The Effect of H_2O_2 and Some Antioxidants on Human Sperm Parameters *in vitro*

Faris, N.A.A1-Hady
College of Science / Babylon University
Sahib, Y.H.Al-Morshidy
College of Science / Kufa University
Mufeed, J.Ewadh
Karbala University

Abstract

This study was performed to determine the effect of H_2O_2 addition to the semen specimens to stimulate the formation of free radicals, and treated with antioxidants. This study involved ten semen specimens collected from fertile men. Seminal fluid analysis was performed to estimation sperm parameters and Malondialdehyde (MDA) concentration. Also seminal fluid analysis and MDA level was determination after adding of H_2O_2 and then antioxidants (VE, VC, VE+VC, glutathione and seminal plasma).

The results revealed a significant decrease ($p<0.05$) in sperm motility percent, grade activity and sperm viability percent, and significant increase ($p<0.05$) in MDA concentration by addition 0.1% of H_2O_2 to semen specimens compared to those values with normal saline. The addition of antioxidants (0.6mg/ml VE, 0.4mg/ml Vc + 0.6mg/ml VE, 0.4rng/ml glutathione and seminal plasma) to the semen specimens contain 0.1% H_2O_2 caused significant increase($p<0.05$) in sperm motility percent, grade activity and sperm viability percent , and significant decrease ($p<0.05$) in MDA level compared to those values in semen samples contain 0.1% H202 alone. While VC caused insignificant differences ($p>0.05$) in all sperm parameters and MDA concentration.

الخلاصة

اجريت هذه الدراسة لتحديد تأثير اضافة بيروكسيد الهيدروجين الى عينات السائل المنوي لحث انتاج الجذور الحرة ومن ثم اضافة مضادات الاكسدة لتخفيف ضرر الجذور الحرة. شملت هذه الدراسة عشر عينات مني جمعت من اشخاص خـصيبين. تـم اجراء فحص السائل المنوي لحساب معالم النطف بالاضافة لقياس تركيز المالونداي الديهايد. كذلك فقد اجريت الفحوصات نفـسها بعد اضافة ٠,١% بيروكسيد الهيدروجين لوحده وكذلك بعد اضافة مضادات الاكسدة (فيتامين E وفيتامين C وفيتامين C+ فيتامين E والكلوتاثيون والبلازما المنوية) الى عينات المني الحاوية على بيروكسيد الهيدروجين.

اشارت النتائج حصول انخفاض معنوي ($P<0.05$) في كل من النسبة المئوية للنطف المتحركة ودرجـة نـشاط النطـف والنسبة المئوية لحيوية النطف وزيادة معنوية ($P<0.05$) في تركيز المالونداي الديهايد لدى اضافة OH بيروكـسيد الهيـدروجين مقارنة بقيمها في عينات المني المضاف اليها المحلول الفسيولوجي.

سببت اضافة اضافات مضادات الاكسدة (٠,٦ملغم/مل فيتامين E و ٠,٤ ملغم/مل فيتامين C +٠,٦ ملغم/مل فيتـامين E و ٠,٤ ملغم/مل كلوتاثيون والبلازما المنوية) الى عينات المني الحاوية على بيروكسيد الهيدروجين حصول زيادة معنوية ($P<0.05$) في كل من النسبة المئوية للنطف المتحركة ودرجة نشاط النطف والنسبة المئوية لحيوية النطف ونقص معنـوي ($P<0.05$) فـي تركيز المالونداي الديهايد مقارنة بقيمها في عينات المني الحاوية على بيروكسيد الهيدروجين لوحده. اما فيتامين C فلم يظهـر أي فرق معنوي في معالم النطف كافة وكذلك مستوى المالونداي الديهايد.

Introduction

The spermatozoa produced few amount of Reactive Oxygen Species (ROS) in specific physiological condition which are necessary for capacitation, acrosorne

reaction and fertilization (Griveau and Le Lannou, 1997). While the large amount of ROS produced by immature sperm and leukocytes caused harmful effects on normal spennatozoa as a result of lipid oxidation activity (Agarwal *et al.,* 2003). The seminal fluid contains molecules with high molecular weight and low molecular weights called Antioxidants or Scavengers system protect the seminal fluid from the ROS (Pasqualotto *et al.,* 2000).

There are a balance between the ROS production and defense mechanism of Antioxidants in male reproductive tract, although may be increased the production of ROS (Sikka, 2004), or the reduction of Antioxidants should be caused to Oxidative Stress (Momen *et al.,* 1999). The Oxidative Stress considered a very important factor in male infertility, because the increasing of Oxidative Stress related negatively with normal sperm parameters (Agarwal and Said, 2003).

The goals of this study are showed the relationship between ROS and sperm parameters, and trial to repair the defects in sperm parameters result from the ROS by Antioxidants addition.

Materials and Methods

Ten semen specimens were collected by masturbation from fertile men after 3-5 days of sexual abstinence. The specimens were allowed to liquefy at 37C.

The semen specimens were divided into equal seven splits and adding normal saline to the 1st split, 0.1% of H_2O_2 to the 2nd split, 0.1% H_2O_2+0.4mg/ml VC to the 3rd split, 0.1% H_2O_2+0.6mg/rnl VE to the 4th split, 0.1% H_2O_2+ 0.4mg/mi VC+ 0.6mg/ml VE to the 5th split, 0.1% H_2O_2+ 0.4mg/ml glutathione to the the split, and 0.1% H_2O_2+ seminal plasma to the split.

All splits were incubated at 37 C for 15 minutes. Seminal fluid analysis was performed after the incubation, and then each split was centrifuged at 3000 rpm for 15 minutes to obtain the seminal plasma. MDA concentration was estimation due to the technique recorded by Muslih *et al.,* (2002).

The results were statistically analyzed by using F- test and Least Significant Difference (LSD) to comparison between the groups (Sorli, 1995).

Results

The addition of 0.1% H_2O_2 to the semen specimens of fertile men caused significant increase ($p<0.05$) in MDA concentration and significant decrease ($p<0.05$) in sperm motility percent, grade activity, and sperm viability percent compared to control (semen specimens with normal saline). While there are insignificant differences ($p>0.05$) in sperm concentration, normal sperm morphology percent and leukocytes concentration (Table-1-).

The treated of semen specimens contain 0.1% H_2O_2 with several antioxidants caused significant improvement in sperm quality. The addition separately of 0.6mg/ml of VE, 0.4mg/ml of VC + 0.6mg/ml of VE, 0.4mg/ml of glutathione, and seminal plasma, caused significant decrease ($p<0.05$) in MDA concentration and significant increase ($p<0.05$) in sperm motility percent, grade activity, and sperm viability percent compared to those values in semen specimens with 0.1% H_2O_2 only. While there are insignificant differences ($p>0.05$) in sperm concentration, normal sperm morphology percent and leukocytes concentration. Also there are insignificant differences ($p>0.05$) in MDA concentration and all sperm parameters mentioned above by using 0.4mg/ml of Vc (Table-1-).

Discussion

The results of this study showed that adding of H_2O_2 to the semen specimens of fertile men caused significant increase of MDA concentration compared to the control, this result may be referred to the increasing of ROS due to adding of H_2O_2 to the semen specimens caused imbalance between ROS and antioxidants resulting of oxidative stress. The oxidative stress caused increased of lipid peroxidation in seminal fluid and spermatozoa result to increased MDA concentration as end product of lipid peroxidation, this result agreement with Saonka and Kurpisz (2004) study, which are revealed that high concentration of H202 activated the lipid peroxidation in semen. The incubation of human spennatozoa with H_2O_2 caused reduction of Glutathione Peroxidase (GPX) enzyme and Superoxide Dismutase (SOD) enzyme (Griveau *et al.,* 1995).

Table-1 - Sperm parameters and Malondialdehyde (MDA) concentration after H202 addition alone and with some antioxidants to the semen Specimens of fertile men.

Parameters	Normal Saline	H_2O_2 0.1%	Antioxidants + H_2O_2 (0.1%)				
			VE (0.6mg/ml)	Vc (0.4 mg/ml)	VE+Vc 0.6+0.4 mg/ml	Glutathione (0.4mg/ml)	Seminar plasma
Sperm concentration (X106/ml)	a 75.30±4.42	a 75.30±4.38	a 75.20±4.37	a 74.90±4.39	a 75.00±5.10	a 75.10±4.50	a 75.10±4.24
Sperm motility Percent	a 58.20±4.19	b 31.30±3.49	c 41.9±2.49	b 32.90±3.18	c 43.90±2.79	c 41.80±2.92	c 46.20±2.98
Sperm grade Activity	a 3.21±0.18	b 1.74±0.17	C 2.24±0.13	b 1.73±0.17	c 2.63±0.16	c 2.33±0.17	c 2.98±0.22
Sperm viability percent	a 85.10±2.97	b 56.90±4.11	c 70.50±3.19	b 59.20±3.18	c 75.50±2.92	c 73.70± 3.11	c 80.20±2.97
Normal sperm morphology percent	a 68.30±5.31	a 67.70±5.08	a 68.30±5.38	a 67.80±5.30	a 69.10±5.30	a 68.40±5.34	a 68.60±5.25
Leukocytes concentration	a 0.70±0.26	a 0.70±0.26	a 0.50±0.22	a 0.60±0.26	a 0.50±0.16	a 0.50±0.26	a 0.6±0.26
MDA concentration	a 5.06±0.69	b 8.55±0.48	c 5.81±0.67	b 7.50±0.67	c 5.75±0.65	c 5.80±0.70	c 5.68±0.69

Number of specimens 10
Different letters indicate to significance ($p<0.05$)

The present study revealed that semen quality (sperm motility percent, grade activity, and sperm viability percent) were decreased when incubation the semen samples with 0.1% of H_2O_2. This result may be refer to the role of H_2O_2 in peroxidation of lipid composed the plasma membrane of spermatozoa result to loss the function of plasma membrane deal with the control the ions transportation result to the defect of sperm motility.

The addition of VE caused significant improvement in sperm parameters and MAD level, these results agreement with other study (Donnelly *et al.,* 1999), revealed that VE addition to the media used for in vitro sperm activation caused decrease the ROS production which are stimulated by addition of H_2O_2 to the media in Percol gradient technique. Vitamin E can soluble in the lipid, so that it can pass through the plasma membrane of the spermatozoa, and then inhibit the harmful effect of the ROS

and maintain the nature of spermatozoa, plasma membrane and prevent MDA formation result to increase the sperm motility and spermatozoal efficiency to penetrate the ovum layers.

Agarwal (2004) revealed that the mixing of antioxidants is very benefit for treatment of infertile men. The treatment of fertile patients with VE, VC, and glutathione for two months caused significant improvement in sperm parameters and MDA level. The glutathione posses a protective role for the sperms versus the increasing of lipid peroxidation stimulated by Polyinorphonuclear Leukocytes (PML) (Baker *et al.,* 1996).

The seminal plasma contains many enzymatic antioxidants like SOD and GPX, in addition to non-enzymatic antioxidants like VE, VC, glutathione, pyruvate and carnitin (Saleh and Agarwal, 2002), The antioxidants in seminal fluid act to remove the free radicals and protect the spermatozoa from the risk of oxidative stress increasing (Smith *et al.,* 1996). So that the decreasing of MDA level and improvement of sperm parameters with addition of seminal plasma to the semen specimens in our study may be refer to the protective role of antioxidants present in the seminal plasma to reduce the ROS level. In addition to that, the seminal plasma contain several energy sources like fructose and pyruvate which are necessary to maintain the sperm motility and viability.

It was concluded that is very important to adding the antioxidants to the media used for in vitro sperm activation in the cases in which the semen specimens contain high level of ROS.

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