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This issue is a new experience evading the e-mailed and hard copy submissions. It demands your impressions and evaluation to better meet your needs moving forward. We hope this gets us one step closer to providing the convenient environment and handy tools worthy of our authors. Many thanks to all the innovators who shared the launching of the Editorial Manager and the adjoining tutorial website.
Varicocele impact on testicular size of infertile men in unilateral or bilateral associated cases

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Aim
To assess varicocele impact on the testicular size of infertile men in unilateral-associated or bilateral-associated cases.

Patients and methods
A total of 250 infertile men were investigated subdivided into; men with left-sided varicocele (\(n = 100\)), men with bilateral varicocele (\(n = 100\)), and men without varicocele (\(n = 50\)). They were subjected to history taking, clinical examination, scrotal color Doppler for testicular size, and semen analysis.

Main outcome measures
Testicular size(s) in relation to varicocele association.

Results
There was a significant decrease in the mean testicular size in infertile men with unilateral or bilateral varicocele compared with infertile men without varicocele. Testicular volume discrepancy was more evident in infertile men with left-sided varicocele compared with infertile men with bilateral varicocele or without varicocele.

Conclusion
Infertile men with varicocele have a significant decrease in ipsilateral as well as total testicular size and significant increased testicular size discrepancy.

Keywords:
color Doppler, male infertility, testicular size varicocele, testis

Introduction
Varicocele is present in approximately 15\% of men, being one of the most well known diagnosed causes of male infertility [1,2]. Various mechanisms were suggested to account for the testicular dysfunction and negative impact on semen parameters associated with varicocele, for example, hyperthermia, retrograde flow of toxic metabolites, venous stasis with hypoxia, alterations in the hypothalamic–pituitary–gonadal axis, and increased oxidative stress [3–7].

The negative impact of varicocele on testicular size had been shown decades ago in both adolescents and adult males depending on its clinical grade [8–11]. Zini et al. [12] showed that clinical left varicocele can negatively impact left testicular size and that subclinical varicocele is associated with decreased left testicular size.

In addition, a discrepancy between the sizes of the two testes secondary to varicocele had also been demonstrated [13,14], suggesting that testicular size could be a prognostic factor for successful varicocele repair [15–17]. Patel and Sigman [18] added that testicular size discrepancy is two times more common in infertile men with varicocele than in men without varicocele and that testicular size discrepancy with a smaller left testicle is more common than size discrepancy with a smaller right testicle, regardless of the side of varicocele.

This study aimed to assess the effect of associated varicocele on testicular size in infertile men.

Patients and methods
A total of 250 infertile men were investigated, recruited prospectively from the Andrology Department, University Hospital, after Institutional Review Board approval and informed consents. They were divided into three groups, matched in terms of age and semen parameters: men with left-sided varicocele (\(n = 100\)), men with bilateral varicocele (\(n = 100\)), and men without varicocele (\(n = 50\)). Exclusion criteria were hypogonadism, subclinical varicocele, orchitis, undescended testis, and testicular atrophy.

All men were subjected to history taking, genital examination, and semen analysis [19]. Scrotal color Doppler ultrasound was performed using a 7.5 MHz high-resolution linear array transducer (Sonoline Versa plus; Siemens Medical System, Erlangen, Germany) with pulsed and color Doppler capabilities. Testicular size was automatically calculated by the length \(\times\) antero-posterior \(\times\) transverse dimensions \(\times\) 0.52 (ml).

Statistical analysis
The data were analyzed and expressed as mean values ± SD, using SPSS version 17 (SPSS Inc., Chicago, Illinois,
Varicocele & testicular size Taha et al. 77

Table 1 Characteristic data of the studied groups

<table>
<thead>
<tr>
<th></th>
<th>Infertile men with left-sided varicocele</th>
<th>Infertile men with bilateral varicocele</th>
<th>Infertile men without varicocele</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>100</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.35 ± 7.35</td>
<td>35.51 ± 7.72</td>
<td>35.72 ± 7.72</td>
</tr>
<tr>
<td>Sperm count (10⁶/ml)</td>
<td>13.1 ± 2.2</td>
<td>12.7 ± 1.9</td>
<td>13.8 ± 2.4</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>27.4 ± 4.1</td>
<td>25.4 ± 3.8</td>
<td>28.1 ± 4.3</td>
</tr>
<tr>
<td>Sperm abnormal forms (%)</td>
<td>42.3 ± 4.4</td>
<td>47.9 ± 7.6</td>
<td>38.1 ± 3.2</td>
</tr>
<tr>
<td>Left testicular size (ml)</td>
<td>12.78 ± 2.94</td>
<td>10.07 ± 2.40^a</td>
<td>17.74 ± 2.45^b</td>
</tr>
<tr>
<td>Right testicular size (ml)</td>
<td>15.34 ± 3.09</td>
<td>11.76 ± 2.79^a</td>
<td>16.81 ± 1.82^b</td>
</tr>
<tr>
<td>Total testicular size (ml)</td>
<td>28.12 ± 5.83</td>
<td>21.83 ± 4.82^a</td>
<td>34.55 ± 3.93^b</td>
</tr>
<tr>
<td>Testicular size difference (ml)</td>
<td>2.96 ± 1.57</td>
<td>1.89 ± 1.99^a</td>
<td>0.93 ± 1.77^a,b</td>
</tr>
</tbody>
</table>

^aSignificant difference from infertile men with left-sided varicocele.
^bSignificant difference from infertile men with bilateral varicocele.

USA). An unpaired t-test was used to compare variables between groups. P < 0.05 was set as statistically significant.

Results
Infertile men with bilateral varicocele demonstrated a significant decrease in left or right mean testicular sizes, total testicular size as well as testicular size difference compared with infertile men with left-sided varicocele or without varicocele. Infertile men with left-sided varicocele demonstrated a significant decrease in left or right mean testicular size and total testicular sizes compared with infertile men without varicocele. The mean testicular size demonstrated a significant increase in infertile men with left-sided varicocele compared with infertile men with bilateral varicocele or without varicocele (Table 1). Testicular size demonstrated a significant positive correlation with sperm concentration (r = 0.532, P = 0.001) and a nonsignificant correlation with sperm motility (r = 0.049, P = 0.472) as well as percentages of sperm abnormal forms (r = −0.035, P = 0.317).

Discussion
In the current study, infertile men with left-sided varicocele showed a significant decrease in ipsilateral testicular size compared with infertile men without varicocele. In addition, infertile men with bilateral varicocele showed a significant decrease in the size of both testicles compared with the other studied groups. Previously, Lipschultz and Corriere [20] had observed progressive testicular hypotrophy in patients with varicocele compared with healthy controls, where Sigman and Jarow [21] observed that 50% of infertile patients with unilateral varicocele have ipsilateral testicular hypotrophy. Zini et al. [12] were the first to show the negative influence of varicocele on the testicular size with ultrasound-derived measurements. Zini et al. [13] confirmed this, adding that the degree of unilateral testicular hypotrophy was proportionate to the clinical grade of varicocele as well as the maximum internal spermatic vein diameters.

There was a significant increase in the mean testicular size discrepancy in infertile men with unilateral compared with bilateral varicocele and those without varicocele. Pinto et al. [22] reported that the mean testicular size difference for fertile men without varicocele was significantly lower than in fertile men with varicocele or infertile men with varicocele. Sakamoto et al. [14] showed that fertile patients with left-sided varicocele had a larger percentage of testicular size differences than infertile patients or fertile patients without scrotal abnormalities. They also added that the relative decrease in left testicular size was not restricted to patients with varicocele as infertile patients had smaller mean left and/or right testicular sizes than fertile patients, irrespective of the presence of varicocele.

These relationships may actually have an impact on fertility. Sigman and Jarow [21] showed that infertile patients with testicular hypotrophy with unilateral varicocele have worse semen parameters than those without hypotrophy. Preston et al. [23] added that patients who show a testicular volume discrepancy that is steadily increasing should be considered for surgical intervention. Hence, the testicular trophic healing observed in 81.5% of the operated patients by Messina et al. [24] led to the belief that an early correction can allow a rapid volumetric increase and an improved function of the gonad. Sakamoto et al. [25] confirmed these findings, demonstrating that left-sided varicocele repair could lead to increased left testicular size concomitant with an improved semen profile.

Conclusion
Infertile men with varicocele have significant decreases in ipsilateral and total testicular size and significant increases in testicular size discrepancy. Future studies should compare these data with fertile men with/without varicocele to assess whether decreased testis size is because of varicocele impact or the infertility status.

Acknowledgements
Conflicts of interest
There are no conflicts of interest.

References


Prevalence of *Chlamydia trachomatis* asymptomatic urethritis among infertile men with oligoasthenozoospermia

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**Purpose**

To investigate the prevalence of *Chlamydia trachomatis* in a group of infertile patients with oligoasthenozoospermia and asymptomatic urethritis and to assess the relationship between chlamydial infection and semen quality.

**Patients and methods**

Two hundred and fifty male patients with a history of primary infertility and idiopathic oligoasthenozoospermia were recruited from the Andrology and STD's outpatient clinic, Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University. The exclusion criteria included any evidence of urinary tract infection or any clinical symptoms of urethritis. Urine examination and detection of *C. trachomatis* by nested-PCR was performed for cases with the positive first glass.

**Results**

The incidence of asymptomatic urethritis among infertile men was 20% (50 patients out of 250), and this urethritis-positive group showed chlamydial DNA in the first voided urine in 30% of the urethritis-positive infertile men (15 patients). Urethritis-positive infertile men had a significantly lower sperm motility and sperm count and significantly higher abnormal forms.

**Conclusion**

Infertility in the couples with proved chlamydial infection may be related to the microbiological pathogen. Asymptomatic urethritis can be a factor in the deterioration of semen parameters, whether the cause of urethritis is *Chlamydia* spp. or another pathogen; however, an accurate diagnosis of *Chlamydia* spp. by nested PCR is necessary to guide specific effective therapy.

**Keywords:** asymptomatic urethritis, *Chlamydia* spp., nested PCR, oligoasthenozoospermia

**Introduction**

Male infertility accounts for 40–50% of the infertility in humans [1,2]. It is commonly due to deficiencies in the semen quality, which is used as a surrogate measure of male fecundity [3]. It is estimated that 15% of male infertility is related to genital tract infection [4].

*Chlamydia trachomatis* is among the most prevalent sexually transmitted pathogens that can affect sperm function and male fertility. The potential mechanisms of the effect of *C. trachomatis* on fertility may be a direct negative effect of *Chlamydia* spp. on the spermatogenesis or an indirect effect, through infection leading to inflammatory obstruction of the tubules and/or epithelial damage that result in impaired spermatogenesis [5,6]. A role for *C. trachomatis* in male factor infertility is not yet proven. A direct interaction between sperm and *Chlamydia* spp. has been shown; however, this leads to a subsequent increase in DNA damage. A number of studies show that the impact of *Chlamydia* spp. on the semen quality is controversial; in-vivo studies of *C. trachomatis* in men have provided conflicting evidence as to whether it is associated with reduced fertility. In contrast, in-vitro studies have shown that coincubation of spermatozoa with *Chlamydia* spp. causes a significant decline in the numbers of motile sperm and results in premature sperm death [5,7]. The reported incidence rates of genital chlamydial infections in the population are likely underestimated because of the highly asymptomatic nature of the pathogen [8].

Asymptomatic men infected with *C. trachomatis* are younger than their symptomatic counterparts. This also emphasizes the need for better screening or prevention practices, because although up to 13.3% of young men may have a genital chlamydial infection, only half of them present with any symptoms and even fewer are likely to pursue treatment [9]. Hence, to make tests easier and with a higher specificity, nucleic acid amplification tests such as PCR may be used as the technique of choice for *Chlamydia* spp. assessment in asymptomatic male partners.

**Patients and methods**

**Inclusion criteria**

This study was carried out on 250 male patients recruited from the Andrology and STD's outpatient clinic, Kasr
Al-Ainy Hospital, Cairo University, with a history of primary infertility and oligoasthenozoospermia.

**Exclusion criteria**
The exclusion criteria included any evidence of urinary tract infection or any clinical symptoms of urethritis, for example, discharge, dysuria, frequency, pain, and pruritus.

**Ethics**
All participants gave their written informed consent before their entry into the study. The study was approved by the Andrology Department Ethics Committee, and was conducted in accordance with ‘good clinical practice’ and all applicable regulatory requirements.

**Evaluation methods**
Complete history was taken, including sexual history (for example, frequency, erection, libido, ejaculation, orgasm, painful ejaculation) and a history of sexually transmitted infections or genitourinary disorders. A general and local examination of the penis, testicles, epididymis, and vas was performed. The following investigations were conducted: semen analysis was performed for all patients as an integral part of the infertility evaluation. An accurate measurement of the semen volume, motility, percentage of morphologically normal sperm, and the number of leukocytes was carried out according to the WHO criteria 2001 (WHO, 2001) [10]. A urine examination was performed to detect asymptomatic urethritis, which was diagnosed if there were greater than 10 leukocytes/high power field (HPF) in the first glass. Detection of *C. trachomatis* by nested-PCR was performed for patients with the positive first glass.

**Two-glass urine tests**
Patients were asked to urinate their first 20 cc urine into a sterile glass, and 20 cc of their midstream urine into another sterile glass. These samples were called VB1 and VB2: VB1, voided bladder 1, representing the urethra; VB2, voided bladder 2, representing the bladder. The presence of greater than 10 leukocytes/HPF in the first glass indicated urethritis.

**Detection of Chlamydia trachomatis by nested PCR**
DNA extraction from 10 ml of the urine sample was performed; in the first PCR reaction (Kits were supplied from Bio Sewoom, Seoul, Korea), 15.0 μl of the first PCR mixture was mixed with 0.5 μl of the first PCR enzyme for each sample in 200-μl PCR tubes, and then 4.5 μl of extracted DNA was added. The solution was mixed well. Positive control was started similar to the first PCR, except for annealing at 64°C. The Band presence for *C. trachomatis* in the agarose gel (2%) was detected using ultraviolet transillumination. Data are illustrated in Fig. 1.

**Statistical analysis**
Data were statistically described in terms of range, mean ± SD, median, frequencies (number of cases), and percentages when appropriate. Comparison of numerical variables between the study groups for independent samples was performed using the Leven test for equality of variance and the *t*-test for equality of means. For comparing categorical data, the χ² test was performed. A *P* value less than 0.05 was considered statistically significant. All statistical calculations were performed using computer programs Microsoft Excel 2007 (Microsoft Corporation, New York, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, Illinois, USA) version 15 for Microsoft Windows.

**Results**
With regard to the results of urine sample examination of the 250 infertile men, only 50 patients were found to suffer from asymptomatic urethritis, and they were further studied for the presence of *C. trachomatis* infection.

Semen analysis of the cases studied is depicted in Table 1.

On comparing the sperm count and the motility between infertile men with and without urethritis, a lower statistically significant value was observed in the asymptomatic urethritis cases: *P*<0.0005. In contrast, a higher

**Table 1 Clinical and semen characteristics among infertile patients**

<table>
<thead>
<tr>
<th></th>
<th><em>n</em></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count (million/ml)</td>
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<td>15.00</td>
<td>6.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Motility (%)</td>
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<td>50.00</td>
<td>18.6</td>
<td>9.7</td>
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<tr>
<td>Abnormal forms (%)</td>
<td>250</td>
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<td>90.00</td>
<td>44.8</td>
<td>14.3</td>
</tr>
<tr>
<td>Pus cells in urine/HPF (%)</td>
<td>50</td>
<td>11.00</td>
<td>27.50</td>
<td>16.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Age (year)</td>
<td>250</td>
<td>20.00</td>
<td>49.00</td>
<td>31</td>
<td>5.9</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>250</td>
<td>1.00</td>
<td>8.00</td>
<td>2.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>
The presence of Chlamydia Trachomatis infection in infertile men with asymptomatic urethritis was confirmed in 30% of the cases (15 out of 50 patients with asymptomatic urethritis) who were Chlamydia positive by nested-PCR. A significant reduction in the sperm count and the motility was found in Chlamydia-positive cases (P = 0.002 and 0.028, respectively) (Table 3).

There was no significant difference regarding sperm count, motility, and abnormal form on comparing patients with Chlamydia-positive urethritis and those with Chlamydia-negative urethritis (Table 4).

**Discussion**

*Chlamydia trachomatis* is among the most prevalent sexually transmitted pathogens. Thus, it is important to determine the presence of this pathogen [4,6,11]. The present study was conducted on 250 infertile men. We found the incidence of asymptomatic urethritis among infertile men in our sample of patients to be 20% (50 out of 250 patients), and this urethritis-positive group showed chlamydial DNA in the first voided urine (FVU) in 30% of the urethritis-positive infertile men (15 patients). Urethritis-positive infertile men had a significantly lower sperm motility and sperm count and significantly higher abnormal forms.

In the literature, many studies have reported a high incidence of the chlamydial urethritis among infertile male patients; some studies proved the deleterious effect of chlamydial infection on semen parameters and other studies could not prove this. In a large study of 627 semen samples, 136 of which had evidence of chlamydial infection, the presence of *Chlamydia* spp. reduced the normal sperm morphology by 14.4%, volume by 6.4%, count by 8.3%, motility by 7.8%, and velocity by 9.3% [12].

Hosseinzadeh et al. [13] studied the semen quality of men with asymptomatic chlamydial infection and suggested that the exposure of spermatozoa to elementary bodies of *C. trachomatis* can lead to sperm death over a number of hours of incubation. The ejaculates of 642 men without symptoms of genitourinary infections were examined. Nested PCR was performed on the ejaculate to detect the presence of *C. trachomatis* DNA. A total of 31 semen specimens (4.9%) were found to be positive, and in 28 of these, the diagnosis was confirmed using the ligase chain reaction. Men whose ejaculates were PCR positive for chlamydial DNA had a significantly higher mean concentration of leukocytes (1.71 ± 2.20 × 106/ml) and a higher mean ejaculate volume (3.45 ± 1.52 ml) than those whose ejaculates were PCR negative (leukocyte concentration: 0.67 ± 2.59 × 106/ml; volume 2.93 ± 1.38 ml). Leukocytospermia was twice as common in men who were PCR positive for chlamydial DNA, but it was not always associated with the presence of chlamydial DNA in the semen. However, there was no difference in the mean percent motility between the two groups. We observed comparable results concerning the mean concentration of...
leukocytes as patients with *Chlamydia* spp. had a higher leukocyte concentration than those without *Chlamydia* spp.

Another study conducted by Gdoura et al. [14] investigated 104 male patients who did not present any clinical symptoms of infection of the lower genital tract, and apart from their infertility, were healthy individuals. Detection of *C. trachomatis* DNA in the semen and the corresponding FVU specimens by PCR revealed that a total of 45 out of 104 (43.3%) patients were positive for *C. trachomatis* in the semen or the FVU specimens. They reported that the concordance between the detection of *C. trachomatis* DNA in semen specimens and corresponding FVU specimens of infertile men was 95.2%. A very good agreement between the two specimens was found [14].

Joki Korpela et al., [15] studied the association between plasma antibodies including IgG and IgA antibodies to *C. trachomatis* and male infertility; the prevalence of plasma IgG antibodies to *C. trachomatis* was higher among men from infertile couples than in control men, and men with chlamydial antibodies had lower sperm counts than those without. This might explain why, usually, most of the IVF centers in Egypt use a doxycycline antibiotic course for couples before starting the intracytoplasmic sperm injection cycle. Ouzounova Raykova et al. [16] also reported that *C. trachomatis* may be the cause of male infertility in infertile couples.

In contrast, Vigil et al. [17] reported that the incidence of *C. trachomatis* infection among the male partners of infertile couples was 38.6%, with no significant differences between the infected and the noninfected infertile men in any of the sperm parameters assessed. In the present study, it is likely that infertility in the couples with proved chlamydial infection may be related to the microbiological pathogen, but the search for other factors that can contribute to infertility should continue. The presence of *C. trachomatis* in an infertile couple does not exclude the possibility of other factors that may be responsible for the development of infertility [17].

Although the present study revealed that asymptomatic urethritis can be a factor in the deterioration of semen parameters, whether the cause of urethritis is *Chlamydia* spp. or another pathogen, yet the accurate diagnosis of *Chlamydia* spp. by PCR is necessary to guide specific effective therapy.

**Conclusion**

In our study, the incidence of asymptomatic urethritis among infertile men was 20%, and because of this very high incidence, we recommend that infertile men with oligoasthenozoospermia to be screened by examining the FVU, and the positive cases should be subjected to further tests to diagnose the etiology of this urethritis and treat the couple before entering any further steps for managing their infertility.

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**References**

Effect of L-carnitine on the sperm motility in ejaculated semen and cervical mucus
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Introduction
Medical treatment of male infertility encompasses specific and empirical approaches. Specific approaches depend on treating the cause and involve a thorough patient appraisal. In contrast, empirical approaches do not address a specific pathophysiology and do not require accurate patient selection.

Whereas idiopathic male infertility comprises a large sector of male infertility cases [1], the main effective solution by far remains assisted reproduction, a costly option that does not resolve the original pathology. The search for the etiology and an effective treatment for idiopathic male infertility is still ongoing.

One of the medications proposed for treating male infertility is L-carnitine [2]. This proposal was based on the fact that L-carnitine represents a cofactor in the transport of long-chain fatty acids inside the mitochondria and their subsequent oxidation, thereby providing the sperm with an energy substrate necessary for propulsion [3].

Several controlled and uncontrolled studies support a potential positive effect of therapy with L-carnitine and its acyl derivatives in treating male subfertility [4–10]. However, Agarwal and Said [11] stated that although many clinical trials have been performed in this domain, the majority of these studies lack a placebo-controlled, double-blind design, making it difficult to reach a definite conclusion.

Spermatozoa in the semen use both glycolysis and fatty acid oxidation for generating energy. In contrast, sperm cells in the cervical mucus rely on fatty acid oxidation solely [3]. As a cofactor in fatty acid oxidation, L-carnitine may be valuable in enhancing sperm activity in the cervical mucus, an understudied assumption.

This work aimed at studying the effect of L-carnitine on sperm parameters in the ejaculated semen and the cervical mucus, through a randomized, double-blind, placebo-controlled design, in cases of male infertility of various etiologies and seminal parameters of various forms.

Methods
The study was presented to and accepted by the internal review board beforehand. A written informed consent was obtained from each patient. Thirty infertile male patients were recruited, and randomized into two groups, one receiving L-carnitine (2 g/day, per oral) and the other receiving placebo for a period of 3 months. Both the investigators and the patients were blinded to the type of therapy administered. All patients provided written informed consents to the experimental nature of the study.

Purpose
This work aimed at studying the effect of L-carnitine on sperm parameters in the ejaculated semen and the cervical mucus, through a randomized, double-blind, placebo-controlled design, addressing idiopathic and varicocele cases.

Methods
Patients were randomized into two groups, one receiving L-carnitine and the other receiving placebo. Pretreatment and posttreatment semen and postcoital cervical mucus samples were evaluated and compared.

Results
Posttreatment semen samples showed that L-carnitine resulted in a statistically significant increase in sperm concentration in the cervical mucus in the study group compared with the placebo, although not in terms of motility or morphology, whether in the semen or in the cervical mucus. This effect was restricted to cases of idiopathic infertility and not in cases with varicocele, and to cases of oligoaesthenozoospermia rather than cases with isolated asthenozoospermia.

Conclusion
L-Carnitine can have a positive effect on sperm concentration within the cervical mucus in cases of idiopathic oligoaesthenozoospermia, possibly because of an enhanced cervical mucus penetration capability.

Keywords:
carnitine, cervical mucus, motility, postcoital, sperm

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The selection criteria included an age range of 25–40 years (to exclude asthenozoospermia resulting from infrequent coitus in the older age groups) and primary infertility for two years. Selection criteria also entailed that semen analyses were to show asthenozoospermia repeatedly whether isolated or associated with other semen parameter disorders, in the absence of seminal antisperm antibodies and leukocytospermia. A scrotal Duplex study was used to exclude cases with abnormality in testicular size or echogenicity, and to determine the presence or absence of varicocele, excluding cases with grade III varicocele that would possibly indicate surgery rather than medical treatment. The female partners were to have normal ovulation, patent tubes, normal pelvic ultrasound, and favorable cervical mucus. A 3-month washout period separated the onset of the study from prior therapy for infertility. Three serial semen analyses, 1 month apart, and a postcoital test preceded and followed treatment. The average of the three samples was adopted as the fertility status to avoid bias generated by the natural variations in semen parameters, a drawback in many studies in this domain. Pretreatment and posttreatment semen analyses and postcoital tests were compared in both groups. Analysis was performed according to the WHO guidelines [12].

Results
Posttreatment semen analyses showed that L-carnitine therapy resulted in a nonsignificant increase in the mean concentration, immediate forward progressive motility, and 2-h forward progressive motility, and a decrease in the mean of abnormal forms, which was also nonsignificant compared with the placebo (Table 1).

In contrast, after treatment, the postcoital cervical mucus samples showed significant increases in the mean of the total sperm/high power field (HPF) compared with the placebo (Table 2). This significant positive effect of L-carnitine on sperm cells in the cervical mucus was reproducible in cases of idiopathic infertility (Table 3), in contrast to cases with varicocele (Table 4), where the findings were nonsignificant. No statistical significance was found for the effect on forward progressive motility and sperm morphology in the cervical mucus. The significant positive effect of L-carnitine on sperm cell concentration in the cervical mucus was also reproducible in cases of oligoasthenozoospermia (Table 5), rather than cases with isolated asthenozoospermia (Table 6), where the improvement was nonsignificant.

Discussion
In 1965, Marquis and Fritz [13] demonstrated that the epididymis contains an extremely high concentration of carnitine and that spermatozoa have a high activity of carnitine acetyl transferase, which is an effect of testosterone. Lenzi et al. [3] suggested the value of L-carnitine as a cofactor in the transport of long-chain fatty acids inside the mitochondria and their subsequent oxidation, thereby enhancing one of the two pathways for energy generation: fatty acid oxidation and glycolysis. Whereas epididymal spermatozoa use fatty acid oxidation for their energy metabolism, ejaculated sperms use the glycolytic process. In contrast, in the cervical mucus, lipids are an important energy source for sperms, and to metabolize these lipids, intrasperm L-carnitine is essential. Mazzilli et al. [14] thus reported the possible mechanism of action of L-carnitine as a sperm motility enhancer. In addition to the aforementioned, a secondary role of carnitine as an antioxidant [15] may add to its value in enhancing sperm cell function. Sperm cells take up relatively large amounts of carnitine during their epididymal transit. By the time spermatozoa reach the cauda epididymis, they have lost the ability to take up

| Table 1 Effect of L-carnitine vs. placebo on sperm characteristics in the semen |
|------------------|------------------|------------------|------------------|
|                  | semen parameters before treatment | semen parameters after treatment |
| Group            | Conc | IFPM | 2HFPM | ABF |
| L-Carnitine (N=15) | Minimum | 5 | 5 | 0 | 20 |
|                  | Maximum | 88 | 22 | 17 | 77 |
|                  | Mean | 31.67 | 14.87 | 3.93 | 46.40 |
|                  | SD | 23.114 | 5.423 | 4.891 | 14.099 |
| Placebo (N=15)   | Minimum | 10 | 7 | 1 | 18 |
|                  | Maximum | 80 | 25 | 25 | 60 |
|                  | Mean | 35.40 | 13.60 | 1.07 | 39.87 |
|                  | SD | 21.006 | 5.396 | 2.344 | 12.112 |
| Total (N=30)     | Minimum | 5 | 5 | 0 | 18 |
|                  | Maximum | 86 | 25 | 17 | 77 |
|                  | Mean | 33.5 | 14.2 | 2.5 | 42.3 |
|                  | SD | 21.7 | 5.3 | 4.0 | 13.1 |

Effect of L-carnitine on semen parameters

<table>
<thead>
<tr>
<th></th>
<th></th>
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<tr>
<td>P value</td>
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<td>0.2061</td>
<td>0.3591</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ABF, abnormal forms; Conc, sperm concentration in million/ml; 2HFPM, forward progressive motility after 2 h; IFPM, immediate forward progressive motility; PCT, postcoital test.
Several studies supported a potential positive effect of therapy with L-carnitine and its acyl derivatives in male infertility [4–10]. However, the issue remains controver-

### Table 2 Effect of L-carnitine vs placebo on sperm characteristics in cervical mucus

<table>
<thead>
<tr>
<th>Group</th>
<th>Total sperm/HPF</th>
<th>Active motile sperm/HPF (%)</th>
<th>Total sperm/HPF</th>
<th>Active motile sperm/HPF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Minimum</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>3</td>
<td>100</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Mean</td>
<td>1.33</td>
<td>31.07</td>
<td>1.47</td>
<td>30.86</td>
</tr>
<tr>
<td>SD</td>
<td>1.175</td>
<td>46.198</td>
<td>1.272</td>
<td>44.186</td>
</tr>
</tbody>
</table>

| L-Carnitine | 15 | 15 | 13 | 13 |
| Minimum | 0 | 0 | 1 | 0 |
| Maximum | 5 | 66 | 5 | 76 |
| Mean | 1.87 | 11.73 | 3.31 | 31.62 |
| SD | 1.246 | 24.482 | 1.377 | 32.961 |

| Total | 30 | 30 | 27 | 27 |
| Minimum | 0 | 0 | 0 | 0 |
| Maximum | 5 | 100 | 5 | 100 |
| Mean | 1.60 | 21.40 | 2.15 | 31.22 |
| SD | 1.221 | 37.634 | 1.657 | 38.442 |

**Items compared**

- PCT posttreatment: total sperm/HPF
- PCT posttreatment: % of active motile sperm/HPF

**Effect of L-carnitine on postcoital test**

- $P$ value: 0.0093

**Effect of placebo on postcoital test**

- $P$ value: 0.375

- $P$ value: 0.469

HPF, high power field; PCT, postcoital test.

### Table 3 Effect of L-carnitine on postcoital test in varicocele-free cases

<table>
<thead>
<tr>
<th>Nonvaricocele</th>
<th>Total sperm/HPF</th>
<th>Active motile sperm/HPF (%)</th>
<th>Total sperm/HPF</th>
<th>Active motile sperm/HPF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Minimum</td>
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<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Maximum</td>
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<td>66</td>
<td>5</td>
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<tr>
<td>Mean</td>
<td>1.89</td>
<td>12.89</td>
<td>3.43</td>
<td>37.86</td>
</tr>
<tr>
<td>SD</td>
<td>0.928</td>
<td>25.887</td>
<td>1.272</td>
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<tr>
<td>Median</td>
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<td>0.00</td>
<td>4.00</td>
<td>40.00</td>
</tr>
</tbody>
</table>

**Items compared**

- PCT posttreatment: total sperm/HPF
- PCT posttreatment: % of active motile sperm/HPF

**Effect of L-carnitine on postcoital test in varicocele cases**

- $P$ value: 0.047

**Effect of placebo on postcoital test**

- $P$ value: 0.066

HPF, high power field; PCT, postcoital test.

### Table 4 Effect of L-carnitine on postcoital test in varicocele cases

<table>
<thead>
<tr>
<th>Varicocele</th>
<th>Total sperm/HPF</th>
<th>Active motile sperm/HPF (%)</th>
<th>Total sperm/HPF</th>
<th>Active motile sperm/HPF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
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<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>5</td>
<td>60</td>
<td>5</td>
<td>66</td>
</tr>
<tr>
<td>Mean</td>
<td>1.83</td>
<td>10.00</td>
<td>2.17</td>
<td>14.33</td>
</tr>
<tr>
<td>SD</td>
<td>1.722</td>
<td>24.495</td>
<td>1.602</td>
<td>28.296</td>
</tr>
<tr>
<td>Median</td>
<td>1.50</td>
<td>0.00</td>
<td>3.00</td>
<td>20.00</td>
</tr>
</tbody>
</table>

**Items compared**

- PCT posttreatment: total sperm/HPF
- PCT posttreatment: % of active motile sperm/HPF

**Effect of L-carnitine on postcoital test in varicocele cases**

- $P$ value: 0.102

**Effect of placebo on postcoital test**

- $P$ value: 0.285

HPF, high power field; PCT, postcoital test.

carnitine, but they now contain high concentrations of carnitine and acetyl carnitine and a high carnitine acetyl transferase activity [16].
sial and open-ended due to contradictory results of those studies in addition to some methodological errors such as a lack of placebo controls in some and ignoring the natural spontaneous variations in semen parameters in others. These shortcomings were well documented by Agarwal and Said [11].

One of the controlled studies affirmed the efficacy of combined treatment with L-carnitine and L-acetyl carnitine in improving sperm motility, especially in patients with lower baseline levels [17]. In contrast, Lenzi et al. [10] demonstrated that even though increases were seen in all semen parameters after carnitine treatment, they were nonsignificant, which is similar to our finding. In contrast, Sigman et al. [18] stated that carnitine supplementation demonstrated neither a significant nor a nonsignificant effect on sperm motility or total motile sperm concentrations in men with idiopathic asthenospermia.

On the scale of sperm-cervical mucus interaction, and in agreement with the current results, Mazzilli et al. [14] stated that a strict correlation was found between the L-carnitine content and the sperm motility in the cervical mucus.

The current study confirms the value of L-carnitine in enhancing sperm concentration in the cervical mucus by a double-blind placebo-controlled study, despite its nonsignificant effect on sperm parameters in the semen. In addition, this work is, to our knowledge, the first to demonstrate that L-carnitine therapy is effective in idiopathic, varicocele-free cases, with a resultant recommendation to restrict the use of L-carnitine to cases with idiopathic infertility.

In our study, cases with isolated asthenozoospermia had an associated varicocele; we therefore cannot confirm the inefficacy of carnitine in cases of isolated asthenozoospermia, but can confirm its value in cases of idiopathic oligoasthenozoospermia.

In our opinion, the enhancing effect on sperm concentration could be due to better cervical mucus penetration on account of better metabolism of the cervical mucus lipids as an energy substrate, despite an apparent lack of improvement of forward progressive motility that may be hindered by the nature of the mucus.

In conclusion, and according to the results shown, we recommend the utility of L-carnitine in the management of infertile men with idiopathic oligoasthenozoospermia.

### Acknowledgements

### Conflicts of interest

There are no conflicts of interest.

### References

Intratubular germ cell neoplasia in testicular biopsies of nonobstructive azoospermic patients
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Purpose
To assess the prevalence of intratubular germ cell neoplasia (ITGCN) in testicular biopsies of nonobstructive azoospermic (NOA) patients.

Materials and methods
The files of 415 NOA cases were revised retrospectively, in which the following were recorded: age, infertility duration, testicular sperm extraction (TESE), and histopathology. Suspected ITGCN slides were sent for placental alkaline phosphates (PLAP) stain evaluation.

Results
Out of 415 patients reviewed, ITGCN was confirmed in four patients (0.96%) with age range 25–35 years. These cases presented with primary infertility and unsuccessful TESE in their testicular biopsies; two of them showed severe hypospermatogenesis and two patients showed germ cell arrest.

Conclusion
NOA patients should be subjected to testicular biopsy evaluation for CIS, especially for the patients at high risk, to allow earlier detection of such threatening conditions. Also, it is recommended for proved CIS patients to have a biopsy from the other side.

Keywords:
azoospermia, carcinoma in situ, male infertility, neoplasia, spermatogenesis, testis

Introduction
Carcinoma in-situ testis (CIS), also known as intratubular germ cell neoplasia (ITGCN), is a preinvasive precursor of testicular germ cell tumors, the most common cancer type in male adolescents and young adults, except for spermatocytic seminoma in elderly men and yolk sac tumors and mature teratomas in infants [1,2]. The assumption that a CIS cell is the precursor of TGCTs is supported by the frequent observation of CIS in testicular parenchyma surrounding the invasive cancer and the development of invasive TGCTs in patients in whom CIS has been diagnosed previously [3]. ITGCN can be found in testicular tissues adjacent to GCTs in approximately 90% of adult cases and is found in all groups at risk for testicular cancer; cryptorchid testes, prior testicular cancer, and individuals with abnormal sexual differentiation [4–6].

Testicular ITGCN may be diagnosed in different clinical scenarios: the contralateral testis of a man with a GCT, the testis of a man who presents with an extragonadal GCT, a cryptorchid testis (regardless of orchidopexy), or in the normal contralateral testis, the testes of infertile men, and dysgenetic gonads. A multitude of additional risk factors have also been described, including testicular atrophy, microlithiasis, low birth weight, Down syndrome, and gestational estrogen excess [2]. CIS cells can also be detected in the adjacent parenchyma of most invasive tumors, and are more frequently associated with nonseminomatous germ cell tumors than with seminomas [7].

This work aimed to assess the prevalence of ITGCN among testicular biopsies from a group of nonobstructive azoospermic (NOA) patients.

Materials and methods
This is a retrospective study carried out on testicular biopsies performed on 415 infertile NOA Egyptian patients scheduled for testicular sperm extraction (TESE) in the University Hospital after IRB approval during the period from Jan 2005 to Jul 2008. All patients were of Caucasian ethnic origin. The following data were retrieved from their files: age, duration of infertility, TESE, and histopathology results. The testicular biopsy paraffin blocks (one side only) selected fulfilled the inclusion criteria of being >3 × 3 mm in diameter and containing a minimum of 30–40 tubules in a histological cross-section to be representative of the whole testis according to Holstein and Lauke [8].

The slides were examined microscopically for histopathologic evaluation, and suspected cases of ITGCN were recorded and sent for PLAP (placental alkaline phosphatase) stain evaluation. According to histopathology [9],
the cases were divided into normal spermatogenesis \((n = 9)\), hypospermatogenesis \((n = 44)\), germ cell arrest \((n = 174)\), Sertoli cell-only pattern \((n = 153)\), and tubular hyalinization \((n = 22)\).

Immunohistochemical staining with PLAP was carried out using a mouse monoclonal antibody of IgG1 designed for the specific localization of p21 receptors in frozen, formalin-fixed and paraffin-embedded tissue (Santa Cruz Biotechnology, Santa Cruz, California, USA). Glass slides treated with 3-amino-propyl-triethoxy saline were used. Paraffin blocks were cut by a microtome at 5 μ thickness. The sections were mounted on glass slides, and then incubated at 37°C overnight for accurate adhesion of the section to the slide. The slides were washed in distilled water for 2 min. Endogenous peroxidase activity was blocked by incubation in a freshly prepared 10% hydrogen peroxide in methanol for 30 min and it was then subjected to immunohistochemical staining [10].

**Discussion**

Among 415 patients, ITGCN examination was confirmed in four Egyptian patients (0.96%) with age range 25–35 years. Huyghe et al. [11] reported that testicular cancer is a common neoplastic malignancy during 20–34 years. The incidence of CIS among our examined biopsies was within average among other studies carried out in different countries, being 0.6% in Switzerland, 0.77% in Germany [12], 0.7% in Spain [13], 0.39% in UK [14], 1.0% in Denmark [15], and 1.8% in USA [13]. Prener and Ostlind [16] considered that the variation in different countries may reflect the selection of patients for testicular biopsies.

All participants selected in this study were Caucasian in origin, which showed the average incidence within similar studies. Huyghe et al. [11] demonstrated that the geographical and ethnic differences have been noted for testicular cancer, with an unexplained high prevalence among Caucasians living in well-developed countries and a notably lower prevalence among men of African descent and Asians, even inhabiting the same countries. CIS cells probably originate from primordial germ cells early during embryogenesis as a consequence of an endocrinological imbalance; an excess of estrogen during early embryonic life might stimulate primordial germ cells to acquire the tumorigenic potential of CIS cells, explaining the higher incidence of CIS among fertile men [4,17].

There was no history of undescended testis within our NOA diagnosed CIS cases as among the more common urogenital abnormalities; undescended testis is the best documented risk factor for testicular neoplasia. Pereira Arias et al. [18] reported that undescended testes have a 3.5–5 times higher risk of progressing to malignancy compared with normal descended testes. Parkinson et al. [19] determined, by meta-analysis, the relative risk of testicular cancer in patients with a history of cryptorchidism to be 4.8.

The histopathology of the diagnosed CIS cases was spermatogenic maturation arrest. A hypothesis was put forward that CIS originates from arrested fetal germ cells, and thus, testicular cancer is a developmental disease of germ-cell differentiation. The arrest of germ-cell differentiation is thus the key first event followed by malignant transformation and overt germ-cell cancer in young adults. In most cases, the arrest of delay of germ-cell

---

**Table 1 Data of the confirmed carcinoma in-situ testis cases**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Residence</th>
<th>Occupation</th>
<th>Infertility</th>
<th>Sperm count</th>
<th>Testicular size (ml)</th>
<th>Serum FSH (mIU/ml)</th>
<th>TESE result</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td># 1</td>
<td>25</td>
<td>Benha</td>
<td>Farmer</td>
<td>Primary</td>
<td>Azoospermia</td>
<td>17.0</td>
<td>7.0</td>
<td>Unsuccessful</td>
<td>Severe hypospermatogenesis</td>
</tr>
<tr>
<td># 2</td>
<td>27</td>
<td>Fayoum</td>
<td>Worker</td>
<td>Primary</td>
<td>Azoospermia</td>
<td>18.0</td>
<td>6.5</td>
<td>Unsuccessful</td>
<td>Severe hypospermatogenesis</td>
</tr>
<tr>
<td># 3</td>
<td>30</td>
<td>Cairo</td>
<td>Client</td>
<td>Primary</td>
<td>Azoospermia</td>
<td>16.0</td>
<td>7.8</td>
<td>Unsuccessful</td>
<td>Germ cell arrest</td>
</tr>
<tr>
<td># 4</td>
<td>35</td>
<td>Cairo</td>
<td>Client</td>
<td>Primary</td>
<td>Azoospermia</td>
<td>15.0</td>
<td>6.3</td>
<td>Unsuccessful</td>
<td>Germ cell arrest</td>
</tr>
</tbody>
</table>

FSH, follicle-stimulating hormone; TESE, testicular sperm extraction.
Recently, Stoop cytology for detecting CIS was not confirmed [24]. Of testicular specimens from fine needle aspiration, applying these maneuvers on testicular biopsies is advisable when the reliability of PLAP immunostaining is demonstrated to focus on the differentiation. PLAP monoclonal antibodies, a tissue-specific alkaline phosphatase with unknown biological function in CIS cells, used in this study in addition to the standard phosphatase with unknown biological function in CIS, proved CIS patients to have a biopsy from the other side.

It is concluded that NOA patients should be subjected to testicular biopsy evaluation for CIS, especially for the patients at high risk, to allow earlier detection of such threatening conditions. Also, it is recommended for proved CIS patients to have a biopsy from the other side.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

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Personality profile, anxiety, and sexual satisfaction: a comparison between implanted penile prosthesis and intracorporeal injection

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Purpose of the study
To evaluate personality profile, anxiety, patient satisfaction in patients with erectile dysfunction treated with penile prosthesis as compared to those on Intracorporeal Injection (ICI) home therapy.

Patients and methods
A total of 40 male patients complaining of erectile dysfunction were classified into two equal groups according to the line of treatment: Intracorporeal Injection (ICI) and penile prosthesis. Patients were subjected to the following questionnaires: Beck Anxiety Scale, Eysenck Personality Questionnaire, sexual Satisfaction Scale and International Index of Erectile Function Questionnaire (IIEF-5).

Results
Results for Beck Anxiety Scale showed statistically significant difference between both groups ($P = 0.000$), while for Eysenck Personality Questionnaire, no significant difference was found in Psychoticism ($P = 0.056$), Neuroticism ($P = 0.169$), in Extraversion ($P = 0.225$), Lie scale ($P = 0.159$), and in Criminality ($P = 0.378$).

Statistically significant difference between both groups was found in the results of sexual Satisfaction Scale, ($P = 0.013$) and for the results of International Index of Erectile Function Questionnaire (IIEF-5), ($P = 0.000$).

Conclusion
Patients who underwent penile prosthesis implantation showed lower level of anxiety, better sexual satisfaction and erectile function, while no significant difference was found when comparing personality profile five dimensions Psychoticism, Neuroticism, Extraversion, Lie scale and Criminality.

Keywords:
anxiety, Beck, erectile dysfunction, Eysenck, intracorporeal injection, International Index of Erectile Function, penile prosthesis, personality profile, sexual satisfaction

Introduction
Erectile dysfunction (ED) is defined as the consistent inability to achieve or to maintain an erection sufficient for satisfactory sexual activity [1].

The ability to perform sexually and satisfy a partner is frequently perceived by men as defining their masculinity. ED can have a profoundly adverse effect on their psychological well-being, besides a loss of self-esteem and depression. Increasing erection hardness and the frequency of successful intercourse attempts has positive effects on a man’s psychological health and quality of sex life. The result is an increased desire for sexual intimacy that improves a man’s relationship with his partner and overall life satisfaction [2].

Patients and methods
The study included 40 male patients complaining of ED for at least six months, recruited from the Andrology outpatient clinic of Kasr El Aini University Hospital. The patients were divided into two groups; group (I) included 20 patients successfully managed by intracorporeal injection (ICI) home therapy. Group (II) included 20 patients who underwent penile prosthesis implantation after failure of other treatment modalities (Table 1).

Inclusion criteria
The inclusion criteria were as follows: age above 21 years, informed written consent as approved by the ethical committee of the Department of Andrology, Kasr El Aini Faculty of Medicine, regular sexual practice after implantation of penile prosthesis or ICI home therapy for at least 3 months.

Exclusion criteria
The exclusion criteria were as follows: age less than 21 years, single patients, and patients without a regular sex partner/coital activity.

Evaluation methods
Patients were subjected to complete history taking and a general and local examination; the following questionnaires

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were used to assess anxiety, personality profile, and sexual satisfaction:

1. Beck Anxiety Scale [3]: The Beck Anxiety Inventory has been designed to differentiate between behavioral, emotional, and physiological symptoms in individuals with anxiety and depression. To achieve this goal, the authors incorporated items that are specific to the physiological and cognitive symptoms widely used to measure the severity of anxiety by self-report.

2. Eysenck Personality Questionnaire [4]: The Arabic version of the Eysenck Personality Questionnaire was used. It is composed of 90 statements to which participants respond by ‘yes’ or ‘no’. It has five scales that measure the dimensions of extraversion–introversion, neuroticism, psychoticism, and psychopathic deviation in addition to a lie scale.

3. Sexual Satisfaction Scale [5].

4. International Index of Erectile Function Questionnaire (IIEF-5) [6]. The Arabic validated version was used.

Statistical analysis
Data were statistically described in terms of range, mean ± SD, median, frequencies (number of cases), and percentages when appropriate. A comparison of numerical variables between the study groups was performed using Mann–Whitney U-test for independent samples. For comparing the categorical data, a χ² test was performed. Exact test was used instead when the expected frequency was less than 5. A P value less than 0.05 was considered statistically significant. All statistical calculations were performed using computer programs Microsoft Excel 2007 (Microsoft Corporation, New York, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, Illinois, USA) version 15 for Microsoft Windows.

Results
The mean Beck anxiety inventory score for the ICI group and the penile prosthesis group were 16 ± 7.8 and 4 ± 5.4, respectively, with a high statistical significance (P < 0.005). The mean sexual satisfaction scale score was 37 ± 8 for the ICI group and 43.5 ± 3.6 for the penile prosthesis group, a statistically significant difference (P < 0.05). The mean IIEF-5 score for the ICI and the penile prosthesis groups were 19 ± 4.2 and 25 (maximum score), respectively, a statistically significant finding (P < 0.005).

Regarding the Eysenck personality profile questionnaire, there was no statistically significant difference in psychopathic deviation (C) (P = 0.378), extraverison (E) (P = 0.225), lie scale (L) (P = 0.199), and criminality (C) (P > 0.05) (Table 2).

Discussion
ED has a major impact on the lives of millions of men and their partners, affecting sexual and psychological health and quality of life [7]. Beyond the direct effect on sexual
function, ED has been shown to lead to depressive symptoms, low self-esteem, and other signs of psychological distress [8]. The primary objective in the management of patients with ED is to restore a satisfactory sexual relationship for both partners with minimal expense and risk.

In the current study, ICI therapy was associated with significantly higher anxiety levels than was the case with penile implants, as detected by the Beck Anxiety Scale. This could be explained by the assumption that patients with a prosthesis implanted could be more confident with their sexual potency and their concern about obtaining and maintaining an erection during sex was significantly alleviated, with possibly higher libido, not to mention that patients with an implanted prosthesis can enjoy a higher frequency of intercourse than possible by injection, the utility of which should be regulated to avoid fibrosis. This was confirmed by Tefilli et al. [9] who also demonstrated an improvement in the individual domains of the Beck Anxiety scale: sadness, depression, anxiety, anger, frustration, and embarrassment related to sexual activity; and they were able to document significant psychosexual improvement up to 1 year after insertion of the penile prosthesis. In the same study, self-esteem was noted to improve quickly. At 3 months after penile prosthesis implantation, 68.6% of the patients reported high self-esteem. This increased to 71.4% at 6 months and 85.7% at 1 year. The authors reported that penile prosthesis surgery did not merely make sexual intercourse possible, but also reduced patient suffering and anxiety [9].

Scores of the sexual satisfaction scale were also higher in the penile prosthesis group than in the ICI group. There are previous reports that highlight dissatisfaction and discontinuation of ICI therapy for reasons such as drug side effects, aversion or fear of injection, pain, which happens to be a primary reason for discontinuing ICI therapy, not to mention reasons independent of the therapeutic modality such as lack of a partner, loss of interest, cost of ICI therapy [10], and drug ineffectiveness [11], which may possibly set in after initial success because of the persistence of risk factors for ED. A greater number of patients treated with penile prosthesis believed that the treatment considerably or completely met their expectations compared with ICI. Prior studies have shown that 15% of patients offered ICI do not accept it, 40% discontinue it within 3 months of starting therapy, and only 20–30% continue to use it after 3 months [12]. Sexton et al. made a comparison of long-term outcomes of penile prosthesis and ICI therapy. They found that patients undergoing insertion of a penile prosthesis remained sexually active significantly longer than those patients who chose ICI [10].

Rajpurkar and Dhabuwala made a comparison of satisfaction rates in patients treated with ICI and penile prosthesis. Total EDITS (Erectile Dysfunction Inventory of Treatment Satisfaction) and EDITS Index scores were significantly higher in patients who underwent penile implant surgery. Of 90.63% of the patients who underwent penile prosthesis were satisfied compared with 36.36% on ICI therapy (P < 0.001) [11]. Other studies reported the satisfaction rate following prosthesis implantation to range from 85 to 90% [13,14]. In the current study, IIEF-5 scores were significantly higher in patients who underwent penile prosthesis therapy than in those on ICI. This may be due to the increased reliability, longevity, and satisfaction with penile prosthesis, where rigidity is granted, unlike ICI therapy where occasional or gradual and persistent failure may set in because of various reasons such as aggravation of ED, faulty injection technique, and unsuitable storage conditions of the material injected, among other possible reasons.

In the last three decades, mechanical and material design changes have caused marked improvements in the function, reliability, and safety of penile prosthesis [15]. Atul and Chirpriya found that the EFD of IIEF scores were significantly higher in patients who underwent penile prosthesis than in those on ICI [7]. Carson et al. studied the efficacy, safety, and sexual satisfaction outcomes of the AMS 700 inflatable penile prosthesis; they found that the results reveal excellent function, reliability, and patient satisfaction; overall, 82% of the patients were satisfied with the penile function [16]. Goldstein et al. evaluated the Mentor three-piece inflatable prosthesis and reported that expectations were realized 9 days after surgery in 89%, and the overall function satisfaction with the implant was greater than 80% [17]. Moreover, Sexton et al. clearly showed that long-term use, satisfaction, and function are significantly better for penile implants than for pharmacological injection therapy [10].

According to the results of the present study, ED patients treated with penile prosthesis implantation showed an improvement in subjective symptoms such as erectile confidence, erectile function, and sexual satisfaction, with a lower level of anxiety when compared with intracavernosal injection therapy.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

**References**

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Heavy metal assay in the serum and the semen of infertile patients with and without varicocele compared with fertile controls
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Purpose
To evaluate the differential concentration of lead and cadmium in seminal plasma, relative to its serum concentration in infertile men with and without varicocele compared with fertile healthy controls, and to correlate these levels with the conventional sperm parameters and sperm vitality.

Methods
This study is a case-control study. Sixty infertile male patients, including 30 infertile men with palpable varicocele (group 1) and 30 men with idiopathic oligozoospermia and/or asthenozoospermia (group 2), in addition to 30 healthy fertile men without palpable varicocele (group 3) were included randomly in the study. Patients and controls included in the study were subjected to measurement of lead and cadmium levels in the serum and the seminal plasma, conventional semen analysis, and sperm vitality with a hypoosmotic-swelling test.

Results
Despite the lack of significant difference in the serum lead and cadmium concentrations between the three groups, there was a significant increase in both the seminal and the differential seminal concentrations of lead and cadmium among infertile men with and without varicocele in comparison with fertile controls. There were significant negative correlations between differential seminal concentrations of lead and cadmium on the one hand, and progressive sperm motility and vitality (hypoosmotic-swelling test) in infertile men of both groups in the other.

Conclusion
This study shows that, infertile men with and without varicocele tend to have a relatively higher semen concentration of heavy metals in comparison with fertile men, despite the lack of a significant difference in the corresponding serum levels. This differential elevation is correlated with the derangement of semen parameters, especially sperm motility and vitality.

Keywords:
cadmium, hypoosmotic-swelling test, lead, male infertility, varicocele

Introduction
Human fecundity appears to be on the decline, a situation that cannot be attributed solely to an increase in contraception [1]. Rather, a body of data suggests that poor semen quality, especially a progressive decrease in the sperm concentration, is likely to be a contributing factor [2].

Men seem to be more susceptible than women to the effects of occupational or environmental exposures to reproductive toxicants [3], and it is not surprising that environmental agents have been postulated to be contributory to the deteriorating semen quality and a decline in male reproductive health [4].

Results of several studies suggest that the testis and reproductive organs may be exquisitely sensitive to cadmium, with cadmium exposure leading to profound testicular damage and irreversible infertility without affecting any other organ system [5]. Cadmium and lead were found to be elevated in the seminal plasma and testes of infertile men with varicocele and in the testes of oligozoospermic and oligoasthenozoospermic men with or without varicoceles [6–8].

Previous studies provided some evidence suggesting that, not only occupational exposure but also geographic variations (and thus environmental exposures) contribute to the blood cadmium and lead levels [9]. To the best of our knowledge, no published studies have been conducted in our community to explore the levels of heavy metals in the semen among infertile men.

A varicocele is observed in 10–20\% of the general population, in 35–40\% of men with primary infertility, and in up to 80\% of men with secondary infertility [10]. Cadmium exposure through smoking was found to
potentiate the hazardous effect of varicocele on spermatogenesis and consequently the semen parameters [11]. Cadmium and lead were found to be elevated in the seminal plasma and testes of infertile men with varicocele [6–8]. Geographic variations (and thus environmental exposures) contribute to the blood cadmium and lead levels [9]. Thus, it is necessary to perform studies in different communities to evaluate the heavy metal levels, its reflection on the fertility potential, and the added effect of these agents on different genital pathologies including varicocele.

The largest group of men attending fertility clinics (about 25% of men referred to specialized infertility clinics) are classified to have idiopathic male infertility, a diagnosis made by exclusion, after elimination of all other possible or probable causes of infertility [12]. In idiopathic male infertility, the female partner is evidently free from any cause of infertility, semen parameters are subnormal, and a clinical examination of the men reveals no specific etiology such as varicocele, maldescended testes, male accessory gland infection, and hypogonadism, etc. [13]. They are classified according to semen findings by the terminology of idiopathic oligo, astheno, or teratozoospermia [12,14]. The purpose of this study was to evaluate the differential concentration of lead and cadmium in the seminal plasma relative to its serum concentration in infertile men with and without varicocele compared with fertile healthy controls, and to correlate these levels with the conventional sperm parameters and sperm vitality.

Methods

Inclusion criteria

This study included two groups of patients:

Group 1: Thirty male patients having clinical (palpable) varicocele.

Group 2: Thirty male patients having idiopathic male infertility (idiopathic oligo and/or asthenozoospermia).

Exclusion criteria

Patients with other specific genital causes that may impair the reproductive capacity, such as genital infection, undescended testis, etc., were excluded. Patients with other systemic diseases that may impair the reproductive capacity, such as hepatic, renal, endocrine, autoimmune diseases, etc., were also excluded. In addition, those with a significant female factor infertility were excluded.

Controls (group 3)

Thirty men with proven fertility served as the control group. They were healthy men without a medical or genital condition that may affect their reproductive capacity adversely.

Both patients and controls were selected from the population of Assiut city. All participants showed no special habits or occupational exposure to heavy metals to limit the results of the study to the effect of basic environmental exposure.

Participant recruitment

Patients (groups 1 and 2) were recruited randomly from the Andrology clinic of Dermatology and Andrology Department, Assiut University Hospital. Controls were recruited from the Dermatology clinic of Dermatology and Andrology Department, Assiut University Hospital. Both patients and controls were invited to participate in the study, and the steps and the aim of the research were explained to the participants before signing an informed consent. The study was carried out after the approval by the institutional review board of Assiut Faculty of Medicine.

All participants (patients and controls) included in the study were subjected to the following:

History taking and clinical examination

Each participant completed an extensive questionnaire regarding his occupation, residence, social status, diet, water source, and smoking habits. Complete detailed medical history was taken from all participants, with special emphasis on the reproductive history. They were also subjected to a thorough general medical and genital examination.

Serum metal analysis

As described by Inhorn et al. [15], approximately 2 ml of whole blood were drawn from all consenting participants and frozen for later heavy metal analysis; then it was lysed by freezing and thawing; a known volume was digested, as semen samples, twice with 5 ml of an acid mixture (6HNO3 : 1HClO4) in a glass tube (semen samples with an inadequate volume were diluted and multiplied by the dilution factor). The residue was dissolved in 1 ml of 1% HNO3, and then applied to an air-acetylene flame atomic absorption spectrophotometer (Buck model 210 VGR Buck Scientific, Inc., Norwalk, CT, USA) with a hollow cathode lamp (8 mA) current for detection of cadmium and lead.

Wavelengths: lead 283.2 nm and cadmium 228.9 nm.

Two determinations were made for each sample. The accuracy and precision of the analytical methods were tested with standard reference materials.

Conventional semen analysis

All samples were collected by masturbation in polypropylene containers after three to five days of sexual abstinence. After liquefaction at 37°C, conventional semen analysis was carried out according to the WHO guidelines 1992 [16] (concerning sperm morphology) and the WHO guidelines 1999 [17] (concerning other semen parameters).

The evaluation included liquefaction time, pH, odour, viscosity, presence of pus or epithelial cells, sperm motility, sperm morphology, and sperm concentration. The semen findings were categorized as follows: oligozoospermia, a sperm concentration of less than 20 × 10⁶/ml; asthenozoospermia, lesser than 50% progressive motile sperm; and oligoasthenozoospermia, including both criteria.
The hypoosmotic-swelling (HOS) test was performed according to the method described by Jeyendran et al. [18].

**Seminal plasma metal analysis**

As described by Pant et al. [8], approximately 1 ml of seminal plasma was digested twice with 5 ml of an acid mixture (6HNO₃: 1HClO₄) in a glass tube. The residue was dissolved in 1 ml of 1% HNO₃, and then applied to a flame atomic absorption spectrophotometer (Buck model 210 VGP) with air-acetylene flame and a hollow cathode lamp current (8 mA) for the detection of cadmium and lead.

Wavelengths: lead 283.2 nm and cadmium 228.9 nm.

Two determinations were made for each sample. The accuracy and precision of the analytical methods were tested with standard reference materials.

**Statistical analysis**

Data were analyzed and expressed as mean values ± SD. SPSS version 16 (SPSS Inc., Chicago, IL,USA) program was used for data processing.

The differential semen concentration of lead and cadmium was determined by dividing their seminal concentrations by the serum concentration in the same individual. Unpaired t-test was used for the comparison of numerical parametric data between the patient and the control groups. Mann–Whitney test was used for the comparison of numerical nonparametric data between the patient and the control groups. Pearson correlation test was applied to analyze the correlations between different quantitative variables within each group. Values were considered significant when P-values were equal or less than 0.05.

**Results**

**Sociodemographic data**

The three groups were comparable in terms age (33.87 ± 5.9, 34.87 ± 6.2, and 32.26 ± 6.1 years, respectively), BMI (24.84 ± 3.52, 23.72 ± 2.6, and 25.17 ± 2.29 kg/m², respectively), and social status. They all live in Assiut city.

**Conventional semen parameters and sperm hypoosmotic-swelling test**

There was no significant difference in the semen parameters between infertile men with and without varicocele. However, the two groups of infertile men had a significantly lower total sperm count (P<0.05, P<0.01), progressive sperm motility, normal sperm morphology, and sperm vitality percentages (P<0.001 for each) in comparison (Table 1).

**Serum, semen, and differential semen lead and cadmium levels**

There was no significant difference in the serum plasma levels of lead and cadmium among the three groups. There was also no significant difference in the seminal plasma levels of lead and cadmium among the two groups of infertile patients. However, both infertile men with and without varicocele had significantly higher levels of both semen and differential semen lead and cadmium in comparison with the fertile controls (P<0.001 for each) (Table 2).
Despite the lack of any significant correlation between the serum lead and cadmium levels and the semen parameters, there are significant correlations between the differential semen lead and cadmium concentrations and some other semen variables (Table 3 and Figs. 1 and 2). There were significantly negative correlations between the differential semen lead and cadmium concentrations, and each of progressive sperm motility and sperm vitality (HOS) percentages in both groups of infertile men but not in the fertile individuals.

**Discussion**

Recently, the role of environmental toxins has been increasing with a worldwide trend towards an impaired human sperm quality and male fertility capacity [19,20]. Lead and cadmium are among the heavy metals to which humans are exposed occupationally and environmentally [21,22]. The absence of a significant difference in the serum plasma levels of lead and cadmium among the

### Table 2 Serum, semen, and differential semen concentrations of lead and cadmium in the infertile men with or without varicocele and the fertile controls

<table>
<thead>
<tr>
<th>Heavy metals level</th>
<th>(Group 1)</th>
<th>(Group 2)</th>
<th>(Group 3)</th>
<th>1 vs. 2</th>
<th>1 vs. 3</th>
<th>2 vs. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum lead (µg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>18.67–34.21</td>
<td>20.52–32.11</td>
<td>13.0–30.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.89 ± 5.06</td>
<td>26.39 ± 4.05</td>
<td>25.06 ± 4.09</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Semen lead (µg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>11.21–20.11</td>
<td>10.8–19.2</td>
<td>5.91–13.34</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>15.1 ± 3.17</td>
<td>15.27 ± 2.6</td>
<td>8.5 ± 2.36</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Differential semen lead concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.506–0.685</td>
<td>0.526–0.658</td>
<td>0.263–0.457</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.584 ± 0.047</td>
<td>0.578 ± 0.033</td>
<td>0.339 ± 0.067</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum cadmium (µg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4.8–7.9</td>
<td>4.88–7.96</td>
<td>4.28–7.81</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>6.01 ± 0.82</td>
<td>6.37 ± 1.01</td>
<td>6.133 ± 0.84</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Semen cadmium (µg/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.4–5.3</td>
<td>3.22–5.97</td>
<td>2.2–3.38</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.02 ± 0.55</td>
<td>4.4 ± 0.87</td>
<td>2.81 ± 0.51</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Differential semen cadmium concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.552–0.821</td>
<td>0.534–0.878</td>
<td>0.288–0.623</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.874 ± 0.077</td>
<td>0.693 ± 0.103</td>
<td>0.466 ± 0.1</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 3 Correlations between the differential semen metal concentrations and the semen parameters

<table>
<thead>
<tr>
<th>Progressive sperm motility (%)</th>
<th>Lead group 1</th>
<th>Lead group 2</th>
<th>Cadmium group 1</th>
<th>Cadmium group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>−0.11</td>
<td>−0.13</td>
<td>−0.15</td>
<td>−0.17</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sperm HOS test (%)</td>
<td>−0.4</td>
<td>−0.11</td>
<td>−0.29</td>
<td>−0.1</td>
</tr>
<tr>
<td>r</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HOS, hypoosmotic-swelling test.

**Figure 1**

Correlation between the differential semen lead concentration and sperm vitality in infertile men with varicocele. HOS, hypoosmotic-swelling test.
three study groups may indicate a general environmental pollution involving all people.

In contrast, the results of our study revealed a significant increase in lead and cadmium levels in the semen of infertile patients with or without varicocele in comparison with the fertile controls. These results may indicate a pooling of heavy metals in the testes and the accessory sex glands. The reasons and factors governing this pooling are not clear. In general, our results are consistent with the previous studies that stated that, cadmium and lead were found to be elevated in the seminal plasma and testes of infertile men with or without varicocele [6,7]. It is also consistent with another study performed by Pant et al. [8] among the Indian general population, and another study performed by El-Zohairy et al. [23] among urban Egyptian fertile and infertile environmentally-exposed and occupationally-exposed individuals. Similar results were reported in a Singaporean general population by Xu et al. [21] with regard to cadmium but not lead.

The mean seminal levels of lead in the infertile patients and the fertile controls in our study were lower than the mean seminal levels in the infertile and the fertile environmentally-exposed and occupationally-exposed individuals, in a study published by El-Zohairy et al. [23]. However, our mean levels were higher in both the seminal lead and the cadmium in the three groups compared with those shown in the Singaporean general population [21]. At the same time, Abou-Shakra et al. [24] reported mean seminal lead concentrations of 0.8 µg/dl in normospermic (comparable with that of our control group), and 1.2, 0.6, and 1.0 µg/dl in oligospermic, severely oligospermic, and azoospermic men, respectively, in a fertility clinic (lower than the mean levels in the infertile patients with and without varicocele in our study). These differences may be due to variations in the environmental exposure, diet, heterogeneity of the population, and different analytical methods of detection.

The current study showed no significant difference between patients and controls with regard to the serum level of lead and cadmium. Moreover, we did not find any significant association between heavy metals in the blood and reduced sperm parameters, despite the presence of significant negative correlations between its differential semen levels and sperm parameters in the infertile patients. This is consistent with a previous study by Inhorn et al. [15]. This latter study suggested that heavy metal concentrations in the blood of Lebanese men did not diminish their fertility in any significant way, where none of the infertile groups included in their study had significantly higher or lower whole blood concentrations of heavy metals when compared with the fertile controls. Furthermore, in previous studies, Benoff et al. [25,26] stated that the blood plasma cadmium levels are in the normal range in infertile patients with varicocele irrespective of whether or not the reproductive tract cadmium concentrations are elevated. Moreover, Benoff et al. [25] stated that no correlation was detected between the blood plasma cadmium and the seminal plasma cadmium in infertile patients with varicocele. Thus, the analysis of blood plasma alone, the traditional method of biological monitoring of environmental and occupational exposures to toxic metals, may fail to detect specific alterations in trace metal levels in the semen [25].

It is clear that patients and controls included in our study show no significant difference with regard to the serum lead and cadmium, whereas semen levels of both metals are significantly elevated among infertile patients. This would lead us to the following question: Does pooling of lead and cadmium in the semen lead to male infertility by testicular dysfunction or is seminal pooling of heavy metals a consequence of testicular dysfunction (de-novo dysfunction or secondary to varicocele)? Some authors stated that the testes and the reproductive organs may be exquisitely sensitive to cadmium, which may lead to profound testicular damage and irreversible infertility even without affecting any other organ system [5]. In contrast, others believe that a damaged blood testis barrier and a malfunctioning testicular vascular endothelium provide a route of entry for toxic metals such as cadmium, rendering the testis able to accumulate cadmium more rapidly [26].

In this study, we focused on the effect of differential semen concentrations of lead and cadmium (rather than their rough semen levels) on the sperm parameters. We found significant negative correlations between these differential semen concentrations of both metals and progressive sperm motility and vitality. Most of the published studies focused on the relation between the rough semen levels of lead and cadmium and the sperm parameters. These studies showed conflicting results in healthy and infertile men of the general population or those with an occupational exposure [8,21,27–32].
Our finding of a significantly negative correlation between differential semen concentrations of lead and cadmium levels on one hand, and progressive sperm motility on the other, in patients with or without varicocele raises the suspicion about the role of elevated semen levels of these metals in infertile men on impairing the sperm motility. Similar results were obtained in another study [8]. In addition, Leoni et al. [33] suggested that sperm motility is a parameter sensitive to cadmium toxicity. Different suggestions were mentioned in previous studies about how lead and cadmium could affect sperm motility. Cadmium was shown to disturb microtubule sliding and assembly [34–36] and mitochondrial function and structure [37]. Furthermore, it has been established that cadmium competes with calcium for calmodulin binding [38], which results in a decrease in sperm motility [39]. Cadmium and lead may also induce oxidative stress due to Reactive Oxygen Species accumulation, mostly superoxide anion radical, hydrogen peroxide, and hydroxyl radical [37,40]. Reactive Oxygen Species may affect sperm motility by peroxidation of membrane lipids, reducing the phosphorylation of axonemal proteins [41] or by reducing ATP levels [42]. Moreover, cadmium may also lead to premature acrosome reaction in sperms [33]. Cadmium’s competition for calcium-binding sites may explain the premature acrosome exocytosis in sperms, which affects sperm motility [37].

The presence of a significantly negative correlation between sperm vitality as measured by HOS on one hand, and differential semen concentrations of both lead and cadmium on the other in patients with or without varicocele in our study is consistent with prior findings of the effect of cadmium on sperm viability in vitro [33], which showed that the vitality of ram sperm was significantly affected by exposure to cadmium. Furthermore, the oxidative stress induced by lead and/or cadmium may explain the reduction in sperm vitality [40,43].

Only a few studies were concerned with the correlation between varicocele and heavy metals and their impact on male fertility. The study published by Benoff et al. [25] revealed that the levels of seminal plasma cadmium in varicocele patients were significantly higher than the maximal cadmium concentration in seminal plasma from fertile men without varicocele. Importantly, seminal plasma cadmium levels in the latter patients were equivalent to those of the fertile donors. This is contradictory to the results of our study, where no significant difference was detected between infertile patients with and without varicocele with regard to lead and cadmium levels in the semen. This can be due to the lack of a significant difference in the semen parameters between infertile patients with and without varicocele among our patients. Another study by Benoff et al. [26] stated that the blood plasma cadmium levels are in the normal range in infertile patients with varicocele irrespective of whether or not the reproductive tract cadmium concentrations are elevated, which is consistent with our results. We assume that various causes of testicular dysfunction that lead to infertility (e.g. damaged blood testis barrier) might play a role in lead and cadmium accumulation in the testes of infertile patients rather than varicocele alone. Hence, we think it is probable that the infertile patients without varicocele included in our study might also have testicular lesions that lead to lead and cadmium accumulation in their seminal plasma, and they may lead to impaired spermatogenesis. The data described in our study makes us believe that pooling of heavy metals in the genital tract is not among the means by which varicocele affects male fertility. The major limitation in our study is the lack of a group of fertile varicocele patients. Inclusion of this group may explain whether these heavy metals may have effects on the fertility potential of varicocele patients.

Conclusion
This study shows that infertile men with and without varicocele tend to have a relatively higher semen concentration of heavy metals in comparison with fertile men, despite the lack of a significant difference in the corresponding serum levels. This differential elevation correlated with the derangement of semen parameters, especially sperm motility and vitality. These finding may indicate environmental pollution. Further studies should be carried out on a larger number of infertile men with different diagnoses to determine whether this elevation is a direct cause of impaired fertility capacity in the affected patients, or the result of inherent defects in the natural barriers in the testes and/or dysfunction in the male accessory glands.

Acknowledgements
There are no conflicts of interest.

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Seminal plasma S-adenosylmethionine and S-adenosylhomocysteine associations in infertile men

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Purpose
To assess seminal plasma S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) association in infertile men.

Materials and methods
In all, 95 infertile men were investigated and classified in terms of diagnosis into asthenozoospermia, atheteratozoospermia and oligoatheteratozoospermia compared with 22 healthy fertile controls. They were subjected to semen analysis, assessment of seminal SAM, SAH, malondialdehyde (MDA), acrosin activity, α-glucosidase, and sperm DNA fragmentation.

Results
In healthy fertile men, seminal SAM, SAM/SAH ratio, α-glucosidase, and acrosin activity were significantly increased, whereas seminal SAH, MDA, and sperm DNA fragmentation percentage were significantly decreased compared with other investigated infertile groups. Seminal SAM and SAM/SAH ratio showed a significant positive correlation with semen parameters, acrosin activity, and α-glucosidase and a significant negative correlation with seminal MDA.

Conclusion
Alteration in the seminal transmethylation pathway in infertile men is associated with increased oxidative stress, sperm DNA fragmentation and decreased sperm acrosin activity, seminal α-glucosidase.

Keywords:
DNA fragmentation, male infertility, methylation, oxidative stress, semen, sperm

Introduction
DNA methylation influences chromatin structure and gene expression, and methylation of the cytosine residues by DNA methyltransferase is considered one of the major epigenetic mechanisms that control gene expression and imprinting [1]. Hypomethylation of DNA has been shown to be associated with gene transcriptional activity, whereas DNA hypermethylation has been shown to be associated with gene silencing [2,3]. In humans, the methylation pattern of the paternally imprinted gene is erased in early fetal life; remethylation is initiated as spermatogonia enter meiosis and is effectively complete by the primary spermatocyte stage of differentiation. It has been shown that sperm pathology within the testicular or the epididymal environment has the potential to disrupt the establishment of normal sperm DNA methylation patterns but spermatogenesis-specific genes can undergo late epigenetic re-programming while maturing in the epididymis [4,5].

Sperm abnormalities have been shown to play a role in the majority of epigenetic defects observed in pregnancies through in-vitro fertilization, linking sperm DNA hypomethylation with reduced pregnancy rates [6]. Also, aberrant sperm DNA methylation is more commonly seen in semen samples of infertile men compared with normozoospermic ones [7,8]. An underlying mechanism for abnormal sperm DNA methylation in infertile men was identified by DNA sequence variations in the gene encoding the DNA methyltransferase enzyme in infertile men associated with abnormal paternal DNA methylation. Other possible mechanisms for sperm DNA hypomethylation may exist such as defects in the folate/homocysteine pathway and oxidative stress [6]. Oxidative stress is detrimental to the spermatozoa, causing damage of sperm DNA and plasma membrane through lipid peroxidation that alters sperm membrane fluidity, leading to dysfunctional metabolism and disrupting acrosome reaction reactivity [9,10].

Moreover, infertile men have substantially more damaged sperm DNA than fertile men, which adversely affects their reproductive outcomes [11,12]. Sperm DNA damage is proposed to be due to testicular gonadotoxins, hyperthermia, genital tract infection, varicocele, increased oxidants, aberrant protamine expression, excessive reactive oxygen species (ROS), or abortive apoptosis during spermatogenesis [13–15]. Oxidative attacks were demonstrated to form DNA base adducts such as 8-hydroxy-2'-deoxyguanosine and O6-methylguanine, which interfere with the ability of DNA to act as a substrate for DNA methyltransferases.
and inhibit methylation of adjacent cytosine residues, resulting in global DNA hypomethylation [16–18].

This study aimed to assess the association of seminal S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in infertile men.

Materials and methods
In all, 95 men recruited from the Andrology Department, University Hospital, were included in this study after Institutional review board approval and informed consent. They were divided on the basis of diagnosis into asthenozoospermia (n = 20), asthenoteratozoospermia (n = 20), and oligoasthenoteratozoospermia (n = 55). They were compared with 22 healthy fertile men as controls. Men with varicocele, leukocytospermia, exposed to radiation, consumed cytotoxic drugs, or smokers were excluded. History taking, clinical examination, and semen analysis were carried out.

Semen samples were obtained in sterile plastic containers by masturbation after 5 days of abstinence. After liquefaction, semen analysis was performed according to the WHO guidelines [19], and sperm morphology was evaluated using a phase-contrast microscope and Spermac stain (Fertipro Pro, NV, Beernem, Belgium); leukocytospermia was excluded by a myeloperoxidase staining test [20]. The sperm fraction was subjected to assessment of DNA fragmentation and acrosin activity and the seminal plasma was subjected to estimation of malondialdehyde (MDA), SAM, and SAH.

Sperm DNA fragmentation analysis [21]
Sperm DNA fragmentation analysis was carried out using the enhanced apoptotic DNA ladder detection kit (BioVision Research Products, Mountain View, California, USA). Sperm pellet with 5–10 × 10⁵ cells in a 1.5 ml microcentrifuge tube was washed with PBS and centrifuged for 5 min at 500 g, and the cells were lysed with 35 µl TE lysis buffer. Five microliters of enzyme A solution was added and mixed with gentle vortexing and incubated at 37 °C for 10 min; then, 5 µl of enzyme B solution was added to each sample and incubated at 50 °C for 30 min. Also, 5 µl ammonium acetate solution and 50 µl isopropanol were added and mixed well. The DNA pellet was washed with 0.5 ml 70% ethanol and air dried. The DNA pellet was dissolved in 30 µl of the sample was added on a 1.2% agarose gel containing 0.5 µg/ml ethidium bromide. Fifteen to 30 µl of enzyme A and B were added to the gel and the running buffer. The gel was run at 5 V/cm for 1 h. Ethidium bromide-stained DNA was visualized by transillumination with ultraviolet light and photographed (Fig. 1).

Estimation of seminal malondialdehyde [22]
The proteins of the seminal plasma were precipitated by adding trichloroacetic acid, which reacts with MDA to form a thiobarbituric acid-reactive product that was measured at 534 nm.

Figure 1
Genomic sperm DNA fragmentation in different groups by agarose gel electrophoresis. Lane 1: 100-bp ladder; lane 2: No DNA fragmentation, lanes 3, 4, 5, 6, 7: DNA fragments (DNA ladder).

Seminal S-adenosylmethionine and S-adenosylhomocysteine estimation [23]
Seminal plasma was mixed 1 : 2 with 0.4 mol/l HClO₄ for 30 min and centrifuged at 10 000 g for 15 min at 4 °C and then the supernatant was filtered. Twenty microliters of the acid extract was used directly for high-performance liquid chromatography (HPLC). SAM and SAH standards (Sigma, St Louis, Missouri, USA) were dissolved in water at a concentration of 1 mmol/l and then diluted with 0.4 mol/l HClO₄ to the final concentration for HPLC analysis. Twenty microliters of standard solutions containing 50–11 000 pmol were injected onto the HPLC equipped with a variable ultraviolet detector (Hewlett Packard, 1050 series, Palo Alto, California, USA) set to a wave length of 254 nm. The separation was carried out on a reversed phase column (Hibar, Merck, Darmstadt, Germany). The mobile phase consisted of 40 mmol/l NH₄H₂PO₄, 8 mmol/l 1-heptanesulfonic acid (Sigma), and 18% (v/v) methanol (HPLC grade), pH 3.0, with HCl. HPLC analysis was conducted at a flow rate of 0.7 ml/min room temperature on the basis of integration of peak areas compared with the standard calibration curves.

Determination of sperm acrosin activity [24]
Gelatin-covered slides were prepared by spreading 20 µl of 5% gelatin (Merck, Darmstadt, Germany) in distilled water on the slides. The slides were then air-dried, stored at 4 °C overnight, fixed, and washed in PBS. Purified spermatozoa were diluted 1 : 10 in PBS containing 15.7 mmol/l α-D-glucose. Semen samples were smeared on the prepared slides and incubated in a moist chamber at 37 °C for 2 h. The halo diameter around any 10 spermatozoa was measured in phase contrast using an eyepiece micrometer. The rate of halo formation was calculated per slide as the percentage of spermatozoa showing a halo. One hundred spermatozoa were evaluated. An acrosin activity index was calculated by multiplying the halo diameter by rate of the halo formation.

Seminal α-1,4-glucosidase estimation [25]
Seminal α-1,4-glucosidase was estimated using a commercial kit (Epi screen; Fertipro, Ghent, Belgium) by
assessing the intensity of color change induced by the reaction between β-glucosidase and 0.125 of 0.09% Na-azide added to 0.125 ml thawed seminal plasma. The mixture was mixed well by pipetting, one diagnostic tablet (p-nitrophenyl-β-glucopyranoside) was then added, and the mixture was remixed, vortexed for 60 s, and incubated for 4 h at 37°C. Then, 3 ml 0.02 mol/l NaOH was added and centrifuged for 6 min at 3000g. The absorbance value, obtained by reading the supernatant against reagent 2 as a blank, was calculated at 405 nm.

**Statistical analysis**

Statistical analysis was carried out using SPSS program version 17 (SPSS Inc., Chicago, Illinois, USA). The parametric data were expressed as mean ± SD. The nonparametric data were expressed as median and range. The Mann–Whitney test was used as a test of significance for comparison of two groups. The Spearman rank correlation coefficient (r) was calculated to study the relation between the investigated variables. P value less than 0.05 was considered as significant.

**Results**

Semen parameters of the different investigated groups are presented in Table 1. Seminal SAM, SAM/SAH ratio, acrosin activity, and β-glucosidase were significantly increased, whereas seminal SAH and MDA were significantly decreased in healthy fertile men compared with the other groups. Sperm DNA fragmentation percentage showed a significant increase in asthenoteratozoospermia and oligoasthenoteratozoospermia men compared with the fertile controls.

Semen samples with sperm DNA fragmentation showed a significant decrease in sperm count, sperm motility, sperm velocity, linear velocity, linearity index, sperm normal morphology, acrosin activity, β-glucosidase, seminal SAM, and SAM/SAH ratio and a significant increase in seminal SAH and MDA compared with semen samples without sperm DNA fragmentation (Table 2).

Semenal SAM and the SAM/SAH ratio showed a significant positive correlation with sperm count, sperm motility, sperm velocity, linear velocity, linearity index, sperm normal morphology, acrosin activity, and β-glucosidase and a negative correlation with seminal SAH and MDA. Seminal SAM demonstrated the inverse significant correlations (Table 3).

**Discussion**

Several studies have reported that spermatozoa from infertile men are more likely to express aberrant DNA methylation patterns [6–8]. This study describes the

### Table 1: Seminal tested parameters of the different groups investigated (median, range)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 22)</th>
<th>A (n = 20)</th>
<th>AT (n = 20)</th>
<th>OAT (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (million/ml)</td>
<td>73.1 (44.8–96)</td>
<td>53.3 (24.9–672)</td>
<td>29.9 (20.8–53.2)</td>
<td>9.3 (1.5–18.8)</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>61.5 (51–60)</td>
<td>38.5 (35–45)</td>
<td>26.0 (2–40)</td>
<td>15.0 (4–40)</td>
</tr>
<tr>
<td>Sperm velocity (µm/s)</td>
<td>77.2 (63.4–86.6)</td>
<td>74.1 (32.1–85.3)</td>
<td>36.9 (176–99.9)</td>
<td>36.1 (8.8–70.5)</td>
</tr>
<tr>
<td>Linear velocity (µm/s)</td>
<td>60.6 (42.9–70.7)</td>
<td>47.5 (18.1–68.2)</td>
<td>23.0 (11.1–49.4)</td>
<td>21.4 (5.7–52.6)</td>
</tr>
<tr>
<td>Linearity index (%)</td>
<td>81.2 (675–86.7)</td>
<td>65.8 (43.3–77.6)</td>
<td>61.5 (48.7–86.5)</td>
<td>52.2 (38.6–94.7)</td>
</tr>
<tr>
<td>Sperm normal morphology (%)</td>
<td>64.0 (58–65)</td>
<td>44.0 (34–65)</td>
<td>12.0 (2–20)</td>
<td>4.0 (0–20)</td>
</tr>
<tr>
<td>SAM (nmol/l)</td>
<td>103.6 (76.8–160.0)</td>
<td>96.7 (80.4–115.7)</td>
<td>84.3 (66.1–108.6)</td>
<td>74.3 (46.9–99.0)</td>
</tr>
<tr>
<td>SAH (nmol/l)</td>
<td>278.9 (35.9)</td>
<td>278.9 (11.6–38.0)</td>
<td>34.3 (23.5–51.9)</td>
<td>40.4 (30.1–54.7)</td>
</tr>
<tr>
<td>SAM/SAH</td>
<td>4.2 (1.27–9.02)</td>
<td>3.7 (2.1–7.9)</td>
<td>2.48 (1.57–3.16)</td>
<td>1.91 (1.08–2.64)</td>
</tr>
<tr>
<td>Acrosin activity index (%)</td>
<td>12.3 (8.9–19.4)</td>
<td>10.7 (5.5–13.9)</td>
<td>5.4 (0.94–11.1)</td>
<td>1.23 (0.55–9.5)</td>
</tr>
<tr>
<td>MDA (nmol/10⁹ sperm)</td>
<td>23.5 (12.2–76.8)</td>
<td>36.5 (20.1–143.7)</td>
<td>71.2 (21.6–166.4)</td>
<td>285.7 (77.6–2281.9)</td>
</tr>
<tr>
<td>DNA fragmentation (ratio, %)</td>
<td>3/22 (13.6%)</td>
<td>4/20 (25%)</td>
<td>8/20 (40%)</td>
<td>29/55 (52.7%)</td>
</tr>
</tbody>
</table>

A, Asthenozoospermia; AT, Astheno-teratozoospermia; MDA, malondialdehyde; OAT, oligoasthenoteratozoospermia; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine.

*aSignificance compared with the controls.

*bSignificance compared with the A group.

*cSignificance compared with the AT group.

### Table 2: Tested parameters in semen samples with/without sperm DNA fragmentation (median, range)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Samples with sperm DNA fragmentation (n = 44)</th>
<th>Samples without sperm DNA fragmentation (n = 73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (million/ml)</td>
<td>22.72 (4.48–83.2)</td>
<td>48.0 (1.5–96)</td>
<td>0.0107*</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>30.0 (4–60)</td>
<td>38.0 (2–65)</td>
<td>0.0469*</td>
</tr>
<tr>
<td>Sperm velocity (µm/s)</td>
<td>43.1 (8.8–85.3)</td>
<td>68.7 (9.2–86.6)</td>
<td>0.0461*</td>
</tr>
<tr>
<td>Linear velocity (µm/s)</td>
<td>25.8 (8.3–68.4)</td>
<td>44.2 (5.7–70.7)</td>
<td>0.0289*</td>
</tr>
<tr>
<td>Linearity index (%)</td>
<td>50.3 (38.6–74.7)</td>
<td>71.0 (38.6–86.7)</td>
<td>0.0485*</td>
</tr>
<tr>
<td>Sperm normal morphology (%)</td>
<td>12.0 (0–64)</td>
<td>42.0 (0.0–86)</td>
<td>0.0137*</td>
</tr>
<tr>
<td>SAM (nmol/l)</td>
<td>77.23 (46.9–120)</td>
<td>91.4 (677–160)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>SAH (nmol/l)</td>
<td>36.3 (21.4–51.9)</td>
<td>30.2 (9.5–54.7)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>SAM/SAH</td>
<td>1.99 (1.07–4.87)</td>
<td>3.17 (1.58–9.02)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Acrosin activity</td>
<td>1.06 (18.96)</td>
<td>9.3 (0.55–19.5)</td>
<td>0.0099*</td>
</tr>
<tr>
<td>MDA (nmol/10⁹ sperm)</td>
<td>41.7 (12.2–967)</td>
<td>87.4 (179–1264.9)</td>
<td>0.0431*</td>
</tr>
<tr>
<td>β-glucosidase (µmol/ml)</td>
<td>44.2 (18.4–90.7)</td>
<td>32.2 (18.4–83.9)</td>
<td>0.0048*</td>
</tr>
</tbody>
</table>

MDA, malondialdehyde; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine. 

*P < 0.05 = significance.
There are no conflicts of interest.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

Table 3 Correlations of SAM, SAH, and SAM/SAH ratio with the semen parameters studied

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAM</th>
<th>SAH</th>
<th>SAM/SAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (million/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.597</td>
<td>-0.620</td>
<td>0.717</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>0.551</td>
<td>-0.579</td>
<td>0.678</td>
</tr>
<tr>
<td>r</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Sperm normal morphology (%)</td>
<td>0.589</td>
<td>-0.639</td>
<td>0.731</td>
</tr>
<tr>
<td>r</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Acrosome activity index</td>
<td>0.505</td>
<td>-0.603</td>
<td>0.664</td>
</tr>
<tr>
<td>r</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>α-glucosidase (m U/ml)</td>
<td>0.568</td>
<td>-0.554</td>
<td>0.662</td>
</tr>
<tr>
<td>r</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>MDA (nmol/10⁹ sperm)</td>
<td>-0.537</td>
<td>0.544</td>
<td>-0.641</td>
</tr>
<tr>
<td>r</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>P</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

MDA, malondialdehyde; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine. r, Correlation coefficient; P<0.05 = significance.

abundance of seminal plasma S-adenylated amino acids in seminal plasma with the amount of aberrations in sperm parameters. Depending on the degree of sperm aberrations in general, there was a trend toward higher levels of S-adenylated homocysteine and lower levels of S-adenylated methionine in infertile men compared with the fertile controls. In addition, seminal SAM and the SAM/SAH ratio were positively correlated with sperm parameters. Dhillon et al. [26] showed that infertile men are more prone to inefficient folate cycle recovery of homocysteine into methionine as polymorphisms in their methylenetetrahydrofolate reductase gene are more common. Kelly et al. [27] added that the adverse effects of methylenetetrahydrofolate reductase deficiency on spermatogenesis are mediated by alterations in the transmethylation pathway.

There was a significant increase in seminal SAH and MDA in infertile men compared with healthy fertile controls. The link between oxidative DNA damage and hypomethylation was established for somatic cells reporting a link between oxidative DNA adducts and impaired DNA methyltransferase activity [28]. Also, the presence of oxidative stress in a significant proportion of infertile men was believed to be a cause of sperm DNA fragmentation, which plays a role in sperm DNA hypomethylation [29,30]. Tunc and Tremellen [28] added that oxidative damage to sperm DNA is responsible for sperm global DNA hypomethylation, with a significant correlation between total semen ROS production and sperm DNA methylation. This would imply that spermatozoa themselves are the primary source of ROS production interfering with the DNA methylation process and thus, biologically, intrinsic ROS production within the sperm cytoplasm is more likely to interfere with this process in the adjacent nucleus.

Also, cases with sperm DNA fragmentation were associated with decreased sperm parameters, seminal SAM, and SAM/SAH ratio. Tavaalee et al. [31] and Benchai et al. [32] reported a significant negative correlation between sperm DNA fragmentation and sperm DNA methylation, suggesting that hypomethylated spermatozoa are more prone to DNA damage. As normally methylated sperm DNA is less susceptible to DNA damage, it might be hypothesized that DNA methylation protects it from apoptotic and/or oxidative damage, the two principal causes of sperm DNA damage.

Seminal epididymal marker α-glucosidase showed a positive correlation with seminal SAM and SAM/SAH ratio and a negative correlation with seminal SAH level. The epididymis plays a crucial role in the maturation of spermatozoa and their acquisition of progressive motility and fertilizing capacity, with a significant relation between α-glucosidase activity and semen parameters [33,34]. Ariel et al. [4] and Xie et al. [35] added that sperm remethylation is a part of the process of sperm maturation that occurs in the epididymis.

Seminal SAM and SAM/SAH ratio were shown to have a positive correlation with sperm acrosin activity. Chaudhury et al. [36] considered acrosin activity a sensitive biochemical marker for the clinical evaluation of unexplained male infertility, whereas Chen et al. [37] correlated its activity with semen quality, reflecting aspects not diagnosed by routine semen analysis or acrosome ultrastructure. Therefore, there is an inverse correlation between affected sperm acrosin activity and deleterious factors that could affect sperm DNA integrity such as sperm hypomethylation, DNA fragmentation, oxidative stress, varicocele, or smoking [12,38–40].

A negative link was suggested between sperm DNA methylation status and the likelihood of pregnancy [6,41]. A complicating factor in determining the direct effect of sperm DNA methylation on pregnancy outcome is its positive association with sperm DNA integrity but it is not possible to determine whether sperm DNA fragmentation alone or hypomethylation is primarily responsible for pregnancy outcome [42]. Le Bouc et al. [43] suggested that unfaithful maintenance of DNA methylation marks following fertilization involves the dysregulation of a trans-acting regulatory factor that could be altered by assisted reproductive technology. It has been reported that quality control of intracytoplasmic sperm injection sperm through detection of its epigenetic factors, such as methylated DNA, is essential for reducing its genetic and epigenetic risk [44].

A limitation in this study is that sperm DNA methylation was not measured to correlate reduced SAM or SAM/SAH levels with hypomethylation in the same samples.

It is concluded that alteration in the seminal transmethylation pathway in infertile men is associated with increased oxidative stress, sperm DNA fragmentation and decreased sperm acrosin activity, seminal α-glucosidase.
References


Role of asymptomatic genital tract inflammation in normozoospermic infertile men undergoing intracytoplasmic sperm injection: Erratum

Hum Androl 2011; 1:108

In the article by El-Tonsy et al. that appeared in the August 2011 issue [1], there is an error on page 39 in the authors and affiliations section.

It should read:

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Reference