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Ameliorative Effects of Corn Oil against *Hydrogen Peroxide*-Induced Oxidative Stress in Rabbits *Sperm* Characteristics

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Abstract : hydrogen peroxide (H_2O_2), which is one of the reactive oxygen species (ROS), count the most destructive to human spermatozoa. Unsaturated fatty acids of Semen are oxidized at preservation resulting in ROS. These ones oxygen species (OS) are very effective on the level of cellular causing many degrees of harm to the cells of sperm. The adding corn oil is related to have antioxidant properties that assist to confrontation the deleterious effects of excess ROS. The *in vitro* study has been aimed to investigate the effect of escalating concentrations of corn oil in the presence of different concentrations of hydrogen peroxide in diluent of male rabbits' semen on sperm characteristics (motility, viability, grade activity and malondialdehyde (MDA) concentration in seminal plasma).The characteristics of semen, inclusive motility, viability, and grade activity, were higher in the groups that were treated with corn oil and H_2O_2 in comparison to the group that treated only with H_2O_2. While the levels of malondialdehyde in control (0.9% Physiological solution), and the corn oil with H_2O_2 groups were lower than only H_2O_2 group. These results demonstrate that corn oil can Reduces the harmful changes in some characteristics of semen induced by hydrogen peroxide.
Keywords : Corn oil, antioxidants, hydrogen peroxide, rabbits, sperm parameters.

Introduction

The production of reactive oxygen species are result from the oxygen metabolism (1,2). Animals may possess the ability to utilize advanced defense mechanisms to mitigate the ROS production as a result of ROS reaction with molecules in a non-targeting method (3).There are two kinds of mechanisms, enzymatic and non-enzymatic, which used the antioxidants to dominance ROS (4), and it can cause hurtful effects on viability and function of sperm due to the peroxidation of membrane lipids (5). Lipids of Spermatozoa are the prime substrates for peroxidation, which may induce acute functional unrest of sperm. The sharp functional disorder of sperm can be stimulated by spermatozoa's lipids which behold the main substrates for peroxidation. furthermore, the low levels of lipid peroxidation reverberate the influence of ROS on sperm metabolism enhancing human spermatozoa capacity to interact with zona of pellucid (6). Number of pathological situations can be connected with oxidative stress which result from reactive oxygen species production (7), However, a relationship between infertility of male and oxidative stress was not noted before the 1980s. The major causes for the occurrence of oxidative stress are depletion of seminal antioxidants and an overabundant free radicals production via the spermatozoa themselves(8). (9), showed that there is an opposite connection between the generation of ROS in semen and the *in vitro* and *in vivo* fertility. (10), have been appeared that the level of reactive oxygen species linked inversely with the motile spermatozoa percentage. generally, the reactive oxygen species have been prevented from being formed by antioxidant systems, or eliminate them before they can disadvantage vital of the cell components (11). Otherwise, the natural antioxidant substances perform a important role in interfering jointly with the process of oxidation by inhibiting of free radicals. thus, the natural

antioxidant of plants have been choosed from consumers for the stabilization of both fats and oils against oxidative process (12). Oil of many various plant materials have been utilized for antioxidant activity source as (13), and because its antioxidants are substantial in fatty acids steadiness (14). The health advantages of corn oil are related to their matchless phyto-chemical composition (15), which the main constituents of corn oil (co): polyunsaturated fatty acids($PUFA_S$), linoleic acid and a small amount of linolenic acid (together -54%), oleic acid monounsaturated (-25%), saturated of palmitic acid and stearic acid (-10% and <2%) respectively, total compose of triglyceride (~95%). Lin *et al*.(2016), the ratio 1:1 of n-6/n-3 of fatty acids caused increased in testis index, total sperm number per, and Sperm density of breeding boars. (16), have been shown that corn oil was used exceedingly without accident as a vehicle to leader test chemicals through gavage in a set of toxicity tests and researchers of dietary also has generally been presupposed to be biologically static simultaneously with regard to developmental situation and reproductive performance. Recently, numerous studies have discovered the powerful antioxidant possibility particularly in corn oil (17).

2.Materials and Methods

2.1Experimental of animals

Ten (10) of laboratory male rabbits between the ages of 4 months with a weight of 1.513 g average, which have been placed in the a cage under the control of the case of water, diet, and the duration of light (12hour light-12hour dark). The semen of male rabbits were collected twice a week into a graduated collection tube by utilize an artificial vagina. First: the diluent samples of semen were divided into six(6) equal parts; the first part has been added 0.9% of physiological solution and the others parts have been added the escalating concentration of (0.2, 0.4, 0.6, 0.8, and 1% H_2O_2). After that the samples of semen were left in the incubator into temperature of 37^0C for 30 minutes. And the concentration of malondialdehyde in seminal plasma, sperm motility percent, grade activity, and sperm viability percent were measured. Second: after choosing the most influential concentration depending on the results of the statistical analysis of the five concentrations of H_2O_2, these men samples divided into eight (8)equal parts, and hydrogen peroxide was added to part one and promised the control group, while the upward concentrations of (1.5, 3, 4.5, 6, 7.5, 9, and 10.5 mg/ml) of corn oil were added to the rest of these men parts. Then the treatment samples were left in incubator at a temperature of 37^0C for 30 minutes, and the optimal concentration of the seven corn oil concentrations have been chosen depending on the results of the statistical analysis. Third: after choosing the most effective concentration of hydrogen peroxide and the corn oil, semen samples were divided into three (3) equal parts, and have been added toits physiologicalsolution0.9%, physiologicalsolution0.9%andhydrogen peroxide, and corn oil and hydrogen peroxide. The samples were left in the incubator at a temperature of 37^0C for 30 minutes, the sperm parameters have been studied.

Table 1.Sperm parameters after the addition of H_2O_2alone and with corn oil to diluents of the male rabbits' semen at a temperature of $37c^0$ and incubated period for 30 minutes.

Sperm parameters	0.9% Ph. S.	1% H_2O_2	10.5 C.O.
Sperm concentration (million/ml)	90.00±0.094a	86.67±0.072a	89.33 ±1.089a
spermmotility (%)	64.00±0.471a	39.00±0.471b	48.33±0.720c
spermviability(%)	89.33±1.186a	60.00±2.357b	76.00±2.357c
spermabnormality(%)	15.67±0.720a	16.67± 0.720a	15.67±0.720a
progressively motile sperm (a ± b) (%)	46.00±2.357a	27.00±0.943b	33.67±0.544c
topicalmotile sperm c (%)	18.00±0.0170a	12.00±0.471b	15.33±0.720c
non-motile sperm (d)	31.00±0.471a	49.67±0.720b	40.67±0.544c

Ph. S.: Physiological solution; H_2O_2: hydrogen peroxide, C.O.: corn oil.
P<0.05, Different Letters Indicate Significant at P<0.05, Mean ± SE.

2.2 Measurement of Lipid Peroxidation (LPO):

The process of Lipid peroxidation is determined in seminal plasma of male rabbits by method of the thiobarbit**uric acid** (TBA) which assessments the formation of malondialdehyde according to (19).

3.Statistical analysis

All the data that have been obtained from the statistical analysis were normally distributed. The differences between the treated groups and the control groups were statistically evaluated by using a Student's F-test. And all the data are expressed as the mean values±SE, with significant values at $p<0.05$.

4.Results

The results have been demonstrated, that the addition of (1% and 0.8% of H_2O_2) for samples of diluted semen during the period of 30 minutes incubation at 37^{o}C, a significant decrease ($P <0.05$) in sperm motility, viability, and the percentage of progressively motile sperm (a+b), also a significant increase ($P <0.05$) in the concentration of malondialdehyde in seminal plasma at concentration of (1% of H_2O_2) compared to the control group of the physiological solution, while the other concentrations of hydrogen peroxide have been revealed insignificant decline ($P> 0.05$) in the above-mentioned of sperm parameters, and insignificant increased ($P>0.05$) of MDA concentration in seminal plasma as compared to group of the control.

A decrease in concentration of MDA have been appeared after the addition of upward concentrations of corn oil, where the statistical analysis revealed that the concentration of9 mg/ml and 10.5mg/ml of corn oil were caused a clear alleviation ($P <0.05$) in malondialdehyde concentration when compared to the group of the control which is only hydrogen peroxide, also a significant elevation ($P> 0.05$) in motility of sperm, viability, and the grade activity were observed after the addition of10.5 mg/ml of only corn oil compare to diluted sample of semen that contain only hydrogen peroxide.

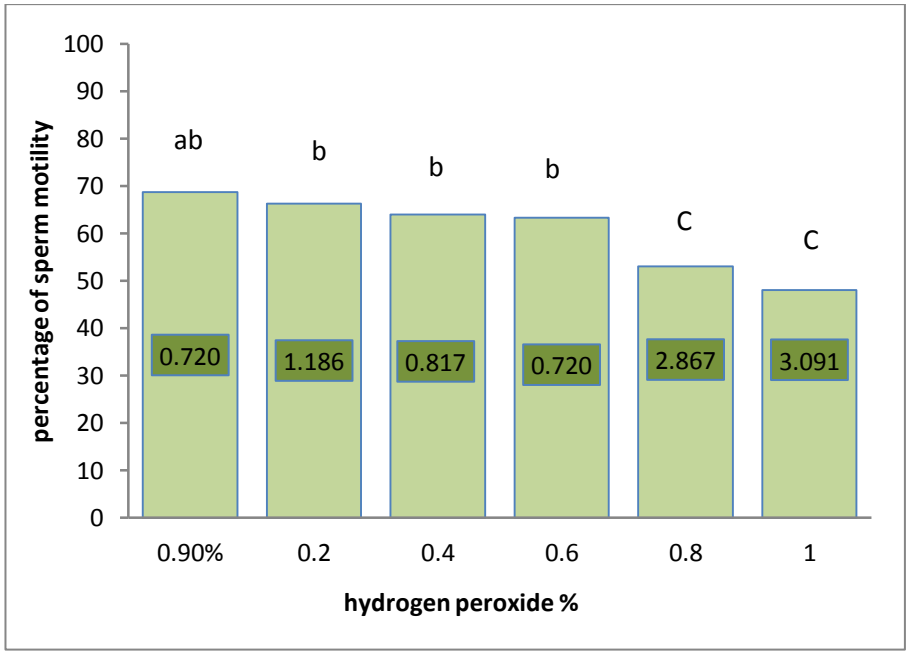

Figure 1. The percentage of sperm motility after the addition of escalating concentrations of H_2O_2 in diluents of male rabbits' semen at a temperature of 37^{0}C and incubated period for 30 minutes. Different Letters Indicate Significant at P<0.05, Mean ± SE.

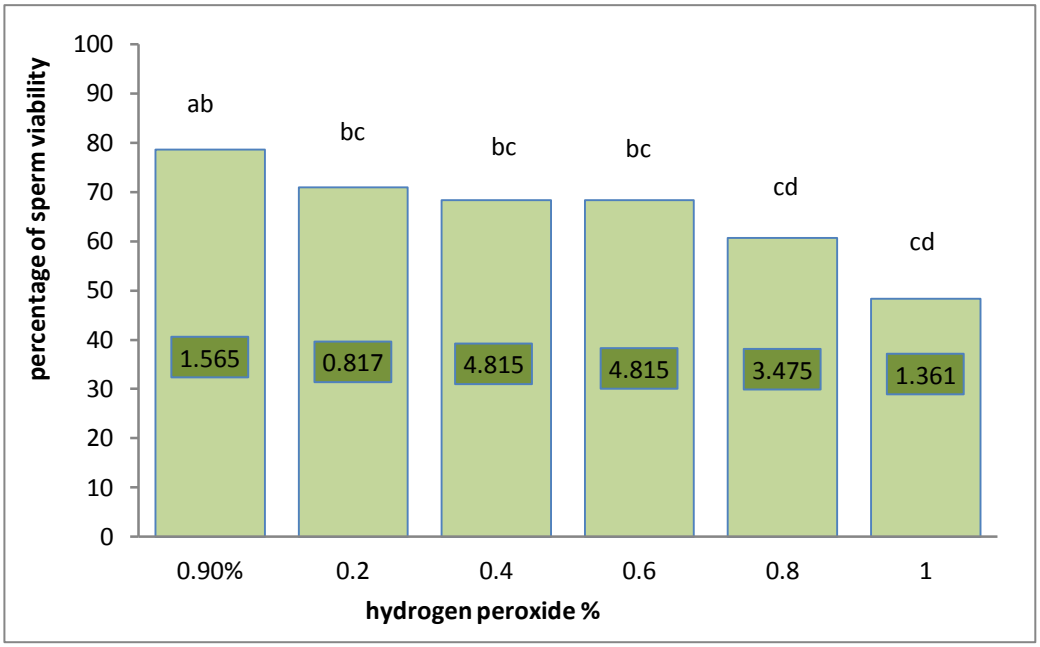

Figure 2. The percentage of sperm viability after the addition of escalating concentrations of H_2O_2 in diluents of male rabbits' semen at a temperature of 37^0C and incubated period for 30 minutes. Different Letters Indicate Significant at P<0.05, Mean ± SE.

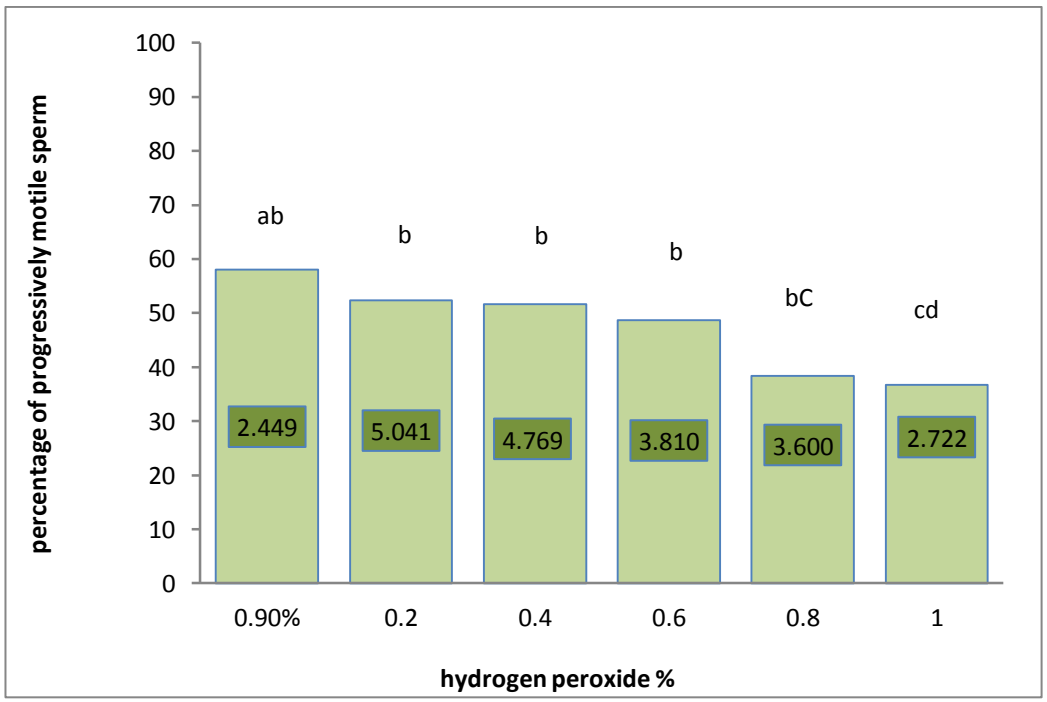

Figure 3.The percentage of progressively motile sperm (a ± b) after the addition of escalating concentrations of H_2O_2 in diluents of male rabbits' semen at a temperature of $37c^0$ and incubated period for 30 minutes. Different Letters Indicate Significant at P<0.05, Mean ± SE.

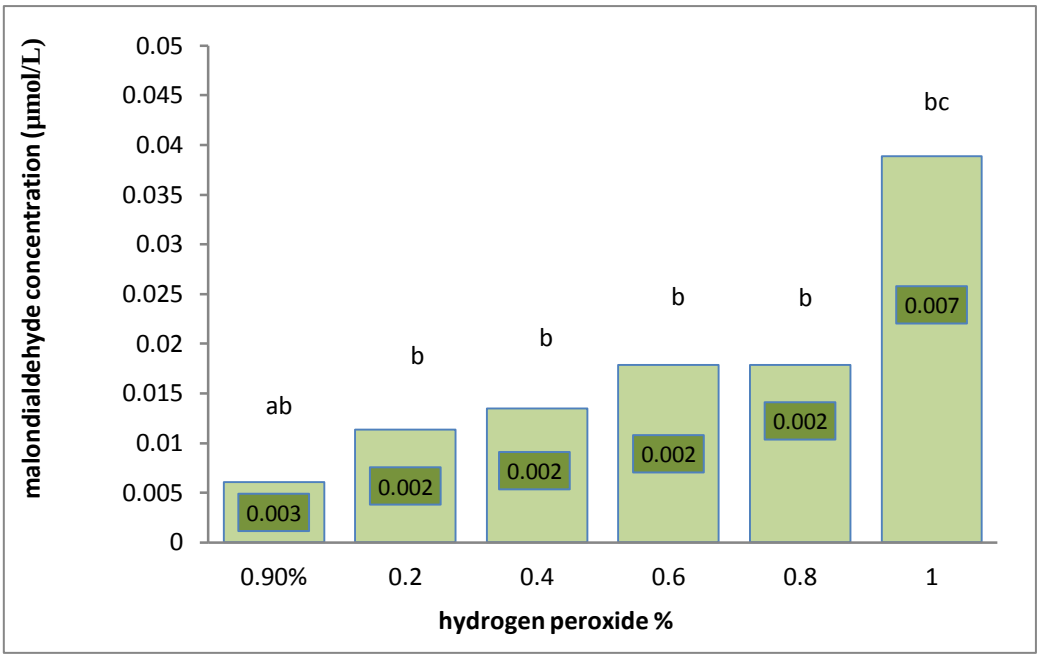

Figure 4.The malondialdehyde concentration after the addition of escalating concentrations of H_2O_2 in diluents of male rabbits' semen at a temperature of 37^0C and incubated period for 30 minutes. Different Letters Indicate Significant at P<0.05, Mean ± SE.

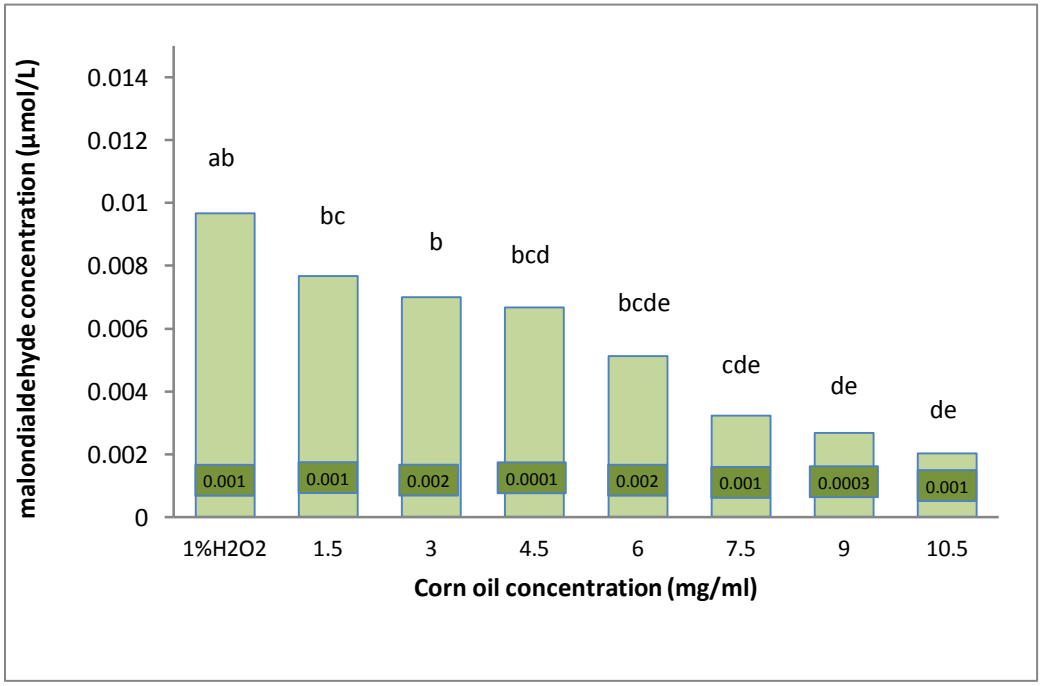

Figure 5.the malondialdehyde concentration after the addition of escalating concentrations of corn oil in diluents of male rabbits' semen at a temperature of 37^0C and incubated period for 30 minutes. Different Letters Indicate Significant at P<0.05, Mean ± SE.

5.Discussion

The production of reactive oxygen species caused oxidative damage which primarily take place through that are generated during the react and reactivity with biological molecules, eventually deleterious membranes and other tissues (20,21). Because ROS are extremely reactive, when they reach the levels of pathological, they exert significant disadvantage on biological molecules, such as lipids, proteins and nucleic acids (22). (23), has been shown that the extent of this harm depends on many agents such as types of ROS and

its concentration, duration of exposure, as well as external factors like tension of oxygen, temperature and levels of antioxidant. The plasma membrane of sperm is highly be composed of polyunsaturated fatty acids (PUFAs) which are exposure to damage by oxidative because of its presence of double bonds (24). The presence of PUFAs in membranes of cell is substantial to keep the lipid bilayer soundness (25). The spermatozoa lipidsare very substantial for the flexibility and fluidity of spermatozoa as been shown in the study of (26). However, high concentrations of PUFAs in the membrane of the spermatozoa make it to be highly extremely susceptible to ROS which leads to lipid peroxidation of plasma lipoproteins and cell membranes (27). Spermatozoa are well armed with an arrangement of antioxidants against ROSs, but an imponderables between ROS production and the obtainable defense of the antioxidants consequences in oxidative stress (28). In many feeding experiments, PUFAs have been provided to change fatty acid composition of the sperm membrane in order to ameliorate the fertility and the quality of sperm. Indeed, the profile of fatty acid in sperm membranes can be modulated with diet and an improvement of sperm quality has been spotted in many of livestock species (29,30,31,32). Thence, to reduce oxidative stress that have been stimulated by free radical, the natural antioxidants in the oils of vegetable with ω-3 fatty acids or ω-6 fatty acids have been much more attention (15). Therefore, the research was to examine the effects the rabbits semen supplementing with corn oil at various concentrations on some sperm characteristics after adding the hydrogen peroxide in diluents of white rabbits' semen. And the present study has been concluded that concentration of 1% H_2O_2 in diluents samples caused a decrease in sperm motility, viability, and the percentage of progressively motile sperm, and caused an increase in MDA concentration of seminal plasma. The cells of sperm are very oversensitive to peroxidation of lipid by free radicals such as hydroxyl radical, superoxide anion, hydrogen peroxide, and whose could later caused to deterioration in structure of membranes of the sperm through the aerobic storage of sperm (33). The technique by which the free radicals activate their injury was related to their capability to catch the electrons of nucleic acids, proteins and lipids give rise to damage to the cell (34).Due to the high movement of the sperm, relying on the energy generation via oxidative phosphorylation in the mitochondrial of the mid-piece, a rise in the concentration of free radicals are created outside and inside the cells of sperm (35,36). The increase of ROS production might deterioration the membrane of sperm cell causing a decrease sperm survival and motility after stock pilling at low temperatures (37).the composition of lipid, the degree of polyunsaturated fatty acids, and the ratio of sperm polyunsaturated fatty acids have been appeared to affect sperm quantity (38). The most important character of the rabbit semen lipid is the considerably high proportions of long chain PUFAs in the phospholipids fraction of spermatozoa, which is necessity antioxidant in order to keep the properties of specific membrane (fluidity, flexibility, etc) (39,40,41). So the addition of corn oil in diluted semen induced by H_2O_2 caused a clear reduction in malondialdehyde concentration, and improvement in motility of the sperm, viability, and grade activity. In the study of (42), have been noted that no effects of corn oil on motility of sperm, but the progress motion of greatest motility of spermatozoa has been founded in groups of corn oil (80.2%). An interesting finding in viability of the sperm examination was that corn oil increased sperm viability compared to control group. Since the usage of corn oil antioxidants has been shown to enhance the viability of sperm (18), it is agreeable to suggest that corn oil helps antioxidant system of sperm to improve its viability. (41), have been showed that the supplement of cooked oil as a source of unsaturated fatty acids in the diet rooster village, the rapid motility of spermatozoa and linearity movement have been significantly increased. Also (43), has been appeared that intake of corn oil is potentially useful in increasing the fertility of male rabbits by increasing sperm concentration, motility, grade activity, viability, and reduced abnormality. (44), hypothesized that dietary lipids may effect on membrane structure of sperm, sperm tendency to peroxidation, and fluidity or all three, by changing fatty acids, specific phospholipids, or n-6:n-3 ratios, or the all of three. The study of (45), have been showed that the increasing in ratio of n-3/n-6 PUFA, sperm motility and density were increased, and the deformity rate of sperm tended to decrease. Furthermore, the ratio of n-3/n-6 PUFAs in sperm of boar were positively correlated with sperm viability, motility, normal plasma membranes, and normal morphology (46), and excessive supplementation with n-3 PUFA decreased the motility of sperm and sperm density in the experiment, which referenced the importance ratio of the n-6/n-3 PUFA in quality of sperm. (47), found the proportion of n-3 fatty acids in spermatozoa from Japanese male quail nourish with corn oil was 4.3% and that of n-6 fatty acids was 33.3%. In (48), have been concluded that addition of PUFAs and antioxidants may be of very importance for normal spermatozoa morphology properties in young domestic cock of Potchefstroom Koekoek cockerels. The study of (49), has been founded that the oil of fish or corn caused amelioration in the concentration of sperm, motility, and the morphology of infertile men with idiopathic oligoasthenoteratospermia.

6.Conclusion

The *in vitro* addition of 10.5 mg/ml of corn oil in diluent of the adult male rabbits' semen is potentially useful in decreasing the oxidative stress induced by hydrogen peroxide, and increasing motility, grade activity, and viability.

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