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Correlation between Seminal Parameters and Response to *In Vitro* Sperm Activation According to Age and Type of Infertility

Sanabel H. Hilo^{1*}, Muhammad-Baqir M-R Fakhrildin², Sabah N. Alwachi³

¹Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

²High Institute for Infertility Diagnosis and ART, Al-Nahrain University, Baghdad, Iraq.

³American university, Beirut, Lebanon.

Abstract

This study was aimed to investigate the effect of sperm parameters on the response to *in vitro* sperm activation (ISA) and to compare sperm parameters according to the age and type of infertility.

One hundred twenty-eight subjects employed in this study, with age range (18-49) years and duration of infertility (1-28) years. From each subjects semen sample was obtained and seminal fluid analysis (SFA) was done. Simple medium for assisted reproductive techniques; (SMART) was used for *in vitro* sperm activation by direct swim-up technique and centrifugation Swim-Up technique. Sperm concentration, sperm motility (%), sperm grade activity (%), normal sperm morphology (%) and sperm agglutination (%) were assessed. Crude data were statistically analyzed. From the results of present study, it was appeared that most sperm parameters were significantly ($P<0.05$) enhanced post-activation *in vitro* as compared to pre-activation using SMART medium by direct swim-up technique and centrifugation Swim-Up technique. Sperm concentration for post-activation was significantly reduced ($P<0.05$) compared to pre-activation groups and the percentages of progressive sperm activity with grade A, total progressive sperm motility (grades A and B) and normal sperm morphology were significantly increased ($P<0.05$). Based on the results of this study, it can be concluded that sperm motility and concentration were considered most important parameters that have the importance of activation and sperm morphology one of the sperm parameters that have role of the influence on the sperm qualities after activation.

Keywords: Male infertility, sperm parameters, *in vitro* sperm activation.

العلاقة بين متغيرات النطف والاستجابة إلى تنشيط النطف البشرية بناء على العمر ونوع العقم

سنابل حسين حلو^{1*}، محمد باقر محمد رشاد فخر الدين²، صباح ناصر العلوجي³

¹قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

²المعهد العالي لتشخيص العقم والتقنيات المساعدة على الإنجاب، جامعة النهرين، بغداد، العراق.

³الجامعة الأمريكية، بيروت، لبنان.

الخلاصة

صممت هذه الدراسة لتقييم مدى تأثير متغيرات النطف على الاستجابة للتنشيط خارج الجسم ولمقارنة متغيرات النطف مع العمر ونوع العقم. شارك في هذه الدراسة مئة وثمانية وعشرون شخص، وكان متوسط أعمارهم (18-49) وفترة العقم (1-28) سنة. أخذت عينة السائل المنوي من كل مريض واجري لها فحص

السائل المنوي. استعمل الوسط الزرعي البسيط للتقنيات المساعدة على الإنباب لتنشيط النطف خارج الجسم باستخدام تقنية سباحة النطف للأعلى مباشرة وتقنية الطرد المركزي. تم تقييم تركيز النطف والنسبة المئوية للنطف المتحركة والنسبة المئوية لنشاط النطف والنسبة المئوية لشكل النطف الطبيعي والنسبة المئوية لتكامل النطف. كما تم تحليل النتائج الأولية إحصائياً. أظهرت نتائج الدراسة الحالية تحسن معنوي ($P < 0.05$) في اغلب متغيرات النطف بعد التنشيط خارج الجسم عند المقارنة مع قبل التنشيط باستخدام وسط سمات وتقنية سباحة النطف للأعلى مباشرة وتقنية الطرد المركزي. تركيز النطف بعد التنشيط انخفض بشكل معنوي ($P < 0.05$) عند المقارنة مع قبل التنشيط والنسب المئوية لكل من الحركة التقدمية (A) للنطف والحركة التقدمية الكلية (A+B) للنطف وشكل النطف الطبيعي أظهرت زيادة معنوية ($P < 0.0$). لذلك نستنتج اعتماداً على معطيات هذه الدراسة بأن حركة النطف وتركيزها من أهم الصفات التي لها أهمية بالتنشيط خارج الجسم وشكل وشكل النطف الطبيعي واحد من الصفات التي لها دور بالتأثير على صفات النطف بعد التنشيط خارج الجسم.

Introduction

The World Health Organization (WHO) defines the infertility as an inability of a couple to achieve conception or bring a pregnancy to term after one year or more of regular unprotected sexual intercourse. Conception is normally achieved within 12 months in 80%–85% of couples using no contraceptive measures [1]. Infertility is a health concern affecting nearly one in six couples of childbearing age and approximately 10% of the global population. Male-factor infertility accounts for %50 of all cases [2, 3, 4]. Male infertility is due to the complete absence of sperm in the ejaculate and is relatively uncommon. Male subfertility can be due to low numbers of sperm, a low percentage of sperm with effective progressive movement or abnormalities in the sperm's ability to fertilize an egg [5]. Semen quality is usually considered to be a proxy measure of male fertility, and changes in semen quality can occur after exposure to toxic agents, or from host factor effects such as age [6]. Basically, sperm count, motility and percentage of normal sperm morphology are conventional criteria for semen quality [7]. Male infertile patients are often classified as oligozoospermic, asthenozoospermic, or teratozoospermic on the basis of concentration, motility, morphology or any of these combination [8]. The cause of infertility in infertile men with normal semen parameters could be related to abnormal sperm DNA [9].

Asthenozoospermia is therefore one of the major causes of infertility or reduced fertility in men [10]. Motility is the prime functional parameter that determines the fertilizing ability of spermatozoa, the cause underlying loss of sperm motility may be either hormonal, biochemical, immunological or infection [11].

Materials and methods

One hundred twenty-eight were involved in this study. Age range for subject was (18-49) years and duration of infertility from (1-28) years. Subjects were instructed to collect a semen sample by masturbation for all specimens, seminal fluid analysis was done according to WHO criteria [1]. 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 37°C for 30 minutes to allow the semen liquefaction [12]. The liquefied semen is then carefully mixed by glass Pasteur pipette for few seconds to homogenize, and then subjected to both macroscopic and microscopic examinations. The standard criteria of WHO [1] was used to record parameters of the seminal fluid analysis and two technique used in this study to prepare semen sample, direct Swim-up technique and centrifugation swim-Up technique.

Statistical analyses

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago). Sperm parameters, pre and post activation assay were analyzed using complete randomized design (CRD) (one way ANOVA). The mathematical model was

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where

Y_{ij} = dependent variables (sperm parameters pre and post activation ultra sound digenesis and hormonal assay).

μ = overall mean.

T_i = effect of treatments (activation technique, age groups, BMI groups, infertility groups and infertility duration groups).

e_{ij} = error term.

Differences among means were compared using the Duncan multiple ranges test [13].

The correlation between pre and post activation was carried out using spearman correlation.

Results and Discussion

One hundred twenty-eight males were involved in this study, with the range age of subjects was 18-49 years old. However, the range duration of infertility was more than 1 to 28 years.

From table-1, there was significant increased ($P < 0.05$) between pre-and post- activation in the most sperm parameters for asthenozoospermia group. However, a significant decreased ($P < 0.05$) in the sperm concentration and percentages of immotile sperm (grade D) was noticed after sperm activation as compared to pre- activation.

Non-significant ($P > 0.05$) differences in the progressive motile sperm (%) (grade B) and non-progressive motile sperm (%) (grade C) were noticed between pre- and post-activation for oligozoospermia group . While, significant ($P < 0.05$) decrease in the sperm concentration and immotile sperm (%) (grade D) between pre- and post- activation. Also, significant ($P < 0.05$) increase were found in percentage of sperm motility, progressive sperm motility (grade A), total progressive sperm motility (grades A and B) and normal sperm morphology as a compared between pre- and post-activation Table-2.

Table-3 shows that the sperm concentration and percentage of immotile sperm (grade D) for asthenooligozoospermia group were improved significantly ($P < 0.05$) decreases as a compared between pre- and post-activation. However, The percentage of sperm motility, progressive sperm motility (grade A),(grade B), total progressive sperm motility (grades A and B) and normal sperm morphology Significant ($P < 0.05$) increase as a compared between pre- and post-activation. While, there was no significant ($P < 0.05$) differences in the non-progressive motile sperm (%) (grade C) were noticed between pre- and post-activation.

In the present study, also direct sperm technique was applied for sperm preparation from asthenozoospermic patients. In an attempt to prevent damage by centrifugation and generation of reactive oxygen species (ROS), the method of sperm preparation has been developed using direct swim-up from original sperm sample[14]. In the efficiency of this method for preparing normozoospermia specimens. However, centrifugation swim-up technique was as effective as direct swim up technique but the latter in an easier way and this agreed with Siam[15]. Generally, sperm motility increased with the use of *in-vitro* culture because of their aqueous nature with lower viscosity than of seminal plasma resulted in making spermatozoa move more freely[16].

A significant improvement in the certain sperm function parameters was recorded after activation. This finding may be related to the fast movement of normal spermatozoa from seminal plasma into layer of culture medium, and consequently elicited from impact of some seminal plasma components like leukocytes, round cell and others leading to keep the sperm out of stress factor and ROS production that responsible for DNA damage [17]. The same observation was noticed by other studies (18, 19). It was noticed that, the centrifuge technique have efficacy in elimination of agglutinated sperm and round cell. Moreover, these result can be explained by the effect of centrifugation step in which remove the debris, bacteria and other cells (effect centrifuge power) [20].

After *in vitro* sperm activation (ISA), all sperm parameters for all group in this study enhanced except sperm concentration and immotile sperm (%). Sperm concentration for all groups post-activation was significantly reduced ($P < 0.05$) compared to pre-activation groups and this is due to inability of dead and abnormal sperms morphology with poor motility to swim-up and migrate into upper layer of culture media as certified by [19]. However, sperm motility (%) and progressive sperm motility (%) for all groups post-activation was significantly increased ($P < 0.05$) compared to pre-activation groups. Really, enhancement sperm parameters may be considered as normal response for sperm physiology after the removal of seminal plasma, pus cells and agglutinated spermatozoa using sperm preparation techniques. Furthermore, it was reported that

only the active motile sperms will swim-up to the upper layer of culture medium in vitro human sperm activation [21,22].

However, post-activation using centrifugation swim up technique resulted in a significant increment ($P < 0.05$) in the percentage of normal sperm morphology, this is because the sperm preparation techniques for ART were developed to separate the motile morphologically normal spermatozoa and excluded leucocytes, bacteria, and dead spermatozoa produce oxygen radicals that negatively influenced the ability to fertilize the egg [23].

Table 1-Sperm parameters of pre- and post- *in vitro* sperm activation for men with asthenozoospermia.

Sperm parameters		Pre- activation	Post- activation
Sperm concentration (millions/mL)		45.775 a ±1.936	19.483 b ±1.11
Sperm motility (%)		51.175 b ±1.29	85.408 a ±1.39
Grade sperm activity (%)	Grade A (%)	2.042 b ±0.35	29.075 a ±1.40
	Grade B (%)	20.825 b ±0.75	38.983 a ±1.38
	Grade C (%)	27.975 b ±1.05	17.267 a ±0.98
	Grade D (%)	48.992 a ±1.29	14.675 b ±1.40
Progressive sperm motility grade (A+B) (%)		22.867 b ±0.77	68.183 a ±1.81
Normal sperm morphology (%)		38.833 b ±0.79	55.958 a ±1.08

*Data are Mean ±S.E.

*Means with different superscripts within each row are significantly different ($P < 0.05$).

*Means with similar superscripts within each row are non-significantly different ($P > 0.05$).

*Number of subjects: 120.

Table 2-Sperm parameters of pre- and post- *in vitro* sperm activation for men with oligozoospermia.

Sperm parameters		Pre- activation	Post- activation
Sperm concentration (millions/mL)		11.600 a ±0.68	5.000 b ±1.30
sperm motility (%)		65.200 b ±5.67	90.000 a ±2.24
Sperm grade activity (%)	Grade A (%)	2.000 b ±2.00	26.000 a ±11.22
	Grade B (%)	46.200 a ±5.53	53.000 a ±7.52
	Grade C (%)	19.000 a ±4.00	11.000 a ±4.00
	Grade D (%)	32.800 a ±3.99	10.000 b ±2.24
Progressive sperm motility grade (A+B) (%)		48.200 b ±7.30	79.000 a ±4.30
Normal sperm morphology (%)		34.600 b ±2.27	49.000 a ±6.60

*Data are Mean ±S.E.

*Means with different superscripts within each row are significantly different ($P < 0.05$).

*Means with similar superscripts within each row are non-significantly different ($P > 0.05$).

*Number of subjects:5

Table 3-Sperm parameters of pre- and post-in vitro sperm activation for men with asthenooligozoospermia.

Sperm Parameters		Pre- activation	Post- activation
Sperm concentration (millions/mL)		11.333 a ±0.67	4.000 b ±1.00
sperm motility (%)		51.667 b ±1.67	90.000 a ±2.89
Sperm grade activity (%)	Grade A (%)	0.000 b ±0.00	13.333 a ±3.33
	Grade B (%)	18.333 b ±9.28	53.333 a ±14.24
	Grade C (%)	33.333 a ±8.33	23.333 a ±10.93
	Grade D (%)	48.333 a ±1.67	10.000 b ±2.89
Progressive sperm motility grade (A+B) (%)		18.333 b ±9.28	66.667 a ±10.93
Normal sperm morphology (%)		30.000 b ±0.00	38.333 a ±1.67

*Data are Mean ±S.E.

*Means with different superscripts within each row are significantly different (P<0.05).

*Means with similar superscripts within each row are non-significantly different (P>0.05).

*Number of subjects:3

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