Research Journal of Animal, Veterinary and Fishery Sciences _____ISSN 2320 – 6535
Vol. **8(1),** 1-7, May **(2020)** *Res. J. Animal, Veterinary and Fishery Sci.*

Omega-3 poly unsaturated fatty acids improves semen quality in adult male rats

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Available online at: www.isca.in, www.isca.me
Received 19th June 2019, revised 12th March 2020, accepted 10th April 2020

Abstract

In order to assess the effect of Omega-3 PUNFs on the quality of semen in albino rats in order to get benefit from its use. Fifty mature male albino rats weighing 250-300g and 4-6 months of age were allocated randomly into 2 equal groups; group A that kept as control (n=25) and group B (n=25) where, rats subjected to oral administration of omega-3 for 2 months at 375mg/kg body weight daily. Semen was collected from both groups after 8 weeks for detecting the sperm motility, count and abnormalities. Sera were used for determination of testosterone level. In addition, testis homogenate was used for measuring catalase and malondialdehyde activity. The body weight and sex organs (testis, prostate gland and seminal vesicles) weights were recorded in both groups. Histological examination of testis was performed. We found a significant increase in sperm motility, count, testosterone and catalase concentrations with a decrease in sperm abnormalities percentage and malondialdehyde concentration in omega-3treated group as compared with control. No change in both body and sex organs weights was observed in treated group. In addition, numerous sperms in the lumen of the seminiferous tubules with numerous active spermatocytes were observed in the testis of omega-3treated group. It was concluded that Omega-3PUNFs had a positive and useful effects on the male fertility and could improve semen quality.

Keywords: Omega-3 PUNFs, semen parameters, testosterone, antioxidants, Male fertility.

Introduction

Infertility is considered one of the important problems of human society, where it is influenced by many environmental, behavioral, genotoxic and genetic factors causing impaired spermatogenesis at various stages and male infertility[1]. Several chemical drugs were used to treat infertility but some had a side effect, so the researchers are looking for using drugs with less adverse effects and toxicity[2].

Nowadays, It was found that the differences in food habits could be a cause for the reduction in antioxidants and micronutrients in take that considered essential for the process of sperm maturation and hence inhibition of oxidative stress[3].

Seminal oxidative stress is resulting from an increase in the release of free radicals and reactive oxygen species (ROS) with lower amount of antioxidant causing a damage for lipids, proteins and DNA, which is reflected by increased lipid per-oxidation products as malondialdehyde (MDA) in the blood and tissues. Furthermore, ROS caused great damage for spermatozoa[4]. So it was suggested to use antioxidant therapy to improve the semen quality and treat infertility problems in male[5]. Omega-3 PUFAs considered one of the important antioxidants that had a great safety profile, so it could be used t to improve the semen quality[6].

These polyunsaturated fatty acids considered essential fatty acids, where, they not synthesized by the body and obtained from dietary sources such as fish oil them possess[7].

Omega-3 PUFAs involved in human physiology are α- linolenic acid (ALA) in plant oils, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in marine oils[8].

The physiologic benefits of the high omega-3 FAs intake is due to their ability to modulate cellular metabolic functions and gene expression[9]. In addition, the anti-inflammatory effect of omega-3 PUFAs occur via different mechanisms including the eicosanoid metabolites and inhibiting the genes responsible for the inflammatory process[10].

Omega-3 PUFAs can improve endothelial function by inducing endothelial relaxation and arterial compliance, possibly through improved endothelium mediated vasodilatation induced by altered nitric oxide production[11].

Both omega-3 and omega-6 PUFAs can affect the reproductive processes, where they provide the precursors for prostaglandin synthesis, which is important for all cell membranes. Moreover, they improve both sperm motility and viability associated with acute doxorubicin-induced injury[12]. In addition, it was indicated that supplementing infertile men with omega-3 fatty acids resulted in a significant improvement in sperm motility and concentration of DHA in seminal plasma[13].

The levels of PUFAs in the reproductive tract's tissues indicated the dietary consumption[14].

It was reported that omega-3 PUFAs could improve the decrease in the sex organs weight and protect against the impairment in sperm motility, count, viability and abnormalities and these favorable effects were attributed to their antioxidant properties[15].

Moreover, omega-3 PUFAs reduce the lipid peroxidation level and improve oxidative stress that impair the sperm motility and availability[16]. So, this work was assessed to evaluate the effect of omega-3PUFAs on semen parameters including (sperm motility, sperm cell concentration and abnormalities), serum testosterone level, testicular catalase and malondialdehyde concentrations. Body weight and sex organs (testis, prostate gland and seminal vesicles) weights. Histological studies were performed using testes.

Materials and methods

Ethical approval was taken from the Animal Welfare and Research Ethics Committee, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Animals and diets: In the current study 50 healthy adult male albino rats weighing 200±20g with average age 3-4 months were used. The experiment was carried out in research unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. Water and food available ad-libitum. Rats were fed for 8 weeks on normal diet (18% protein, 14% fiber, 2% fat and 2600Kcal/Kg) according to NRC[17].

Drugs: Omega-3PUFAs were obtained from SEDICO pharmaceutical company (6 October City – Egypt), in the form of capsule each capsule contains (180mg of EPA + 120mg of DHA), so one fish oil capsule or pill is composed of 30% omega-3 PUFAs.

Experimental protocol: Rats were allocated into 2 equal groups. i. Group A (n=25), where the rats received sterile saline and kept as control. ii. Group B (n=25) where, rats subjected to oral administration of omega-3PUFAs at 375mg/kg body weight daily for 8 weeks using stomach tube[15]. Samples were taken from both treated and control animals at the end of the experiment.

Cauda Epididymal spermatozoa collection: 0.1gm of the cauda epididymis was put in 2ml warmed saline, then the contents were mixed by sterilized scissor to be obtained in a form of suspension that used as the semen[18].

Semen analysis: i. Individual Motility (%): Sperm motility was assessed as soon as possible after extraction. Single drop of the semen suspension was placed on a clean, warmed glass slide then covered by a glass cover slip. Percentage of forward progressive motile spermatozoa was recorded through examination under high power objective lens (40x) of light microscope[19]. ii. Sperm cell concentration: the spermatozoa were counted by hemocytometer counting chamber[20]. iii. Sperm Abnormalities: The percentage of abnormal spermatozoa was determined using Eosin- Nigrosin[21].

Organs: Testis, seminal vesicle and prostate gland were obtained, weighed, then one testis was fixed in Bouin's solution for further histological examination, and other testis was put in ice-cold saline for preparation of tissue homogenates.

Preparation of tissue homogenates: Immediately after removal, one testis from each rat was put in ice-cold saline, then mixed with 0.1M Tris buffer (pH 7.4) in Glas- Col motor driven homogenizer (USA).

The obtained tissue homogenate is used for detection of antioxidant enzymes as catalase and malondialdehyde (MDA) concentrations[22].

Serum for hormonal assay: Whole blood was collected by heart puncture into clean tubes free from anticoagulant. Blood samples were allowed to clot at room temperature for 20-30 minutes then centrifuged at 3000 rpm for 15 minutes. The obtained sera were kept in clean sterile tubes at -80ºC until used for determination of testosterone hormone.

Measurement of serum testosterone level: Serum testosterone was carried out using a commercial kit* Testosterone enzyme immunoassay (EIA) DSL-10-4000 (Sigma Aldrich)[23].

Determination of catalase concentration: Catalase level was assessed by a colorimetric assay kit (Sigma Aldrich)[24,25].

Determination of lipid Peroxidation (Malondialdehyde concentration): Malondialdehyde concentration was measured by colorimetric assay kit (Sigma Aldrich). It depends on the colorimetric reaction with thiobarbituric acid (TBA) to produce pink colored product in acidic medium (pH 2-3) and at temperature 90-100^{o}C for 15 minutes. The pink colored product can be measured by spectrophotometer at 532nm[26].

Body weight and Sex organs (testis, prostate gland and seminal vesicles) weights: were recorded in both control and treated group at the end of the experiment.

Histological examination of testis: The routine histological examination for specimens from the testes from both groups were performed then five-micron thick paraffin sections were obtained and stained with hematoxylin and eosin (HE) dyes, then examined microscopically[27].

Statistical analysis: The obtained data were statistically analyzed by using (t-test) according to Tamhane and Dunlop[28]. The results were expressed as means ± S.E.M (standard error of means). Data were considered significant at P-values ≤ 0.05.

Results and discussion

Table-1: Changes in semen parameter of adult male rats supplemented with omega-3PUFAs.

Parameters	Group A	Group B
Sperm Motility (%)	86.67±1.67[b]	97.00±0.77[a]
Sperm Count (x10^6/ml)	55.00±2.89[c]	95.17±1.76[a]
Sperm abnormalities (%)	30.00±0.58[a]	17.67±0.49[c]

[a-c] Different superscripts in each row were significantly differ.

It is obvious from Table-1 that group B revealed elevation in the sperm motility (97.00±0.77%) as compared with group A (86.67±1.67%), a significant increase in sperm cell concentration (95.17±1.76x10^{6}ml) as compared with group A (55.00±2.89x10^{6}ml) and a significant reduction in the percentage of sperm abnormalities (17.67±0.49%) as compared with group A (30.00 ± 0.58%).

Table-2: Changes in serum testosterone, testicular catalase and testicular malondialdehyde concentrations of adult male rats supplemented with omega-3PUFAs.

Parameters	Group A	Group B
Serum testosterone (ng/ml)	5.33±0.09c	11.60±0.21[a]
Testicular Catalase (U/g)	0.40±0.00c	0.66±0.00[a]
Testicular malondialdehyde (nmol/ml)	4.77±0.35a	2.78±0.09[b]

[a-b] Different superscripts in each row were significantly differ.

Concerning, the results from Table-2, group B revealed a significant increase in serum testosterone level (11.60± 0.21ng/ml) as compared with group A (5.33±0.09ng/ml), a significant increase in level of testicular catalase (0.6±0.00U/g) as compared with group A (0.40±0.00U/g) and a significant decrease in level of malondialdehyde (2.78±0.09nmol/) as compared with group A (4.77 ± 0.35nmol/g).

Table-3: Changes in body weight and sex organs weight of adult male rats supplemented with omega-3PUFAs.

Parameters	Group A	Group B
Body weight (g)	432.33±28.67[a]	350.33±19.54[a]
Testis weight (g)	1.65±0.03[a]	1.60±0.04[a]
Prostate gland weight (g)	0.52±0.01[a]	0.81 ±0.12[a]
Seminal vesicle weight(g)	2.19 ±0.19[a]	1.82±0.22[a]

[a-b] Different superscripts in each row were significantly differ.

Body weight and sex organs weight (testis, prostate gland and seminal vesicle) showed no change in group B (350.33±19.5, 1.60±0.04, 0.81±0.12, 1.82±0.22) as compared with group A (432.33±28.67, 1.65±0.03, 0.52±0.01, 2.19±0.19) as observed in Table-3.

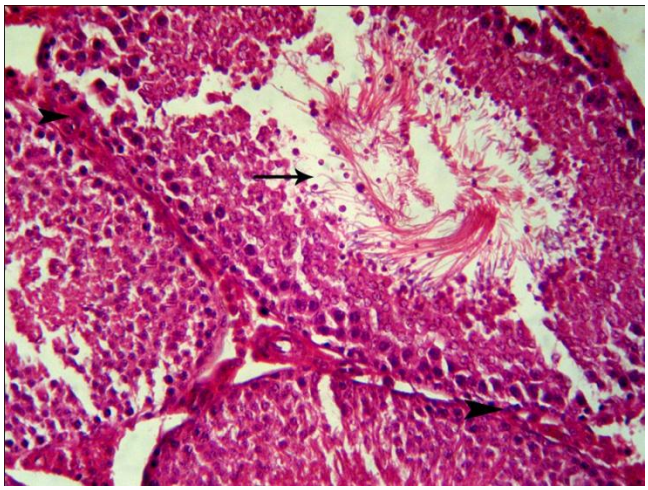

Figure-1: Testis of control rats (group A) shows few round cells among the spermatozoa in the lumen of the seminiferous tubules (arrow) and normal Leydig cells in the interstitium (arrowheads). HE x 400.

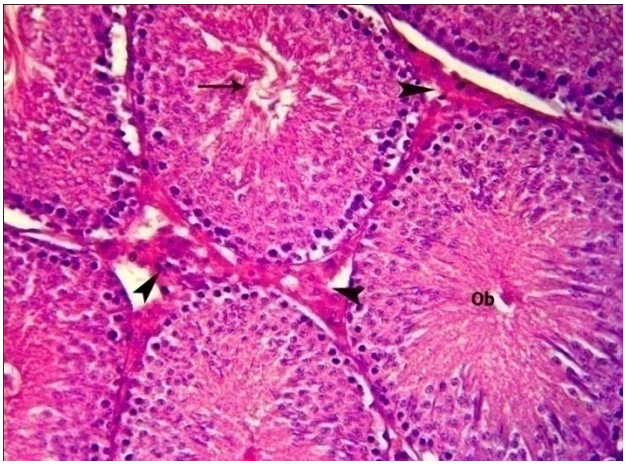

Figure-2: Testis of Omega-3 PUFAs treated rats (group B) shows increase the thickness of the seminiferous tubules by proliferated spermatocytes, narrowing the lumen (ob) (arrow) with huge numbers of spermatozoa (arrow) and increase of the Leydig cells in the interstitium (arrowheads). HE x 200.

Discussion: Omega-3 PUFAs play an important role in the fertility process of both human and animal, especially EPA and DHA improve the motility and morphology of the sperm, where many studies revealed a positive correlation of semen quality and food enriched with omega-3PUFAs. Lipids of the membrane of spermatozoa plays important role in its functions, where, the deficiency of DHA in human spermatozoa produced infertility[5].

It is obvious from Table-1 that the sperm motility and concentration were increased significantly and the sperm

abnormalities percentage was decreased after omega-3 administration, this coincide with Saravana et al.[15] who revealed that omega-3 PUFAs protect against the impairment in different sperm characteristics owing to its the anti-oxidant properties.

Furthermore, the semen quality might be improved when the concentration of PUFA, in the sperm membranes was increased following supplementation with long–chain omega-3PUFAs, where, the total sperm number and sperm motility were improved when boars were supplemented with fish oil[29].

In the same respect, it was concluded that treatment with omega-3 PUFAs increased the sperm cell concentration and reduced the percentages of sperm with abnormal tails and bent tails without any effect on sperm morphology[30].

It had been reported that diet containing high DHA concentrations increased certain spermatozoa characteristics, including percentage of motile cells and cells with normal acrosome scores in stallions[31]. Furthermore, Brinsko et al.[6] showed that feeding a DHA-enriched diet to stallions caused an improvement in motion characteristics of stallions spermatozoa. Also, the high concentration of DHA in both ejaculate and spermatozoa had a positive influence on membrane fluidity that necessary for sperm motility in humans[32].

Similarly Elelaimy et al.[33] revealed that treatment with omega-3PUFAs completely reduced the percentage of sperm abnormalities induced by azathioprine. On other hand it was found that omega-3 PUFA had no effect on sperm concentration, sperm viability and sperm motility in boars[34].

It is obvious from Table-2 that testosterone concentrations were significantly increased in omega-3 treated group. This result confirms the previous results of Saravana et al.[15] who found that the levels of testosterone increased gradually with omega-3 fatty acids treatment. In addition, Ismail et al.[35] found an increase blood testosterone levels in following omega-3 treatment in rats. Additionally, it was found that intake of DHA influenced serum testosterone concentration in men[36] and in rats[37].

The sperm cells are highly sensitive to environmental hazards as compared with other cells, this could attributed to the high amount of PUFAs in the cell membrane of spermatozoa[8].

The harmful effect of ROS is due to its ability to reduce axonemal protein phosphorylation which accompanied with low membrane fluidity via PUFA hydro peroxidation propagation. In addition, it can inhibit the activity of glucose-6-phosphate dehydrogenase (G6PD) that controls the intracellular viability of NADPH-dependent antioxidant enzymes[39].

Lipid per-oxidation lead to damage of the lipid matrix in the sperm cell membrane , which caused death of germ cell during the different stages of development, loss of motility with impairment of spermatogenesis, so antioxidant therapy act as a protective defense against oxidative stress and so improve the fertility parameters[40].

It is obvious from the data in Table-2 that catalase concentration showed significant increase in omega-3 treated group. This agree with Rezk et al.[41] who revealed that oral administration of omega-3 caused a significant increase in the activity of antioxidant enzymes GPX, SOD, and CAT. In addition it was reported that catalase level was significantly low in infertile patients[42]. Also, it was found that omega-3PUFAs and selenium lowered lipid peroxidation and keep normal level of catalase[43]. Additionally, Martínez-Sotoet al.[44] concluded that DHA dietary supplementation could improve seminal antioxidant status and reduced sperm DNA fragmentation.

On the other hand Tabei et al.[45] revealed that catalase activity was significantly decreased in the heart and liver of diabetic rats and supplementation with omega-3PUFAS induced no change in catalase activity.

Concerning, Malondialdehyde concentration, the results observed in Table-2 are agree with previous finding of Ismail et al.[35]; Meydan et al.[46] Furthermore, it was demonstrated that omega-3PUFAs treatment significantly decreased oxidative injury[47]. In the same respect Rezk et al.[41] revealed that oral administration of omega-3PUF As supplement induced a significant decrease in MDA content in testis and cerebral cortex tissues in rats.

The present study revealed no changes in body weight and sex organ (testis, prostate gland and seminal vesicles) weights of adult male rats treated with omega-3PUFAs (Table-3). In this regard, Christian et al.[48] demonstrated that omega-3 did not affect the body and reproductive organ weights. Also, there was no change in sex organ weights after omega-3 treatment[49]. But Zidkova et al.[50] revealed that diets contain high cod liver oils produced a decrease in sex organs weight.

Histological picture of testes showed numerous sperms in lumen of the seminiferous tubules with numerous active spermatocytes in omega-3PUFAs treatment, this could be attributed to the effect of increased testosterone level, where androgens possess anabolic activities, which is essential for the development, growth and normal function of the gonads and production of normal spermatozoa[51]. Also, omega-3 PUFAs were expected to protect testicular tissue due to their antioxidant properties[15]. In addition, Ismail et al.[35] reported that omega-3 fatty acids protect against testicular damage and increase testosterone concentration.

Conclusion

Our results suggested that omega-3PUFAs had a positive and beneficial effects on the male fertility as represented by an increase in the sperm motility, sperm cell concentration, with a reduction of the sperm abnormalities. These improvements may be attributed to the increase in testosterone level, which

stimulates spermatogenesis, testicular function and heightened the male copulatory behavior. In addition the results showed a significant increase in catalase activity indicating anti-oxidant properties of omega-3 and significant decrease in MDA levels, suggesting its effect on decreasing lipid peroxidation. So it could be recommended the use of omega-3PUFAS for improving the semen quality, fertility and reproductive performance of males. Improve the fertility, sexual function of the male and semen quality.

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