

EFFECT OF NATURAL ANTIOXIDANTS TOCOPHEROL AND ASCORBIC ACIDS IN MAINTENANCE OF SPERM ACTIVITY DURING FREEZE-THAW PROCESS

H. A. ASKARI
J. H. CHECK
N. PEYMER
A. BOLLENDORF

The University of Medicine and Dentistry of New Jersey,
Robert Wood Johnson Medical School at Camden,
Cooper Hospital and University Medical Center, Department
of Obstetrics and Gynecology, Division of Reproductive
Endocrinology & Infertility, Camden, New Jersey, USA

Excessive generation of reactive oxygen species and increased peroxidation of the phospholipids in the membrane is proposed as a biochemical basis for the reduced activity of spermatozoa in cryopreserved semen. The possible role of α -tocopherol (VE) and ascorbic acid (VC) in improving the quality of frozen semen was investigated. Semen samples were divided into aliquots and frozen in freezing media with and without VC or VE. Sperm parameters, including motility after thawing and also at 2 h, % grade A motility (rapid and linear forward progression), and hypoosmotic swelling (HOS) scores, were measured in all samples. Vitamin C did not result in any improvement in sperm parameters studies. Vitamin E improved the post-thaw motility minimally. This improvement, although statistically significant, was actually very small. There were, however, wide variations between the samples. The motility improvement was considered in 5 of 12 samples, and HOS improved in some of the samples tested with VE. Future studies are needed to determine if these antioxidants can produce a consistent improvement in some individuals rather than help the majority of patients.

Keywords tocopherol, ascorbic acid, sperm cryopreservation, antioxidants

Cryopreservation of human sperm is commonly used for artificial insemination with donor sperm. It is also used for long-term preservation of sperm prior to procedures that may jeopardize a man's future fertility. In spite of considerable improvement achieved with the use of egg yolk glycerol media, the procedure is still associated with the loss of 50 to 60% or more of prefreeze motility [3, 5]. The deleterious effect of freeze-thawing on sperm is reflected in the many reports of lower fertilization rate with frozen-thawed sperm [3, 8, 13]. The structural damage is principally in the peripheral sperm membrane and quite often there is considerable damage to the acrosome with the leakage of the acrosome contents [2, 9]. It is presumed that

Address correspondence to Jerome H. Check, MD, 7447 Old York Road, Melrose Park, PA 19126, USA.

accumulation of the free reactive oxygen species (ROS) by increased peroxidation of the membrane lipids might damage the membrane [12].

The purpose of this study was to investigate the possible protective role of natural antioxidants α -tocopherol (vitamin E) and ascorbic acid (vitamin C) freezing media in maintenance of sperm quality following the freeze-thaw process.

MATERIALS AND METHODS

Semen obtained from the healthy normal volunteers was divided into equal aliquots, which were mixed with an equal volume of one of the following media:

- (1) Freezing medium containing glycerol-test-yolk-buffer (TYB) (Irvine Scientific, Irvine, CA, USA)
- (2) TYB medium containing 10 mMol of vitamin E, (α -tocopherol, Sigma Chemical Co.)
- (3) TYB containing 10 mMol of vitamin C (ascorbic acid, Sigma Chemical Co.)

The freezing medium cryoprotectant was slowly added dropwise while vortexing at a low setting and then was transferred into 1-mL Nunc vials, which were placed into a 500-mL beaker of 37°C water and then transferred to a 2–6°C refrigerator. After 90 min in the refrigerator, vials were placed in liquid nitrogen (LN2) vapors, 1 in. above the LN2 surface for 60 min. The vials were then plunged into LN2. Vials were kept frozen for a minimum of 24 h and a maximum of 2 weeks; thawing was performed at 37°C. Semen variables of motility, motile density, viability, and hypoosmotic swelling were measured in all post-thaw samples. Sperm parameters in samples containing vitamin E (VE) or vitamin C (VC) were compared with the controls (plain TYB), using the analysis of variance (ANOVA).

RESULTS

Mean values for semen post-thaw characteristics in samples containing tocopherol and ascorbic acid were compared with the samples frozen in plain TYB. Results are shown in Table 1 and Figures 1 and 2. Addition of ascorbic acid did not result in improvement in any of the parameters studied. α -Tocopherol-containing media affected post-thaw motility. The improvements, as reflected in the mean value were minimal, but statistically significant. There were, however, wide individual differences and some samples showed considerable improvement in maintenance of post-thaw motility, with some not showing any improvement.

DISCUSSION

Lower conception rate with cryopreserved semen is attributed to the changes in the sperm metabolic function [3, 6] and damage to its ultrastructure [2], probably a consequence of the former. These are associated with a decrease in fertilizing ability and shortened life span of the sperm [5, 6]. The major cause of this reduced potency seems to lie in a disturbance in the integrity of the plasma membrane and alteration in acrosome membrane and matrix [2], the crucial part of ova penetration. It is shown that loss of motility in sperm samples and in individual spermatozoa in the same sample is related to the accumulation of ROS in the spermatozoa [1, 4]. The antioxidant systems normally present in spermatozoa and probably also in seminal plasma help to prevent this accumulation [10]. Additives like pentoxifylline and IBMX, which are claimed to improve motility, are antioxidants [7]. On the other hand, the freeze-thaw process results in partial destruction of some of the enzyme systems that are

TABLE 1 Semen Parameters for Each Sample

Sample	Motility		Quality A		2-h Motility		HOS		Viability	
	Control	Vita- min E	Control	Vita- min E	Control	Vita- min E	Control	Vita- min E	Control	Vita- min E
1	46	67	40	20	38	44	59	65	62	69
2	36	31	50	50	25	31	43	39	62	51
3	20	26	20	35	8	9	40	25	53	45
4	40	45	50	20	35	45	60	39	63	50
5	28	57	0	50	15	20	30	25	43	57
6	26	28	50	40	16	15	31	31	31	30
7	22	22	50	50	15	21	40	46	44	48
8	23	26	25	30	17	19	35	36	56	52
9	50	55	30	60	35	42	60	57	61	65
10	38	39	65	70	31	35	52	50	56	57
11	34	36	54	40	8	8	44	40	62	69
14	38	46	43	31	22	44	55	50	56	62
17	33	29	65	63	29	23	56	62	81	77
12	40	35	44	50	21	22	44	54	50	56
13	33	35	50	38	21	20	54	51	54	52
15	31	36	66	50	15	14	33	37	62	60
16	36	48	48	71	33	30	36	47	53	67
18	30	32	37	60	12	18	38	42	60	60
19	35	28	38	50	12	12	48	50	48	53
20	10	13	33	50	5	5	27	27	53	48
21	37	39	82	71	15	11	11	15	38	38
22	36	38	41	46	18	18	34	43	68	69
23	59	66	71	67	22	30	50	48	63	75
24	55	57	57	70	24	45	51	57	76	78
25	67	67	67	58	22	24	47	47	67	68
Mean	36.12	40.04	47.04	49.60	20.56	24.20	43.12	43.32	56.88	58.24
SD	10.91	13.82	17.68	15.43	9.23	12.73	12.19	12.47	11.11	12.04
<i>p</i> value	0.02		0.48		0.01		0.89		0.34	

involved in neutralizing ROS [12]. This could be one of the major reasons for rapid loss of the activity that occurs after thawing [6]. It is shown that α -tocopherol (VE), a natural antioxidant, could improve the motility of spermatozoa in animals [11] and improves the heterogeneous ova penetration (SPA) ability of human spermatozoa [1].

In this study, we found some minimal improvement in maintaining the motility when the freezing media contained VE. It would be interesting to study changes in other criteria of sperm activity, such as SPA and acrosome reaction, which might be associated with VE addition and also to measure if these actually are associated with a change in the ROS. The relatively wide difference in response to VE might also indicate that the quality of sperm samples from some individuals might be improved with the addition of VE to freezing media. It would be interesting to study whether keeping the thawed sperm in media containing vitamin E might be effective in preventing the rapid loss of motility that normally occurs after thawing [12].

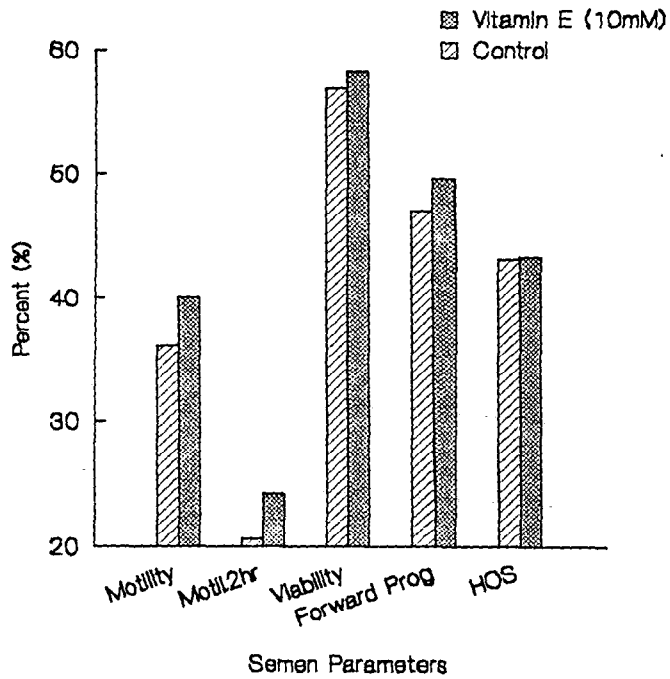


FIGURE 1 Comparison of semen parameters by additives used.

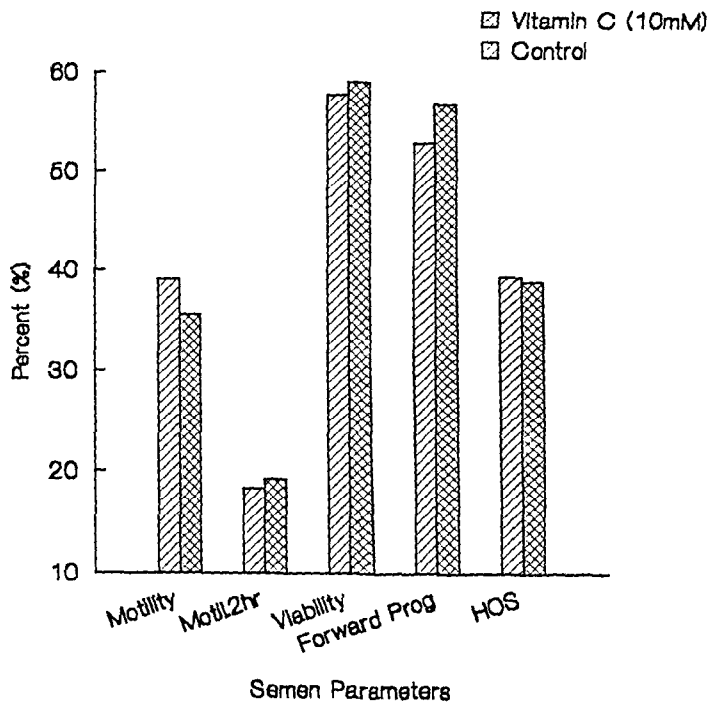


FIGURE 2 Comparison of mean semen parameters by additives used.

REFERENCES

1. Aitken RJ (1988): Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. *J Androl* 9:367-376.
2. Barthelemy D, Royere D, Hammah S, Lebos C, Tharanne MJ, Lansac J (1990): Ultrastructural changes in membrane and acrosome of human sperm during cryopreservation. *Arch Androl* 25:29-40.
3. Brotherton J (1990): Cryopreservation of the human semen. *Arch Androl* 25:181-195.
4. Erasmus EL, Oettle EE, Franken DR, Kruger TF, Oehninger SC (1992): Analysis of association between reactive oxygen species and semen quality. *J Androl Abstr* P-18:3.
5. Keel BA, Webster BW (1989): Semen analysis data from fresh and cryopreserved ejaculates. *Fertil Steril* 52:100-105.
6. Keel BA (1987): Effects of cryopreservation on motility characteristics of spermatozoa. *J Reprod Fertil* 81:213-220.
7. Kolon TK, Phillips K, Buch JP (1992): Pentoxifyline enhancement of post-thaw motility in cryopreserved sperm. *J Androl* P-35-72.
8. Morshedi M, Oehninger S, Veeck LL, Ertunc H, Bocca S, Acosta AA (1990): Cryopreserved/thawed semen for IVF. *Fertil Steril* 54:1093-1099.
9. Nagae T, Yanagimachi R, Srivastava PN, Yanagimachi H (1986): Acrosome reaction in human spermatozoa. *Fertil Steril* 45:701-707.
10. Rhemu JPT (1992): The principal anti-oxidants in human semen, uric acid and vitamin C. *Hum Reprod* 7(2):10-11.
11. Singh P (1989): Effect of vitamin E on lipid peroxidation in buffalo sperm. *Indian J Exp Biol* 27:14-16.
12. Storey BT, Alvarez JG (1992): Peroxidative damage to cryopreserved sperm is consequent to partial inactivation by superoxide dismutase induced by freeze-thaw process. *J Androl Abstr* P-18:3.
13. Yavetz H, Lessing JB, Niv Y, et al (1991): The efficacy of cryopreserved semen versus fresh semen in IVF. *J Vitro Fertil Embryo Transfer* 8:145-148.