Plant Archives Vol. 20, Supplement 1, 2020 pp. 243-246 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF OMEGA 3,6,9 SUPPLEMENTATION TO SEMEN EXTENDER ON SPERM PARAMETERS POST CRYOPRESERVATION

Ali Abdaljabbar Ibrahim[1,*], Ahmed Younis Saieed[2] and Ali S. Amin Altawash[1]

[1]Department of Animal Production, Agricultural Collage, University of Al-Qadisiyah, Iraq.

[2]General Directorate Vocational Education, Ministry of Education, Iraq.

*Corresponding author: ali.abdaljabbar@qu.edu.iq ali.amin@qu.edu.iq

Abstract

This study aim was investigated the effect of different concentrations of omega 9, 6, 3 (2, 1, 0.5, 0%) on the quality of the rams semen after freezing. The semen collected using the artificial vagina from four rams and evaluated, then semen samples were diluted using tris extender containing egg yolk (10%) and DMSO (5%). The samples of semen were cooling at 4 °C gradually for 2 hours. Then transported to liquid nitrogen vapor for 10 minutes and immersed in liquid nitrogen. After one month, the samples were thawed at 37 °C for 5 minutes. Then evaluated the sperm motility, sperm plasmid membrane integrity, sperm mortality, sperm abnormality, sperm DNA fragmentation and sperm mitochondrial apoptosis, (0.5 and 1%) of omega 9,6,3 recorded the highest percentage of movement and safety of the plasma membrane compared to the other concentrations (43.20, 42.80%), respectively. No significantly differences were recorded ($P \geq 0.05$) between the concentrations used in the study of dead, deformed and fragmented sperm (0.5%) had the lowest percentage of apoptosis of mitochondria (66.80%), while no significant differences were found between the other concentrations. The results indicate that omega can be used for low concentrations in semen extenders on cryopreservation conditions.

Keywords : Omega 3, 6, 9, semen sperm, cryopreservation.

Introduction

Sheep are important farm animals in Iraqi, so Sheep are important farm animals, so improving their reproductive performance is critical to increasing their numbers and thus increasing their productivity (Al-Haboby *et al.*, 2003). The main problem faced by livestock projects in Iraq is the low productivity of one head of sheep due to genetic factors, spread of diseases, low level of management and care provided to them (Yimer *et al.,* 2014). Improved genital rams efficiency is increased by increasing sperm able to reach the fertilization site depending on the quality and quantity of semen produced (Saacke *et al.,* 1994). The process of conservation of biomaterials at low temperatures is a technique found to have a number of applications in the field of medicine and biotechnology, and several types of cells and tissues can be stored almost indefinitely in liquid nitrogen (-196 m), back to the physiological nature of temperature (Woods *et al.,* 2004).The freezing of semen for the bulls has been very successful in various countries of the world, including Iraq, but this technique did not achieve the same success for the rams, this may be due to the sperm rams nature, characterized by high sensitivity to temperature changes during freezing and thawing, high unsaturated fatty acids and low cholesterol in sperm membrane (Salamon and Maxwell 1995, Bansal and Bilaspuri 2011). The long-acting unsaturated fatty acids form 60% of the plasma membrane of the sperm, for its direct role in increasing the pliability and elasticity of the plasma membrane, increasing receptor effectiveness and increasing the hormonal regulation of the cell, thereby improving cell efficiency (Poulos *et al.*, 1973; Calder, 2011).

The role of omega in sperm resistance for refrigeration and storage is different (Castellano *et al.*, 2010). In rabbits, the association of dietary-3 fatty acids with vitamins E and C enhancing quality of sperm during storage of semen (Castellini *et al.*, 2003). In buffalo, feeding sunflower oil or sunflower seeds has improved the quality of sperm (Adeel *et al.*, 2009). In the other hand, the addition of fish oil to the diet was not improve the quality of freezing pigs sperm (Castellini *et al.*, 2003). In addition, some recent experiments have found no improvement in the quality of stored sperm when adding poly unsaturated fatty acids to a diet for pigs (Maldjian *et al.*, 2005), rabbits (Gliozzi *et al.*, 2009), and horses (Grady *et al.*, 2009). Also, supplemented swine semen with ducoshexeneic acid (DHA) enriched egg yolk did not increase sperm resistance to freezing (Wathes *et al.*, 2007). this study objective was to determine the possibility of freezing the semen awassi rams by using different concentrations of Omega 9.6.3 and its effect on the motility and integrity membrane, dead and abnormal sperm, mitochondrial apoptosis and DNA fragmentation of the sperm ram after thawing.

Materials and Methods

This study was conducted in the Department of Agricultural Research / Ministry of Agriculture in cooperation with the Higher Institute of Infertility and Reproductive Assisted Technologies, University of Nahrain for the period from the beginning of April 2015 to the end of June 2015. The experiment used 4 rams ranging between 2.5-3 years and 75- 80 kg, semen samples were collected using the sheep and goat artificial vagina (Badawy *et al.*, 1975).

The semen samples were diluted with the Tris extender and prepared extender was accorded to Evans and Maxwell (Evans and Maxwell, 1987), with 5% egg yolk and 5% dimethyl sulfoxide (DMSO) and 1: 1 dilution with the gear dilution. And then complete the dilution by 1:10 Omega 9, 6,3concentrations were added (1.5, 1, 0.5, 0%) and egg yolk and dimethyl sulfoxide, and then cooled samples and transferred to the Institute of higher diagnosis of infertility and assisted reproductive technologies using thermos Fill the semen with Cryovial and leave it at 5 °C for one hour and then transport it to the liquid nitrogen bath. The samples were subjected to nitrogen vapor for 10 min in the nitrogen vapor (-75 m) and then plunged in liquid nitrogen-196 m and left for 1 month, than throwing process was accord by removing

the cryovial from the liquid nitrogen, then the Cryovial was placed in the water bath (Water bath) at 37 °C for 5 min to evaluate the sperm motility, sperm plasmid membrane integrity, sperm mortality, sperm abnormality, sperm DNA fragmentation and sperm mitochondrial apoptosis.

The individual movement of the sperm after melting was estimated by placing a diameter of thawed semen on a warm slice at a temperature of 37 °C and measured at 400x magnification (Graham and Maki-Laurila, 1970)

The Membrane Integrity (Membrane Integrity), which is called the water test, has been estimated to estimate the percentage of plasma membrane integrity based on what it says (Lomeo and Giambersio, 1991). Sperm mortality% test according by Swanson and Beardon (1951) DNA fragmentation was estimated using the orange acardine dye (Tejada *et al*., 1984). The programmed death of mitochondrial apoptosis was tested according to Ying Chen *et al*. (2001).

data were statistically analyzed using the Statistical Analysis System, using full randomized design (CRD), and differences between the means were measured using Duncan's Multiple Range test (Duncan, 1955).

Results and Discussion

Adverse cooling and cryopreservation effects on quality of semen have already been described (Castellini *et al.*, 2003; Maldjian *et al.*, 2005.). In this study, also observed that chilling and thawing leaded up to decrease in parameters of sperm standards that included viability and motility. This study results also revealed that the omega-3, 6 and 9 addition Omega fatty acids to the semen dilator mitigated the harmful effects of freezing and thawing processes on sperm quality as assessed by mobility, viability, abnormality, membrane integrity and Mitochondrial apoptosis.

In this study the results of effect of addition omega 3, 6, 9 to semen extender on sperm motility (%) showed in (figure1) there were significantly increase (P≤0.05) on sperm motility (%) when addition omega 3, 6, 9 in concentrations (0.5 and 1). While there were significantly decrease (P≤0.05) on sperm motility (%) when addition omega 3,6,9 in concentrations 0 control group and group 2.

Figure (2) showing results of effect of addition omega omega 3, 6, 9 to semen extender on sperm membrane integrity (%) there were significantly increase on sperm membrane integrity (%) when addition omega 3,6,9 in concentrations (0.5 and 1). While there were significantly decrease(P≤0.05) on sperm membrane integrity (%) when addition omega omega 3, 6, 9 in concentrations 0 control group and 2.

Although both positive and negative actions for Omega fatty acids are theoretically possible, their overall effects on fertility are not fully understood. The effects of Omega fatty acids on sperm quality appear to depend not only on the Omega fatty acid type but also on their long-chain content (Castellano *et al.*, 2010). Furthermore, animal species and the Omega fatty acids addition to a diet or extenders semen were considered important factors in explaining the effects of Omega fatty acids on sperm quality. The Holstein bulls feeding using DHA improved the new semen standards, but this amelioration was not noted in semen thawing (Castellano *et al.*, 2010).

Effect of addition omega 3 to extender of semen on the percentage of sperm mitochondrial apoptosis. Summarizing in (figure 3) there were significantly decrease (P≤0.05) on sperm membrane integrity (%) when addition omega omega 3, 6, 9 in concentrations 0.5.

Addition omega omega 3, 6, 9 to extender noted non-significant different (P>0.05) on the results of percentage of sperm mortality (figure 4).

Percentage of sperm abnormality presented non-significant different (P>0.05)when comparing between control group with the other treatments (figure 5).

Addition omega omega 3, 6, 9 to extender observed non-significant different (P>0.05) on the results of percentage of sperm DNA fragmentation (figure 6).

The addition of fish oil as a source of omega-3 fatty acid for pig feeding has not affected the purity of the frozen sperm (Castellano *et al*, 2010). while, the addition of omega-3 to the diet noted significant improvement in sperm motility and integrity of sperm membrane in pigs (Aksoy *et al.*, 2006; Am-in *et al.*, 2011).

furthermore, Previous studies have noted that there is a significant negative correlation between omega-6/omega-3 ratios and motility of sperm and sperm abnormality. In fact, sterile men have a high content of omega-6 fatty acids in semen (Aksoy *et al.*, 2006). Omega-6 fatty acids higher level reduce semen concentration, motility% and abnormality%. A diet that contains omega-3 and omega-6 acids may alter the levels of unsaturated fatty acids in semen by omega-fatty acids transferring to the membrane of sperm (Safarinejad *et al.*, 2010).

These fatty acids increase the of the sperm membrane fluidity in the semen and are responsible for increasing the resistance of the cooling semen. Unsaturated fatty effect on other physical properties does not, such as permeability, temperature, which can occur when a change in the phosphorus phase of the membrane (Safarinejad *et al.*, 2010.). However, omega fatty acids also attack reactive oxygen species that initiate a chain of lipid oxidation and seriously threaten the functional integrity of sperm. Research has shown that antioxidants in extracellular vitamin E serum have the potential to reverse the negative effect of omega supplements (Wathes *et al.*, 2007).

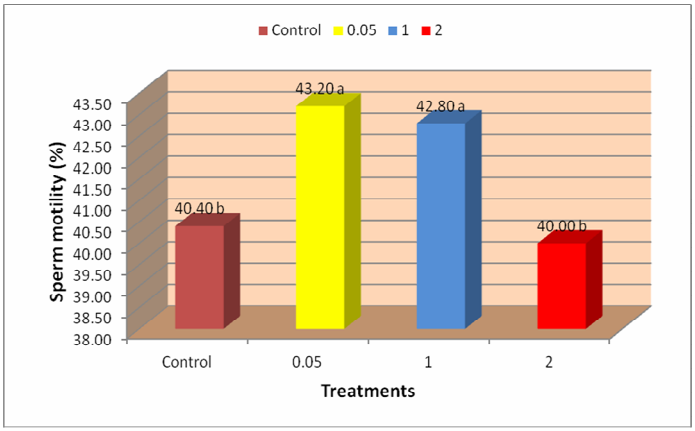

Fig. 1 : Effect of addition omega 3 to extender of semen on the percentage of sperm motility.

Different letters significant differences (P< 0.05).
Similar letters no significant differences (P >0.05).

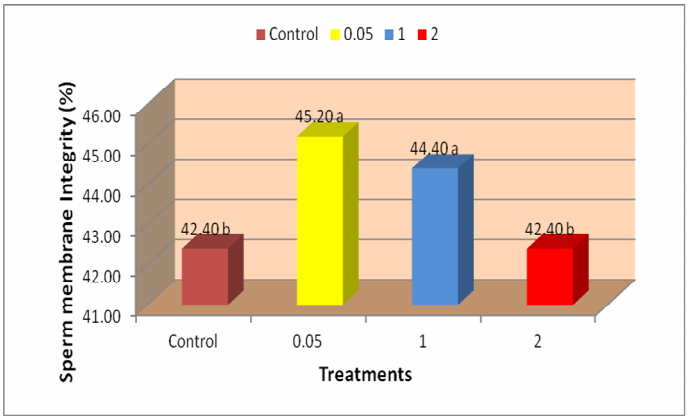

Fig. 2 : Effect of addition omega 3 to extender of semen on the percentage of sperm membrane Integrity.
Different letters significant differences (P< 0.05).
Similar letters no significant differences (P >0.05).

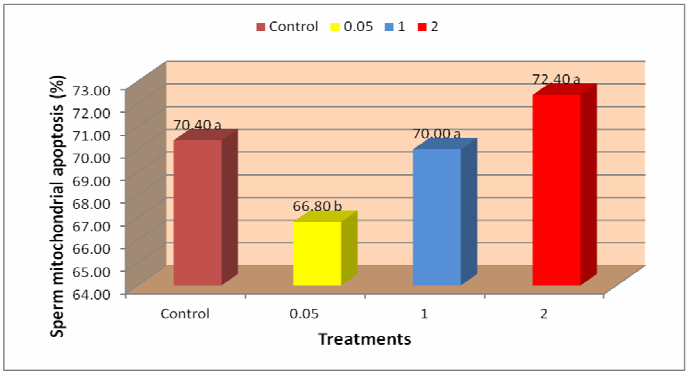

Fig. 3 : Effect of addition omega 3 to extender of semen on the percentage of sperm mitochondrial apoptosis.
Different letters significant differences (P< 0.05).
Similar letters no significant differences (P >0.05).

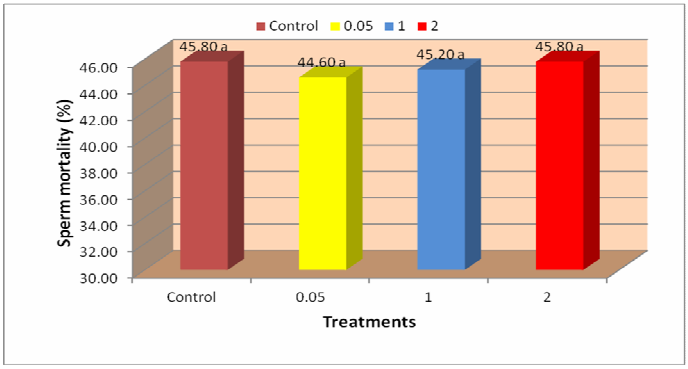

Fig. 4 : Effect of addition omega 3 to extender of semen on the percentage of sperm mortality.
Different letters significant differences (P< 0.05)..
Similar letters no significant differences (P >0.05).

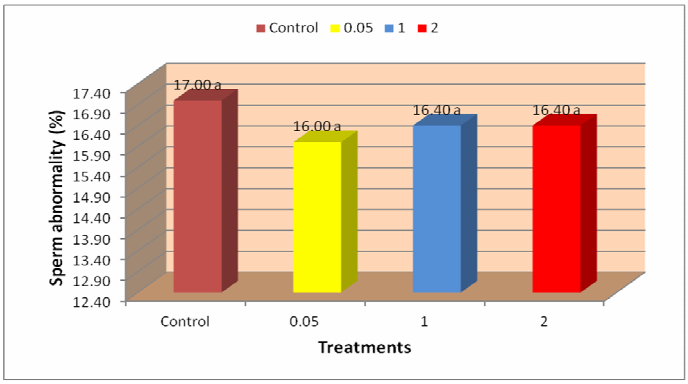

Fig. 5 : Effect of addition omega 3 to extender of semen on the percentage of sperm abnormality.
Different letters significant differences (P< 0.05).
Similar letters no significant differences (P >0.05).

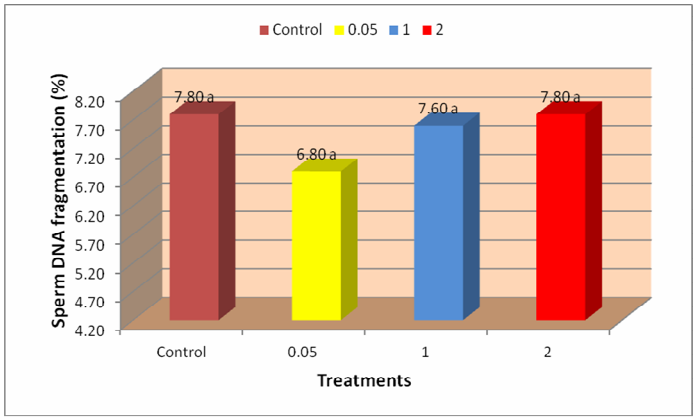

Fig. 6 : Effect of addition omega 3 to extender of semen on the percentage of sperm DNAfragmentation.

Different letters significant differences (P< 0.05).
Similar letters no significant differences (P >0.05).

References

Adeel, M.; Ijaz, A.; Aleem, M.; Rehman, H.; Yousaf, M.S.b. and Jabbar, M.A. (2009). Improvement of liquid and frozenthawed semen quality of Nili-Ravi buffalo bulls (*Bubalus bubalis*) through supplementation of fat. Theriogenology.; 71(8): 1220-1225.

Aksoy, Y.; Aksoy, H.; Altinkaynak, K.; Aydin, H.R. and Ozkan, A. (2006). Sperm fatty acid composition in subfertile men. Prostaglandins Leukot. Essent. Fatty acids 75: 75-79.

Al-Haboby, A.; Hamra, A.H.A and Mabdi, A.K. (2003). Effect of Licorice Extract on semen Quality and Libido in Awassi Rams. Arab Authority for Agricultural Investment and Development. first number.

Am-in, N.; Kirkwood, R.N.; Techakumphu, M. and Tantasuparuk, W. (2011). Lipid profiles of sperm and seminal plasma from boars having normal or low sperm motility. Therigenology, 75: 897-903.

Badawy, A.; Yaseen, A.; El-Bashary, A. and Ibrahim, M. (1975). Effect of sexual preparation on some characteristics of the semen of buffaloes and cattle bulls. Alexandria J. Agric. Res., 21: 185-191. (Anim. Breed. Abstr., 43: 1055).

Bansal, A.K. and Bilaspuri, G.S. (2011). Impacts of oxidative stress and Antioxidants on semen Function. Review Article. Veterinary Medicine International Volume, Article ID 686137, 7 pages.

Calder, P.C. (2011). Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol; 668 Suppl. 1:S 50–8.

Castellano, C.A.; Audet, I.; Bailey, J.L.; Laforest, J.P. and Matte, J.J. (2010). Dietary omega-3 fatty acids (fish oils) have limited effects on boar semen stored at 17ºC or cryopreserved Theriogenology.; 74: 1482-1490.

Castellano, C.A.; Audet, I.; Bailey, J.L.; Laforest, J.P. and Matte, J.J. (2010). Dietary omega-3 fatty acids (fish oils) have limited effects on boar semen stored at 17 °C or cryopreserved. Theriogenology, 74: 1482-1490.

Castellini, C.; Lattaioli, P.; Dal Bosco, A.; Minelli, A. and Mugnai, C. (2003). Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. Reprod Nutr Dev.; 43(1): 91-103.

Duncan, D.B. (1955). Multiple range and multiple F tests. Biometrics, 11: 1–42.

Evans, G. and Maxwell, W.M.C. (1987). Salomon's Artificial Insemination of Sheep and Goats. Butter worth's, Sydney, 194.

Gliozzi, T.M.; Zaniboni, L.; Maldjian, A.; Luzi, F.; Maertens, L. and Cerolini, S. (2009). Quality and lipid composition of spermatozoa in rabbits fed DHA and vitamin E rich diets. Theriogenology.; 71(6): 901-909.

Grady, S.; Cavinder, C.A.; Brinsko, S.P.; Forrest, D.W.; Sawyer, J. and Scott, B. (2009). Dietary supplementation of two varying sources of n-3 fatty acids and subsequent effects on fresh, cooled, and frozen seminal characteristics of stallions. Prof Anim Sci.; 25(6): 768-773.

Graham, E.; Schmehl, M. and Maki-Laurila, M. (1970). Some physical and chemical methods of evaluating semen. In: Proc. 3rd NAAB Tech. Conf. Artif. Insemin. Reprod., 12–14 April Milwaukee, WI. National Association of Animal Breeders, Columbia, MO, 44-48.

Lomeo, A. and Giambersio, A. (1991). Water-test: a simple method to assess sperm-membrane integrity. Int J Androl, 14: 278-282.

Maldjian, A.; Pizzi, F.; Gliozzi, T.; Cerolini, S.; Penny, P. and Noble, R. (2005). Changes in sperm quality and lipid composition during cryopreservation of boar semen. Theriogenology.; 63(2): 411-421.

Maldjian, A.; Pizzi, F.; Gliozzi, T.; Cerolini, S.; Pennya, P. and Noble, R. (2005). Changes in sperm quality and lipid composition during cryopreservation of boar semen. Theriogenology, 63: 411-421.

Poulos, A.; Darin-Bennett, A. and White, L.G. (1973). The phospholipid-bound fatty acids and aldehydes of mammalian spermatozoa. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 46: 541-549.

Royere, D.; Barthelemy, C.; Hamamah, S. and Lansac, J. (1996). Cryopreservation of spermatozoa: a review. Hum. Reprod. Update, 2: 553-559.

Saacke, R.G.; Nadir, S. and Nebel, R.L. (1994). Relationship of semen quality to sperm transport, fertilization and embryo quality in ruminants. Theriogenology, 41: 45-50.

Safarinejad, M.R.; Hosseini, S.Y.; Dadkhah, F. and Ali Asgari, M. (2010). Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: A comparison between fertile and infertile men. Clin. Nutr., 29: 100-105.

Salamon, S. and Maxwell, W.M.C. (1995). Frozen storage of ram semen II. cause of low fertility after cervical insemination and methods of improvement. Anim. Reprod. Sci.; 38: 1-36.

Swanson, E.W. and Beardon, H.J. (1951). An eosin nigrosin stain differentiating live and dead bovine spermatozoa. J. Anim. Sci., 10: 981-987.

Tejada, R.; Mitchell, J. and Norman, A. (1984). A test for the practical evaluation of male fertility by acridine orange (AO) fluorescence. Fertil. Steril. 42(1): 87-91.

Wathes, D.C.; Abayasekara, D.R. and Aitken, R.J. (2007). Polyunsaturated fatty acids in male and female reproduction. Biol. Reprod.; 77(2): 190-201.

Wathes, D.C.; Abayasekara, D.R. and Aitken, R.J. (2007). Polyunsaturated fatty acids in male and female reproduction. Biol. Reprod., 77: 190-201.

Woods, E.J.; Benson, J.D. and Agca, Y. (2004). Fundamental cryobiology of reproductive cells and tissues. Cryobiology., 48(2): 146-156.

Yimer, N.; Noraisyah, A.H.; Rosnina, Y.; Wahid, H.; Sarsaifi, K. and Hafizal, A.M. (2014). Comparison of Cryopreservative Effect of Different Levels of Omega-3 Egg-Yolk in Citrate Extender on the Quality of Goat Spermatozoa. ISSN: 0253-8318 (PRINT), 2074-7764

Ying Chen, Y.; Debora, L. and Kramer, P. (2001). Apoptotic Signaling in Polyamine Analogue-treated SK-MEL-28 Human Melanoma Cells1. Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York. 61: 6444-6437.