ISSN 1991- 8690 الترقيم الدولي ١٩٩١ - ٨٦٩٠
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The effect of varicocele treatment on human sperm plasma membrane integrity and pregnancy rate in infertile men after varicoceloctomy

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Abstract

This study was designed to evaluate the results of semen parameters and hypo-osmotic swelling (HOS) test for semen samples of infertile patients with and without varicocele undergoing intra-uterine insemination (IUI) techniques. Twenty-four semen sample from infertile patients with varicocele and fifty-four without varicocele were collected by masturbation and evaluated after and before *in vitro* sperm activation.

Spermatozoa prepared by conventional layering technique by using 1ml of the liquefied semen were layered beneath a culture medium (IVF medium). The HOS test was performed after and before *in vitro* sperm preparation by mixing 0.1 ml of semen with 1.0 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution. Sperm function tests (SFT) including sperm concentration, sperm motility (%), progressive sperm motility (%), and normal sperm morphology (%) were evaluated according to standard World Health Organization (WHO) criteria and subjected to HOS test. For IUI, the sperm prepared and incubated for 30 minute in 5% CO_2 at 37ºC after *in vitro* sperm preparation.

The results of the present study reported that successful pregnancy rates was more chance for semen sample with normal HOS test scores than abnormal HOS test. Eighteen clinical pregnancy rates were achieved for non-varicocelic infertile patients with normal HOS test scores and have SFT recovered than *in vitro* sperm activation as compared to varicocelic infertile patients with abnormal HOS test scores. Therefore, it was concluded that the use of HOS-test as an easy and reliable test to identify successful pregnancy after IUI technique. Further studies are recommended to assess the presence of varicocele on the percentage of hamster ova penetration assay after IVF-ET.

Introduction

The presence of varicocele in the general male population is 15-20% (1). Also, varicocele causes infertility in 30-50% of all marriages (2). An abnormal spermogram is more frequent in men with varicocele than in those with normal parameter findings (3). According to WHO (4), the infertility of a great number of couples is caused by varicocele, which is the main cause of male infertility (30-50%). Moreover, Varicocele is associated with a decrease in fertility and testicular functions. If varicocele is a consequence of a congenital lack in the vein drainage of the testicular groove, it can appear between the ages of 13 and 15 (5) and its occurrence is caused by the presence of vein reflux (6). In general, damage to spermatogenesis caused by the negative influence of varicocele occurs as the result of the integrated effect of increased testicular temperature and the slower flow and stagnation of blood in the pampiniform plexus due to the effect of prostaglandins and seronine. In the pathogenesis of varicocele, gradual damage is done to the germ cell epithelia and Leydig cells (7).

The sperm functions tests are the best way to investigate male fertility and considered the most important factors in male reproduction potential (8). In general, semen analysis is the cornerstone and primary laboratory test for the evaluation of male fecundity (9), and useful in predicting the results of assisted reproductive technology (10). Sometimes, fertilization occurs despite an abnormal semen analysis, or it fails to occur when analysis values are normal. Moreover, sperm functions tests are based on the detection of sperm count, percentage of sperm motility, progressive sperm motility (%), percentage of normal sperm morphology (%) and sperm viability (%) (11, 12).

The Hypo-osmotic swelling test is a simplest and less expensive test for assessing the functional integrity of sperm plasma membrane (13). The HOS test introduced by Jeyendran *et al*. (1984) has added a new parameter for the evaluation of human spermatozoa. The functional integrity of sperm plasma membrane is an important factor in sperm metabolism, capacitation, acrosome reaction, and binding of spermatozoa to the egg surface (14). Nevertheless, in the presence of normal semen parameters, an HOS test less than 50% is rarely associated with pregnancy *in vivo* (15). The HOS test was demonstrated as an important test and indicator for male factor infertility and success of intra-uterine insemination. However, HOS test predict pregnancy rate and outcome in couples undergoing IVF and IUI procedure (16, 17). Therefore, in the current study, sperm function test (SFT), sperm HOS test, and IUI were applied to observe the diagnostic tools of HOS test in predicting pregnancy rates and outcome in couples undergoing IUI performance.

Materials and Methods

1. Subjects

Twenty-four infertile patients with varicocele and fifty-four infertile patients without varicocele were obtained from IVF Institute of Embryo Research and Infertility Treatment/Al-Nahrain University. The study extended from September, 2006 to March, 2007.The selection of varicocelic infertile patients was based on the physical examination performed with the patients in both recumbent and upright position. The varicocele was confirmed by palpable on physical examination of the secrotum by the clinical evaluation in order to measure the severity of varicocele. Therefore, ancillary diagnostic measures, such as scrotal ultrasonography and Doppler examination were done.

2. Laboratory assessments

1. Semen analysis and collection

The sample of seminal fluid was collected after 3-5 days of abstinence directly in a clean, dry and sterile disposable Petri-dish by

masturbation in a private and quite room adjacent to the semen analysis laboratory. The container labeled with the following information, name, age, abstinence period and time of sample collection. The specimens were placed in an incubator at 37ºC for 30 minutes to allow the semen liquefaction (18). The liquefied semen is then carefully mixed for few seconds, and then specimen was examined in details by microscopic and macroscopic examinations including semen volume, semen liquefaction time, semen viscosity, semen pH, sperm concentration, sperm motility (%), progressive sperm motility (%), normal sperm morphology (%). The WHO criteria for normal semen values were applied (19).

2. *In vitro* sperm activation and preparation

Sperm processing for intra-uterine insemination were done using a conventional layering technique. For this, 1ml of the liquefied semen was layered beneath a culture medium (IVF medium, Medi-cult, Jyllinge, Denmark), after incubation for 30 minute in 5% CO_2 at 37ºC.The supernatant was removed and was used for treatment. Intra-uterine insemination was performed using a Portex catheter. The cervix was exposed and the catheter was passed into the uterus to about 0.5 cm from the top of the uterine cavity. The sperm were then expelled. However, the sperm parameters were examined after 10 minutes of incubation and a one drop (10µl.) of the mixture was added to a clean class and examined at light microscope under 400X objective for assessment of HOS test score for both groups of infertile patients.

3. Hypo-Osmotic Swelling (HOS) Test

The HOS test was performed before and after *in vitro* sperm preparation technique and examination of standard semen parameters by mixing 0.1 ml of semen with 1.0 ml of a 150 mOsm/ L NaCl as a hypo-osmotic solution (20). The mixture was incubated for 30 minute at 37 ºC in 5% CO_2 in accordance with a previously described technique (21). One hundred spermatozoa were examined, and the morphological changes in sperm tail were classified according to the types described by Jeyendran *et al.* (22). The overall rate of sperm swelling was calculated. Finding at least 50% swollen spermatozoa was considered normal.

4. Statistical analysis of the data

Statistical analysis was performed with the SPSS version 12.00 by the Statistical Package for Social Sciences Software. The data analysis was done using paired sample t-test to assess the statistical differences in the results of SFTs and sperm HOS test for both varicocelic and non-varicocelic infertile patients. Mean and standard error of mean (S.E.M) obtained from crude data to compare between Pre-and Post-activation for semen parameters. P-value < 0.05 was used as a level of statistically significant.

5. Results

Twenty-four varicocelic infertile patients with mean age (30.83 ± 1.33 years) and duration of infertility (5.66 ± 0.33 years) and fifty-four infertile patients without varicocele with mean age (31.24 ± 0.89 years) and duration of infertility (3.16 ± 0.21 years) were involved in the present study. The results of the present study showed that varicocelic infertile patients have least value for sperm functions and sperm HOS test (36.40 %) as compared to non varicocelic infertile patients (51.29 %) before *in vitro* sperm preparation. However, it was noticed a highly significant ($P<0.001$) differences in sperm functions were assessed post *in vitro* sperm activation for both groups as compared to pre-activation. Meanwhile, significant ($P<0.001$) and markedly reduction in sperm concentration were observed for *in vitro* post-activation. In contrast, a highly significant ($P<0.001$) differences in the percentage of sperm motility (%), progressive sperm motility (%), normal sperm morphology (%), and HOS test were noticed after sperm activation and processing. In general, in the present study, the best results for clinical

pregnancy rates were observed in the infertile patients without varicocele (18%) after IUI techniques. It was advisable, clinical pregnancies were achieved for infertile couples where their male have normal percentages of the sperm HOS test scores as compared to male partners have abnormal HOS-test scores with varicocele, who have no clinical pregnancy rates were achieved in the present study.

Table (1): Sperm functions tests and sperm hypo-osmotic swelling test pre-and post-activation *in vitro* for human spermatozoa for non-varicocelic infertile patients* undergoing IUI technique

Parameters	Conventional layering technique	
	Pre-activation *in vitro*	Post-activation *in vitro*
Sperm Concentration ($\times 10^6$ sperm/ml)	59.18±2.37	32.64±1.48 a
Sperm Motility (%)	63.05±0.96	85.92±0.89 a
Progressive sperm Motility (%)	39.64±0.99	60.38±1.24 a
Normal Sperm Morphology (%)	54.25±1.46	86.20±0.85 a
Sperm HOS test (%)	51.29±1.01	66.05±1. 07 a

Number of infertile patients=54
Values are Mean ± S.E.M

a: Means a highly significant (P< 0.001) difference between pre-and post-activation *in vitro* for human spermatozoa.

Table (2): Sperm functions tests and sperm hypo-osmotic swelling test pre-and post-activation *in vitro* for human spermatozoa for varicocelic infertile patients* undergoing IUI technique

Parameters	Conventional layering technique	
	Pre-activation in vitro	Post-activation in vitro
Sperm Concentration ($\times 10^6$ sperm/ml)	18.20±2.94	9.75±1.04 a
Sperm Motility (%)	43.54±1.54	71.50±1.82 a
Progressive sperm Motility (%)	26.08±1.48	51.87±1.83 a
Normal Sperm Morphology (%)	43.64±1.70	83.54±1.14 a
Sperm HOS test (%)	36.40±1.24	45.58±1.04 a

Number of infertile patients=24
Values are Mean ± S.E.M

a: Means a highly significant (P< 0.001) difference between pre-and post-activation *in vitro* for human spermatozoa.

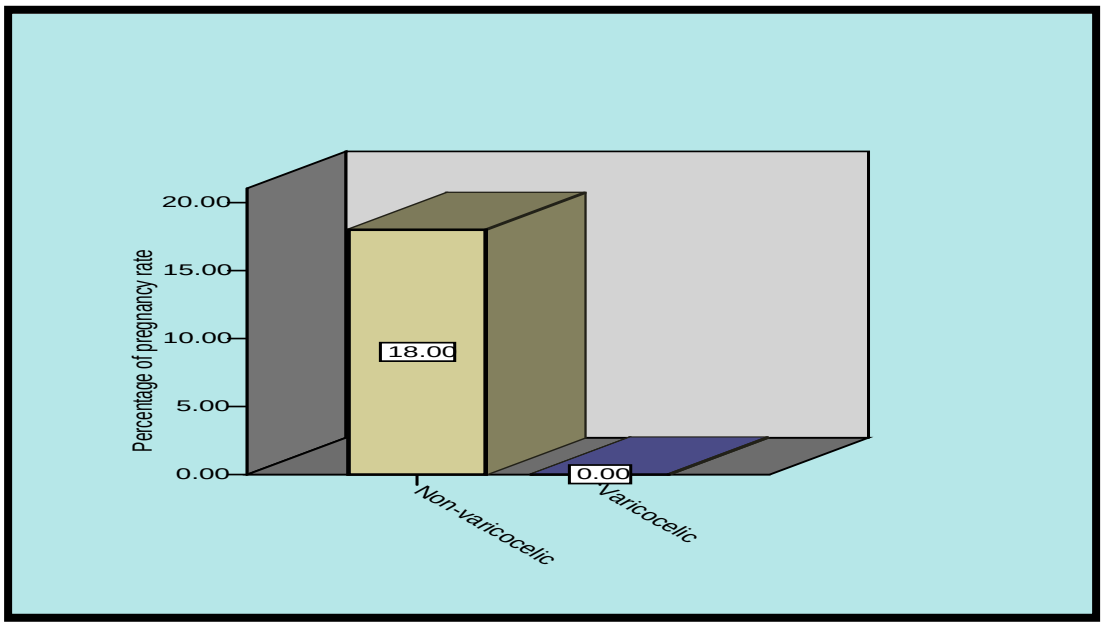

Figure (1): Outcomes of intra-uterine insemination for varicocelic and non-varicocelic infertile patient enrolled in this study

Discussion

In the present study, sperm functions tests, hypo-osmotic swelling (HOS) test, and IUI were evaluated to scrutinize the competence of HOS test in predicting pregnancy rate and outcome in varicocelic and non-varicocelic infertile patients undergoing intra-uterine insemination. A highly significant ($P<0.001$) differences in sperm concentration, percentage of sperm motility (%), progressive sperm motility (%), normal sperm morphology (%) were observed as compared to pre-activation for both study groups. However, only active motile mature normal sperm were able to swim to upper layer of the culture medium. A highly reduction in sperm concentration post *in vitro* sperm activation as a result of sperm preparation techniques and selection of highly recovery of spermatozoa.

The percentage of HOS-test statistically significant ($P<0.001$) differences between pre-activation and post-activation of human spermatozoa for varicocelic and non-varicocelic infertile patients (Table 1, 2), but the best results and response were reported in non-varicocelic infertile patients. The peroxidative damage of unsaturated fatty acids in sperm plasma membrane induced by reactive oxygen species (ROS) lead to low scores of HOS test and defective sperm function in patients with varicocele as compared with non-varicocelic infertile patients (23). However, ROS is associated with varicocele and correlate with fertility potential. A significantly elevated ROS levels and depressed total antioxidant capacity levels for infertile patients with varicocele related infertility as compared to those without varicocele (24). The markedly increased ROS generation in infertile patients with varicocele compared with without varicocele who suggesting a possible association of ROS with fertility (25).

The percentage of swollen spermatozoa was lower in the infertile patients with varicocele than without varicocele (26). Varicocele may cause injury to the sperm plasma membrane

integrity and lead to low scores of HOS test. Also, may cause deleterious effects by affecting sperm penetration and motility in the female genital tract which in turn may affect gamete interaction (27). In addition, improvement in sperm membrane swelling was detected in a group of non-varicocelic infertile patients who achieved pregnancies as compared with those who failed to achieve pregnancies (28). In contrast, significantly better semen characteristics (concentration, motility, and normal morphology) as well as enhanced acrosin activity for non-varicocele infertile patients (29). Varicocele impairs and alters the mechanism of sperm binding to zona pellucida (ZP) and gamete membrane fusion, resulting in impaired potential fertilization (30). Additionally, the numbers of sperm cells bound to the zona pellucida (ZP) are significantly lower as compared with non-varicocelic infertile patients (31). The semen parameters for non-varicocelic infertile patients have SFTs and HOST better than post-activation *in vitro* of varicocelic infertile patients (Table 1).

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تاثيرعلاج دوالي الخصية على كفاءة الغشاء البلازمي للنطفة البشرية ومعدلات الحمل لمرضى العقم بعد اجراء التدخل الجراحي

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الخلاصة

هدف هذه الدراسة الى تقييم معايير السائل المنوي و فحص كفاءة غشاء النطفة البلازمي لمرضى المصابين وغير المصابين بدوالي الخصية الذين تم ادخالهم في برنامج الاستمناء داخل الرحم. تضمنت هذه الدراسة(٢٤) مريضاً مصاباً بدوالي الخصية و(٥٤) مريضاً غير مصاب وجمعت العينات بطريقة الاستمناء وتم فحصها قبل وبعد اجراء عملية التنشيط خارج الجسم.عينات السائل المنوي تم تحضيرها و تنشيطها بوساطة التقنية الطباقية التقليدية. تم أجراء فحص كفاءة غشاء النطفة البلازمي قبل وبعد التنشيط. اختبارات وظائف النطف والمتضمنة تركيز النطف, النسبة المئوية لحركة للنطف, النسبة المئوية للحركة التقدمية للنطف, والنسبة المئوية للنطف السوية شكلياً تم تقييمها وفقاً لمقررات منظمةالصحة العالمية. سُجلت افضل النتائج الخاصة بالنسب المئوية للحمل للعيينات ذات فحص كفاءة غشاء النطفة البلازمي الطبيعي مقارنة بغير الطبيعي. ثماني عَشَرة حالة حمل تم الحصول عليها بالنسبة للمرضى غير المصابين بدوالي الخصية و ذات فحص كفاءة غشاء النطفة البلازمي طبيعي مقارنة بالمرضى المصابين بدوالي الخصية و ذات فحص كفاءة غشاء النطفة البلازمي غير طبيعي. لذلك نستنتج انه بالامكان استعمال فحص كفاءة غشاء النطفة البلازمي كاختبار ذات كفاءة عالية لمعرفة معدلات الحمل بعد اجراء الاخصاب داخل الرحم.