*Jordan Medical Journal, Volume 57, No.2 2023*
DOI: https://doi.org/10.35516/jmj.v57i2.1364

# Omega-3 Fatty Acids as Supplements and Cryoprotectants and Positive Outcomes for Human Seminal Cryopreservation: A Study in the Iraqi Context

***Mustafa M. Al-Obeidy,[1] Sameh S. Akkila[1] and Khalida I. Noel✉[1]***

**Abstract**

**Background**: With the increasing deterioration in male fertility rates over the past few decades, assisted reproductive therapy via sperm banking has gained considerable attention from different health authorities worldwide. The process of sperm cryopreservation exerts certain harmful effects on sperm quality parameters. The aim of the current study was to examine the effect of omega-3 on human sperm cryopreservation as a dietary supplement and as a cryoprotectant stimulant.

**Methods**: From healthy men, 120 seminal fluid samples were randomly collected for cryopreservation (for 30 days). All samples were divided into three groups (40 in each). The supplement group (SG) (first group) had been given dietary supplements of omega-3 (30% EPA: 20% DHA) for eight weeks before sample collection. The samples of the cryoprotectant group (CG) (second group) were treated with omega-3 additive in the diluent at the time of being collected and before we cryopreserved them. The samples from the control group (third group) were cryopreserved without any dietary or cryoprotectant supplementation. Initial seminal analysis was recorded and post-thawing assessment of sperm motility, vitality (using eosin test) and oxidative stress (using a nitro blue tetrazolium (NBT) test) were compared.

**Results and Conclusion:** SG samples had greater initial seminal fluid volumes, as well as better sperm motility and vitality, but the oxidative stress assessment did not differ significantly pre-cryo. Post-thawing assessment revealed that the CG group had the best parameters of motility, vitality and oxidative stress. These results may be related to the positive effects of omega-3 fatty acids on reproductive glands, hormonal milieu, sperm function and structure, making it a suitable biostimulant for improving the outcome of human sperm banking. Results were comparable to multiple previous animal studies.

**Keywords**: Male fertility, sperm cryopreservation, omega-3, polyunsaturated fatty acids, motility, vitality, oxidative stress

*(J Med J 2023; Vol. 57 (2): 182-192)*

| *Received* | *Accepted* |
|---|---|
| *August 27, 2022* | *January 15, 2023* |

## INTRODUCTION

Human reproductive health is an integral part of the survival of the species, and the decline in male fertility over the past years (or possibly decades) has drawn considerable attention from numerous authorities. The observed worldwide deterioration in various sperm parameters may be related to different factors including lifestyle (e.g., stress) [1], diet and increased rates of obesity [2] and/or exposure to environmental toxins [3]. Efforts to combat this problem have led to rapid and continuous advances in assisted reproduction techniques (ART), including sperm cryopreservation [4]. Gestation rates using

[1] Human Anatomy Dept., College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq.
✉Corresponding author: dr.khalidanoel@uomustansiriyah.edu.iq

cryopreserved sperms have improved with progress in these technologies, especially intracytoplasmic sperm injection (ICSI), in which one viable spermatozoon is required for fertilization [5]. Initially, sperm banking was used to treat infertile couples with onco-medical conditions, but the scope of application has increased nowadays to include homologous family planning, donor insemination, serodiscordance for viral infection and even posthumous reproduction [6].

After sperm procurement, the process of sperm cryopreservation typically consists of both slow-cooling and rapid-freezing methods, preferably using nitrogen vapor to limit viral cross-contamination encountered with liquid nitrogen [7]. When cryopreserved properly, the sperms remain in a state of metabolic arrest that prevents cellular aging and retains the fertilizing capacity of the sperm for an almost unlimited period [8]. Nevertheless, the freezing-thawing processes cause deterioration in sperm viability due to osmotic or oxidative stress [9], cryoprotectant toxicity and DNA fragmentation [10], physical cellular damage [11], or a combination of these factors. Permeating (e.g., glycerol) and non-permeating (e.g., proteins) cryoprotective agents (cryoprotectants) are used to minimize damage from freezing and thawing [12]. Several studies have examined and shown the benefits of adding further cryoprotective agents, such as minerals, vitamins, proteins and fatty acids to protect the integrity of stored sperms [13].

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs) that encompass a large family of essential fatty acids. The three main types of omega-3 fatty acids involved in human physiology are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is found in plants, while DHA and EPA are found in marine food sources [14]. EPA and DHA can be synthesized in the liver from ALA, but because of low conversion efficiency, the consumption of foods rich in EPA and DHA is recommended [15]. The ratio and rate of production of eicosanoids, a group of hormones closely involved in inflammatory and homeostatic processes, are greatly affected by the omega-6 to omega-3 ratio, since both eighteen-carbon PUFAs compete for the same metabolic enzymes [16].

Fatty acids are essential ingredients of the spermatozoan membrane and also function as sources of energy [17]. Diets low in PUFAs or with an unbalanced omega-6 to omega-3 ratio reduce sperm quality, whereas dietary PUFA supplementation or corrected ratios reverse this effect [18]. Studies have shown that omega-3 fatty acid supplements are associated with better seminal fluid analysis (SFA) parameters and could improve fertility rates among infertile men [19].

The aim of the current study is to examine the possible effect of omega-3 fatty acids as cryoprotectants and compare it to the effect of omega-3 oral supplements on various aspects of sperm quality.

## METHODOLOGY

### *Study Scope/Ethical Issues*

This prospective experimental analytic study was conducted over nine months (September 2021 to June 2022) at the Postgraduate Studies Lab, Al-Mustansiriyah College of Medicine, in Baghdad, Iraq. The study protocol was approved by the Scientific Committee and Editorial Board of the Department of Human Anatomy (No. ECP:21/3B, July 22, 2021).

The study participants were randomly selected from Kamal Al-Samarrai Hospital's Fertility Center in Baghdad. The study aim and protocol were clearly explained to all participants and ethical consent was obtained. All unused or remaining samples were disposed of in accordance with the Regulations of the National Biomedical Waste Disposal Program [20].

### *Study Design*

The minimum sample size for this study was calculated using Fisher's formula for sample size estimation with a type one error rate of 5% statistical assumptions, 60% condition knowledge, and ±5% points precision [21].

The study enrolled 120 randomly selected men who were having routine seminal fluid

analysis due to their spouses not conceiving, despite otherwise normal male-related factors (i.e., not infertile or subfertile). All participants had baseline seminal analysis that excluded male-related infertility/ subfertility. Inclusion criteria included: age <45 years (range 24–42 years [34.4±10.2]), no chronic/ hereditary disease affecting fertility, being a non-smoker, having no current or previous history of drug or alcohol consumption/ dependence, no current or recent genito-urinary tract inflammation or infection, and being on a normal balanced diet (not taking omega supplements). Participants were instructed to be sexually abstinent for three days before providing a seminal sample. All samples were obtained by traditional masturbation at the lab.

Using simple random allocation, the participants were divided into three groups (40 samples each):

1. Supplement group (SG): these participants were given omega-3 oral supplements for eight weeks. The supplement was provided as 1,000 mg Adrien Gagnon© (Canada) OMEGA-3 concentrate soft gel capsules of marine fish oil (containing 200 mg DHA and 400 mg EPA). Participants were instructed to take three soft gels daily before the main meal. Seminal samples were collected eight weeks afterwards for cryopreservation.

2. Cryoprotectant group (CG): the seminal samples of these participants were supplemented with 1 mg omega-3 (30% EPA: 20% DHA in 50 ml diluents, Biomedical Pharma©, Italy) according to previously approved techniques [22], and then cryopreserved.

3. Control group: the seminal samples of these participants were cryopreserved with no dietary or sample intervention.

***Cryopreservation/ thawing***

After collection of the samples and recording for seminal fluid analysis, a cryopreservation medium (SpermFreeze™ SSP, FertiPro, Belgium) was mixed with each seminal fluid specimen in a 3:1 seminal fluid: medium ratio (after the medium had been warmed to room temperature). For equilibration, the cryo vials containing the mixture were left for ten minutes at room temperature. Afterwards, liquid nitrogen vapor was used to freeze each cryo vial vertically for 15 minutes, and they were then rinsed and stored in liquid nitrogen (at -196˚C) for 30 days [23].

At the time of thawing, frozen cryo vials were placed in tap water for five minutes and the contents were then carefully mixed with a flushing medium (FertiCult, FertiPro, Belgium) in 3:0.5 ml (medium: semen) ratio. The resulting mixture was centrifuged for 15 minutes and the supernatant cast off, and then the pellet [i.e., sperms] was re-suspended in 0.5 ml flushing medium (FertiCult, FertiPro, Belgium) and examined.

***Study parameters***

1. Seminal fluid volume was recorded at the procuring of each seminal sample only prior to cryopreservation. All other parameters were recorded before cryopreservation and post-thawing.

2. A spermatic motility assessment: high power field magnification (x400) was used to determine sperm motility. Spermatozoa were divided according to their movement into either motile or non-motile and the motility percentage was calculated by examining 100 spermatozoa throughout ten fields [24].

3. A spermatic vitality assessment was recorded using Eosin Y (Sigma-Aldrich©, USA). Under high power field magnification (x 400), 20 µl of semen was mixed with 20 µl of the stain and examined microscopically. The heads of viable spermatozoa were not stained with eosin, but the heads of dead sperms were stained red or pink. Vitality was determined by calculating the percentage of viable (unstained) spermatozoa [25].

4. A reactive oxygen species (ROS) assessment was performed using a nitro blue tetrazolium (NBT) test, according to previous literature [24]. After washing with phosphate buffer saline (PBS) and centrifugation of seminal samples, the resulting pellet was resuspended in equal volumes of PBS and 0.1% NBT. Then, after incubation of the mixture, KOH and DMSO were used to solubilize the resulting formazan crystals (as a result of the

reduction of NBT by the intracellular ROS of the sperms). An ELISA plate reader (ELISA BioTek, ELx 800) was used to measure the resulting mixture photometrically at a wavelength of 630 nm.

***Data analysis***

Data were analyzed using SPSS-26 and Microsoft Excel 2019. Data were presented as mean ± standard deviation (SD) with a paired *t*-test assessment of the cryopreservation effects and a two-way ANOVA comparison of the three groups. The power of statistical significance was tested at *p*-value ≤0.05 with a 95% confidence interval.

**RESULTS**

Pre-cryopreservation analysis of seminal fluid volume revealed a statistically significant difference ($p$=0.009), with the supplement group (SG) having significantly greater volumes than the other two groups (Figure 1). Pre-cryopreservation parameters of motility and vitality were closely related in the three groups, but the SG samples showed significantly higher levels of pre-cryopreservation sperm motility and vitality levels (Figure 2). Pre-cryopreservation oxidative stress was also lowest in the SG samples, but the difference was not statistically significant ($p$>0.05).

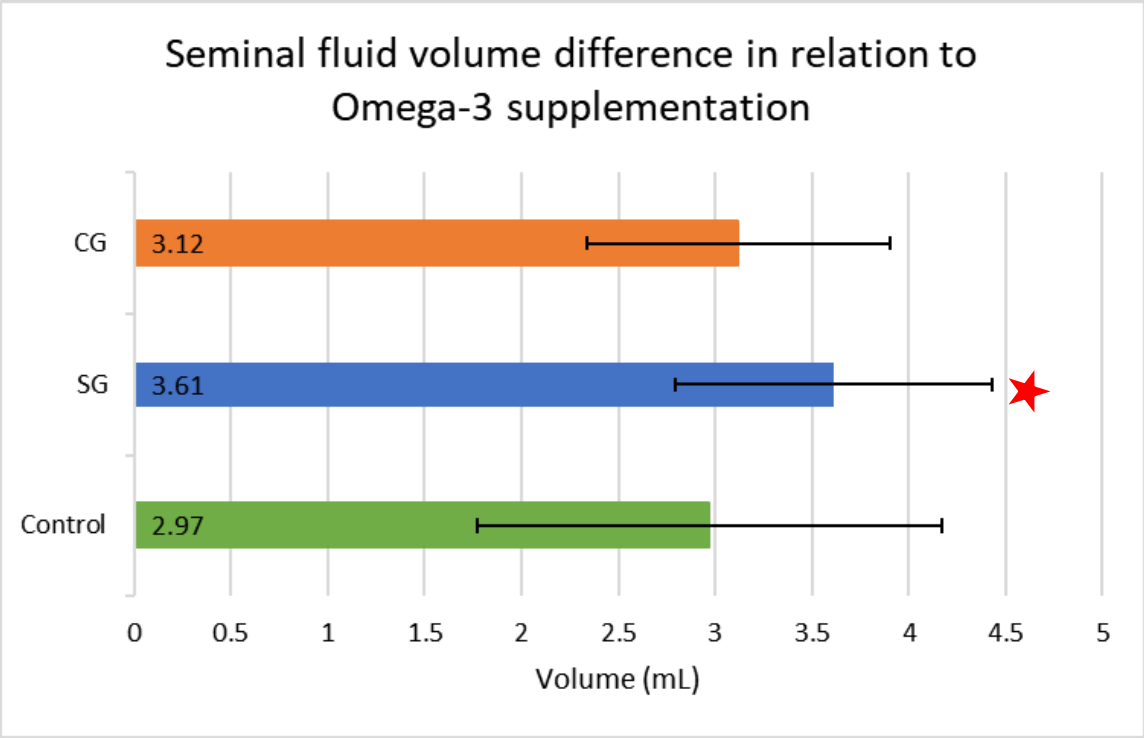


**Figure 1: Pre-cryopreservation difference in seminal fluid volume for the three groups. SG: supplemented group. CG: cryoprotectant group. Bars represent means; error bars represent standard deviation. Red star indicates statistically significant difference $p$<0.05**

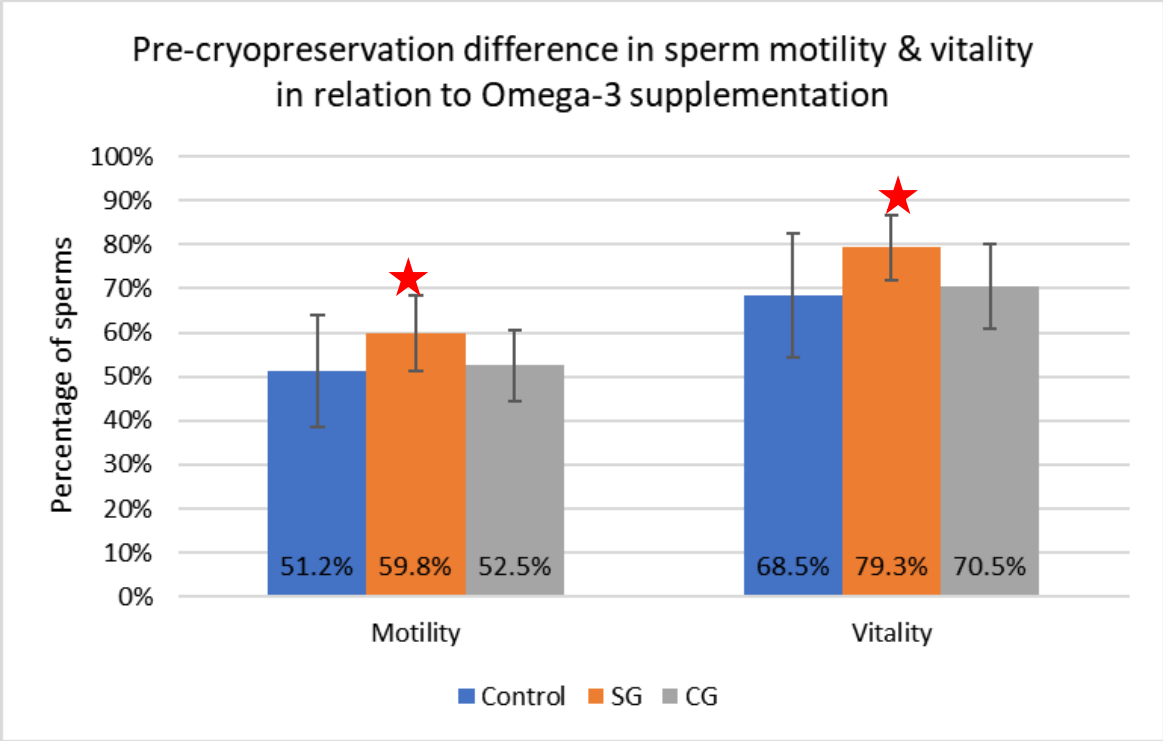


**Figure 2: Pre-cryopreservation difference in sperm motility and vitality of the three groups. SG: supplemented group. CG: cryoprotectant group. Columns represent means; error bars represent standard deviation. Red star indicates statistically significant difference $p<0.05$**

Sperm motility and vitality parameters decreased significantly after cryopreservation for all study groups ($p<0.001$). However, post-thawing analysis of variance of groups showed that the greatest decrease was seen in the control group and the least in the cryoprotectant group. Similarly, post-thawing oxidative stress increased significantly in all groups, but the least oxidative stress was seen in cryoprotectant group, while the greatest increase in ROS was seen in the control group. These results are summarized in Table 1.

**Table 1: Pre-cryopreservation and post-thawing values for sperm motility, vitality and oxidative stress for the three groups. SG: supplemented group. CG: cryoprotectant group. * indicates a significant difference $p<0.05$**

| **Sperm motility %** | | |
|---|---|---|
| **Groups** | Pre-cryopreservation | Post-thawing |
| **Control** | 51.2±12.6 | 17.5±7.1 |
| **SG** | 59.8±8.6* | 25.9±7.3 |
| **CG** | 52.5±8.1 | 37.4±6.3 |
| **Sperm vitality %** | | |
| **Groups** | Pre-cryopreservation | Post-thawing |
| **Control** | 68.5±14 | 45.7±12.5 |
| **SG** | 79.3±7.5* | 55.9±10.4 |
| **CG** | 70.5±9.6 | 68.7±14.7 |

| **Oxidative stress (NBT photometric test)** | | |
|---|---|---|
| **Groups** | Pre-cryopreservation | Post-thawing |
| **Control** | 0.2377±0.089 | 0.3903±0.057 |
| **SG** | 0.2120±0.058 | 0.2875±0.051 |
| **CG** | 0.2265±0.049 | 0.2405±0.033 |

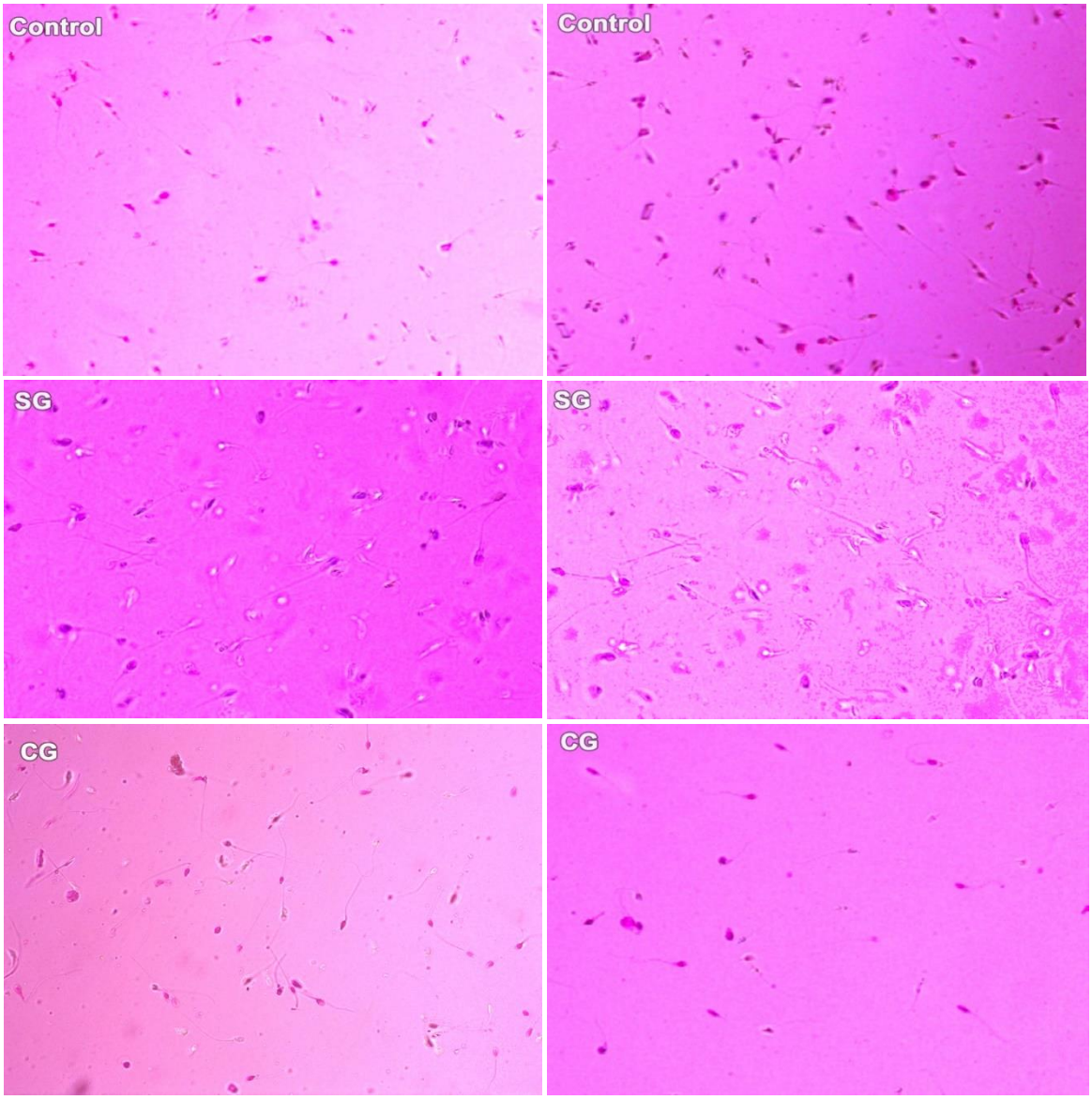


**Figure 3. Microscopic difference in pre-cryopreservation and post-thawing sperm motility shown using an eosin test. Dead sperms appear stained red and are obviously more numerous in the control groups, and least seen in the cryoprotectant group (CG)**

## DISCUSSION

Sperm cryopreservation is becoming an integral part of ART techniques and the process begins with the obtaining of a proper sample of

seminal fluid. A larger sample volume may increase the probability of obtaining a larger number of active vital sperms.

The current study revealed that omega-3 supplementation was associated with a significantly larger seminal fluid volume. Up to 75% of seminal fluid volume depends on the secretions from the seminal vesicles, the remainder being composed of contributions from the prostate, bulbourethral glands, epididymis and testis, in descending order [26]. Omega-3 PUFAs may improve the function of the seminal vesicles and prostate by affecting the mitochondrial energetic and steroidogenic cellular pathways of the male reproductive system, as well as provide precursors for testosterone production that positively influences the male secretory function [27]. Recent research has also revealed that dietary supplements of omega-3 or omega-6 modulate the testicular levels of adipokines in a way that improves the antioxidant and anti-inflammatory status of the reproductive system [28].

Omega-3 oral supplements increased pre-cryopreservation sperm motility and vitality with statistical significance and lower pre-cryopreservation oxidative stress in the current study. Spermatic plasma membrane fluidity is essential for its fertilization capacity and the fluidity of this membrane is greatly affected by its content of PUFAs such as DHA and EPA [29]. Being an integral part of the plasma membrane, omega-3 fatty acids may affect the length of the flagellar segments and the size of the accessory structures, including axonemes and outer dense fibers, which physically influence sperm motility and vitality [30]. Omega 3 fatty acids have anticoagulant properties and can cause a rise in cytoplasmic and peroxisomal catalase levels, which in turn enhances antioxidant properties [31]. Reducing oxidative stress and increasing membrane fluidity are possibly the key elements to increasing sperm vitality. Moreover, low ROS levels are associated with enhanced mitochondrial membrane structure and function, an effect that is essential for better sperm motility [27].

Sperm banking via cryopreservation entails sperm damage that may be caused by the formation of ice crystals, oxidative stress, cryocapacitation and/ or the toxicity of cryoprotectants [32]. The current study showed that, while pre-cryopreservation supplementation with omega-3 improves seminal parameters, adding omega-3 as an adjuvant cryoprotectant greatly improves post-thawing outcomes regarding sperm motility and vitality and reduces the oxidative stress of cryopreservation. While previous animal studies have shown that supplementing sperm sample diluent with omega-3 fatty acids can improve chilled and cryopreservation capacity [33], other animal studies have concluded that this addition is not beneficial and may even have a detrimental effect [34]. The cause of this discrepancy may be species-related or affected by dose concentration and/ or cryopreservation period and method. To our knowledge, no human study has been conducted to enable the in vitro results of the current study to be compared. As a biostimulant, omega-3 seems to have a greater effect as an additive to semen samples than as a dietary supplement [35]. This could be related to the fact that live spermatozoa may have a greater affinity to incorporate DHA/ EPA into their cellular components than when supplied via diet and having to cross the blood testis barrier [36]. In addition to the aforementioned biochemical properties of omega-3, being lipids, PUFAs may provide sperms with greater resistance to the physical detrimental effects of the freezing-thawing cycles [37–38].

How omega-3 supplements affect the viability of sperm has been considered in the literature, with previous studies demonstrating that omega-3 supplementation increases antioxidant activity in human seminal fluid and improves the number, motility, and shape of sperm [39–40]. Additionally, studies on the functions of fatty acids in the cryopreservation of sperm have been focused on animal sperm not human [41].

The limitations of the current study may be related to sample size and dose of cryprotecant. Further studies using larger populations, different doses and different cryopreservation periods will provide deeper insight.

## CONCLUSION

Omega-3 DHA/ EPA PUFAs have a positive influence, pre-cryopreservation and post-thawing, on sperm quality parameters, both as dietary supplements and biostimulant additives to diluents of sperm cryopreservation. The effect as direct biostimulant additives seems to exceed that of dietary supplements.

**Competing Interest Statement**

The authors have declared no competing interest.

**Funding Statement**

This clinical trial was funded by the personal efforts of the authors.

**Acknowledgements**

The authors acknowledge the postgraduate lab. in the College of Medicine, Al-Mustansiriyah University, Jordan, for permission to use the facilities.

## REFERENCES

1. Lacqua A, Izzo G, Emerenziani GP, Baldari C, Aversa A. Lifestyle and fertility: the influence of stress and quality of life on male fertility. Reprod Biol Endocrinol 2018 Nov 26; 16(1):115.
2. Mann U, Shiff B, Patel P. Reasons for worldwide decline in male fertility. Current opinion in urology 2020 May 1; 30(3):296-301.
3. Giwercman A, Bonde JP. Declining male fertility and environmental factors. Endocrinology and metabolism clinics of North America 1998 Dec 1; 27(4):807-30.
4. Grötter LG, Cattaneo L, Marini PE, Kjelland ME, Ferré LB. Recent advances in bovine sperm cryopreservation techniques with a focus on sperm post-thaw quality optimization. Reproduction in Domestic Animals 2019 Apr; 54(4):655-65.
5. Martini AE, Doyle JO. Fertility preservation before deployment: oocyte and sperm freezing in members of the active-duty military. InSeminars in Reproductive Medicine 2019 Sep ; 37( 05/06):232-238).
6. Ginsburg ES, Srouji SS. Donor insemination. UpToDate, Waltham, MA. Accessed 2020; 3(22):17.
7. Abram McBride J, Lipshultz LI. Male fertility preservation. Current Urology Reports 2018 Jul; 19(7):1-1.
8. Mocé E, Fajardo AJ, Graham JK. Human Sperm Cryopreservation. EMJ 2016; 1[1]:86-91.
9. Chatterjee S, Gagnon C. Production of reactive oxygen species by spermatozoa undergoing cooling, freezing, and thawing. Mol. Reprod Dev 2001; 59, 451–458.
10. Huang C, Tang YL, Hu JL, Zhou WJ, Huang ZH, Luo XF, *et al*. Update on techniques for cryopreservation of human spermatozoa. Asian Journal of Andrology 2022 Apr 1.
11. Rios AP, Botella IM. Causes and Impact of Cryopreservation-Associated Damage on Different Parameters of Human Spermatozoa and its Clinical Impact. Reproductive Health 2019; 5[1]:100-109.
12. Sieme H, Oldenhof H, Wolkers WF. Mode of action of cryoprotectants for sperm preservation. Anim Reprod Sci 2016 Jun; 169:2-5.
13. Rozati H, Handley T, Jayasena CN. Process and Pitfalls of Sperm Cryopreservation. J Clin Med 2017 Sep 19; 6(9):89.
14. Ameur A, Enroth S, Johansson A, Zaboli G, Igl W, Johansson ACV, *et al*. Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. Am J Hum Genet 2012; 90(5):809-820.
15. Burdge G. α-Linolenic acid metabolism in men and women: nutritional and biological implications. Curr Opin Clin Nutr Metab Care 2004; 7(2):137-144.
16. Hanson S, Thorpe G, Winstanley L, Abdelhamid AS, Hooper L. Omega-3, omega-6 and total dietary

polyunsaturated fat on cancer incidence: systematic review and meta-analysis of randomised trials. British journal of cancer 2020 Apr; 122(8):1260-70.

17. Amaral A, Castillo J, Estanyol JM, Ballescà JL, Ramalho-Santos J, Oliva R. Human sperm tail proteome suggests new endogenous metabolic pathways. Mol Cell Proteomics 2013 Feb; 12(2):330-42.
18. Ferramosca A, Zara V. Diet and Male Fertility: The Impact of Nutrients and Antioxidants on Sperm Energetic Metabolism. International Journal of Molecular Sciences 2022 Feb 25; 23(5):2542.
19. Salas-Huetos A, Rosique-Esteban N, Becerra-Tomas N, Vizmanos B, Bullo M, Salas-Salvado J. The effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and meta-analysis of randomized clinical trials. Adv Nutr 2018; 9, 833–848.
20. Iraqi Wakae' Journal. Federal Law 31-01-2011. Vol. 4175. p.1.
21. Noordzij M, Dekker FW, Zoccali C, Jager KJ. Sample size calculations. Nephron Clinical Practice. 2011;118(4):c319-23.
22. Rateb SA. Influence of omega-3 incorporation in sperm preservation medium on physical and kinematic properties of chilled and cryopreserved ram spermatozoa. Reproduction in Domestic Animals 2018 Dec; 53(6):1506-16.
23. Anger JT, Gilbert BR, Goldstein M. Cryopreservation of sperm: indications, methods and results. J Urology 2003; 170 (4 Pt 1): 1079-84.
24. World Health Organization (WHO). "Chapter -2- Basic examination/ 2.4. Examination and post examination procedures". In: WHO "WHO Laboratory Manual for the Examination and Processing of Human Semen". 6th edition; 2010. World Health Organization; p. 15-63.
25. Kashou AH, Sharma R, Agarwal A. Assessment of oxidative stress in sperm and semen. Methods Mol Biol 2013; 927:351-61.
26. Noda , Ikawa M. Physiological function of seminal vesicle secretions on male fecundity. Reproductive medicine and biology 2019 Jul; 18(3):241-6.
27. Arab A, Rafie N, Mansourian M, Miraghajani M, Hajianfar H. Dietary patterns and semen quality: A systematic review and meta-analysis of observational studies. Andrology. 2018; 6:20–28.
28. Moustafa A. Effect of Omega-3 or Omega-6 Dietary Supplementation on Testicular Steroidogenesis, Adipokine Network, Cytokines, and Oxidative Stress in Adult Male Rats. Oxidative Medicine and Cellular Longevity 2021 Jun 28; 2021.
29. González-Ravina C, Aguirre-Lipperheide M, Pinto F, Martín-Lozano D, Fernández-Sánchez M, Blasco V, *et al*. Effect of dietary supplementation with a highly pure and concentrated docosahexaenoic acid (DHA) supplement on human sperm function. Reproductive Biology 2018 Sep 1; 18(3):282-8.
30. Gu NH, Zhao WL, Wang GS, Sun F. Comparative analysis of mammalian sperm ultrastructure reveals relationships between sperm morphology, mitochondrial functions and motility. Reproductive Biology and Endocrinology 2019 Dec; 17(1):1-2.
31. Mohammadi H, Golbabaei F, Dehghan SF, Imani H, Ramezani Tehrani F, *et al*. The Influence of Vitamin E and Omega-3 Fatty Acids on Reproductive Health Indices Among Male Workers Exposed to Electromagnetic Fields. American journal of men's health 2022 Feb; 16(1):15579883221074821.
32. Benko F, Lenický M, Lukáč N, Tvrdá E. Negative Impact Of Cryopreservation And Cryodamage On Bovine Spermatozoa: A Review. Slovak Journal of Animal Science 2021 Sep 30; 54(03):151-6.
33. Salman RS. Evaluation of physiological parameters and the quality of chilled bull semen after adding different concentrations of omega-3 to tris-egg-yolk extender. EurAsian Journal of BioSciences 2020; 14(1):2379-84.
34. Kaka A, Haron W, Leghari RA, Memon MI, Kaka U, Mirani AH, *et al*. Effect of in-vitro supplementation of polyunsaturated fatty acids on

frozen-thawed bull sperm characteristics using Bioxcell® extender. Pure and Applied Biology (PAB) 2021 Oct 22; 5(3):399-405.

35. El-Amrawi GM, Metwally KK, El-Halag B. Improvement of Semen Quality in Rams by Adding Some Biostimulants During Cooling. Alexandria Journal for Veterinary Sciences 2021 Apr 1; 69(1): 136-142.
36. Wang S, Chen Q, Zhang Y, Zheng F, Xue T, Ge X, *et al*. Omega-3 polyunsaturated fatty acids alleviate hydrogen sulfide-induced blood-testis barrier disruption in the testes of adult mice. Reproductive Toxicology. 2020 Dec 1;98:233-41.
37. Al-Jeburi NJ, Al-Saadoon AA. Cryopreservation of goat epididymal sperms under freezing condition using omega 3, 6 and 9. Plant Arch 2019; 19:211-6.
38. Hameed AF, Ajeel IS, Noel KI, Hassan KM. Multifactorial etiology of male and female infertility/ assisted reproductive technique. Magna Scientia Advanced Biology and Pharmacy. 2022; 07(01): 001–008.
39. Safarinejad MR, Safarinejad S. The roles of omega-3 and omega-6 fatty acids in idiopathic male infertility. Asian J Androl 2012 Jul; 14(4):514-5. doi: 10.1038/aja.2012.46. Epub 2012 Jun 4. PMID: 22659579; PMCID: PMC3720081.
40. Falsig AML, Gleerup CS, Knudsen UB. The influence of omega-3 fatty acids on semen quality markers: a systematic PRISMA review. Andrology 2019; 7:794-803. https://doi.org/10.1111/andr.12649.
41. Yimer N, Kaka A, Yusoff R, Haron A. The Roles of Antioxidants and Fatty Acids in Sperm Cryopreservation. In F. Marco-Jiménez, and H. Akdemir (Eds.) 2016, Cryopreservation in Eukaryotes. IntechOpen. https://doi.org/10.5772/65571.

# أحماض أوميغا 3 الدهنية كمكملات غذائية وواقية من التجمد لتحسين الحفظ بالتبريد للسائل المنوي البشري: دراسة على عينة من السكان العراقيين

***مصطفى العبيدي[1]، سامح عقيلة[1]، خالدة نوئيل[1]***

[1] قسم التشريح البشري، كلية الطب، الجامعة المستنصرية، بغداد، العراق.

**الملخص**

**الخلفية:** مع التدهور المتزايد في معدلات خصوبة الذكور على مدى العقود القليلة الماضية ، اكتسب العلاج الإنجابي المساعد الذي ينفذ بنك الحيوانات المنوية اهتمامًا كبيرًا من السلطات الصحية المختلفة في جميع أنحاء العالم. تؤدي عملية تجميد الحيوانات المنوية إلى العديد من الآثار الضارة على معايير جودة الحيوانات المنوية. الهدف من الدراسة الحالية هو فحص تأثير أوميغا 3 كمكملات غذائية وكمحفزات واقية من التجمد على حفظ الحيوانات المنوية البشرية.

**الطريقة:** تم جمع 120 عينة من السائل المنوي من الرجال الأصحاء بشكل عشوائي للحفظ بالتبريد (لمدة 30 يومًا) ، وتم تقسيم جميع العينات إلى ثلاث مجموعات (40 لكل مجموعة). تم توفير مجموعة المكملات) (SG) المجموعة الأولى) مسبقًا عن طريق المكملات الغذائية من30 Omega-3 ٪ EPA: 20 ٪ ((DHAلمدة 8 أسابيع قبل جمع العينة ، تم علاج عينات مجموعة الحماية من التجمد) (CG) المجموعة الثانية) باستخدام أوميغا 3 مضافة إلى المادة المخففة في وقت جمع عينة السائل المنوي وقبل أن نقوم بحفظها بالتبريد ، وتم حفظ عينات المجموعة الضابطة (المجموعة الثالثة) بالتبريد دون أي مكملات غذائية أو مواد واقية من التجمد. تم تسجيل التحليل المبدئي المنوي وقارن تقييم ما بعد الذوبان لحركة الحيوانات المنوية وحيويتها (باستخدام اختبار اليوزين) والإجهاد التأكسدي (باستخدام اختبار تترازوليوم النيترو الأزرق.((NBT)

**النتائج والخلاصة:** عينات SG تحتوي على كميات أكبر من السائل المنوي الأولي ، وحركة وحيوية أفضل للحيوانات المنوية، لكن تقييم الإجهاد التأكسدي لم يختلف بشكل كبير قبل التجميد. أظهر تقييم ما بعد الذوبان أن مجموعة CG لديها أفضل معايير الحركة والحيوية والإجهاد التأكسدي. قد تكون النتائج مرتبطة بالتأثيرات الإيجابية لأحماض أوميغا 3 الدهنية على الغدد التناسلية ، والبيئة الهرمونية، ووظيفة الحيوانات المنوية وهيكلها مما يجعلها مرشحًا جيدًا كمحفز حيوي لتحسين نتائج بنك الحيوانات المنوية البشرية. كانت النتائج قابلة للمقارنة مع العديد من الدراسات الحيوانية السابقة.

**الكلمات الدالة:** خصوبة الرجال ، تجميد الحيوانات المنوية ، أوميغا 3 ، PUFA ، الحركة ، الحيوية ، الإجهاد التأكسدي.