Impact of Nano-Selenium and Vitamin E Supplementation on Sperm Abnormalities in Holstein Bulls.

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Abstract
This study aimed to know the Effect of dietary supplementation of nano-selenium and vitamin E on sperm abnormalities in Holstein bulls. Twelve Holstein bulls were randomly divided into three groups (n=4/group). For three months, they were treated twice a week with the standard diet (control=C) and the second group included the standard diet + 0.1 mg green synthesis nano selenium + 300 IU vitamin E/kg dry matter, and the third group included the standard diet + 0.3 mg green synthesis nano selenium + 300 IU vitamin E/kg dry matter. Semen samples were collected after one month of feeding and every other week (four times apart during feeding and four weeks after feeding). Abnormal sperm were evaluated fresh (37 °C), chilled (5 °C), and following cryopreservation (-196 °C). The results showed no significant improvement in the percentage of abnormalities of the head, mid-piece, and tail between treatments during and after feeding and at periods fresh (37 °C), chilled (5 °C), and following cryopreservation (-196 °C). The results of our study indicate that there is no significant improvement in sperm abnormalities when using nano-selenium and vitamin E.

Keywords: Nano-selenium, Sperm abnormalities, Holstein bulls, Vitamin E.

Introduction

Artificial insemination technology has provided many advantages, e.g., decreasing sexually transmitted diseases, maximizing reproductive efficiency, reducing the bull-to-cow ratio, genetic improvement enhancements, and reducing breeding burdens [7]. The most crucial stage of this technology is the process of sperm cryopreservation [30]. During cryopreservation, additional sources of different types of reactive oxygen species (ROS) cause damage to sperm and make them more susceptible to oxidative stress, On the other hand, the liquefaction process also leads to deformities and, thus, a decrease in their ability for egg fertilization [32]. Sperm abnormalities are classified according to the location of the damage into head, midpiece, and tail abnormalities. Since the head of the sperm contains the genetic material and enzymes necessary for fertilization, it may be responsible for the most significant decrease in fertility [23]. Midpiece and tail abnormalities arise during development in the epididymis. In general, sperm with such abnormalities are either immobile or have abnormal motility [6]. To overcome these defects, researchers tended to use nutritional supplements, as antioxidant materials [29], to enhance reproductive properties and increase sperm vital activities [12], such as selenium (Se) [19], and vitamin E [34].

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Selenium significantly improves reproductive characteristics represented in the quantity and quality of semen and reduces the percentage of deformed sperm [2]. A study conducted on rams by [11] concluded that adding selenium to the diet resulted in a significant decrease observed in selenium groups in sperm abnormalities compared to the control. These data concluded that selenium is important in reducing sperm abnormalities [19]. Low levels of vitamin E also negatively affect semen quality, in addition to increasing the percentage of deformed sperm [16]. A study was conducted on rams to show the effect of vitamin E on ram semen. It is concluded that sperm abnormalities showed an improvement in the percentage of deformed sperm, including head, midpiece, and tail, compared to the control when Iraqi Awassi rams were treated with vitamin E twice a week at 175.70 mg/ram [3]. This is consistent with [31] and [4]. The integrated relationship between them contributes greatly to playing complementary roles in the mechanism of suppressing and resisting the negative results due to oxidative stress [21]. It has been noted that selenium has an essential role in fertility, in addition to its complementary role to vitamin E as an antioxidant and enhances the immune system [17]. Selenium nanoparticles may be used due to their positive effect on sperm parameters and low health risks [28]. Nano selenium is much more effective than conventional selenium and has a low toxic effect due to its effective lifespan, which has been shown to be shorter than conventional selenium [33]. When the nanomaterial is in combination with another material, it may give better results or vice versa [1].

There were no studies that addressed the synergistic effect of nano-selenium and vitamin E on the head, midpiece, and tail abnormalities in artificially inseminated Holstein-Friesian bulls. Therefore, our study aimed to investigate the effect of dietary supplementation of nano-selenium and vitamin E on sperm abnormalities in Holstein bulls.

Material and Methods

Experiment design:

All experimental Ethical protocols were previously approved according to the ethical approval by the Department of Animal Production Council, Al-Qasim Green University (No. 7486, 2023), Al-Qasim Green University, Babylon, Iraq. The current experiment was conducted in the Artificial Insemination Department - Abu Ghraib, Ministry of Agriculture, for the period from 13/11/2023 to 13/4/2024, Twelve Holstein bulls, and their ages ranged from 5-7.5 years and weights ranging from 500-950 kg, were trained for semen collection by using artificial vagina and water was provided freely. All animals were subject to veterinary supervision and were in good health conditions. The experimental animals were randomly distributed into three groups (n=4/group). All bulls were subjected to the same diet, including 5 kg of concentrated feed/bull/day. The percentages of crude protein and metabolized energy were 18% and 2164 kcal/kg, respectively. The roughage consisted of hay from the hay in an amount ranging between 7-9 kg/bull/day with green feed at a rate of 40-50 kg/bull/day. The experimental treatments were prepared by mixing nano selenium and vitamin E with 100 grams of concentrated feed to ensure homogeneity. This mixture was then added to the standard feed shown in Table 1, presented to the bulls according to the groups, were provided twice a week for a period of three months. Semen was collected after one month of feeding at two-

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week intervals (4 weeks during feeding and 4 weeks after feeding) to evaluate the abnormal sperm fresh (37°C), chilled (5°C), and following cryopreservation (-196°C). The animals were fed on the basic diet with or without green synthesis nano selenium and vitamin (With a purity of 50%, produced by Kaesler/Germany). Treatments were divided into: group control (T1) basic diet without additives, the second group (T2) basic diet + 0.1 mg of selenium + 300 IU of vitamin E/kg dry matter, and the third group (T3) basic diet + 0.3 mg of selenium + 300 IU of vitamin E/kg dry matter. Bull feeding (Feed the bulls).

Table 1: Percentages of the components of the concentrated feed provided to the experimental animals.

No.	Ingredients	Percentage in the Feed
1	Barley	35 %
2	Wheat Bran	33 %
3	Yellow Corn	10 %
4	Soybean Meal	20 %
	Limestone	0.5 %
6	Table Salt	0.5 %
7	Vitamins & Minerals	1 %
8	Total	100 %

Semen collection and evaluation:

The process of collecting semen from the artificial insemination bulls began at 7:00 a.m. using an artificial vagina. The temperature at the time of collection was between 41-42°C, with a rate of one ejaculation per bull per week. To increase sexual desire and prepare for ejaculation, the bulls were allowed to perform a false jump. The artificial vagina was equipped with a graduated glass tube in which the semen sample was collected It is placed inside a special container with its opening closed with the thumb to prevent light from reaching it and to maintain its temperature, which prevents it from being exposed to environmental shock. Then it is transferred to the laboratory to be placed in a water bath at a temperature of 37°C and remains there until the sample is evaluated and diluted [5]. Then, the fresh semen (37°C) is evaluated for each sample in terms of abnormalities of the head, middle piece, and tail according to the method described by [13].

Dilution and cooling of semen:

After preparing the diluent (Tris 2.42g, Citric Acid 1.34 g, Fructose 1 g, Distilled Water 73.6 ml, Glycerol 6.4 ml, Egg Yolk 19.2 ml, Gentamicin 0.4 ml, Tylosin 0.08 ml), it is placed in a water bath at a temperature of 37°C, the semen is added to the diluent and not the other way around, using a graduated pipette and gradually to avoid shocking the sperm. Then, the tubes are tightly closed and returned to the water bath, according to the method described by [25]. To ensure the correct temperature gradient, the water in the water bath must cover the semen portion in the tubes. The samples are placed after dilution in plastic containers containing water at a temperature of (32°C) with a mercury editor, then transferred to a special refrigerator at a temperature of (5°C) and the gradual decrease in the temperature of the samples is monitored for a period of time estimated at (1.15 - 1.45) hours approximately until it reaches (5°C) according to the method described by (24). Then, the diluted semen (5°C) is evaluated for each sample in terms of abnormalities of the

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head, the middle piece, and the tail according to the method described by [13].

Preserving semen by freezing

After the temperature of the samples placed in the special refrigerator stabilized at (5°C), they were left for 4 hours until the glycerol was equalized, the cooled samples were filled automatically by the filling machine. In 0.25 ml artificial insemination tubes. After completing the filling process, the tubes were distinguished by colors and signs for the purpose of identifying them; then they were placed on a special holder inside the liquid nitrogen tank at a temperature of (-120°C) and a height of 5 cm above the level of the liquid nitrogen surface for 9 minutes. Then, they were immersed and preserved in the liquid nitrogen tank at a temperature of (-196°C) with a mark bearing the date to distinguish them according to the method described by (24). They were taken out and liquefied after 48 hours for the purpose of assessing the abnormalities of the head, middle piece, and tail according to the method described by (13).

Microscopic tests:

Percentage of deformed sperm

The percentage of abnormal sperm was estimated according to the method described by [13]. A drop of semen was placed on a clean glass slide at 37°C with 10 microliters of 5% eosin and 10% Nigrosin stain placed next to it. The two drops were mixed together gently using another glass slide, followed by drawing the mixture onto a new glass slide at a 45° angle and then left to dry at 37°C. The glass slide was placed under a microscope at a magnification of 40X magnification lens was used to calculate the percentage of abnormal sperm by counting 200 sperm in different microscopic fields, as the percentage of deformed sperm was calculated for each part of the sperm. The abnormalities were classified as follows: Head abnormalities (Pyriform, Dwarf, Narrow, Giant, Twin, Detached), Mid piece abnormalities (Swollen, Twin, Protoplasmic droplet), and Tail abnormalities (Coiled, Twin, Bent).

The percentage of deformed sperm was calculated for each part according to the following equation:

Percentage of abnormal sperm =(Number of abnormal sperm)/(Total number of sperm)×100

Statistical analysis

Statistical Analysis System [26] (User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.) was used to analyze the data to study the effect of treatment and time on the studied parameters according to a completely randomized design (CRD) in the form of rows and columns. The significant differences between the means were compared using (10) multinomial range test, according to the following two mathematical models. The first mathematical model/ to study the effect of treatment for each time.

$Yij = \mu + Ti + eij$

Where:

Yij: The value of observation j for treatment i.

μ: The general average of the studied trait.

Ti: The effect of treatment i for each time.

eij: The random error that is normally distributed with a mean equal to zero and a variance of σ2e.

The second mathematical model/ to study the effect of time for each treatment.

$Yij = \mu + Pi + eij$

Where:

Pi: time effect i for each treatment.

Results and Discussion

The percentage of abnormalities in the sperm head percentage

Table (2) shows that there were no significant differences in the percentage of head

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abnormalities during feeding between groups T1, T2, and T3. However, there were significant differences between treatments in the head abnormalities following feeding (P≤0.05; Table 2). The effect of adding vitamin E and nano selenium after feeding, the results indicated a significant increase (P≤0.05) in the percentage of head abnormalities for treatments T2 and T3 compared to the T1 group. In the period of 37 degrees (Fresh). While the cooling period recorded treatment T3 significant increase (P≤0.05) compared to treatment T1, treatment T2 did not differ from T1. As for the freezing period, treatments T2 and T3 reached a significant increase (P≤0.05) when compared to treatment T1. Regarding the effect of time after 37 degrees (Fresh), after cooling and after freezing, the results of the study showed a highly significant increase (P≤0.01) in the percentage of head abnormalities for each of the cooling and freezing periods compared to after 37 degrees (Fresh) for treatment T1, T2, T3 and the two periods did not differ significantly between them. As for the effect of time among treatments After feeding, the results of treatment T1 and T3 showed a highly significant increase (P≤0.01) in the percentage of abnormalities after cooling and cryopreservation compared to the period of 37 ^{o}C (Fresh). As for treatment T2, there was a highly significant increase (P≤0.01) in the percentage of head abnormalities for the period of freezing compared to the period of 37 degrees (Fresh) and after cooling.

Table 2 Effect of treatment and time on the percentage of head abnormalities during and after feeding

Feeding	Treatments	Mean ± Standard Error (%)			Level of Significance
		37 degrees (Fresh)	5 degrees (Cooling)	-196 degrees (Freezing)	
During feeding	C	0.19±5.7 A a	0.23±8.20 A b	0.23±8.30 A b	**
	T1	0.31±6.10 Aa	0.26±7.91 Ab	0.19±8.42 Ab	**
	T2	0.40±6.25 Aa	0.29±8.25 Ab	0.26±8.45 Ab	**
	Level of Significance	NS	NS	NS	---
After feeding	C	0.19±4.92 Aa	0.24±7.27 Ab	0.14±7.61 Ab	**
	T1	0.11±5.62 Ba	0.15±7.74 ABb	0.23±8.42 Bc	**
	T2	0.25±5.88 Ba	0.24±8.00 Bb	0.22±8.56 Bb	**
	Level of Significance	*	*	*	---

This means that different capital letters within a column and lowercase letters within a row are significantly different from each other. *(P≤0.05), ** (P≤0.01).

The percentage of abnormalities in the middle piece

The results of the current study showed that there was no significant difference between the experimental groups in the percentage of abnormalities of the middle piece during

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feeding, Table 3. As for the effect of time within the unit treatment, the results showed a highly significant difference (P≤0.01) in the abnormalities of the middle piece, as it increased in the post-freezing period in T2 compared to the period after 37 degrees (Fresh) and after cooling respectively. As for the treatments T1 and T2, no significant differences were recorded in the periods after 37 degrees (Fresh), after cooling, and after freezing. As for the deformations of the middle piece after feeding, the results in Table (3) for treatment T2 indicated a significant increase (P≤0.05) in the post-freezing period compared to control T1, and the control treatment did not differ significantly from treatment T3. No significant differences were recorded between them in the period after 37 degrees (Fresh) and after cooling for all experimental treatments. As for the effect of time within the unit treatment, the results of the study showed a highly significant increase in the percentage of deformations of the middle piece for treatment T2 for the period after freezing compared to the two periods after 37 degrees (Fresh) and after cooling. Treatments T1 and T3 did not record any significant effect on the percentage of deformations of the middle piece for each of the periods after 37 degrees (Fresh), after cooling, and after freezing. Table 3 Effect of treatment and time on the percentage of midpiece deformations during and after feeding.

Table 3 Effect of treatment and time on the percentage of midpiece abnormalities during and after feeding

Feeding	Treatments	Mean ± Standard Error (%)			Level of Significance
		37 degrees (Fresh)	5 degrees (Cooling)	-196 degrees (Freezing)	
During feeding	C	0.02±0.02 Aa	0.0 Aa	0.02±0.02 Aa	NS
	T1	0.0 Aa	0.0 Aa	0.03±0.05 Ab	**
	T2	0.0 Aa	0.0 Aa	0.02±0.02 Aa	NS
	Level of Significance	NS	NS	NS	---
After feeding	C	0.0 Aa	0.0 Aa	0.0 Aa	NS
	T1	0.0 Aa	0.0 Aa	0.03±0.08 Bb	**
	T2	0.0 Aa	0.01±0.01 Aa	0.02±0.02 AB a	NS
	Level of Significance	NS	NS	*	NS

This means that different capital letters within a column and lowercase letters within a row are significantly different from each other. *(P≤0.05), **(P≤0.01).

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The percentage of abnormalities in the sperm tail

Tail deformity percentage Table 4 shows that there were no significant differences between treatments T1, T2, and T3 in the percentage of tail abnormalities during feeding. As for tail abnormalities in the post-cooling and post-freezing period, the results of treatment T1 showed a highly significant increase (P≤0.01) compared to the 37°F period (Fresh). As for treatment T2, there was a highly significant increase (P≤0.01) in the percentage of head abnormalities for the cooling and freezing periods compared to the 37°F period (Fresh). As for treatment T3, there was a highly significant increase (P≤0.01) in the percentage of head abnormalities for the cooling and freezing periods compared to the 37°F (Fresh) period. As for tail abnormalities after feeding, the results showed a significant increase (P≤0.05) for treatment T3 for the post-cooling period compared to the control treatment T1 and did not differ significantly with T2. In contrast, the post-37°F (Fresh) and post-freezing periods did not record any significant differences for all experimental treatments. As for the effect of time, the percentage of tail abnormalities increased in treatment T1 in the two periods after cooling and after freezing compared to the period after 37 degrees (Fresh). In treatment T2, tail abnormalities also increased in the two periods after cooling and after freezing compared to the period after 37 degrees (Fresh). As for treatment T3, tail abnormalities increased in the two periods after cooling and after freezing compared to the period after 37 degrees (Fresh).

Table 4 Effect of treatment and time on the percentage of tail abnormalities during and after feeding

Feeding	Treatments	Mean ± Standard Error (%)			Level of Significance
		37 degrees (Fresh)	5 degrees (Cooling)	-196 degrees (Freezing)	
During feeding	C	0.2±4.29 Aa	0.2±5.93 Ab	0.18±8.83 Ac	**
	T1	0.19±4.03 Aa	0.20±6.13 Ab	0.19±8.95 Ac	**
	T2	0.27±4.29 Aa	0.23 ±6.3 Ab	0.27±9.16 Ac	**
	Level of Significance	NS	NS	NS	---
After feeding	C	0.25±3.43 Aa	0.17±5.24 Ab	0.11±8.49 Ac	**
	T1	0.15±3.66 Aa	0.15±5.74 ABb	0.19±8.70 Ac	**
	T2	0.27±3.98 Aa	0.25±5.88 Bb	0.20±9.02 Ac	**
	Level of Significance	NS	*	NS	---

Means with different capital letters within a column and lowercase letters within a row are significantly different from each other. *(P≤0.05), ** (P≤0.01

Discussion

Sperm abnormalities indicate the presence of defects or a defect in the shape of the sperm, which negatively affects its ability to fertilize, The abnormalities may vary between changes in the head, such as the presence of irregular heads, which reduces its ability to penetrate the egg, or in the tail, which affects the efficiency of its movement and makes it

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difficult to reach the egg to complete the fertilization process, It is clear from the above that administration of both nano-selenium at a concentration of 0.1 or 0.3 mg and vitamin E at a concentration of 300 IU/kg dry matter caused an increase in the percentage of abnormalities compared to the control group [8]. showed that the use of vitamin E and selenium at higher doses and ratios can lead to increased sperm damage, although both are nutritional supplements that are beneficial for semen quality. In fact, a study [14] showed that any excess of selenium can reduce sperm motility and increase abnormalities, and very high doses of vitamin E, up to 20 times the normal dose, can cause harmful effects on sperm [9]. Although vitamin E and selenium are essential nutrients that are important components of the antioxidant system, responsible for the defense of tissues and cells. Selenium, a component of the glutathione enzyme, along with vitamin E acts as a biological antioxidant and maintains cell integrity [36]. The reason for these results may be that high doses may create reverse oxidative stress due to excess antioxidants beyond the normal required levels, leading to an imbalance between oxidants and antioxidants in the semen, thus increasing abnormalities and deteriorating sperm quality. According to [27].

A certain level of apoptosis is required to prevent excessive sperm production and remove damaged sperm whose high levels negatively affect ejaculate quality. Dead sperm cells are mainly removed by Sertoli cells. Failure of this mechanism can lead to a high number of abnormal sperm in the semen. Based on our results, we can assume that the ratio of nano-selenium and vitamin E is not sufficient to maintain higher selenium concentrations in the blood for a longer period, thus increasing the overall abnormality rate. In contrast, the results of a number of studies [15,18] were weak, which reported a decrease in the rate of abnormalities in sperm supplemented with vitamin E and nano-selenium and attributed the role of vitamin E in protecting the plasma membrane from peroxides of unsaturated fatty acids, in addition to the role of selenium in reducing the occurrence of abnormalities in the sperm tail and maintaining the integrity of sperm and its motor and metabolic function [35].

Conclusions

This study is the first to show the effect of dietary supplementation of nano-selenium and vitamin E on sperm abnormalities in Holstein bulls. There were no significant effects of nano-selenium and vitamin E on sperm abnormalities during and after feeding and at periods of 37 ^{o}C (fresh), 5 ^{o}C (after cooling), and -196 ^{o}C (after freezing), including abnormalities of the head and middle piece and tail. More studies are warranted to Study the effect of nano-selenium and vitamin E at different concentrations on semen blood parameters.

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