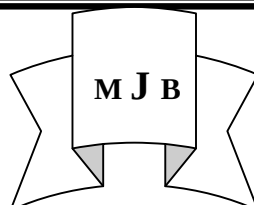


In Vitro Sperm Activation of Infertile Semen Samples Complaining from Varicocele

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Abstract

The goal of the present study is to investigate the effect of *in vitro* sperm washing technique on the semen of men complaining from different grades of varicocele. The study was conducted at the IVF Institute , Al-Nahrain University. Fifty infertile men were divided to three groups according to the grade of varicocele namely; sub- clinical and palpated with Valsalva maneuver (grade I, n=24) palpated without Valsalva maneuver (grade II, n=13) and visible (grade III , n=13). Protocol of wash and spin technique was used for *in vitro* sperm preparation and certain sperm characters were recorded before and following washing technique.

Mean of sperm concentration between the three varicocele groups were shown no significant ($P>0.05$) different before and following washing technique. The percentage of sperm motility was significantly ($P<0.01$) decreased before and after *in vitro* washing technique in men complaining from varicocele grade III compared to men with grade I and II. The grade activity of forward progressive movement (a+b) was significantly ($P<0.05$) lower in the semen of grade III group than that of grade I and grade II groups. There was a significant difference ($P<0.01$) in the percentage of morphologically normal sperm (MNS) between grade III and other grades of varicocele. It was concluded from this study that the grade of varicocele determine the response of the semen to *in vitro* washing technique. Other studies are recommended to improve our results practically in intra-uterine insemination (IUI) and *in vitro* fertilization (IVF).

الخلاصة

تهدف الدراسة الحالية الى بحث تأثير تقنية غسل وتنشيط النطف في الزجاج على مني المرضى الذين يعانون من درجات مختلفة من حالة الدوالي. أجري البحث عام 2005 في قسم فسلجة التناسل السريري والعيادة الاستشارية في معهد أبحاث الأجنة وعلاج العقم – جامعة النهرين. قسم خمسون من الرجال العقيمين الى ثلاث مجاميع طبقا لدرجة الدوالي التي يعانون منها وهي: الدرجة الأولى وتكون الدوالي أما غير منظورة سريريا أو ممكن تحسبها بعد الفحص باللمس وعند الوقوف بأستخدام Valsalva maneuver (n=24). الدرجة الثانية: ممكن تحسبها باللمس دون الحاجة لاستخدام Valsalva maneuver (n=13). الدرجة الثالثة: ممكن مشاهدة الدوالي بالعين المجردة (n=13). استخدمت طريقة غسل ونبد النطف للتنشيط في الزجاج وتم تسجيل نتائج النطف قبل وبعد التنشيط.

لوحظ عدم وجود اختلاف معنوي ($P>0.05$) في معدلات تركيز النطف في مجاميع الدوالي الثلاث قبل وبعد التنشيط. سجل انخفاض معنوي ($P<0.01$) في نسبة حركة النطف قبل وبعد تنشيط النطف للمرضى الذين يعانون من الدوالي درجة الثالثة بالمقارنة مع المرضى الذين يعانون من الدوالي الدرجة الأولى والثانية. كما لوحظ انخفاض معنوي في درجة نشاط النطف ذي الحركة التقدمية الأمامية من الدرجة (a+b) في الرجال الذين يعانون من الدوالي درجة الثالثة بالمقارنة من الدرجة الثانية والأولى. وقد وجد اختلاف معنوي ($P<0.01$) في نسبة شكلية النطف الطبيعية في مني مرضى الدوالي الدرجة الثالثة وبقيّة الدرجات. تشير الدراسة الحالية بأن درجة الإصابة بالدوالي تحدد الاستجابة لتقنية الغسل والتنشيط في الزجاج. ونوصي بأجراء دراسات أخرى لتأكيد نتائجنا عند التلقيح الرحمي أو الإخصاب في الزجاج.

Introduction

Varicocele is considered as a major cause of male infertility[1], and is found in approximately 15% of normal men, 35 % of infertile men with primary infertility and in up to 81 % of men with secondary infertility[2, 3]. Moderate to large varicoceles are found in about 12–25% of men being examined for infertility, and another 15% have small or subclinical varicoceles [4,5]. Men with varicoceles have poorer semen quality than those without varicoceles [5, 6] indicating adverse effects of varicoceles on the testis.

The pathophysiology of varicoceles is not clearly understood and it is probably multifactorial. Theories of pathophysiology of varicoceles include abnormally high scrotal temperature, hypoxia due to venous stasis, dilution of intratesticular substrates (e.g. testosterone), imbalances of the hypothalamic-pituitary-gonadal axis, and reflux of renal and adrenal metabolites down the spermatic vein. In addition, nitric oxide[7], reactive oxygen species[8], and regulators of apoptosis[9] have all recently been implicated in the pathophysiology of varicoceles. The majority of men with varicoceles remain fertile and asymptomatic. Follow-up studies of groups of treated and untreated patients with varicoceles suggest that pregnancies are as frequent without treatment as they are with treatment of the varicocele [4, 10]. On the other hand, most patients choose alternative managements for the infertility, which is sperm preparation *in vitro* and IUI or any other assisted reproductive techniques [11]. However, there are no literatures concerning the effect of *in vitro* preparation techniques on the semen of men complaining from different grades

of varicocele. Therefore, our study aimed to use washing technique *in vitro* for the semen of different grades of varicocele. The results can be utilized in choosing semen samples with positive washing response for IUI and the other varicocele grades with negative response for IVF or other assisted reproductive techniques.

Materials and Methods

The study was conducted at IVF Institute, Baghdad University through year 2005. All patients were assessed for varicocele by physical examination and ultrasound Doppler.

-Physical examination :patients were examined in a warmed room in supine and standing position (for 5 minutes). The scrotal contents were palpated between thumb and forefingers to assess volume, position and consistency of testis and epididymis on both sides. Each spermatic cord was palpated in the standing position and during the Valsalva maneuver. The patients were divided according to the grade of varicocele. The severity of the varicoceles is graded I, II and III using the system of Dubin and Ameral (12) as the followings: grade I (small) varicoceles palpable only during Valsalva maneuver and men with subclinical varicocele were included in this group (n=24), grade II (medium) varicocele palpable in standing position (n=13) and grade III (large) varicocele detectable by visual scrutiny alone (n=13).

Ultrasound Doppler: The Ultrasound study was performed to optimized the detection of blood flow. The examination done with patient in supine and standing position too. Each side of the scrotum was scanned and vascular channels in the spermatic cord were noted, and the vessel diameters were measured by electronic clippers. Any testicular abnormalities,

spermatocele , epididymal lesions and hydrocele were recorded. The Valsalva maneuver was used in the supine and erect positions. The maximal diameter of the scrotal veins was recorded and the presence of the venous plexus was noted. The flow velocity of the main vein was measured before and after the Valsalva maneuver. The sonographic diagnosis of varicocele was based on the detection of two to three venous channels one of which measured $>3\text{mm}$ in diameter and reflux during the Valsalva maneuver. Patients with 2.7mm or less in diameter were regarded as subclinical varicoceles as they were not detected by palpation[13].

***In vitro* sperm preparation:** The semen samples of patients with different grades of varicocele were examined for semen fluid analysis as recommended by WHO [14]. All the semen samples were with normal macroscopic analysis value .The semen volume was ranged between (2-5 ml) ,The pH ranged between (7.4-8) with normal semen viscosity and liquefaction time .Wash and spin technique for sperm preparation *in vitro* was performed as described by Avery and Elder ,[15] using sperm washing medium (Medicult-Denmark).The results of certain sperm function parameters namely; sperm concentration(million/ml), sperm motility percentage(%) , grade activity of forward progressive movement percentage (a and b) and morphologically normal sperm before and following the preparation were recorded.

Statistical analysis was performed using student's t-test .When P reach the value of 0.05 the results considered significant[16].

Results

Data collected revealed that ,the mean of sperm concentration(million/ml) in the semen of men with different grades of varicocele not reach the significant($P>0.05$) difference between before and after *in vitro* preparation (figure 1-A).

There was a highly significant($P<0.01$) decrease in the percentage of sperm motility in semen samples of men complaining from varicocele grade III compared with other grades (I and II) before and following the preparation *in vitro* as shown in figure 1-B. However, ,the mean of sperm motility in men complaining from grades I(80%) ,II(84.5%) and III(52%) following *in vitro* washing technique was highly significantly ($P<0.01$) increase compared with its corresponding groups before sperm washing(45.24%, 48.5% and 36%, respectively).In figure 1-C, the sperm grade activity of forward progressive movement (percentage of a and b) following washing technique was significantly($P<0.01$) higher than that of the samples before washing of men complaining from different grades of varicocele. Percentage of grade activity of grade I (64.8%) and II(58.85%) varicocele groups following the preparation *in vitro* was significantly($P<0.05$) increment compared with grade III(54.37%) varicocele group(figure 1-C).

The percentage of morphologically normal sperms(MNS) in the semen samples of men complaining from different grades of varicocele .There was a significant ($P<0.01$) difference in the percentage of MNS between before and following washing technique *in vitro*. The percentage of MNS group following preparation technique was significantly ($P<0.01$) lower than that of grade I and grade II groups(figure 1-D).

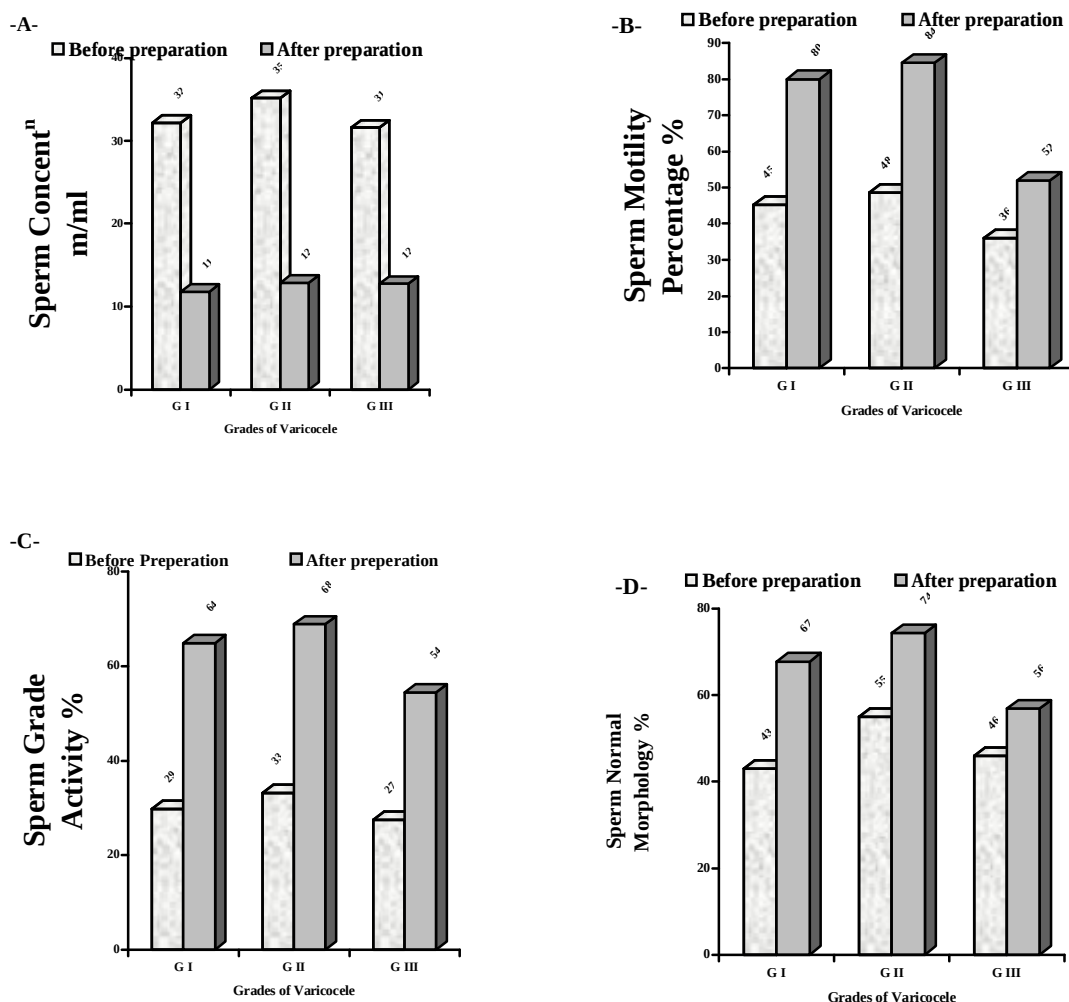


Figure 1 Effect of *in vitro* preparation technique on certain sperm characters of semen in men complaining from different grades of varicocele **A**-sperm concentration **B**-sperm motility **C**-grade activity (a+b) **D** –morphologically normal sperm

Discussion

The effect of different grades of varicocele on certain sperm function characters was clearly observed in this study. It has been found that the quality of semen was inversely associated with the grade of varicocele. This finding is compatible with other studies [17,18]. The mean of sperm motility and percentage of grade activity of forward progressive movement (a +b) with morphologically normal sperm were significantly decreased in all

varicocele men groups may be as a result of high production of free radicals [19] or the imbalance between reactive oxygen species (ROS) production and seminal antioxidants in the semen resulting in oxidative stress leading to spermatozoa damage which in turn causing male infertility [8,20]. *In vitro* washing technique was significantly improved sperm motility and the percentage of grade activity (a+b) of semen complaining from varicocele grade I and II. This result

can be utilized for IUI .At the same time the improvement of motility in varicocele grade III specimens was lower than other grades .This observation may be due to the detrimental effect of ROS on the specimens varicocele grade III semen samples which prevent the chances of comparative materials in the culture media to enhance sperm motility or sperm morphology .Consequently, sperm damage in grade III semen was greater than that of grade I and grade II semen. Although , wash and spin technique eliminate the dead and immotile sperm with semen debris and abnormal forms [15],the severity of varicocele have a detrimental effects following washing the sperm because of irreversible status of damaged sperm resulted from oxidative stress[21] . It was concluded from the present work that not all grades of varicocele response positively to *in vitro* preparation techniques .Therefore it could only be use washing technique for varicocelic semen grades I and II for IUI and grade III varicocelic semen for IVF and ART. Other studies are recommended to improve our results practically in IUI and IVF .

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