Effect Invitro Activation on the Spermiograms of Oligozoospermic Patients

تأثير التنشيط بالزجاج على معالم النطف لمرضى قلة النطف

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الخلاصة :-

الهدف: هدفت الدراسة الحالية الى زيادة معايير وظائف النطف البشرية في الزجاج للمرضى المصابين بقلة النطف او عوامل اخرى عند الذكور باستخدام تقنيات معينة مثل تقنية السباحة للاعلى وتقنية تدرج الكثافات .

المنهجية : تم اجراء هذه الدراسة للمدة من كانون الثاني 2014 الى تشرين الاول 2014 ، حيث شملت الدراسة(30) عينة من السائل المنوي لمرضى قلة النطف الذين يراجعون مركز الخصوبة في مدينة الصدر الطبية في محافظة النجف الاشرف . حيث تم فحص المني المتميع عينياً ومجهرياً بعد مزجه جيداً لثواني قليله . في هذه الدراسة استخدمت طريقتين لتنشيط الحيامن (السباحة للاعلى وتدرج الكثافات) لغرض فصل الحيامن النشطة من البلازما المنوية .تم تحليل النتائج احصائيا باستخدام العامل الاحصائي SPSS Version(20).

النتائج: أظهرت النتائج وجود انخفاض معنوي ($p<0.05$) في كل من تركيز النطف، تركيز كريات الدم البيض، تركيز المالون داي الديهايد (MDA) وتركيز نضج الكروماتين النطفي بعد التنشيط ولكلا التقنيتين (السباحة للاعلى وتدرج الكثافات) مقارنة بما قبل التنشيط .كما أظهرت النتائج حدوث تحسن معنوي ($p<0.05$) في كل من الحركة التقدمية للنطف (A+B) والشكل السوي للنطف بعد التنشيط بكلا التقنيتين مقارنة بما قبل التنشيط. ايضا تبين حدوث تحسن معنوي ($p<0.05$) في الحركة التقدمية للنطف (A+B) من خلال تقنية تدرج الكثافات بشكل أفضل مما في تقنية السباحة للاعلى .

الاستنتاجات : توصلت هذه الدراسة الى ان استخدام تقنية تدرج الكثافات والسباحة للاعلى تظهر تحسنا ملحوظا في معالم النطفالاساسية لمرضى قلة النطف بعد التنشيط .

التوصيات: استخدام تقنية تدرج الكثافات مع تقنية الصنعية (IUI) لمعالجة مرضى قلة النطف .

Abstract:

Objective: The objective of this study was to improve certain sperm function parameters in vitro of oligozoospermic patients and other mild male factors using sperm activation techniques such as swim-up and density gradient.

Methodology: The study was carried out between January 2014 and October 2014 including (30) semen samples of oligozoospermic patients who attended the Fertility Center in AL- Sadr Medical City. The liquefied semen was then carefully mixed for few secondand the semen was analyzed by macroscopic and microscopic examination.Two method of IUI sperm activation have been used in this study to separate the motile spermatozoa from seminal plasma.Data analyzed using the SPSS version(20)

Results: The results of the study showed that there was statistical significant ($p<0.05$) decrease in the sperm concentration, leukocyte concentration, malondialdehyde(MDA) concentration and sperm chromatin maturity after activation by both swim-up and density gradient techniques compared with before activation. Also the results was appeared a significant ($p<0.05$) improvement in progressive motility (A+B) and normal morphology after activation by both techniques while, there was a significant ($p<0.05$) improvement in progressive motility (A+B) was noticed by using density gradient technique compared to swim-up technique.

Conclusions: It was concluded that the density gradient and swim-up techniques were appeared clear improvement in the certain sperm parameters of oligozoospermic patients after activation.

Recommendation: The density gradient techniques with intrauterine insemination to treatment Oligozoospermic patients.

Keywords: in vitro activation, Oligozoospermia, swim- up, density gradient techniques.

INTRODUCTION:

Sperm activation methods for assist reproduction techniques such as intrauterine insemination were perform to isolate progressive motile and normal form of spermatozoa, and eliminate debris, bacteria, immature sperm and leukocytes produce oxidative stress that negatively effect of fertilization. They produce wide range of motile and normal morphology of spermatozoa might affect recovery option and IVI outcome [(1)]. The objective of sperm activation is to isolate the functional sperm from non-functional of the ejaculate and in the condition of intrauterine insemination, to produce many functional motile sperm following activation is a correlation with total number of motile sperm pre-activation is appeared the treatment rate [(2)]. Activation methods that have elevation treatment rates are represented better for IUI. The rates of treatment are important when you compare various sperm activation methods [(3)].The most common sperm activation techniques including: density gradient centrifugation, glass wool columns and Swim-up [(4)].

OBJECTIVES:

The objective of this study was to improve certain sperm function parameters in vitro of oligozoospermic patients and other mild male factors using sperm activation techniques such as swim-up and density gradient.

METHODOLOGY:

Subjects:

The study was carried in the Fertility Center in AL-Sadr Medical City. Semen samples were obtained from (30) oligozoospermic men, were collected by masturbation in to wide – mouth containers after 3-5 days of sexual abstinence in room near the laboratory, and immediately placed in an incubator at 37C˚ till complete liquefaction. After liquefaction time, the liquefied semen was then carefully mixed for few secondand the semen was analyzed by macroscopic and microscopic examination using standardization[(5)].

Sperm activation:

After liquefaction and initial sperm analysis. Two method of IUI sperm activation have been used in this study to separate the motile spermatozoa from seminal plasma. The main IUI outcome measure was the pregnancy rate.

Swim –up technique:

Dilute the semen sample 1:1 with ferticult medium to promote removal of seminal plasma, then transfer the diluted suspension in to centrifuge tubes, centrifuge at 2500 rpm for 5 minutes. The supernatant was discarded, the final pellet was gently covered with 1 ml of medium and incubated for 60 minutes at 37 c˚ in an incubator until used in IUI procedure[(6).]

Density gradient centrifugation technique:

A two gradients 45% and 90% sil select stock medium were used for the procedure. All procedures were conducted under sterile conditions. Media were brought to 37c˚ temperature. Using sterile pipette 2.0 ml of the lower layer (90%) was transferred in to a conical centrifuge tube. Using anew sterile pipette 2.0 ml of the upper Layer (45%) was gently dispensed on top of the Lower Layer. 1 ml of Liquefied semen sample was then placed on top of the upper Layer and the tube was centrifuge at 3000 rpm for 10 min. The supernatant was discarded and the sperm

pellet was suspended in volume 3 ml of ferticult medium, centrifuge for 10 minutes at 3000 rpm. The supernatant was then removed and the final pellet suspended in a volume of 0.5 ml of ferticult medium which used in IUI procedure[6].
Statistical analysis: Analyzed using the SPSS version (20). P value was considered significant if it was less than 0.05.

Determination of malondialdehyde (MDA) concentration

Seminal MDA levels were analyzed according to[7] was assessed using the thiobarbituric acid method.

Procedure

The test tube contain 0.15ml of semen sample, 1ml of 17.5% TCA, and 1ml of 0.6% TBA. The tubes were vortexes and boiled in a water bath for 15min, then allowed to cool. Added 1 ml of 70 % TCA and left the mixture at room temperature for 20min. Centrifuged for 15min at 2000 rpm and the absorbance of supernatant were read on a spectrophotometer at 532nm.

MDA concentration was calculated according the following equation.

$$\text{MDAcon.}\,(\mu\text{mol/L}) = \frac{\text{A532}}{\text{L} \times \text{E}_\text{o}} \times \text{D}$$

Aniline blue staining

Assessment of sperm chromatin maturity using Aniline blue stain. Slides were prepared by smearing 10 µl of semen specimen. The slides are left to dry in air and then fixed with 3%glutaraldehyde in PBS. The smear in stained with 5%aqueous AB solution (PH3.5). sperm head with immature chromatin condensation will stain blue and those with mature chromatin will not be take the stain[8] .

Results:

Table(1) Effect of invitro activation on spermiograms of oligozoospermic semen using swim-up and density gradient centrifugation techniques.

Patients / Variables	Before Activation		After Activation	
	Swim – up n=15	Density gradient n=15	Swim – up n=15	Density gradient n=15
Sperm concentration (x 10^6/ml)	16.46±1.55 ^{a}	16.9±1.48 ^{a}	7.1±0.83 ^{b}	7.6±0.89 ^{b}
Progressive motility percent(A+B)%	57.3±5.65 ^{a}	57.8±5.28 ^{a}	69.9±5.06 ^{b}	79.1±7.66 ^{c}
Normal morphology percent %	42.9±8.76 ^{a}	46.7±7.55 ^{a}	64.6±8.73 ^{b}	65.9±5.35 ^{b}
Leukocyte concentration (x 10^6/ml)	2.5±1.04 ^{a}	2.5±0.63 ^{a}	1.8±0.93 ^{b}	1.2±0.52 ^{c}
MDA concentration (µmol/l)	5.9±1.74 ^{a}	5.1±1.18 ^{a}	4.3±1.38 ^{b}	3.2±0.97 ^{c}
Sperm chromatin maturity percent%	35.7±2.37 ^{a}	34.5±3.09 ^{a}	27.5±2.47 ^{b}	22.2±2.55 ^{c}

Value are expressed as mean ±S.D.

different letters means significant difference (P<0.05).

Table (1) there was a significant (P < 0.05) decline in mean sperm concentration after activation by both (swim-up and density gradient methods) compared to the results before

activation and no significant ($P > 0.05$) difference was noticed when compared between two techniques. As well as a significant ($P < 0.05$) improvement was appeared in progressive motility (A + B) and normal morphology percent following activation by both methods as compared with before activation.Moreover, there was a significant ($P < 0.05$) improvement in progressive motility (A + B) by using density gradient technique compared to swim – up technique while no significant ($P > 0.05$) difference was noticed in normal morphology percent between two methods after activation. Lastly, the results in table (1) was recorded a significant ($P < 0.05$) reduce in the leukocyte concentration and MDA concentration as well as sperm chromatin maturity percent following activation by swim – up and density gradient techniques as compared with before activation and also a significant ($P < 0.05$) improvement was appeared by using density gradient technique compared to swim – up technique.

DISCUSSION:

Sperm activation techniques play major role in improvement sperm ability of fertilization for infertile patients before artificial insemination [(1).] Table(1) shown a significant decrease ($P < 0.05$) in sperm concentration, leukocyte concentration, MDA concentration, and sperm chromatin maturity following activation by both swim – up and density gradient techniques compared to before activation. Also the results of present study was appeared a significant improve ($P < 0.05$) in progressive motility (A + B) and normal morphology after activation by both methods compared with before activation.

The sperm concentration was significant reduced after activation by both methods. This due to that non – active or immotile sperms remain in the bottom of activation tube and only sperms have excellent motility and normal morphology which able swim – up to upper layer. While, the density gradient technique has been separate cells of seminal fluid according density, therefore sperm have move actively and have normal form will reach to pellet while the immotile sperms and other cells will reach to the upper layer of activation tube. The same observation was showed by several studies[(9,5)].

Improvement of sperm motility occur because the culture media contain special components lead to increase number of motile sperms and normal form during activation. The benefits of in vitro culture media that decrease the viscosity of the seminal fluid and promote sperms move more freely and finally increase sperm motility and grade activity [(10)]. Therefore, sperm activation techniques should use culture media involve buffers and protein source to promote sperm by peractivation and capacitation, also added glucose, pyruvate and sodium lactate to generate of energy[(11)].Further significant improvement in the percentage of normal morphology after activation. This due to eliminate leukocytes, round cells, debris and others leading to reduce ROS production that induce DNA damage therefore, normal spermatozoa move fasting from seminal plasma into layer of culture media [(12)]. In the same portion, [(13)] proposed the decapacitation factors in the seminal plasma that inhibition spontaneous capacitation, which impact on sperm function.

Increase seminal leukocytes particularly macrophage and neutrophils that stimulate male subfertility through damaging sperm by the yield of ROS and apoptosis [(14)].Therefore, in the circumstance of male infertility, seminal oxidative stress, sperm DNA damage and apoptosis are interlinked and establish a unified pathogenic molecular mechanism [(15)]. Consequently culture media contain essential components may maintain sperm membranes from effect of ROS by generate adenosine triphosphate (ATP) during transport fatty acids into the mitochondria therefore DNA damage is reduce [(16)].

Data in the current study was noted improvement of chromatin maturity following activation by both swim – up and density gradient techniques with increase of normal morphology and progressive motility. This may to be the affection of both techniques (swim – up and density gradient) which used to the isolation of normal sperms from abnormal as well as removal leukocyte is the major source of ROS, all of these will be given good results by reduction the percentage of chromatin maturity. Also other causes that could impact positively on the improvement in the percentage of sperm chromatin maturity in this study. This due to various dilutions obtain through activation leading to absent in leukocyte and decreased abnormal cells and other cell may yield ROS.This finding agrees with many studies that proposed activation by density gradient and swim – up that increase sperm quality with normal percentage of sperm chromatin [(17)]. [(18)]concluded the relationships between chromatin condensation and morphological alteration of the acrosomal vesicle through spermatogenesis because impairment of condensation lead to failure association of acrosome to the nucleus.

The current study used two techniques (swim – up and density gradient) were given more effective in certain sperm parameters were improved through activation by density gradient technique more than by swim – up technique. These data similar study by [(19)] which suggested the density gradient technique was showed more benefit in sperm treatment than swim – up technique, density gradient method is provide greater number of motile sperms and decrease the contamination of bacteria and procedure time. Moreover, density gradient method have given a greater percentage of normal morphology of sperm treatment with best DNA integrity and chromatin maturity [(20)].

CONCLUSION

It was concluded that the density gradient and swim-up techniques were appeared clear improvement in the certain sperm parameters of oligozoospermic patients after activation .

RECOMMENDATION:

The density gradient techniques with intrauterine insemination to treatment Oligozoospermic patients.

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