The role of Cucumic Melo Varieta Agrestis, Vitamin C and Zinc on Sperm Activity and Oxidative Stress during Cryopreservation in Infertile Male

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Abstract
The objective of this study was to investigate the role of Cucumic melo varagrestis, Vitamin C and zinc on human spermatozoa and oxidative stress during cryopreservation in asthenozoospermia. One hundred males were participated in this study at Infertility Clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al- Nahrain University. There ages were (31.86 ±0.76) years. Semen samples were collected and seminal fluid analysis was done according to WHO (1999). Cryopreservation kept for one month in liquid nitrogen (LN2) at (-196◦C 1ml semen sample with 1ml of sperm freeze medium with zinc, vitamin c , cucmic melon extraction). After one month; 0.5 ml of Ferticuilt Flushin was added to pallet and incubation 37◦C\30min and Microscopic sperm parameters Examination had conducted. The oral administration of antioxidant combination of Cucumic melo var. agrestis, vitamin C and zinc improves sperm characteristics in asthenozoospermia.

Keywords: Cucumic melo varieta agrestis, Vitamin C, Zinc, infertile male

How to cite this article: Abd MW, Rasool Akwasiti EA, et al (2019): The role of Cucumic melo varieta agrestis, Vitamin C and zinc on sperm activity and oxidative stress during cryopreservation in infertile male, Ann Trop Med & Public Health; 22(7): S205. DOI:

Introduction
Infertility and reduced fertility are one of the major problems in medical science. World Health Organization (WHO) considers infertility as an important issue of reproductive health. Approximately 10 to 15 percent of couples are infertile. In about half of these cases, male infertility plays a role (1). Assisted Reproductive Technologies (ART's) have become the treatment of choice in several cases for male and female infertility (2).Thus, the best sperm preparation technique is to achieve the foremost number of morphologically normal, motile spermatozoa in a tiny volume of physiological culture media free from seminal plasma, leukocytes and bacteria (3). Semen cryopreservation is one of the most effective and acceptable methods to maintain male fertility potential and considered a routine procedure in (ART) (4).This technique becomes particularly important in cases of preservation of male fertility before radiotherapy, cytotoxic chemotherapy, or certain surgical treatment (5), herbal drugs from naturopathy (Ayurveda, Sidha, Unani and Chin, Anese traditional medicine) are general to treat male infertility despite the lack of scientific experimentation to measure its effectiveness (6). Cucumic melo var. agrestis is an annual climber belonging to the family Cucurbitaceous. It is commonly called as wild melon, small gourd and wild musk melon (7). This fruit contains carbohydrate, protein, lipids, water soluble vitamins were ascorbic acid, phenyl alanine, glutamine and asparagines, that is mean this fruit contains sufficient amount of all essential nutrients which could become a good source of human food (8). Cucumic melo seeds extract can be used to treat disease caused by free radical (9). Vitamin C is capable of neutralizing hydroxyl, superoxide and hydrogen peroxide radicals thus providing protection for human spermatozoa against endogenous oxidative damage (10). Zinc is an essential trace mineral that is also involved in the metabolism of DNA and RNA and has anti-apoptotic and antioxidant properties with a potential positive effect on spermatogenesis, while its effect on spermatogenesis appears to be protective in order to prevent premature oxidation of sulfhydryl groups during the formation of the outer dense fibers (11).

Materials and methods
This study was carried out in the High Institute for Infertility Diagnosis and Assisted Reproductive

Technologies, Al-Nahrain University. The study included one hundred sample divided into seventy five asthenozoospermia sample and twenty five normozoospermic sample according to standard criteria of WHO (12).The range age of as the nozoosperma was 20 to 42 years; however, the range age of normozoospermic was 23 to 37 years. The semen samples were obtained via masturbation after three to five days of sexual abstinence, collected directly into a clean, dry and sterile disposable plastic wide mouth container in a room especially made for this purpose in the institute. The study includes100semen samples (75 semen samples from asthenozoospermia men and 25 from normozoospermic men). Each group was examined microscopically by light micros cope at x40objactive lens to determine sperms parameters (concentration, motility, and morphologically normal sperms), according to criteria established by WHO (1999). Measurement of spermatozoa malondialdehyde(MDA), Estimation of reduced glutathione (GSH) and mix 1ml of sperm freeze media with 5mg of zinc and 15mg vitamin c and 500mig cucmic melon extraction and adding to 1ml of semen by using millipore 0.45µM and fixed for 7.4-7.8 pH at room temperature, and esterilizad in UV light. Each was cryopreserved (slow techniques) in liquid nitrogen at -196°C. Duration of cryopreservation was one month. After thawing sperm activation by flushing medium microscopic was determined of sperm function parameters. Result was reported according to manual of WHO (12).

RESULTS

The sperm characteristics of study sample are outlined in Table (1), There was highly significant reduction in mean sperm concentration form 56.91 ±11.83 million/ml to 27.45 ±5.27 million/ml (P< 0.001), however, there was highly significant improvement in grade A sperm % from 6.71 ± 3.27 to 19.75 ±7.16 (P < 0.001).In addition, there was highly significant rise in grade B sperm % from 28.02 ±5.87 to 38.38 ±5.59 (P< 0.001), On the other hand there was highly significant reduction in grade C and grade D % from 38.13 ± 7.77 to 27.61 ±6.01 and from 27.10 ± 7.73 to 14.25 ±6.68, respectively (P < 0.01). Moreover, there was highly significant rise in mean normal morphology sperm % from 50.10 ± 9.04 to 57.77 ±7.34 (P < 0.001), as shown in Table (1)

Table (1): Sperm characteristics before and after treatment

Characteristic	Statistical index	Before treatment	After treatment	*P*
Sperm Concentration (million /ml)	Mean ±SD	56.91 ±11.83	27.45 ±5.27	< 0.001 † HS
	Range	35 - 85	17 - 45	
Grade A %	Mean ±SD	6.71 ± 3.27	19.75 ±7.16	< 0.001 † HS
	Range	0 - 35	9 - 50	
Grade B %	Mean ±SD	28.02 ±5.87	38.38 ±5.59	< 0.001 † HS
	Range	12 - 40	29 - 58	
Grade C %	Mean ±SD	38.13 ± 7.77	27.61 ±6.01	< 0.001 † HS
	Range	18 - 48	12 - 39	
Grade D %	Mean ±SD	27.10 ± 7.73	14.25 ±6.68	< 0.001 † HS
	Range	10 - 47	0 - 30	
Normal Morphology %	Mean ±SD	50.10 ± 9.04	57.77 ±7.34	< 0.001 † HS
	Range	5 - 69	37 - 75	

SD: standard deviation; †: Paired sample t-test; HS: highly significant at $P \leq 0.01$

Oxidative markers study

Oxidative markers study included assessment of the concentration of glutathione peroxidase (GSH) enzyme and malondialdehyde (MDA) concentration in the seminal plasma of sub fertile asthenospermic men before and after treatment. The results were outlined in Table (2). There was no significant change in mean seminal plasma was no significant change in mean seminal plasma GSH concentration before and after treatment, 57.77 ±7.34 µmol/L versus 56.41 ±7.70 µmol/L, respectively (P = 0.089). However, there was highly significant reduction in MDA concentration from 2.02 ±0.76 nmol/L to 1.36 ±0.80 nmol/L (P < 0.001), as shown in Table (2).

Table (2): Seminal plasma concentration of oxidative enzymes and MDA in men with asthenozoospermia before and after treatment

Variable	Statistical index	Before treatment	After treatment	*P*
GSH µmol/L	Mean ±SD	57.77±7.34	56.41±7.70	0.089 † NS
	Range	37.02 -75.21	33.21 - 73.09	
	Range	3.01 -25.21	3.23 - 25.29	
MDA nmol/L	Mean ±SD	2.02±0.76	1.36±0.80	<0.001 † HS
	Range	0.72 -3.71	0.38 - 2.90	

SD: standard deviation; †: Paired sample t-test; NS: not significant at $P \leq 0.05$; HS: highly significant at $P \leq 0.01$

Correlations of sperm characteristics to demographic characteristics and oxidative markers

Before treatment sperm concentration positively correlated to MDA, Normal sperm morphology % was positively correlated to GSH, as shown in Table (3).

After treatment, sperm concentration was unrelated to any parameter. Grade A sperm % was unrelated to any parameter. Grade B sperm % was negatively correlated to MDA. Grade D sperm % was positively correlated to MAD. Normal morphology sperm % was positively correlated to GSH, as shown in Table (4).

Table (3): Correlations of sperm characteristics before treatment to demographic characteristics and oxidative markers

Characteristic	Sperm Concentration		Grade A		Grade B		Grade C		Grade D		Normal Morphology	
	R	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	0.150	0.137	0.095	0.347	0.066	0.517	-0.064	0.530	-0.132	0.191	-0.012	0.903
Duration	-0.007	0.948	-0.103	0.309	0.096	0.344	0.105	0.297	-0.014	0.890	-0.039	0.701
BMI	0.069	0.495	0.044	0.665	-0.060	0.554	0.001	0.989	0.027	0.791	0.148	0.142
GSH umol/L	0.130	0.196	0.116	0.251	0.034	0.740	-0.039	0.702	-0.122	0.225	0.736	<0.001**
MDA nmol/L	0.005	0.960	0.187	0.063	0.170	0.092	-0.172	0.087	0.197	0.050*	-0.053	0.598

*significant at $P \leq 0.05$; ** highly significant at $P \leq 0.01$

Table (4): Correlations of sperm characteristics after treatment to demographic characteristics and oxidative markers

Characteristic	Sperm		Grade A		Grade B		Grade C		Grade D		Normal	
	R	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	0.082	0.415	0.133	0.188	0.053	0.600	-0.028	0.781	-0.148	0.143	0.016	0.877
Duration	0.065	0.523	-0.142	0.158	0.002	0.981	0.169	0.093	0.004	0.969	-0.122	0.227
BMI	0.070	0.487	0.006	0.950	0.065	0.521	-0.012	0.905	-0.053	0.601	0.122	0.225
GSH	0.177	0.078	0.003	0.974	0.060	0.553	-0.032	0.749	-0.013	0.896	0.447	<0.001
MDA	-0.001	0.989	0.140	0.165	-0.262	0.008	-0.098	0.334	0.299	0.003*	-0.147	0.143

DISCUSSION

Sperm characteristics before and after treatment

Cucumis melo var. agrestis has been described by a number of authors to improve male fertility according to animal experimental studies (13) and some clinical observations (14). It has been stated that the use of such medical herbs may be associated with increase in sperm production and improvement in sperm quality; however, and following thorough search in available published articles, the authors of the current study failed to find a clinical research that have tested the effect of Cucumis melo var. agrestis specifically in asthenozoospermia men. Raigani *et al* have evaluated the administration of vitamin C to sub fertile men with Oligoasthenoteratozoospermia and found significant rise in sperm motility, a finding that is similar to the finding of the current study; however, *Raigani et al.,* have described, in the contrary to the finding of the present study, a rise in sperm concentration. Probably the addition of L-carnitine is the factor that resulted in rise in sperm concentration in the study of Raigani *et al*. (15). The current study agrees with that of Kobori *et al*. in that anti-oxidant therapy increases sperm motility, but it disagrees that anti-oxidant therapy increases sperm count. Consistent with current observation, it was observed that a diet rich in vitamin C, vitamin E, vitamin D, zinc, foliate, total fiber, polyunsaturated fatty acids, and selenium was significantly associated with a lower risk of asthenozoospermia (16). Vitamin C supplementation (250 mg twice daily) improved sperm motility and normal morphology, but not sperm count, in 115 infertile men (17). A systematic review concluded that seminal zinc levels were lower in infertile men and that zinc supplementation increased semen volume, sperm motility and normal sperm morphology (18). The present study agrees with the later systemic review in that anti-oxidant therapy containing zinc can improve both sperm motility and normal morphology %. In an Iraqi study, semen volume, sperm count, and progressive sperm motility significantly improved following zinc supplementation in asthenozoospermia men (19).

Oxidative markers study

In the current study, oxidative markers study included assessment of the concentration of myeloperoxidase (MPO) enzyme, glutathione peroxidase (GSH) enzyme and catalase enzyme in addition to malondialdehyde concentration in the seminal plasma of sub fertile asthenospermic men before and after treatment. Based on the present study observation, there was no significant change in mean seminal plasma MPO concentration before and after treatment. In addition, there was no significant change in mean seminal plasma GSH concentration before and after treatment. Moreover, there was no significant change in mean seminal plasma catalase concentration before and after treatment. However, there was highly significant reduction in MDA concentration.

Therefore, a suggestion can be made that the particular use of Cucumis melo var. agrestis, vitamin C and zinc resulted in improvement of seminal fluid characteristics because of their own anti-oxidant activities rather than by increasing the activity of endogenous seminal plasma naturally occurring anti-oxidant agents. Therefore, it follows that these agents or some form of their metabolites have reached the seminal plasma and exerted their direct antioxidant activities there. Indeed, it was a limitation in the current study to estimate the seminal plasma levels of zinc, vitamin C and Cucumis melo var. agrestis metabolites before and after treatment. However, several studies has documented that oral zinc administration can cause increment in seminal plasma concentration of zinc (18)(20). On the other hand, vitamin c supplementation also has been shown increase seminal plasma availability of vitamin C particularly if one takes into consideration that vitamin C is a water soluble vitamin (21)(22). In addition, a study reported that vitamin C mitigated the cyclophosphamide-induced reduction in seminal fluid parameters in rats attributed to oxidative stress (23).

Correlations of sperm characteristics to demographic characteristics and oxidative markers

In this study, before treatment sperm concentration was negatively correlated to MPO and positively correlated to catalase. Grade A sperm % was positively correlated to catalase. Grade B % was negatively correlated to MPO and positively correlated to catalase. Grade C sperm % was negatively correlated to catalase and positively correlated to MPO. Grade D sperm % was negatively correlated to catalase and positively correlated to MDA. Normal sperm morphology % was positively correlated to GSH. After treatment, sperm concentration was unrelated to any parameter. Grade A sperm % was unrelated to any parameter. Grade B sperm % was negatively correlated to MDA. Grade C sperm % was positively correlated to MPO. Grade D sperm % was positively correlated to MAD. Normal morphology sperm % was positively correlated to GSH. These correlations findings indicated that better quality seminal fluid is associated with lower concentration of oxidative stress markers, namely MDA, and higher level of endogenous anti-oxidant molecules. Similar findings have been reported by (21) indicating the necessary action of reducing oxidative stress when planning for assisted reproductive technologies.

CONCLUSIONS

The oral administration of antioxidant combination of Cucumis melo var. agrestis, vitamin C and zinc improves sperm characteristics in asthenozoospermia men, The oral administration of antioxidant combination of Cucumis melo var. agrestis, vitamin C and zinc has no effect on the level of endogenous seminal plasma anti-oxidants, but significantly reduces oxidative markers. , and the improvement in seminal fluid characteristics of asthenozoospermia men is due to direct anti-oxidant effect of Cucumis melo var. agrestis, vitamin C and zinc.

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