



Scholars Research Library

Annals of Biological Research, 2011, 2 (5) :351-359
(<http://scholarsresearchlibrary.com/archive.html>)



Scholars Research
Library

ISSN 0976-1233
CODEN (USA): ABRNBW

Human Seminal oxidative stress: correlation with antioxidants and sperm quality parameters

¹Zunjarrao G. Badade, ¹Kavita M. More, ²Jayshree G. Narshetty, ³Vandana. Z. Badade,
¹BirendraKumar Yadav

¹Department of Biochemistry, MGM Medical College, Kamothe, Navi-Mumbai, India

²Department of Obstetrics and Gynecology, MGM Medical College, Kamothe, Navi-Mumbai, India

³Department of Mathematics, ICLES' Motilal Jhunjhunwala College, Vashi, Navi Mumbai, India

ABSTRACT

Oxidative stress (OS) has been known as one of the most important cause of male infertility. The study was aimed to investigate seminal MDA, nitric oxide, TAC and zinc in infertile men and study their relationship with semen parameters. The study comprises total 80 subjects including fertile men (n=30) and infertile men (n=50). Seminal plasma malondialdehyde, nitric oxide, zinc and TAC were evaluated by spectrophotometric methods and correlated with semen parameters. Seminal levels of malondialdehyde and nitric oxide were significantly higher ($p<0.001$) while zinc and TAC were significantly lower ($p<0.001$) in infertile men than fertile men. Elevated seminal plasma MDA, nitric oxide and low TAC and zinc may have significant role in the etiology of sperm abnormality. Negative correlation of sperm parameters with MDA and nitric oxide and positive correlation with TAC and zinc indicates that oxidative stress adversely affects spermatogenesis in male infertility. Evaluation of MDA, nitric oxide, TAC and zinc can be used for diagnosis, prognosis of male infertility. Therapeutic usage of the antioxidants in treatment of male infertility should be studied extensively. A series of clinical trials are needed to investigate the prospect.

Key words – Oxidative stress (OS), malondialdehyde (MDA), Nitric oxide, Total antioxidant Capacity (TAC), zinc.

INTRODUCTION

Infertility is a major clinical problem, affecting people medically and psychologically [1]. Approximately 15% of couples trying to conceive are infertile, in that about 30% cases are due

to males only and in another 20% cases both partners have detectable abnormalities. Thus male factor plays an important role in 50% of infertile couples [2, 3]. Causes of infertility are anatomic defects, endocrinopathies, immunologic problems, gene mutation, radiation, chemotherapy, ejaculatory failures and environmental exposures. [4]

Oxidative stress (OS) precipitates the range of pathologies that currently are thought to affect the reproductive function [5]. It occurs when the generation of reactive oxygen species (ROS) overwhelms the limited body antioxidant defense. Optimum amount of ROS are vital for development of spermatozoa and capable of fertilization [6]. In spite of the antioxidant activity of seminal plasma, epididymis and spermatozoa, OS damages sperm functions and DNA integrity [7, 8].

The main antioxidative defense in seminal plasma includes SOD, CAT, GPx, Vit-C, Vit.-E and Zn. This represents total antioxidant capacity (TAC) [9]. Zinc also plays an important role in testicular development, spermatogenesis and sperm motility. [10] TAC and individual antioxidants are found to be low in infertile men [11].

One of the important markers of oxidative stress is malonaldehyde (MDA), which is an end product of lipid peroxidation [12]. Kumar *et al.* found excess ROS and low antioxidant levels in the semen of infertile oligoasthenozoospermic men [13]

In recent years nitric oxide (NO) has been known as a molecule that plays an important role in regulating the biology and physiology of the reproductive system and we know that it can affect human sperm functions, such as motility, viability and metabolism. At low concentrations it can have a positive effect on cells, but a negative effect at high concentrations [14].

Thus in view of pathological role of oxidative stress in male infertility, the present study was attempt to assess oxidative stress in terms of MDA, nitric oxide, total antioxidant capacity and zinc in infertile and fertile men. We also evaluated the influence of ROS and reactive nitrogen species (RNS) on sperm parameters.

MATERIALS AND METHODS

Present study was carried out in the Department of Biochemistry, Department of Obstetrics and Gynecology, MGM Medical College, Kamothe, Navi Mumbai. Semen samples were obtained from 50 married infertile men aged 21-50 yrs who have not conceived after one year of regular, unprotected intercourse and had an abnormal semen analysis. At first clinic attendance, a detailed background history and physical examination were done on both husband and wife. Wives of the infertile subjects included had no obvious causes of infertility like tubal blockage or ovulation disorders. Patients with Varicocoele, hypogonadism, prolonged illness were excluded from the study.

Thirty fertile males aged 21-45 years, whose partners had conceived within a year and having sperm count more than 20 million/ml with motility more than 50% in forward progression were selected from general population and considered as fertile control group.

Semen samples were analyzed according to WHO criteria. Samples were collected by masturbation in wide mouth sterile plastic container after minimum of three days of abstinence.

The institutional ethical committee clearance was obtained for the present study, on due orientation about the nature of study, a written consent was obtained from healthy individuals & infertile male subjects. After liquefaction, samples were processed by conventional analysis to determine sperm count, sperm motility and sperm morphology according to WHO criteria.

On centrifugation, seminal plasma was used for measurements of malondialdehyde by Satoh K method [15]. Nitric oxide was estimated by Griess reaction. [16] In this kinetic method, nitrate is reduced to nitrite by copper coated cadmium granules. This nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylenediamine.

TAS of semen samples will be determined by using a novel automated measurement method developed by O. Erel, [17] In this method, the hydroxyl radical, the most potent radical, is produced via Fenton reaction and consequently the colored dianisidiny radical cations, which are also potent radicals, are produced in the reaction medium of the assay. Antioxidant capacity of the added sample against these colored potent free radical reactions measured the total antioxidant capacity. The results will be expressed as millimoles of Trolox equivalent per liter. Zinc was estimated by spectrophotometric method (kit by Coral Clinical systems); zinc in alkaline medium reacts with nitro-paraaminophosphosulphate (n-PAPS) to form purple colored complex. The color intensity is directly proportional to the amount of zinc present in the sample.

Statistical analysis:

Statistical analysis of the data was carried out with SPSS, version 16; Data was reported as mean \pm SD. The comparisons between two groups were tested by unpaired t-test. A 95% confidence interval was used. *P* values less than 0.05 were considered statistically significant. Correlation between two continuous outcomes was evaluated using Pearson correlation coefficients.

RESULTS

Results were expressed as mean \pm SD for each parameter. Statistically significant differences among infertile and fertile men are indicated in Table No.1 along with their significant values. Seminal levels of malondialdehyde (4.4 ± 0.19 nmol/l) and nitric oxide (8.28 ± 2.08 μ mol/l) were significantly high ($p < 0.001$) in infertile men than fertile men (1.27 ± 0.56 , 3.42 ± 1.7 respectively). Seminal levels of zinc (14.97 ± 2.83 mg/dl) and TAC (12.3 ± 1.01) were significantly lower ($p < 0.001$) in infertile men than fertile men (20.65 ± 2.3 , 16.66 ± 1.02 respectively).

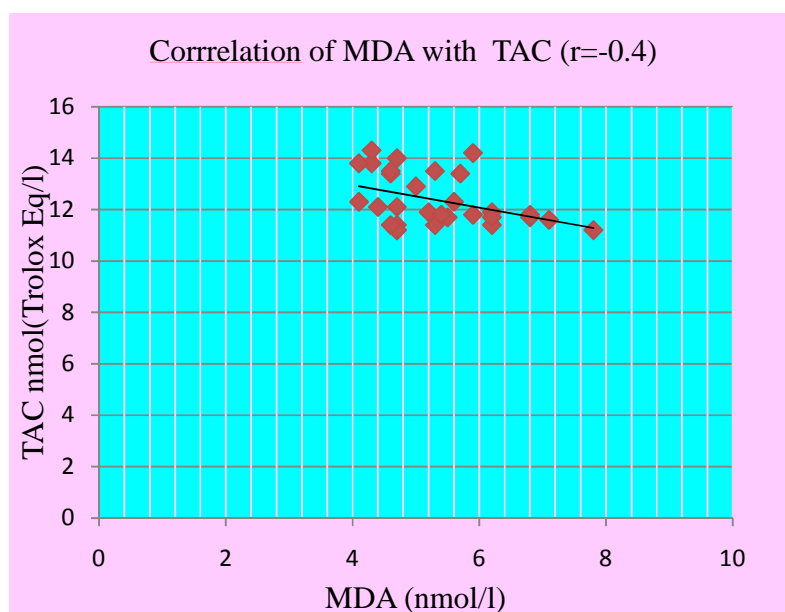
Correlation coefficient of various parameters is indicated in Table No.2 along with their significant values. There was negative correlation of malondialdehyde and nitric oxide with zinc and SOD in infertile men. Seminal plasma malondialdehyde and nitric oxide have a negative correlation with sperm count, motility and morphology. Seminal plasma zinc levels were positively correlated with sperm count, motility, morphology and TAC in infertile patients. There was negative correlation between malondialdehyde and nitric oxide with zinc and TAC in fertile men.

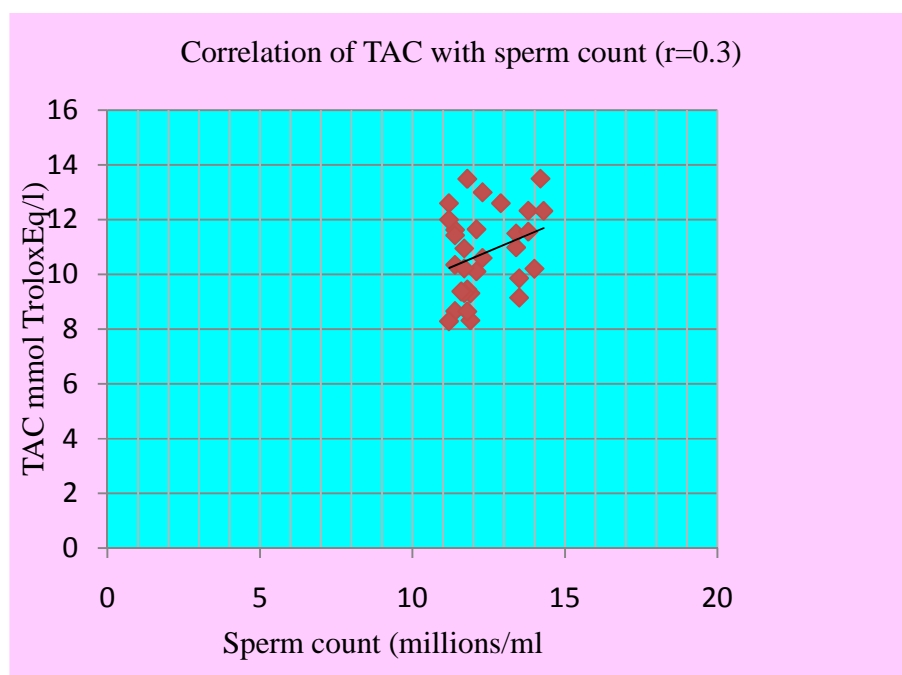
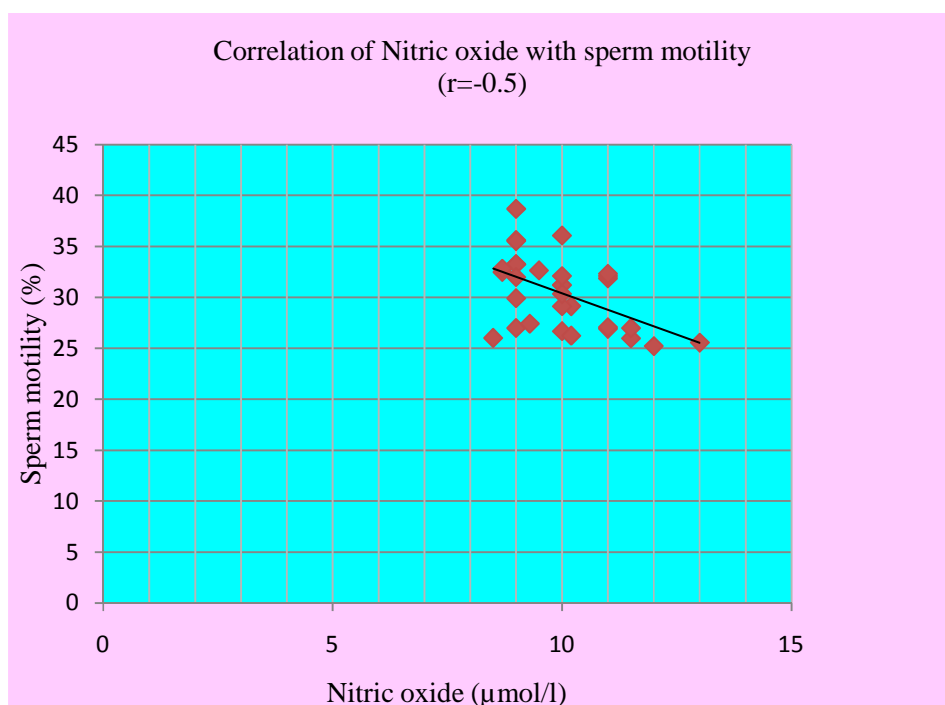
Table No.1 The mean values of sperm count, sperm motility, sperm morphology, seminal MDA, nitric oxide, TAC and zinc in fertile and infertile men

| Parameters (Seminal) | Fertile group (n=30) | infertile group (n=60) | <i>p</i> -Value |
|---|----------------------|------------------------|-----------------|
| Malondealdehyde (nmol/l) | 1.27 ± 0.56 | 4.4 ± 0.19* | <i>p</i> <0.001 |
| Nitric oxide (µmol/l) | 3.42 ± 1.7 | 8.28 ± 2.08* | <i>p</i> <0.001 |
| TAC(mmol trolox Eq/l) | 16.66 ± 1.02 | 12.3 ± 1.01* | <i>p</i> <0.001 |
| Zinc ((mg/dl) | 20.65 ± 2.3 | 14.97 ± 2.83* | <i>p</i> <0.001 |
| Sperm count (10 ⁶ millions/ml) | 71.51 ± 10.8 | 15.65 ± 3.4* | <i>p</i> <0.001 |
| Sperm motility (%) | 70.53 ± 8.36 | 32.25 ± 4.67* | <i>p</i> <0.001 |
| Morphology (%) | 40.12 ± 6.29 | 18.46 ± 3.01* | <i>p</i> <0.001 |

(Mean ± S.D., Comparison with control: **p*<0.001)**Table No. 2.** Correlation coefficient of various parameters studied in infertile men

| Parameters Seminal) | | MDA | NO | Zinc | TAC | Sperm Count | Sperm motility |
|---------------------|----------|---------|--------|--------|--------|-------------|----------------|
| MDA | r- value | - | 0.2* | -0.5** | -0.4** | -0.5** | -0.6*** |
| NO | r- value | 0.2* | - | -0.3** | -0.2* | -0.4** | -0.5** |
| Zinc | r- value | -0.5** | -0.3** | - | 0.1* | 0.4** | 0.6*** |
| TAC | r- value | -0.4** | -0.2* | 0.1* | - | 0.3** | 0.2* |
| Sperm Count | r- value | -0.5** | -0.4** | 0.4** | 0.3** | - | 0.6*** |
| Sperm motility | r- value | -0.6*** | -0.5** | 0.6*** | 0.2* | 0.2* | - |
| Sperm Morphology | r- value | -0.04* | -0.3** | 0.1* | 0.2* | 0.03* | 0.04* |

r = Pearson's correlation co-efficient.; *** Highly significant (*p*<0.001), **Significant (*p*<0.05), * Not significant (*p*>0.05).**Graph 1: Correlation between MDA and TAC in infertile men**

Graph 2: Correlation between TAC and Sperm count in infertile men**Graph 3: Correlation between nitric oxide and Sperm motility in infertile men**

DISCUSSION

In present study, we found that MDA and nitric oxide were increased and TAS and zinc were decreased in infertile men as compared with fertile donors. There was a positive correlation of semen parameters with TAS and Zinc and negative correlation with MDA and Nitric oxide in infertile patients.

Recently the over-production of ROS in the male reproductive tract has become a potential cause of male infertility. Though it has been shown that small amounts of ROS are essential for regulation of normal sperm functions like sperm capacitation, acrosome reaction and oocyte fusion [18], but at high levels they have potential toxic effects on sperm quality and function [19].

Sperm plasma membrane has a high concentration of polyunsaturated fatty acids which makes it susceptible to lipid peroxidation by ROS, this can leads to loss of membrane fluidity and integrity, as a result of this the spermatozoa lose their competence to participate in the membrane fusion events associated with fertilization. Also they can attack DNA, induced strand breaks and oxidative stress damage in spermatozoa [20]

Koksal *et al* [21] study demonstrated that severe pathologic changes in the testicular tissue are associated with high level of lipid peroxidation and suggested that overproduction of ROS may play a role in the mechanism of testicular degeneration associated with infertility. Recent reports [22, 23] indicated that high levels of ROS can be detected in semen samples of 25-40% of infertile men. There are some well known potential sources of ROS production in semen such as immature sperm, morphologically abnormal spermatozoa and peroxidase positive leukocyte [24]. In addition, several clinical entities are also concerned as a cause of oxidative stress in semen such as varicocele, cigarette smoking and spinal cord injury [19].

Several studies have reported that spermatozoa from oligozoospermic or asthenozoospermic men showed a greater production of oxidative stress [11, 25, 26]. In present study, we found high levels of MDA in infertile men as compared to fertile and it was negatively correlated with sperm count and sperm motility and morphology. Our results of MDA are in accordance with studies by Nabil H. *et al* [27], Hsieh YY *et al.* [28] and Fraczek *et al.* [29]. Nabil H. *et al* [27] reported elevated seminal MDA concentration in patients with oligozoospermic and azoospermic groups. Our results are in contrast with Suleiman *et al.* [30]. They demonstrated that MDA concentration in the seminal plasma was not related with the sperm concentration and motility.

The free radical nitric oxide (NO^\cdot) and peroxynitrite anion (ONOO^\cdot) also appear to play significant roles in reproduction and fertility. [14] Oztezcan *et al.* [31] indicate that ONOO^\cdot might cause sperm dysfunction through an increase in lipid peroxidation (LPO) and total sulphydryl group depletion. Amiri *et al.* [32], Sheikh *et al.* [33] reported significantly higher values of seminal nitric oxide in infertile males as compared to fertile males. Giancarlo *et al.* [34] demonstrated same findings in idiopathic asthenospermia and they found significant negative correlation between NO^\cdot concentration and sperm motility. Garg *et al.* [35] found negative correlation between NO^\cdot concentrations with sperm concentration and sperm morphology (%) and study suggests a possible role of NO^\cdot in pathophysiology of male infertility In contrast,

Revelli *et al.* [36] observed that NO[•] concentration in the seminal plasma was not correlated with sperm concentration and motility.

We found significant high levels of nitric oxide in infertile men and negatively correlated with sperm count and motility and morphology. Infertile men have higher concentrations of NO[•] may be due the male genital tract disease and associated factors, such as inflammation and infection, which can lead to NO[•] overproduction.

In present study high levels of MDA and nitric oxide suggests that lipid peroxidation of the membrane lipid may disturb the functions carried out by the sperm membrane and negative correlation with sperm count, motility and morphology indicates free radicals may have role in decrease the fertility potential by altering semen quality parameters.

Zinc plays an important role in normal testicular development, spermatogenesis, sperm motility, and nuclear chromatin decondensation [10] and acrosin activity.[37] Leydig cell synthesis of testosterone depends on adequate dietary zinc [38]. Wong WY *et al.* [39] reported that oral zinc supplementation in men shows proportionate increase in number of spermatozoa and sperm motility. Omu *et al.* [40] had demonstrated that zinc therapy results in significant improvement in sperm quality with increase in sperm density, progressive motility and improve conception and pregnancy outcomes. It appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation [41].

We observed significantly low levels of zinc in infertile group and showed significantly positive correlation with sperm count, sperm motility and morphology. The results obtained in our study were in accordance with the study of Hasan Ali *et al* [42] and Chia SE *et al.*[43]. Chia *et al.* [43] reported significant positive correlation of zinc concentration with sperm density, motility and viability. In our study the low zinc levels in the infertile men might be attributed to disorders in the prostate excretory function or possibly due to asymptomatic prostate infection, as well as it is potent scavenger of superoxide anion may contribute to poor spermatogenesis and poor motility observed in these patients.

We found significantly lower seminal TAC activity in infertile men compared to fertile men. Our results are on par with the Khosrowbeygi A *et al* [44] and Koca *et al.*[45]. Khosrowbeygi A *et al* [44] reported that TAC levels significantly lower in the asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic versus Control group. Koca *et al.* showed that seminal plasma TAC in infertile asthenozoospermic and asthenoteratozoospermic males is lower than fertile men. Sharma *et al.* [46] suggests that ROS production and TAC can be used as a marker of OS in seminal fluid and correlate with male infertility. Nasrin S. *et al.* [33] reported DNA damage was significantly correlated with nitric oxide concentration in infertile men and low levels of TAC. They also observed a positive correlation between seminal plasma TAC and sperm motility. Thus low levels of TAC indicate that antioxidants are utilized to detoxify the excessive amount of ROS and Reactive nitrogen species

Negative correlation of MDA and Nitric oxide with antioxidants and semen parameters as found in our study supports imbalance between oxidants and antioxidants mainly responsible for poor sperm quality which leading to infertility.

CONCLUSION

Our study suggests that increasing seminal plasma MDA, nitric oxide and decreasing TAC and zinc may have significant role in the etiology of sperm abnormality. Negative correlation of sperm parameters with MDA, nitric oxide and positive correlation with TAC and zinc indicates, oxidative stress adversely affects in male infertility. Evaluation of MDA, nitric oxide, TAC and zinc can be used for diagnosis and prognosis of male infertility. Therapeutic usage of the antioxidants in the treatment of male infertility should be studied extensively. A series of clinical trials are needed to investigate the prospect.

Acknowledgement

We are extremely thankful to MGM University of Health Sciences, Kamothe, Navi Mumbai-410209 for the financial support for present work.

REFERENCES

- [1]. I.D. Snarlip, J.P. Jarop, A.M. Belker, *Fertil. steril*, **2002**, 77, 873–82.
- [2]. M.P. Singh, M.B.K. Sinha, R. Sinha, *Medicine Update*, **2005**, 318-322.
- [3]. K. Gopalkrishnan, *ICMR Bulletin*, **1997**, 27, (8).
- [4]. A.J. Campbell, D.S. Irvine, *Br. Med. Bull.*, **2000**, 56, 616-629.
- [5]. S.C. Sikka, *Curr. Med. Chem.*, **2001**, 8, 851–862.
- [6]. R.J. Aitken, H.M. Fisher, N.Fulton, E. Gomez, W. Knox, B. Lewis et al., *Mol Reproduction Dev.*, **1997**, 47,468-482.
- [7]. N. Desai , E. Jr. Sabanegh , T. Kim , A. Agarwal, *Urology*, **2009**, 75, 14-19.
- [8]. F.M. Lanzafame, S.L. Vignera., E. Vicari and A.E. Calogero, *Reprod. Biomed. Online*, **2009**, 19, 638-659.
- [9]. M. Eid. Hammadeh, *Int. J. Fert. and Sterility*, 3, **2009**, 87- 110.
- [10]. H.H. Sandstead, A.S. Prasad, A.R. Schulert et al., *Am. J. Clin. Nutr.*, **1967**, 20, 422-42.
- [11]. F.F. Pasqualotto, R.K. Sharma, D.R. Nelson, A.J. Thomas and A. Agarwal, *Fertil. Steril*, **2000**, 73, 459-464.
- [12]. P.J. Rowe, F.H. Comhaire, T.B. Hargreave, A.M. A. Mahmoud, WHO manual for the Standardized investigations, diagnosis and management of infertile couple. WHO. Cambridge University Press. Cambridge. **2000**, 4th Ed, 8-61.
- [13]. R. Kumar, S. Venkatesh, M. Kumar, M. Tanwar and. M.B. Shasmsi et al., *Indian J. Biochem. Biophys.* **2009**, 46, 172-177.
- [14]. M. Rosselli, P.J. Keller and R.K. Dubey, *Human Reproduction Update*, **1998**, 4,13–24.
- [15]. K. Sathoh, *Clin. Chim. Acta.*, **1978**, 90, 37-43.
- [16]. K.C. Najwa and W.W. Nabil, *Clinical Chemistry*, **1990**, 36, 8, 1440-1443.
- [17]. Ozcan Erel, *Clinical Biochemistry*, **2004**, 37, 277– 285.
- [18]. J.F. Griveau, Le. Lannou D., *Int. J. Androl.*, **1997**, 20, 61-69.
- [19]. M.R. Moein, V.O. Dehghani, N. Tabibnejad, S. Vahidi, *Iranian Journal of Reproductive Medicine*, **2007**, 5, 2, 51-55.
- [20]. F.F. Verit, A. Verit, H. Ciftci, O. Erel, H. Çelik, *Journal of Andrology*, **2009**,30,2,183-189.
- [21]. I.T. Koksai, A. Tefekli, M. Usta, H. Erol, S. Abbasoglu, A. Kadioglu, *BJU Int.*, **2000**, 86,549-552.
- [22]. de E. Lamirande, C. Gagnon, *Hum. Reprod.*, **1995**,10,15-21.

- [23]. O.F. Padron, N.L. Brackett, R.K. Sharma, C.M. Lynne, A.J. Thomas, A. Agarwal, *Fertil. Steril.* **1997**, 67,1115-1120.
- [24]. M. Plante, de E. Lamirande, C. Gagnon, *Fertil. Steril.*, **1994**, 62,387-393.
- [25]. R.K. Sharma and A. Agarwal, *Urology*, **1996**, 48,835– 850.
- [26]. J.F. Griveau and de D. Lannou, *Int. J. Androl.***1997**, 20, 61–69.
- [27]. H.Nabil, A. L. Moemen and M. H. Abu E, *Australian Journal of Basic and Applied Sciences*, **2008**, 2, 3, 773-778.
- [28]. Y.Y. Hsieh, C.C. Chang, C.S. Lin., *Int. J. Biol. Sci.*, **2006**, 2,23-29.
- [29]. M. Fraczek, D. Szkutnik, D. Sanocka, M. Kurpisz. *Ginekol Pol*, **2001**, 72,73-9
- [30]. S.A. Suleiman, M.E. Ali, Z.M. Zaki et al., *J. Androl.*, **1996**, 17, 530-537.
- [31]. S. Oztezcan, U.M. Turkoglu, E. Kervancioglu, T. Kocak, N. Kocak-Toker, G. Aykac-Toker, *Andrologia*, **1999**, 31,195-198.
- [32]. I. Amiri, N. Sheike, R. Najafi, *DARU*, **2006**, 14 ,4, 197-202.
- [33]. N Sheikh, I. Amiri, R. Najafi, M.T. Goodarzi, *Afr. J. of Biotech.*, **2010**, 9,35,5739-5745.
- [34]. G. Balercia, S. Moretti, A. Vignini, M. Magagnini, F. Mantero, M. Boscaro, G. Ricciardo-Lamonica, L. Mazzanti, **2004**, 25, 245–249.
- [35]. V. Garg, S.P. Garg, V. K. Deshpande, D.A. Biswas, A.T. Rawekar, N.R. Akarte, *Journal of the Indian Society of Toxicology*, **2011**, 7, 1.
- [36]. A. Revelli, L. Bergandi, M. Massobrio, B. Lindblom, A. Bosila, D. Ghiyo, *Fertil. Steril.*, **2001**, 76, 3, 469-500.
- [37]. F.S. Steve, M.M. Griffin, E.N. Chantler, *Int. J. Androl.*, **1982**, 5, 401-12.
- [38]. A. Prasad, *Am. J. Clin. Nutri.*, **1991**, 53, 403-12.
- [39]. W.Y. Wong, M.C. Thomas, M.J. Merkus, A.G. Zielhuis, P.R. Steegers-Theunissen, *Fertil. Steril.*, **2000**, 73,3, 435-42.
- [40]. A.E. Omu, H. Dashti, S. Al-Othman, *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **1998**, 79,179-184.
- [41]. M. Gavella, V. Lipovac, *Andrologia*, **1998**, 30,317–323.
- [42]. H. Ali, M. Ahmed, M. Baig, M. Ali, *Pak. J. Med. Sci.*, **2007**, 23,1 , 111-114.
- [43]. S.E. Chia, C.N. Ong, L.H. Chua, L.M. Ho, S.K. Tay, *J. Androl.*, **2000**, 21, 53-57.
- [44]. A. Khosrowbeygi, N. Zarghami and Y. Deldar, *Iranian Journal of Reproductive Medicine*, **2004** , 2, 2,58-64.
- [45]. Y. Koca, O.L. Ozdal, M. Celik, S. Unal, N. Balaban, *Arch. Androl.*, **2003**, 49, 355-359.
- [46]. R.K. Sharma, F.F. Pasqualotto, D.R. Nelson, A.J. Thomas Jr. A. Agarwal, *Hum. Reprod.* **1999**, 14, 2801-2807.