

# The Impacts of in Vitro Improving of Seminal Plasma Quality on Sperms Motility of Asthenospermic Infertile Samples

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**Abstract** Motility of the sperm is the most important independent function of the sperm to reach the oocyte and complete fertilization. The seminal plasma is the media for sperm that contain the nutrient and some activation and inhibition factors. Many studies tried to explain asthenospermia (defective sperm motility) according to findings in the seminal plasma. The aim of this study is to evaluate the effects of improving of seminal plasma quality on sperms motility of asthenozoospermic semen samples. Sperms of 100 seminal samples of infertile patients with asthenospermia with the seminal plasma of 100 healthy normal samples were incubated for 60 minute at 37°C and studied again. This study showed that incubation of sperms with poor motility in a media made from seminal plasma of healthy samples significantly improve the motility and decrease the percentage of immotile sperms in the diseased samples.

**Keywords** Asthenospermia, Seminal plasma, Sperm activation

## 1. Introduction

The spermatozoa are smallest cells in the body that have to fulfil their functional extracorporeal. To perform this, it has a highly efficient mechanism to generate motility and hence, sperm motility is the only natural way through which sperm meet the ova during fertilization [1]. Mammalian spermatozoa are produced in testes and then transferred to epididymides for maturation and storage. It has been considered that most mammalian spermatozoa in the epididymal fluids are mature but nonactivated [2]. The activation of motility of epididymal spermatozoa would not take place unless the spermatozoa were mixed with male accessory gland secretions at the time of ejaculation, or diluted into a buffer solution containing activating factor, depending on type species [3]. Asthenospermia means reduce sperm motility or progressive motility or both [4], and considered one of the most important factors related to male infertility [5]. The seminal fluid is a complex medium containing a great variety of molecules, mainly produced by sex accessory glands, and also cells other than spermatozoa (e.g. leucocytes). This fluid may contain factors that modified the state of sperms motility like antisperm antibodies or infection [6]. Asthenospermia may also be

resulted from a defect in the sperm structure and this is called primary asthenospermia and usually involved high percentage of the ejaculated sperms and the methods of assisted reproductive techniques is the only treatment. While the secondary causes could be of idiopathic causes, prolonged abstinence period, or partial ductal obstruction. Sometimes, drug therapy may result in decreased motility and this can be improved by the removal of the drug, or introduction of specific culture media to the sperm washing procedure [7]. Asthenozoospermia may be managed by *in vitro* sperm activation using different types of stimulators [8], and then in intrauterineinsemination or in vitro fertilization [9].

In this study, we tried to prove that the healthy seminal plasma had properties and dissolved factors that improve sperm motility.

## 2. Samples and Methods

This study was done in the seminal fluid analysis lab in the High institute of infertility diagnosis and assisted reproductive techniques AL-Nahrain University \ Baghdad \ Iraq in the period from September 2014 to February 2015.

Patient included in this study were with history of primary infertility and with regular unprotected sexual contacts and no abnormality in physical and hormonal examination.

The samples were collected in a next private room by masturbation after at least 72 hours of sexual abstinence. Ejaculates were allowed to liquefy at 37°C for 25 minutes up

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to 40 minutes before analysis. Manual semen analysis was performed according to World Health Organization (WHO) guidelines to determine sperm concentration and motility. Then samples with asthenospermia (poor sperm motility) were selected with accepted count and structural character to be used in this study.

Then the samples were re-examined using Computer assisted sperm analysis (CASA) which a digital system designed for automatic analysis of the sperm concentration, motility, and morphology.

Sperms of selected 100 seminal samples with asthenospermia with the seminal plasma of 100 healthy normal samples were used in this study.

The selected seminal sample was centrifuged for 10 min at 3000 revolution per minute (rpm) then the plasma was removed. At the same time a healthy sample with a good count and motility was also centrifuged for 10 min at 3000 rpm in the same way but the plasma will be taken to be centrifuged again for another 10 minutes then the plasma will mixed with the sperms of the asthenospermic sample and incubated for 60 minute at 37°C to be studied again by the same examiner and the same instruments.

Patient's agreement was taken and all the samples were discarded after finishing the study and the patients were informed about the results of studying their samples.

### 3. Statistical Analysis

Statistical analysis was performed by the SPSS 20 statistical software program. Univariate data were summarized using standard descriptive statistics, tabulation of categorical variables and numerical variables. An association between categorical variables was assessed via paired T-test. In all statistical analyses, a  $p$  value  $< 0.05$  was considered as significant.

### 4. Results

There was non-significant increase in the percentage of sperms with active progressive motility while there was a significant increase in the percentage of sperms with sluggish non-progressive sperms. Also there was a significant decrease in the percentage of immotile sperms after treatment.

**Table 1.** A comparison between the percentages of sperms motility character of the asthenospermic infertile samples before and after treatment

Motility state	Before incubation	After incubation	p value
progressive sperm	23.4±11.8*	24.2±15.3	0.23
non progressive sperm	33.6±15.1	42.6±16.1	0.01
immotile sperm	43.3±17.5	33.6±19.1	0.005

\*Mean ± standard deviation.

There was no significant change in the sperm count and the percentage of sperms of abnormal morphology and the computer counter gave the same results before and after incubation.

### 5. Discussion

This study showed that incubation of sperms with poor motility in a media made from seminal plasma of healthy samples (natural media) significantly improve the motility and decrease the percentage of immotile sperms in the diseased samples and that means that there was a significant number of sperms that changed their character of motility from "immotile" to motile (but sluggish) sperms. Also there was an increase in the percentage of progressive motile sperms in the treated sample but not significant. The motility could be structural dysfunction if the sperm was of abnormal shape or tail but in this study we tried to select samples with accepted normal character percentages and the abnormal motility are of unknown cause. Many studies showed the effects of adding certain drugs or electrolytes as chemical activation [10] or heat [11], vibration [12], and laser [13] as physical activation factors and many media nowadays are available for treating defective samples before performing assisted reproductive techniques especially before intrauterine insemination or in vitro fertilization. Despite the development of science and increasingly sophisticated diagnostic methods, in some cases, the etiology and pathogenesis of male infertility are still unknown and constitute the group of idiopathic infertility. Male fertility disorder is attributed to environmental factors such as exposure to certain chemicals, heavy metals, pesticides, and heat, or electromagnetic radiation [14]. Smoking, alcohol abuse, chronic stress, obesity, urogenital trauma, and inflammation in the male reproductive system are also associated with decreased male fertility [15]. The consequence of most of these factors is oxidative stress. Oxidative stress results from the imbalance between production of the reactive oxygen species (ROS) and the protective effect of the antioxidant system responsible for their neutralization and removal. An excess of ROS causes a pathological reaction resulting in damage to cells and tissues. Spermatozoa are particularly vulnerable to the harmful effects of ROS. Oxidative stress affects their activity, damages DNA structure, and accelerates apoptosis, all of which consequently decrease their numbers, hinders motility and development of normal morphology, and impairs function [16]. This might leads to disturbances in fertility or embryo development disorder. The main cellular sources of ROS in the semen are immature sperm cells and white blood cells. The increase in the number of leukocytes may be due to infection and inflammation, but can also be secondary to harmful environmental factors, long sexual abstinence, or varicocele [17]. The protective antioxidant system in the healthy semen is composed of enzymes, as well as

non-enzymatic substances, which closely interact with each other to ensure optimal protection against ROS. Non-enzymatic antioxidants include vitamins A, E, C, and B complex, glutathione, pantothenic acid, coenzyme Q10 and carnitine, and micronutrients such as zinc, selenium, and copper. It seems that a deficiency of any of them can cause a decrease in total antioxidant status and many studies shows that these factors are of abnormal levels in abnormal samples [18] but still need further understanding.

This result of this study disagreed with the hypothesis that state that idiopathic asthenospermia is a structural abnormality rather than a functional defect even in normal looking sperms [19].

It is well known that the mitochondria in the sperm midpiece are the energy generator for mammalian sperm and sperm require a sufficient supply of adenosine triphosphate from mitochondrial oxidative phosphorylation for normal function [20] and each mature mammalian sperm contains approximately 50–75 mitochondria and one copy of mtDNA in each mitochondrion in midpiece [21] and this number of copy was found to be different in further studies [22, 23]. Kao and his team have demonstrated multiple deletions of mtDNA in human sperm with low motility scores [24, 25]. Sun and his co-workers in 2007 studied the ultrastructure of 151 samples with idiopathic asthenospermia and found that there were various mitochondrial pathological changes that exist in idiopathic asthenospermia and there is a close association between the sperm viability and mitochondrial ultrastructure and mitochondrial function [26].

The present study concluded that the healthy seminal plasma contain what we can called "curing factors" that improve the motility the sperms that could be functional problem rather than structural abnormality.

This study suggested further studies on each seminal plasma elements to find the optimal media that resample the natural seminal plasma because the usage of any seminal part in treating another diseased seminal fluid and using it in assisted reproductive techniques might be not accepted socially and religiously.

## REFERENCES

- [1] Henkel R, Defosse K, Weissmann N, and Miska W. Assessment of the energy consumption of human spermatozoa related to motility. *Reprod Dom Animal* 2000; 35: 14–19.
- [2] Mohri H and Yanagimachi R. Characteristics of motor apparatus in testicular, epididymal and ejaculated spermatozoa. *Exp Cell Res* 1980; 127:191-196.
- [3] Si Y and Okuno M. Multiple activation of mouse sperm motility. *MolReprodDev* 1993; 36:89-95.
- [4] Schill WB and Haidl G. Medical treatment of male infertility. In: *Infertility: Male and Female*. Lunenfeld B, Insler V. (Eds.), Churchill Livingstone, Edinburgh 1993: 575–622.
- [5] Sigman M and Howards SS: Male infertility. In: *Campbell's Urology*. Walsh PC, Retik AB, Vaughan ED, Wein AJ. (Eds.). 7th Edition. Saunders Company, Philadelphia, USA. 1998; 1287–1330.
- [6] Bradsbaw KD, Cbantilil SJ, and Carr BR. Diagnostic evaluation and treatment algorithms for the infertile patients. In: *Textbook of Reproductive Medicine*. Bruce RC, Richard EB. (Eds.). 2nd Edition. Appleton and Lange, USA. 1999; Pp: 533–548.
- [7] Ng SC, Bongso A, Sathananthan H, and Ratnam SS. Micromanipulation, Its relevance to human in vitro fertilization. *FertilSteril* 1990; 53: 203–19.
- [8] Micic S, Tulic C, and Dotlic, R. Kallikrein therapy of infertile men with varicocele and impaired sperm motility. *Andrologia* 1990; 22: 179–83.
- [9] Francavilla F, Romano R, Santucci R, and Poccia C. Effect of sperm morphology and motile sperm count outcome of intrauterine insemination on oligozoospermia and/or Asthenozoospermia. *Fertile Steril* 1990; 53: 892–7.
- [10] Sheng Z, Jiang JH, Jin CH, Xu SJ, and Zhu JQ. Effects of environmental factors on sperm motility and fertilization rate of *Phascolosomaesculenta*. *ActaBiol Hung*. 2014 Sep; 65(3):274-84.
- [11] Thijssen A, Klerkx E, Huyser C, Bosmans E, Campo R, and Ombelet. Influence of temperature and sperm preparation on the quality of spermatozoa. *Reprod Biomed Online*. 2014 Apr; 28(4):436-42.
- [12] Isachenko E, Maettner R, Isachenko V, Roth S, Kreienberg R, and Sterzik K. Mechanical agitation during the in vitro culture of human pre-implantation embryos drastically increases the pregnancy rate. *Clin Lab*. 2010; 56(11-12):569-76.
- [13] Salman Yazdi R, Bakhshi S, Jannat Alipoor F, Akhoond MR, Borhani S, Farrahi F, LotfiPanah M, and SadighiGilani MA. Effect of 830-nm diode laser irradiation on human sperm motility. *Lasers Med Sci*. 2014 Jan;29(1):97-104.
- [14] Lahdetie J. Occupation – and exposure–related studies on human sperm. *J Occup Environ Med*. 1995; 37:922–930. [PubMed].
- [15] Tunc O, Bakos HW, Tremellen K. Impact of body mass index on seminal oxidative stress. *Andrologia*. 2011; 43:121–128. [PubMed].
- [16] Aitken RJ. The Amoroso Lecture. The human spermatozoon—a cell in crisis? *J Reprod Fertil*. 1999; 115:1–7.
- [17] Fraczek Mand Kurpisz M. The redox system in human semen and peroxidative damage of spermatozoa. *Postepy Hig Med Dosw (online)* 2005; 59:523–534.
- [18] Walczak-Jedrzejowska R, Wolski JK, and Slowikowska-Hilczek J. The role of oxidative stress and antioxidants in male fertility. *Cent European J Urol*. 2013; 66(1): 60-7.
- [19] Shu-Huei Kao, Hsiang-Tai Chao, Hwan-Wun Liu, Tien-Lin Liao, and Yau-Huei Wei. Sperm mitochondrial DNA depletion in men with asthenospermia. *FERTILITY AND STERILITY*. JULY 2004 VOL. 82, NO. 1.
- [20] deLamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of spermatozoa

- motility. J Androl 1992;13: 379–86.
- [21] Michaels GS, Hauswirth WW, Laipis PJ. Mitochondrial DNA copy number in bovine oocytes and somatic cells. Dev Biol 1982;94:246–51.
- [22] May-Panloup P, Chre'tien MF, Savagner F, Vasseur C, Jean M, Malthie'ry Y, et al. Increased sperm mitochondrial DNA content in male infertility. Hum Reprod 2003;18:550–6.
- [23] . D'iez-Sa'nchez C, Ruiz-Pesini E, Lapen~a AC, Montoya J, Pe'rez-Martos A, Enr'iquez JA, et al. Mitochondrial DNA content of human spermatozoa. BiolReprod 2003;68:180–5.
- [24] Kao SH, Chao HT, Wei YH. Mitochondrial deoxyribonucleic acid 4977-bp deletion is associated with diminished fertility and motility of human spermatozoa. BiolReprod 1995;52:729–36.
- [25] Kao SH, Chao HT, Liu WW, Liao TL and Wei YH. Sperm mitochondrial DNA depletion in men with asthenospermia Fertility and Sterility. 2004; VOL. 82, NO. 1:66-76.
- [26] Sun ZM, Ding CF, Yan ZZ, and Bao YZ. Ultrastructure and function of mitochondria in idiopathic asthenospermia: study of 151 cases. Zhonghuayixuezhazhi [2007, 87(18):1263-1265] (Journal Article, Research Support, Non-U.S. Gov't, English Abstract (lang: china)).