

## Relationships of Osmolality and Oxidative Stress with Semen Quality and Their Effects on Male Fertility

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### Abstract

The objective of this study was to investigate and find the relationships between human semen osmolality and oxidative stress with semen quality. Semen analysis was determined to evaluate semen quality and fertility. The concentration of semen malondialdehyde (MDA) was measured to assess oxidative stress. For this purpose, one hundred seventy healthy adult males were used in this study. The study was conducted from September 2018 to November 2019 in the Infertility care and in vitro fertilization center (IVF) and the Biology department of the College of Science at Salahaddin University in Erbil city. A significant positive correlation was observed between the total sperm motility, grade activity, and sperm motility index with semen osmolality less than 300 mOsm kg<sup>-1</sup> ( $r = 0.62$ ,  $r = 0.64$ , and  $r = 0.75$   $p \leq 0.01$  respectively) and osmolality 300-350 ( $r = 0.53$ ,  $p \leq 0.05$   $r = 0.52$   $p \leq 0.05$ , and  $r = 0.56$   $p \leq 0.01$  respectively). Total sperm motility, grade activity, and sperm motility index are negatively correlated with osmolality 351-400 mOsm kg<sup>-1</sup> ( $r = -0.65$   $p \leq 0.05$ ,  $r = -0.56$   $p \leq 0.05$ , and  $r = -0.67$   $p \leq 0.01$ ) and more than 400 mOsm kg<sup>-1</sup> ( $r = -0.86$ ,  $r = -0.74$  and  $r = -0.88$   $p \leq 0.01$ ). Regarding the relation between oxidative stress and sperm motility kinetics, total sperm motility, grade activity, and sperm motility index is negatively correlated with MDA more than 2  $\mu\text{mol/L}$  ( $r = -0.56$   $p \leq 0.05$ ,  $r = -0.52$   $p \leq 0.05$ , and  $r = -0.67$   $p \leq 0.01$  respectively). No significant correlation was found between semen osmolality and MDA concentration with sperm concentration, total sperm count, sperm viability, and normal sperm morphology.

**Keywords:** Osmolality, Semen Quality, Fertility, Oxidative stress, Semen analysis.

### 1. Introduction

Infertility has become a global health problem in recent years and is affecting 25-30% of reproductive-age couples worldwide to varying degrees [1]. An estimated 70 million people worldwide, suffer from subfertility or infertility [2, 3]. About 15% of couples are infertile and over 10% are subfertile. Comparison with females, males responsible for infertility and subfertility in about 40% to 50% of cases [4, 5]. A meta-analysis of recent studies carried out between 1973 and 2011, that was reported a decrease in sperm counts by more than 50%. The same goes for several other studies that have also recorded a continuous decline in semen quality [6, 7]. Low quality of semen is well known as a large disorder causing male fertility [8, 9]. The study of semen quality is the most important and most commonly used clinical laboratory test to evaluate the capacity of male fertility. In 2010, the World Health Organization (WHO) guidelines lowered the sperm concentration reference interval from 20

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million to 15 million/ml [10]. Osmolality is known as a solution's concentration of osmotically active particles. It is related only to the number of particles per kilogram of solution [11]. Mammalian spermatozoa are mixed with the seminal vesicles, prostate, and bulbourethral gland secretions during ejaculation. Seminal plasma osmolarity has previously been shown to affect sperm motility and activity in invertebrates and vertebrates [12]. The motility of spermatozoa is primarily influenced by changes in the surrounding ionic contents, osmolality, and pH [13, 14]. In sturgeon fish, a negative correlation was found between the osmolality of semen and the percentage of sperm motility [15]. The development of oxidative stress (OS), due to the imbalance between the development of reactive oxygen species (ROS) and antioxidant protection mechanisms [16]. In the male reproductive system, the primary causes of ROS are defective sperm and leukocytes [16, 17]. In the sperm plasma membrane, the high concentration of polyunsaturated fatty acids is vulnerable to ROS, and its attack contributes to lipid peroxidation. Lipid peroxidation takes place in three stages: initiation, progression, and termination. The free radicals combine with fatty acid chains during activation to create the radical lipid peroxy. Besides, peroxy radicals bind with fatty acids to create free radicals, and thereby spread the reaction. In closing, the two radicals connect, allowing lipids to break down [18]. Concentrations of seminal MDA are negatively correlated with sperm count and motility [19]. Malondialdehyde is negatively associated with the main sperm parameters [20]. Impaired sperm quality is accompanied by high oxidative stress [21]. Due to poor data worldwide and no data in Erbil city of Iraq about the relationships between semen osmolality and oxidative stress with semen quality and fertility. The present study was done and aimed to evaluate the influence of seminal plasma osmolality and oxidative stress on semen quality and fertility of adult males.

## 2. Materials Method

### 2.1. Subjects

The study included 170 healthy males and divided into four groups according to the semen osmolality:

- Semen osmolality is less than 300 mOsm kg<sup>-1</sup> (50 males).
- Semen osmolality 300-350 mOsm kg<sup>-1</sup> (45 males).
- Semen osmolality 351-400 mOsm kg<sup>-1</sup> (40 males).
- Semen osmolality more than 400 mOsm kg<sup>-1</sup> (35 males).

Also, the subjects were divided into other groups depending on the seminal fluid concentration of the Malondialdehyde (indicators to oxidative stress) and as follows:

- Seminal fluid with low normal MDA concentration (Less than 2 µmol/L) (110 males).
- Seminal fluid with high abnormal MDA concentration (More than 2 µmol/L) (60 males).

The study was conducted from September 2018 to November 2019 in the Infertility care and in vitro fertilization center (IVF) and the Biology department of the College of Science at Salahaddin University in Erbil city. The ages of the subjects ranged between 30-40 years.

### 2.2. Semen collection

Semen samples were collected in plastic containers after 3 days of abstinence via masturbation and after 30 minutes of the liquefaction of semen samples, the following routine

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parameters were evaluated according to the methods described in the WHO [22, 23]. The parameters were including; the volume of the semen, osmolality of the semen, sperm (count, motility, and morphology).

### 2.3. Seminal fluid analysis

A graduated tube was used to measure the volume of the semen. To determine sperm concentration, 10  $\mu\text{L}$  of semen sample was placed in a Makler chamber and covered by a covered glass, and then analyzed at approximately 200 magnifications [23]. Total sperm count = Sperm concentration  $\times$  volume. A drop of semen, covered with a cover glass and analyzed under a microscope 400x equipped with heat plate 37°C was used to determination of sperm motility. Motility % = number of motile spermatozoa/total number of spermatozoa (motile and immotile)  $\times$  100. Progressive motility was measured by counting the spermatozoa with straight-line forward movement only in the presence of motile spermatozoa [22]. In each sample, sperm motility is graded to 0, 1, 2, 3, or 4, depending on the degree and activity of the movement. Grade 0 represents xero or no movement and grade 4 represents the excellent forward movement of the spermatozoa [24]. The sperm motility index was calculated by multiplying the grading activity with the percentage of motility [25]. For measurement of sperm viability, two drops of 1 % eosin Y solution were mixed with a drop of liquefied semen, then three drops of nigrosin solution 10 % was added, and mixed after 30 seconds. Within 30 seconds of adding nigrosin, a thin smear of the semen–eosin–nigrosin mixture was made and after air-dried, examined under the microscope (1000 $\times$ ). To determine the percentage of live spermatozoa, one hundred sperm were counted. The live spermatozoa are white and the dead are stained red [22]. Sperm vitality = number of viable sperm/total number of spermatozoa  $\times$  100. The normal morphology of spermatozoa was determined by using the hematoxylin and eosin staining procedure [26].

### 2.4. Seminal fluid Osmolality

Osmometer type (Knauer, D- 14163, Berlin, Germany) and the freezing point depression method was used to measure the seminal plasma osmolality. This method requires samples of semen to be centrifuged free of particulate matter. Before the osmolality of the sample was measured, the osmometer must be calibrated between 0 and 400 mOsm  $\text{kg}^{-1}$  using distilled water and standard NaCl solution.

### 2.5. Seminal fluid MDA determination

A procedure described by [27] was used to measure the concentration of the semen MDA. In short; apply the following to 150  $\mu\text{l}$  of semen plasma: 1 ml of trichloroacetic acid 17.5 %, 1 ml of 0.6 % thiobarbituric acid, combined well with vortex, incubated for 15 minutes in a boiling water bath, and then allowed to cool. Then add 1 ml of 70% trichloroacetic acid (TCA), then let the mixture stand at room temperature for 20 minutes, centrifuged for 15 minutes at 2000 rpm, and remove the supernatant for spectrophotometric scanning [28].

The conc. of MDA = absorbance at 532 nm  $\times$  D / L  $\times$  E<sub>0</sub>.

L: light bath (1 cm)

E<sub>0</sub>: extinction coefficient  $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$

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D: dilution factor = 1 ml volume. used in Ref./0.15=6.7

## 2.6. Statistical analysis

The data analysis was performed using SPSS version 17. Pearson's correlation (r) was used to founding the relationships between seminal plasma osmolality and oxidative stress (MDA) with semen analysis parameters (volume of semen, sperm concentration, sperm motility, sperm viability, and morphology). A P-value of less than 0.05 was considered to be statistically significant.

## 3. Results and Discussion

### 3.1. Osmolality and semen quality

Pearson's correlation (r) between semen osmolality and semen quality parameters are presented in **Table (1)**. The volume of semen positively ( $r = 0.55$ ,  $p \leq 0.05$ ) correlated with semen osmolality less than 300 mOsm  $\text{kg}^{-1}$ . While in the osmolality 300-350, 351- 400, and more than 400 mOsm  $\text{kg}^{-1}$  it is negatively correlated ( $r = -0.46$ ,  $r = -0.48$ , and  $r = -0.54$   $p \leq 0.05$  respectively). No correlation was found between semen osmolality with sperm concentration, total sperm count, sperm viability, and normal sperm morphology. The correlation between sperm motility kinetics and semen osmolality is mentioned in **Table (2)**. A significant positive correlation was observed between the total sperm motility, grade activity, and sperm motility index with semen osmolality less than 300 mOsm  $\text{kg}^{-1}$  ( $r = 0.62$ ,  $r = 0.64$ , and  $r = 0.75$   $p \leq 0.01$  respectively) and osmolality 300-350 ( $r = 0.53$ ,  $p \leq 0.05$   $r = 0.52$   $p \leq 0.05$ , and  $r = 0.56$   $p \leq 0.01$  respectively). The increase in the semen osmolality is caused by a decrease in the sperm motility kinetics parameters. Total sperm motility, grade activity, and sperm motility index are negatively correlated with osmolality 351-400 mOsm  $\text{kg}^{-1}$  ( $r = -0.65$   $p \leq 0.05$ ,  $r = -0.56$   $p \leq 0.05$ , and  $r = -0.67$   $p \leq 0.01$ ) and more than 400 mOsm  $\text{kg}^{-1}$  ( $r = -0.86$ ,  $r = -0.74$  and  $r = -0.88$   $p \leq 0.01$ ). The seminal plasma is a complex fluid secreted from the testes, epididymis, and accessory sex glands, that acts as a carrier for spermatozoa from the male testicles to the oocyte, their target, and may influence the morphology of sperm, motility, acrosome reaction, and fertility. Several biochemical components are found in seminal plasma, some of which are relatively unique for sperm function control [29, 30]. It is also recognized that there are substances in the seminal plasma that sustain sperm cells. The osmotic balance is formed by sodium and potassium cations in the seminal plasma, while the basic trace elements are the components of several significant enzymes, calcium is also required to stimulate steroidogenesis in the Leydig cells of the testis [31].

**Table 1.** Pearson's correlation (r) between semen osmolality and semen quality parameters.

Semen quality	Semen osmolality (mOsm $\text{kg}^{-1}$ )			
	Less than 300	300-350	351-400	More than 400
Volume (ml)	0.55*	- 0.46*	- 0.48*	- 0.54*
Sperm concentration ( $\times 10^6/\text{ml}$ )	0.12	0.18	0.22	0.15
Total sperm count ( $\times 10^6/\text{volume of semen}$ )	0.24	0.18	0.21	0.35
Sperm Viability %	0.35	0.14	0.25	0.32
Normal Sperm morphology %	0.14	0.34	0.36	0.18

\*Correlation is significant at  $p \leq 0.05$ , - the negative correlation was found

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**Table 2.** Pearson's correlation (r) between semen osmolality and sperm motility kinetics parameters.

Sperm motility kinetics	Semen osmolality (mOsm kg <sup>-1</sup> )			
	Less than 300	300-350	351-400	More than 400
Total sperm motility %	0.62**	0.53*	- 0.65*	- 0.86 ***
Grade activity	0.64**	0.52*	- 0.56*	- 0.74**
Sperm motility index	0.75**	0.56**	- 0.67**	- 0.88***

\*Correlation is significant at  $p \leq 0.05$ , \*\*Correlation is significant at  $p \leq 0.01$ , \*\*\*Correlation is significant at  $p \leq 0.001$ , - the negative correlation was found.

The seminal plasma is a complex fluid secreted from the testes, epididymis, and accessory sex glands, that acts as a carrier for spermatozoa from the male testicles to the oocyte, their target and may influence the morphology of sperm, motility, acrosome reaction, and fertility. Several biochemical components are found in seminal plasma, some of which are relatively unique for sperm function control [29, 30]. It is also recognized that there are substances in the seminal plasma that sustain sperm cells. The osmotic balance is formed by sodium and potassium cations in the seminal plasma, while the basic trace elements are the components of several significant enzymes, calcium is also required to stimulate steroidogenesis in the Leydig cells of the testis [31]. The results of our study are in agreement with the findings of [15] who observed a significant negative correlation ( $r = -0.893$ ) between semen osmolality and motility percent in sturgeon fish. Also, the study of [12] found that semen osmolality correlates negatively with the kinetic characteristics of sperm motility such as motility percent and grade activity in humans. Besides, when sperm were placed in a solution with an increase in osmolality from 300 to 600 mOsm kg<sup>-1</sup>, kinetic characteristics of sperm motility were gradually decreased and almost arrest when the osmolarity was 600 mOsm kg<sup>-1</sup>. These results are in the line with the findings of our study. With the increased osmolality of the activating solution, the percentage of motile spermatozoa drops, osmolality greater than 400 mOsm kg<sup>-1</sup> should be present in the immobilizing solution in pikeperch [32]. The effect of various osmolalities (240-460 mOsm kg<sup>-1</sup>) on the sperm quality parameters of *Jenynsia multidentata* viviparous fish was evaluated by [33]. The results found that semen motility in osmolalities between 280 and 300 mOsm kg<sup>-1</sup> was higher and the motility observed above 380 mOsm kg<sup>-1</sup> was 0%.

### 3.2. Oxidative stress and semen quality

The correlation between oxidative stress and semen quality parameters is presented in **Table (3)**. No significant correlation was found between semen MDA concentration with semen volume, sperm concentration, total sperm count, sperm viability, and normal sperm morphology. Regarding the relation between oxidative stress and sperm motility kinetics, total sperm motility, grade activity, and sperm motility index were not significantly correlated with semen MDA concentration less than 2  $\mu\text{mol/L}$ , but is negatively correlated with MDA more than 2  $\mu\text{mol/L}$  (total sperm motility  $r = -0.56$   $p \leq 0.05$ , grade activity  $r = -0.52$   $p \leq 0.05$ , and sperm motility index  $r = -0.67$   $p \leq 0.01$ ), **Table (4)**. As showed in **Table (5)**, no correlation was found between seminal plasma osmolality and oxidative stress. The cellular damage of oxidative stress initiate when the reactive oxygen species (ROS) exceeds the body's natural antioxidant defenses. A common disease seen in nearly half of all infertile men is oxidative stress. ROS, described as comprising oxygen ions, free radicals, and peroxides, is produced

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within semen by sperm and seminal leukocytes and produces infertility through two main mechanisms. They damage the sperm membrane first, decreasing the motility of the sperm and its ability to fuse with the oocyte. Secondly, sperm DNA can be changed by ROS, which contributes to the passage of faulty DNA from parents to the fetus [34].

**Table 3.** Pearson's correlation (r) between oxidative stress and semen quality parameters.

Semen quality	Oxidative stress (Seminal plasma MDA concentration)	
	Less than 2 $\mu\text{mol/L}$	More than 2 $\mu\text{mol/L}$
Volume (ml)	0.12	0.18
Sperm concentration ( $\times 10^6/\text{ml}$ )	0.25	0.21
Total sperm count ( $\times 10^6/\text{volume of semen}$ )	0.32	0.34
Sperm Viability %	0.15	0.36
Normal Sperm morphology %	0.14	0.22

No correlation was found between oxidative stress and semen quality

**Table 4.** Pearson's correlation (r) between oxidative stress and sperm motility kinetics parameters.

Sperm motility kinetics	Oxidative stress (Seminal plasma MDA concentration)	
	Less than 2 $\mu\text{mol/L}$	More than 2 $\mu\text{mol/L}$
Total sperm motility %	0.35	- 0.56*
Grade activity	0.32	- 0.52*
Sperm motility index	0.43	- 0.67**

\*Correlation is significant at  $p \leq 0.05$ , \*\*Correlation is significant at  $p \leq 0.01$ , – the negative correlation was found.

**Table 5.** Pearson's correlation (r) between oxidative stress and semen osmolality.

Semen osmolality ( $\text{mOsm kg}^{-1}$ )	Oxidative stress (Seminal plasma MDA concentration)	
	Less than 2 $\mu\text{mol/L}$	More than 2 $\mu\text{mol/L}$
Less than 300	0.42	0.25
300-350	0.35	0.34
351-400	0.18	0.22
More than 400	0.38	0.40

No correlation was found between oxidative stress and semen quality.

Malondialdehyde is one of the lipid peroxidation byproducts. This by-product is a significant marker of oxidative stress, and the degree of lipid peroxidation has been extensively studied for monitoring. Sperm membrane lipid peroxidation can cause changes in sperm and can reduce fertility by affecting sperm motility and sperm-oocyte fusion ability. However, it remains to be determined whether the concentration of MDA seminal fluid influences the consistency or role of sperm [35]. Our results are in agreement in some aspects with the results of [19], who found a negative association between the MDA level of seminal fluid and sperm motility but in contrast with them which record a decrease in sperm count, and sperm morphology. The discovery of a high level of MDA in infertile subjects' semen suggests that infertile subjects' spermatozoa have been subjected to elevated oxidative stress.

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In infertile subjects, the sperm count, sperm morphology, and motility as well as the volume of semen were all found to be significantly lower compared to fertile subjects. It thus means that increased oxidative stress has a detrimental effect on semen quality [36]. Due to ROS-induced peroxidation of lipids in the sperm membrane reduced flexibility has been shown to reduce motility [37]. Mitochondria that produce energy from intracellular ATP stores cover the axosome and related dense fibers of the middle sections of the spermatozoa. ROS is well known to cause axonemal and mitochondrial damage, resulting in the immobilization of spermatozoa [38]. Besides, ROS-induced mitochondrial DNA damage contributes to decreased ATP and energy availability and leads to caspase stimulation and eventually apoptosis, impeding the motility of spermatozoa [39]. Another theory includes a sequence of interrelated events leading to a reduction in ROS motility due to a reduction in phosphorylation of axonemal protein and mitochondrial membrane damage and intracellular enzyme leakage [40].

#### 4. Conclusion

Our results concluded that the total sperm motility, grade activity, and sperm motility index are negatively correlated with osmolality 351-400 and more than 400 mOsmkg<sup>-1</sup>. Seminal plasma MDA concentration of more than 2 µmol/L harms sperm motility kinetics. No significant correlation was found between semen osmolality and MDA concentration with sperm concentration, total sperm count, sperm viability, and normal sperm morphology.

#### 5. Acknowledgement

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#### 6. Conflict of Interest

The authors declare no conflict of interest.

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