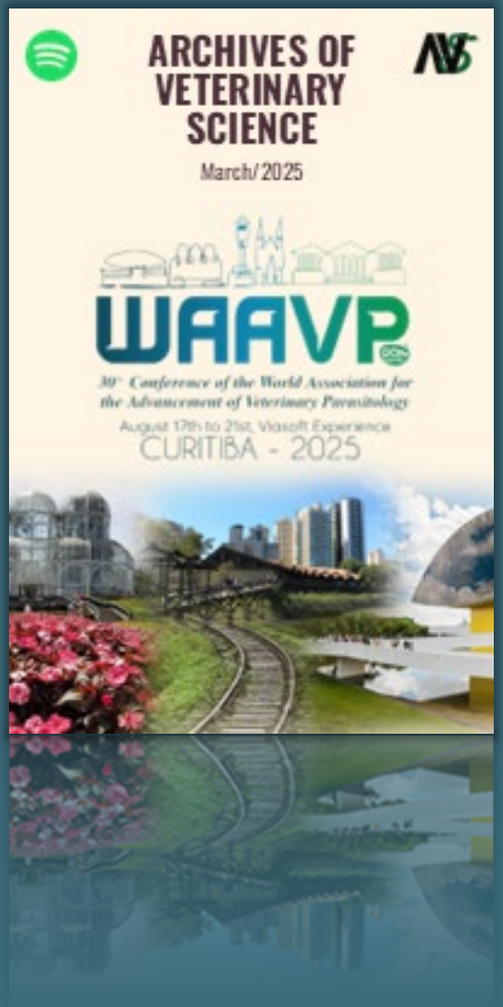
3.1. Total Sperm Motility

The findings of this study demonstrated that total sperm motility was influenced by the addition of varying levels of biotin to the semen extender in a time-dependent manner (Table 1). While the total motility percentage did not show significant changes after 1 hour of semen storage across different treatments, a significant increase in motility was observed at the 25-nanomole biotin level after 24 hours of storage ($P < 0.05$). Moreover, a detailed analysis of total sperm motility percentages over various storage durations (Fig. 1) indicated that the 25-nanomole biotin level consistently preserved optimal sperm motility over time ($P < 0.05$).

Treatments (nmol)	Storage period (hours)				
	1	6	12	18	24
Control	91.25±2.39	76.00±2.42^{b}	60.00±2.89^{bc}	55.00±2.04^{bc}	28.75±4.73^{b}
Biotin 50	89.00±3.54	88.75±1.25^{a}	82.50±3.23^{a}	80.65±3.15^{a}	58.33±4.41^{a}
Biotin 100	87.50±3.23	73.67±2.33^{b}	66.67±7.26^{ab}	55.00±9.35^{b}	25.00±5.24^{b}
Biotin 150	88.75±2.39	75.00±7.64^{b}	61.67±8.33^{bc}	46.25±7.18^{b}	30.12±5.24^{b}
Biotin 200	86.80±4.56	76.67±1.67^{b}	48.33±3.33^{c}	42.50±4.79^{b}	35.06±2.89^{b}
P-value	0.64	0.014	0.029	0.011	0.044

Table 1 – Effect of biotin levels on total sperm motility (%) during chilled semen storage in turkeys. Data are presented as Mean ± SE; means within the same column with different superscripts differ significantly ($P < 0.05$).

http://dx.doi.org/10.5380/avs.v30i1.96899



ARCHIVES OF VETERINARY SCIENCE

March/2025

publish.avs@ufpr.br
editor.avs@ufpr.br

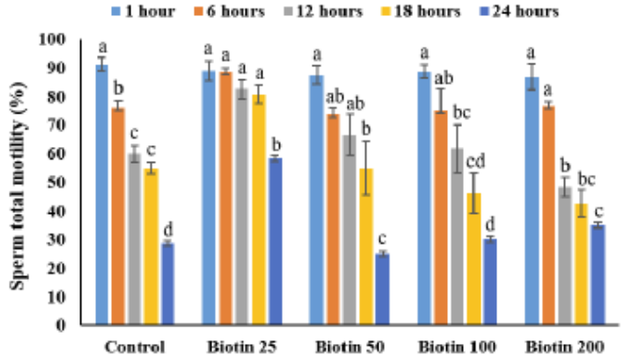


Figure 1 – Effect of biotin levels (nmol) on the sperm total motility over storage time. Data are presented as Mean ± SE; groups with different superscripts differ significantly ($P < 0.05$).

3.2. Progressive Sperm Motility

Table 2 illustrates the impact of different biotin levels on progressive sperm motility. Similar to total sperm motility, initial progressive motility was unaffected by biotin levels. However, prolonged storage of up to 24 hours revealed that a biotin concentration of 25 nmol consistently outperformed other levels in maintaining progressive motility ($P < 0.05$). Notably, higher biotin concentrations (100 and 150 nmol) negatively impacted progressive motility at the 18-hour storage time ($P < 0.05$). Figure 2 shows that the 25 nmol biotin concentration maintained superior performance in preserving progressive sperm motility over time compared to other biotin levels and the control group ($P < 0.05$).

Treatments (nmol)	Storage period (hours)				
	1	6	12	18	24
Control	82.20±3.27	80.50±2.40^{a}	77.25±2.29^{a}	76.00±3.49^{a}	52.67±5.04^{a}
Biotin 50	81.50±3.34	72.12±1.53^{ab}	63.33±6.01^{ab}	49.50±9.67^{b}	22.50±9.51^{b}
Biotin 100	81.00±1.68	67.67±7.31^{b}	53.34±7.69^{bc}	28.35±2.84^{c}	20.00±5.04^{b}
Biotin 150	81.25±4.80	65.40±1.45^{b}	43.67±4.48^{c}	28.00±3.40^{c}	27.67±1.45^{b}
Biotin 200	85.75±2.69	67.50±2.53^{b}	53.20±4.41^{bc}	48.25±2.69^{b}	25.25±3.61^{b}
P-value	0.83	0.03	0.015	0.003	0.026

Table 2 – Effect of biotin levels on sperm progressive motility (%) during chilled semen storage in turkeys. Data are presented as Mean ± SE; means within the same column with different superscripts differ significantly ($P < 0.05$).

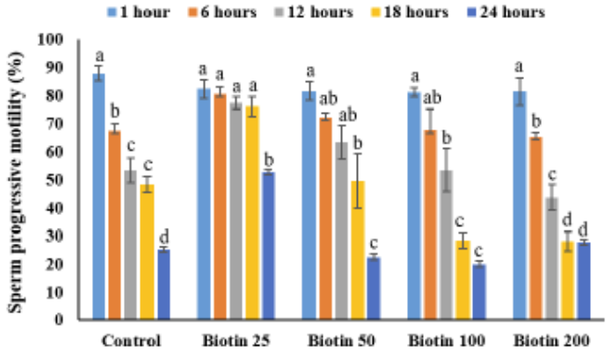
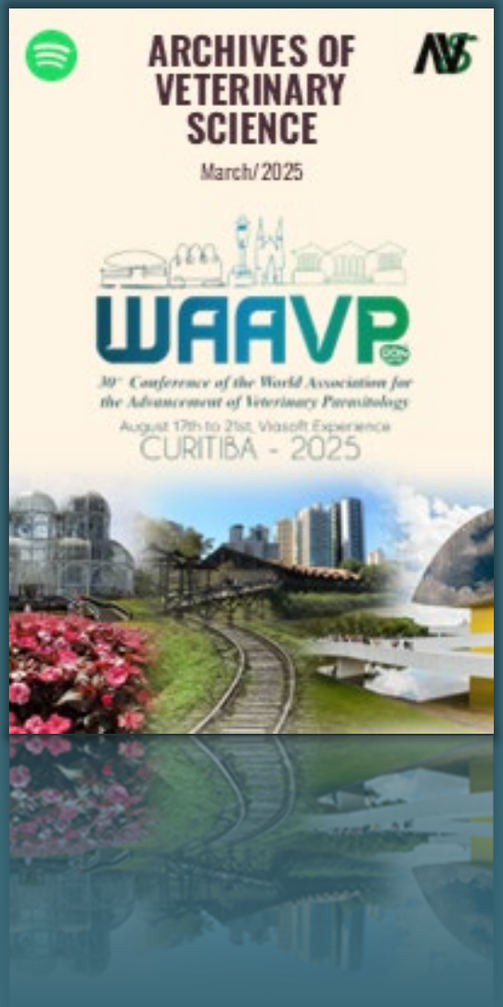


Figure 2 – Effect of biotin levels (nmol) on the sperm progressive motility over storage time. Data are presented as Mean ± SE; groups with different superscripts differ significantly ($P < 0.05$).

http://dx.doi.org/10.5380/avs.v30i1.96899



ARCHIVES OF VETERINARY SCIENCE

March/2025

publish.avs@ufpr.br
editor.avs@ufpr.br

UFPR UNIVERSIDADE FEDERAL DO PARANÁ
50 anos Setor de Ciências Agrárias Universidade Federal do Paraná 1973 - 2023
PPGCV PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS-UFPR

3.3. Sperm Viability

The effect of different biotin levels on sperm viability is presented in Table 3. After one hour of semen storage, no significant differences in sperm viability were observed across the various biotin levels. However, as the storage duration increased, lower biotin levels (25 and 50 nmol) positively influenced sperm viability. Specifically, at the 6-hour, the highest sperm viability percentage was recorded at the 25 nmol biotin level ($P < 0.05$). After 24 hours of storage, the highest sperm viability was associated with the 25 nmol biotin level, while the lowest was observed at the 200 nmol level ($P < 0.05$). Changes in sperm viability percentages over time (Fig. 3) indicate that the 25 nmol biotin level consistently demonstrated superior performance in maintaining sperm viability ($P < 0.05$).

Treatments (nmol)	Storage period (hours)				
	1	6	12	18	24
Control	93.75±4.39	92.70±4.73^{a}	91.25±4.27^{a}	82.50±3.23^{a}	71.25±4.11^{a}
Biotin 50	94.50±3.57	78.33±3.45^{b}	73.35±6.05^{b}	65.33±6.37^{b}	54.33±6.98^{b}
Biotin 100	95.33±2.04	80.15±2.89^{b}	73.80±1.67^{b}	66.67±1.67^{b}	53.40±3.34^{b}
Biotin 150	95.11±3.54	78.26±3.40^{b}	56.25±2.39^{c}	47.57±4.79^{c}	37.13±3.75^{c}
Biotin 200	95.75±1.50	78.20±6.01^{b}	72.50±4.33^{b}	63.75±4.27^{b}	53.20±4.25^{b}
P-value	0.97	0.039	0.002	0.003	0.005

Table 3 – Effect of biotin levels on sperm viability (%) during chilled semen storage in turkeys. Data are presented as Mean ± SE; means within the same column with different superscripts differ significantly ($P < 0.05$).

3.4. Plasma Membrane Integrity

Table 4 shows the impact of different biotin levels on the percentage of sperm with intact plasma membranes. After one hour of semen storage, the sperm plasma membrane integrity was not significantly affected by the biotin treatments. However, at the 6-hour, the highest percentage of plasma membrane integrity was observed with the 25 nmol biotin level ($P < 0.05$). After 24 hours of storage, the highest plasma membrane integrity was associated with the 25 nmol biotin level, while the lowest was observed at the 200 nmol level ($P < 0.05$). Regarding changes in plasma membrane integrity over different storage times, the 25 nmol biotin level consistently demonstrated the best performance (Fig. 4).

Treatments (nmol)	Storage period (hours)				
	1	6	12	18	24
Control	84.50±4.86	83.25±1.25^{a}	84.58±3.33^{a}	75.25±2.06^{a}	61.75±4.17^{a}
Biotin 50	87.33±3.07	69.50±8.18^{b}	66.11±4.58^{b}	50.67±9.40^{b}	44.67±5.36^{b}
Biotin 100	85.74±3.10	70.12±2.89^{b}	66.37±1.04^{b}	47.33±3.93^{b}	38.33±4.41^{b}
Biotin 150	85.00±2.36	71.33±2.40^{b}	48.70±3.80^{c}	29.43±4.21^{c}	26.75±1.97^{c}
Biotin 200	88.90±2.17	70.10±5.03^{b}	62.84±2.63^{b}	46.25±3.73^{b}	41.25±2.06^{b}
P-value	0.85	0.012	0.001	0.009	0.001

Table 4 – Effect of biotin levels on sperm plasma membrane integrity (%) during chilled semen storage in turkeys. Data are presented as Mean ± SE; means within the same column with different superscripts differ significantly ($P < 0.05$).

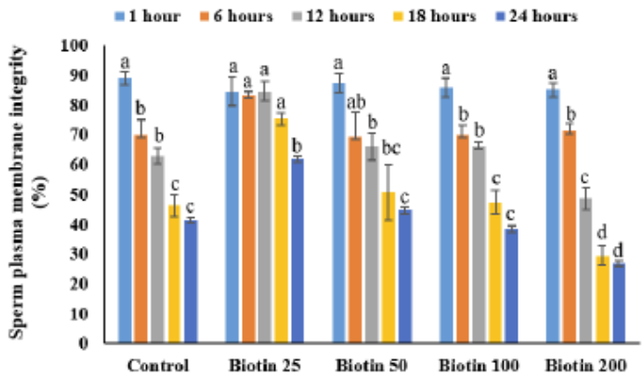
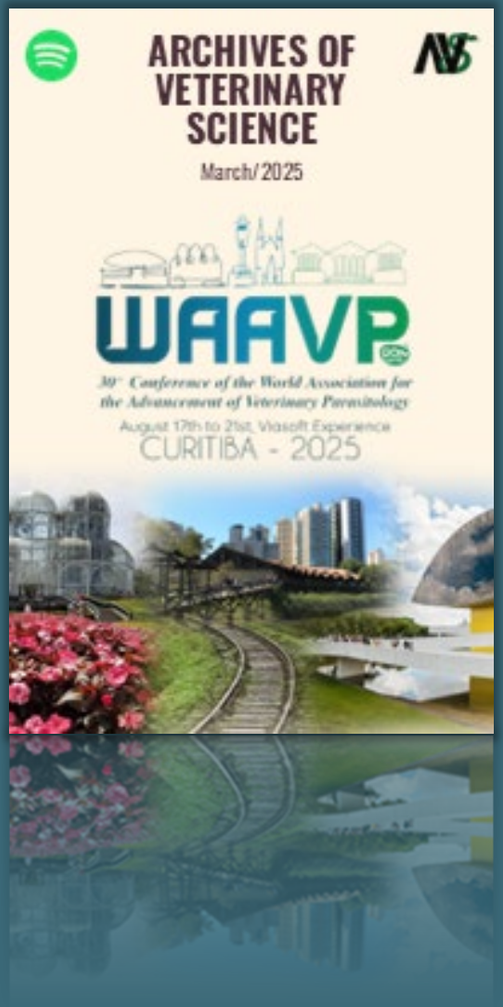


Figure 4 – Effect of biotin levels (nmol) on the sperm plasma membrane integrity over storage time. Data are presented as Mean ± SE; groups with different superscripts differ significantly ($P < 0.05$).

http://dx.doi.org/10.5380/avs.v30i1.96899



ARCHIVES OF VETERINARY SCIENCE

March/2025

publish.avs@ufpr.br
editor.avs@ufpr.br

UFPR UNIVERSIDADE FEDERAL DO PARANÁ
50 anos Setor de Ciências Agrárias Universidade Federal do Paraná 1973 - 2023
PPGCV PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS-UFPR

3.5. Sperm Morphological Abnormalities

According to Table 5, the effect of different biotin levels on sperm morphological abnormalities at 1 and 6 hours of semen storage was not significant. However, after 12, 18, and 24 hours of storage, the percentage of sperm morphological abnormalities at the 25 and 50 nmol biotin was significantly lower compared to the 100 and 200 nmol levels and the control group ($P < 0.05$). Regarding the changes in the percentage of morphological abnormalities over time, the results suggest that biotin has a minimal impact on reducing and improving this parameter. However, the increase in sperm abnormalities over time was slightly less pronounced in the 25 nmol biotin treatment (Fig. 5).

Treatments (nmol)	Storage period (hours)				
	1	6	12	18	24
Control	3.75±0.47	5.25±0.49	8.25±0.45^{b}	9.12±0.48^{b}	12.25±1.17^{b}
Biotin 50	3.50±0.65	5.07±1.03	8.00±0.41^{b}	10.20±0.75^{b}	13.00±0.91^{b}
Biotin 100	3.00±0.71	5.52±0.66	13.75±0.50^{a}	17.75±0.85^{a}	21.50±0.88^{a}
Biotin 150	4.02±0.70	6.04±0.86	13.50±1.19^{a}	18.00±1.08^{a}	21.40±0.75^{a}
Biotin 200	3.50±0.29	5.55±0.40	11.66±0.66^{a}	15.50±1.04^{a}	19.10±1.10^{a}
P-value	0.81	0.82	0.001	0.009	0.0001

Table 5 – Effect of biotin levels on sperm abnormalities (%) during chilled semen storage in turkeys. Data are presented as Mean ± SE; Means within the same column with different superscripts differ significantly ($P < 0.05$).

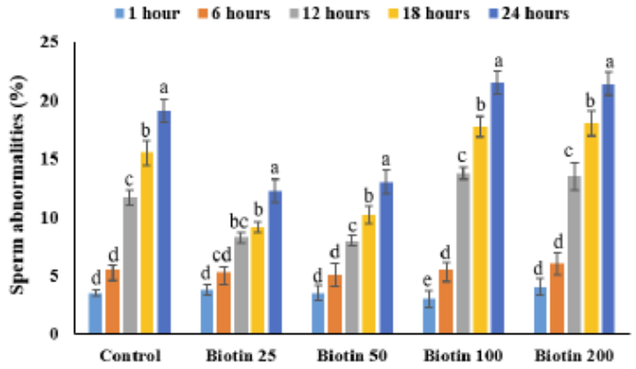


Figure 5 – Effect of biotin (nmol) levels on sperm morphological defects over storage time. Data are presented as Mean ± SE; groups with different superscripts differ significantly ($P < 0.05$).

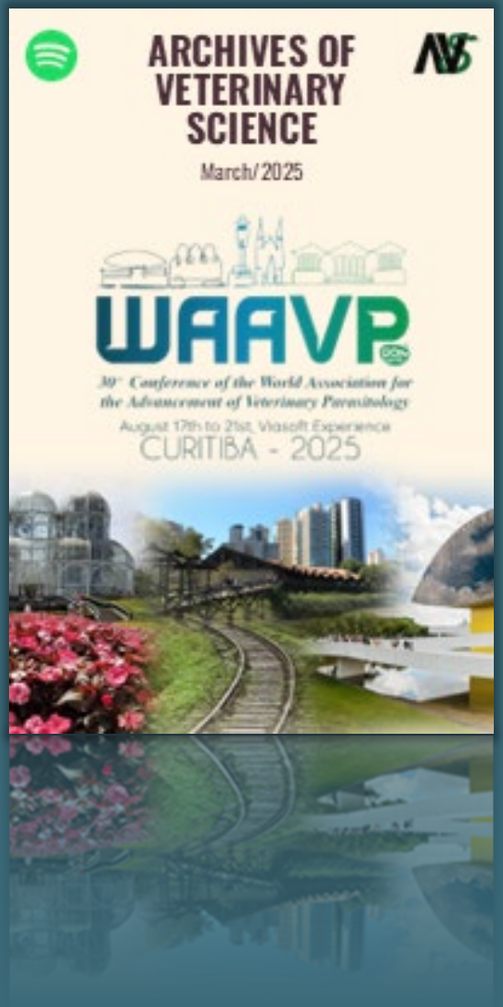
4. Results

In poultry, although artificial insemination using fresh semen yields satisfactory results, techniques and solutions have been proposed to preserve sperm over a longer period for subsequent insemination. By understanding and replicating the natural conditions for sperm, it is possible to maintain sperm viability and fertility outside the body for a certain period. Antioxidants have garnered attention among turkey breeders for preserving semen for artificial insemination (Iaffaldano et al., 2016). While a certain level of reactive oxygen species (ROS) is necessary for normal sperm function, excessive ROS can react with all biological macromolecules, causing oxidative damage and loss of sperm function. Increased free radicals are byproducts of aerobic metabolism and stress signals that, if not neutralized, lead to oxidative damage and disruption of cell structures, including sperm (Baskaran et al., 2021; Di Meo and Venditti, 2020). Oxidative stress is currently considered one of the main causes of infertility, as it increases the risk of DNA fragmentation, reduced motility, and abnormal sperm morphology. Although a certain amount of ROS activity is essential for normal sperm function, excessive levels result in pathological effects on sperm parameters and semen quality, ultimately leading to reduced fertility or male infertility (Kumar and Singh, 2018).

Antioxidants protect cells from damage caused by free radicals by regulating ROS-related enzymes and maintaining cellular health (Sundaram Sanjay and Shukla, 2021). The effectiveness of vitamins and other antioxidant supplements in preserving the quality of human and animal sperm in vitro has been well-documented (Dutta et al., 2019; Ghallab et al., 2017; Kariminezhad et al., 2024).

Biotin is essential for reproductive function in mammals (Yu, 2023). The literature review reveals that research on the impact of biotin on male reproductive performance is mostly limited to humans and laboratory animals, with rare studies on poultry. One study showed that supplementing the sperm environment with various biotin concentrations improved the motility, viability, and

http://dx.doi.org/10.5380/avs.v30i1.96899



ARCHIVES OF VETERINARY SCIENCE

March/2025

publish.avs@ufpr.br
editor.avs@ufpr.br

UFPR UNIVERSIDADE FEDERAL DO PARANÁ
50 anos Setor de Ciências Agrárias Universidade Federal do Paraná 1973 - 2023
PPGCV PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS-UFPR

plasma membrane integrity of goat sperm after freezing and thawing (Ranjan et al., 2022). Khamsuk et al. found that adding vitamin B9 (folic acid) to the sperm freezing medium in humans improved sperm viability and motility (Khamsuk et al., 2014). Supplementing rooster diets with vitamins C and E, antioxidants similar to biotin, improved sperm motility. Progressive sperm motility is a key determinant of fertility and ensures successful and complete fertilization. During cold storage of semen, sperm viability decreases, potentially due to the impact of free radicals on membrane structure (Darestani et al., 2019).

The current study found that biotin, with its antioxidant properties and possibly other vitamin effects, dose-dependently improved sperm quality parameters and semen preservation in turkeys compared to the control under *in vitro* conditions. Montazeri et al. (2021) demonstrated that biotin plays a crucial role in preserving sperm parameters, including chromatin quality, plasma membrane integrity, and sperm viability after freezing and thawing in normozoospermic men. Adding 10 nmol of biotin to human semen diluent significantly preserved sperm motility and viability after freezing and thawing (Kalthur et al., 2012). However, oral biotin supplementation in mice led to reduced motility and morphology parameters compared to controls (Pastén-Hidalgo et al., 2020). Conversely, another study indicated that biotin effectively increased sperm viability and motility in men compared to the control during freezing-thawing (Kalthur et al., 2012). Other research in rams and stallions found that supplementation of vitamin B12 to diluents improved sperm parameters, including viability, progressive motility, plasma membrane integrity, and normal sperm, in a dose-dependent manner (Ahmadi Hamedani et al., 2016). Hosseinabadi et al. (2020) reported that adding specific doses of vitamin B12 to human semen diluents improved total sperm motility. Other studies also showed that vitamin B9 supplementation improved sperm motility parameters in rats and rabbits (Ghadhban et al., 2020; Pietrzik et al., 2010). Aligning with the present results. B vitamins are known to play a role in male fertility and improve sperm quality in individuals with reproductive issues. For instance, B9 and B12 consumption in oligozoospermic men increased the likelihood of returning to normozoospermia. Thus, B vitamins might be considered part of infertility treatments (Tsampoukas et al., 2021).

Adding antioxidants to diluents reduces oxidative stress. Therefore, selecting an appropriate antioxidant level is crucial for balancing ROS production during freezing (Mehdipour et al., 2022). The main challenge in managing oxidative status is distinguishing between natural and abnormal ROS concentrations, complicating the standardization of semen diluents (Kowalczyk, 2022).

Overall, the antioxidant properties of B vitamins include direct inhibition of ROS, especially superoxide, indirect ROS inhibition through glutathione maintenance, protection against oxidative stress responses via cytokines and growth factors, and reduction of oxidative stress from homocysteine (Alzoubi et al., 2014; van de Lagemaat et al., 2019). These vitamins enhance antioxidant capacity and counteract lipid peroxidation through increased glutathione peroxidase activity, leading to improved sperm motility, viability, and plasma membrane integrity (Ogawa et al., 2024). B vitamins are cofactors in many key sperm metabolic enzymes and positively affect sperm nuclei and acrosome function. B vitamin deficiency impairs the activity of the methyltetrahydrofolate reductase enzyme, which converts homocysteine to methionine, resulting in increased homocysteine levels that are associated with reduced sperm quality (Rasul et al., 2022). In the present study, biotin improved sperm quality parameters in turkeys during cold storage, with the most significant effect observed at the 25 nanomole concentration.

5. Conclusion

In general, this study's findings suggest that supplementing the diluent medium with 25 nmol of biotin has the most favorable effect on preserving sperm quality and maintaining viability in turkey semen under cold liquid storage conditions (5°C) compared to the control and other concentrations of this vitamin.

Acknowledgments: The authors gratefully acknowledged the Agricultural Sciences and Natural Resources University of Khuzestan for supporting and funding this research.

6. References

Ahmadi Hamedani M, Tahmasebi AM, Naserian AA, Jafari Ahangari Y. The effect of vitamin B12 on bases of tris extender on protection of Zel ram spermatozoa. J. Rumin. Res., 4(3); 73-91, 2016.

Alzoubi K, Khabour O, Khader M, Mhaidat N, Al-Azzam S. Evaluation of vitamin B12 effects on DNA damage induced by paclitaxel. Drug. Chem. Toxicol., 37(3); 276-280, 2014.

Amini MR, Kohram H, Zare Shahaneh A, Zhandi M, Sharideh H, Nabi MM. The effects of different levels of vitamin E and vitamin C in modified beltsville extender on rooster post-thawed sperm quality. Cell Tissue Bank., 16; 587-592, 2015.

Azubuike US, David O, Ibrahim RP, Sankey RJ. Specific sperm abnormalities observed in rams (*Ovis aries*) following cypermethrin treatment. J. Cell Anim. Biol., 11(1); 1-6, 2017.

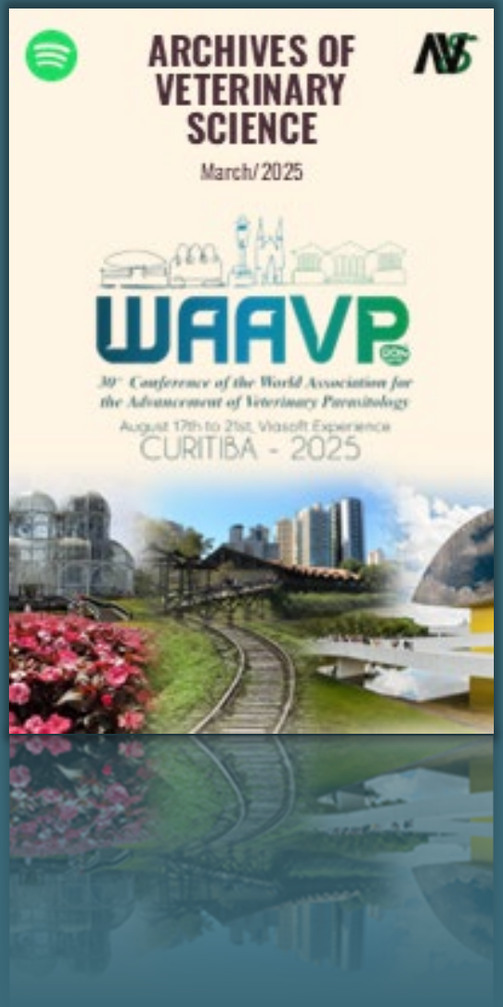
Baskaran S, Finelli R, Agarwal A, Henkel R. Reactive oxygen species in male reproduction: A boon or a bane?. Andrologia, 53(1); e13577, 2021.

Darestani A, Karimi K, Zand K, Khodaei Motlagh M. Effect of vitamins C and E on some sperm characteristics in varamini rooster in cold conditon. Res. Anim. Prod., 10(24); 66-75, 2019.

Di Meo S, Venditti P. Evolution of the knowledge of free radicals and other oxidants. Oxid. Med. Cell. Longev., 9829176, 2020.

Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab. J. Urol., 17; 87-97, 2019.

http://dx.doi.org/10.5380/avs.v30i1.96899



ARCHIVES OF VETERINARY SCIENCE

March/2025

publish.avs@ufpr.br
editor.avs@ufpr.br

UFPR UNIVERSIDADE FEDERAL DO PARANÁ
50 anos Setor de Ciências Agrárias Universidade Federal do Paraná 1973 - 2023
PPGCV PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS-UFPR


UFPR
UNIVERSIDADE FEDERAL DO PARANÁ
50anos
Setor de Ciências Agrárias
Universidade Federal do Paraná
1973 - 2023
PPGCV
PROGRAMA DE PÓS-GRADUAÇÃO
EM CIÊNCIAS VETERINÁRIAS-UFPR