Effect of incubation time on certain sperm function parameters following *in vitro* activation test by FertiCult™ medium

Maha K. Al-Ghazi[1]., 1Khalid S. Al-Azawi[1]. Rana A. Al-Saadi[2]., And Nisreen K. Flayeh.
1 Dept. of Clinical Reproductive Physiology, High Institute of Infertility Diagnosis & ART /Al- Nahrain University
2 Dpet. of Applied Embryology,High Institute of Infertility Diagnosis & ART /Al- Nahrain University.
Corresponding author: e-mail:mahakhalid72@yahoo.com.

Abstract:

Background

There are many factors and media affecting sperm characters which are defined by classical parameters namely, concentration, motility and morphology, according to standard World Health Organization (WHO).

Objectives

To study the effect of incubation time on certain sperm function parameters following *in vitro* centrifugation technique by sperm wash,FertiCult™ medium for the purpose of assisted reproductive techniques (ART) in couples presenting sub-fertility.

Methods:

Two groups of 40 semen samples (group1: fertile men with normal semen parameters and group 2): abnormal semen parameters) underwent sperm activation test (SAT) in the ART laboratory, High Institute of Infertility Diagnosis & ART/Al-Nahrain University. Semen analysis was assessed. In standard WHO (1999).Wash and spin activation technique by FretiCultTM medium was done for all samples. The tubes which was sealed, inclined vertically and kept at 37°C for 30 minute. The supernatant contained actively motile sperms was transferred to another test tube to separate that active sperm from pellet and record sperm parameters after

Results

There is a significant ($p<0.05$) improvement in certain sperm parameters after 30min. of incubation following SAT in both groups compared to before activation and following 30min. incubation in SAT. Certain sperm characters are significantly decline in group 2 after 1 hr. incubation compared to before activation.

Conclusions

Sperm activation test is very important method to perform before undergone artificial insemination (AIH) . Incubation of prepared sperm *in vitro* by sperm wash, FertiCult™ medium is benefit for the first half hour to all semen samples to have a success in artificial insemination .

Key words: Sperm activation test, incubation time, AIH, culture medium

Introduction

Many people assume that infertility is a" woman's "problem, but many cases of infertility is a result of a man factor. Male problems may be a contributing factor in 30 to 50% of couples suffering infertility (1).

There are many factors causing male infertility. The treatment of infertility has undergone phenomenal development & become a highly specialized field involving a multitude of interventions known collectively as assisted reproductive techniques (2).

The success rate of AIH varies considerably, and researches do their best to stand on the causes of low success rate of AIH to find the best procedures that improve male fertility which through the semen must be present in sperm motile, normal morphology and be able to penetrate an ovum (3).

Sperm washing, is usually performed by different media such as Sage ,*In Vitro* life, Global , FertiCult, MediCult and others to prepare the semen sample for

Intrauterine Insemination (IUI) and other ART procedures. Sperm washing achieves two very important goals (4). First, it removes most of the seminal plasma from the semen that would otherwise not react well if directly inseminated into the uterus. Second, it concentrates the sperm density into a small volume that is suitable for intrauterine insemination. In most cases, sperm washing may also enhance the forward progression of the sperm or the percent motility by using proper culture medium (5).This study was designed to found out the effect of incubation time on certain sperm function parameters whenFertiCult™ medium used for preparation and activation *in vitro* of normal and abnormal semen samples.

Materials and Methods

Two groups of 40 semen samples from patients consulting ART laboratory at the High Institute of Infertility diagnosis & ART /Al Nahrain University for SAT were use in this study. Semen analysis was done according to WHO (1999) after 3-7 days of abstinence and liquefied at 37°C for30 min.sperm concentration/ml,sperm motility (%) and normal sperm forms(%) were determined by analyzing 10µLof semen on glass slide used by cover slip (22x22 mm) under 400X microscope (5,7,8,9).Samples were treated with sperm preparation medium (FertiCult™ medium ,Belgium) washed using sperm wash and spin (centrifugation) method (10).

The semen was diluted 1:1 with the buffer of choice (FertiCult™medium, Belgium),mixed and centrifuged at 300xg for 10 minutes. This process was resuspended in the buffer of choice, evaluated after 30 minutes of incubation(11), then separate the supernatant (which contain activated spermatozoa) from pellet, evaluated again after 1hr of incubation.

Statistical analysis:

Crude data were collected and analyzed using SPSS (Statistical program for social studies, Version 12, Illinois, USA) for descriptive statistics involving means and standard error of mean (SEM) and for compare means using paired t- test and ANOVA to detect the significant differences of pre- and post-activation and between groups. P value less than (0.05) was considered as statistically significant (12).

Results:

Results demonstrate a good sperm function parameters improvement after 30 min of incubation which was statistically recorded a significant ($p<0.05$) differences in both groups of the study but it recorded a significant ($p<0.05$) decrease after 1hr in great extent in group 2.

The sperm function parameters of normal semen analysis patients before & after preparation *in vitro* are presented in table (1). There was an increased quality of sperm function parameters after 30 minutes of prepared sample by FretiCultTM medium : number of sperm yielded (25.5±9.40) & high % of progressive motility grade A (GA) (67.25±17.7) & grade B(GB) motility (27.25±14.7) While low % of GC (4.50±6.46) & grade D(GD) motility (2.00±3.76) which were statistically significant ($p<0.05$) compared to before preparation. The normal sperm morphology recorded a significant ($p<0.05$)increasa (58.00±8.51) compared to before activation.

After 1hr of activation, no significant ($p>0.05$) changes were observed in the semen parameters compared to before activation excess GA which decreases significantly ($p<0.05$) & GB increases significantly ($p<0.05$). Table 2 revealed the effect of incubation time of abnormal semen samples on the activation results. There was a significant ($p<0.05$) decrease in the sperm concentration after 30 minutes of activation, with significant ($p<0.05$) decrease in the sperm motility GA and GB after 1 hr. The percentage of sperm morphology was significantly ($p<0.05$) increased in group 2 after activation in both period.

All results showed a significant ($p<0.05$) differences before & after activation in both groups & period times of activation as show in all diagrams (1-6)of this study.

Table 1: Semen parameters in the original (pre activation) and after 30min and 1hr post activation by FertiCult™ medium using wash & spin technique in normal semen samples

Sperm parameters		Before Activation Original	After activation 30 min.	1hr.
Conc.(m/ml)		50.65 ±14.20	25.5 ±9.40	24.65 ±12.36
Sperm Motility	GA%	2.55 ±4.41	67.25 ±17.73	48.50 ±21.58
	GB	34.25 ±9.29	27.25 ±14.73	40.00 ±15.89
	GC%	23.30 ±10.71	4.50 ±6.46	9.00 ±7.71
	GD%	40.90 ±13.19	2.00 ±3.76	2.75 ±4.72
Morphology%		36.65 ±9.18	58.00 ±8.51	56.40 ±9.35

Values are mean ± SDM

Table(2):Semen parameters in the original (pre activation) and after 30min and 1hr post activation by FertiCult™ medium using wash &spin technique in abnormal semen samples.

Sperm parameters		Before Activation Original	After activation 30 min.	1hr.
Conc.(m/ml)		16.8 ±5.15	8.00 ±4.71	4.35 ±3.82
Sperm Motility	GA%	0.85±2.45	24.00 ±24.76	17.00 ±24.60
	GB	8.00 ±9.19	45.00 ±15.12	20.00 ±12.70
	GC%	41.20 ±13.82	21.00 ±15.65	34.00 ±15.95
	GD%	50.10 ±14.40	10.00 ±10.32	29.00 ±14.14
Morphology%		25.80 ±14.57	40.10 ±16.20	41.35 ±12.12

Values are mean ± SDM

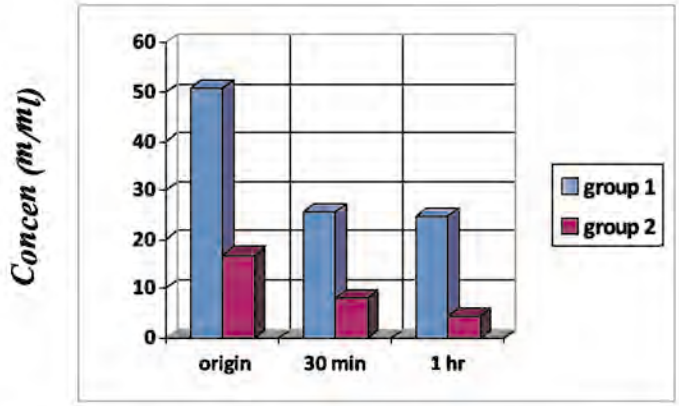

Figure (1): The distribution of mean concentration in normal (group1) & abnormal (group2) seminal fluid analysis patients in different period of incubation .

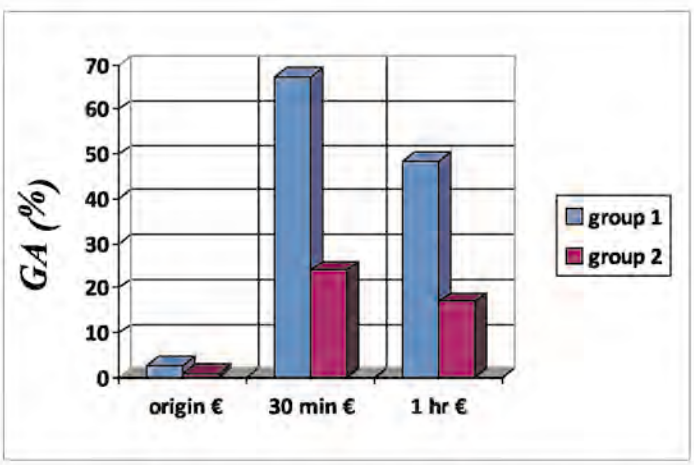

Figure (2): The distribution of mean of GA motility% in normal (group1) & abnormal (group2) seminal fluid analysis patients in different period of incubation.(€)significantly

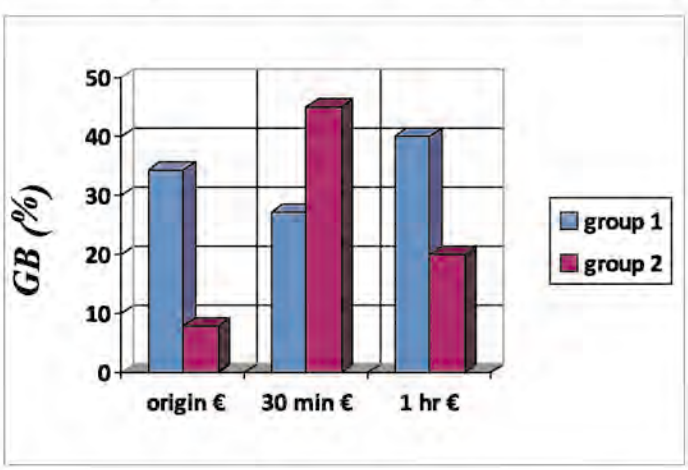

Figure (3): The distribution of mean of GB motility% in normal (group1) & abnormal (group2) seminal fluid analysis patients in different period of incubation (€) significantly

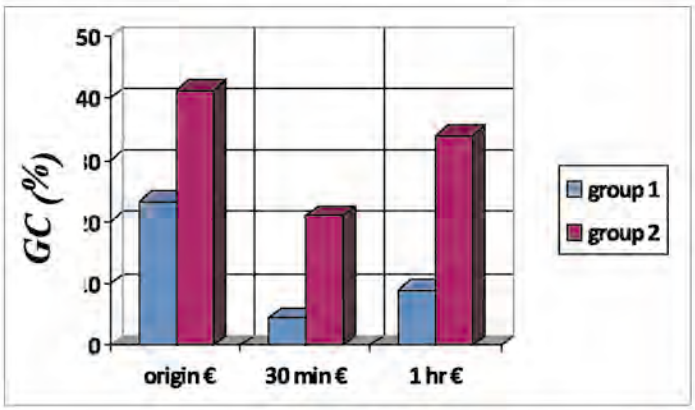

Figure (4): The distribution of mean of GC motility% in normal (group1) & abnormal (group2) seminal fluid analysis patients in different period of incubation (€) significantly different ($p<0.05$)

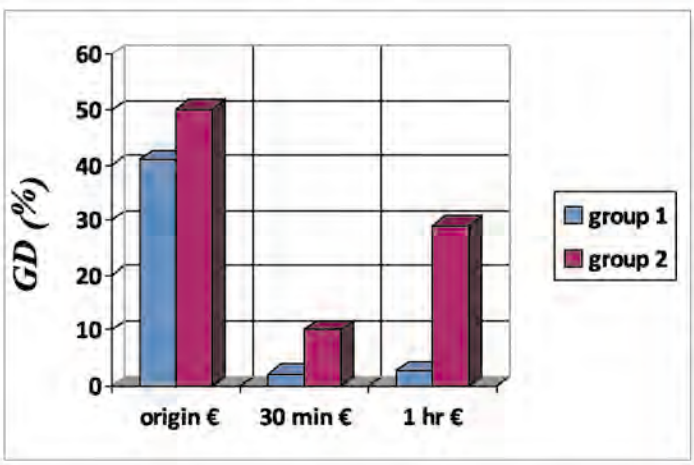

Figure (5): The distribution of mean of GD motility% in normal (group1) & abnormal (group2) seminal fluid analysis patients in different period of incubation (€) significantly

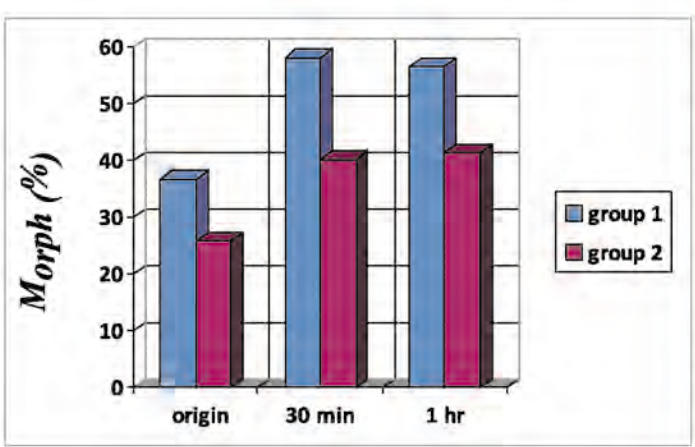

Figure (6): The distribution of mean of normal sperm morphology (%) in normal (group1) & abnormal (group2) seminal fluid analysis patients in different period of incubation.

Discussion

This study was done mainly to find out and prove the importance of SAT to done before assisted reproductive processes to study the type of semen because some samples not respond to activation process by using FertiCult™ medium, there are several harmful effects on certain sperm function when *in vitro* incubated for longer periods (13).

Sperm concentration was decrease after activation for 30 min of semen samples for different time periods compared with before activation because sperm activation techniques separate functional sperms from those that were immotile, have poor morphology and were not capable to fertilize oocytes and dead sperms(14).

Active sperm motility grades A & B were increased following activation for 30 min and 1hr compared with before activation because sperm activation techniques lead to excellent yields in motile spermatozoa (15).

The components of liquid artificial medium(.i.e FertiCult™medium) may enhance the percentage of morphology and progressive motility because it diluted and reduced the semen viscosity (16).

The results of this study indicated the importance of time and sperm parameters after activation. Poor sperm quality is a common problem in male infertility. Good sperm preparation can improve sperm quality by select inghighpercentageofmotile and morphologically normal spermatozoa (9).

References

1. Agarwal A. Treatment of immunological infertility by sperm washing & intrauterine insemination.Arch. Androl. 1992; 29:207-213.
2. Shekarriz M. Thomas AJ. and Agarwal A. Effect of time on reactive oxygen species formation in human. Arch. Androl.1995;34:69-75.
3. Andrews JC and Bavister BD. Capacitation of hamster spermatozoa with the divalent cation chelators D-penicillamine, L-histidine, and L-cysteine in a protein-free culture medium. Gamete Res. 1989; 23: 159-170.
4. World Health Organization. WHO Laboratory Manual for Examination of Human Semen and Semen- Cervical Mucus Interaction, 2nd edition. Cambridge.The Press Syndicate of the University of Cambridge, 1987:62.
5. Sifer, T. Sasportes, V. Barraud, C. Poncelet, J. Rudant, R. Porcher, I. Cedrin-Durnerin, B. Martin-Pont, J.N. Hugues and J.P. Wolf. World Health Organization grade 'a' motility and zona-binding test accurately predict IVF outcome for mild male factor and unexplained infertilities. Oxford Journals Human Reproduction. 2005;20:10. Pp. 2769-2775.
6. Fakhrildin M.B. Improving outcome of in vitro sperm activation using non liquefied versus liquefied semen of oligoasthenozoospermic patients .J.of Family &Reproductive Health.2012;4(1):27-34m.
7. Shekarriz, Anthony J T, and Ashok A. Amethod of human centrifugation to minimize the latrogenic sperm injuries caused by ROS. Eur Urol.1995;28:31-35.
8. Tahir J.Sperm swim-up: a simple and effective technique of semen processing.J.of Pakistan medical association(JPMA).2008;58:71.
9. Mamas Comparison of fallopian tube sperm perfusion and intrauterine tuboperitoneal insemination: a prospective randomized study, Mamas L. Ferti steril, 2006; 735-740 851(3).
10. Panayota N. Z, Panayiotis M. Can the method of sperm preparation for intrauterine insemination affect subsequent pregnancy rates? comparison between the sperm preparation and the traditional double wash method. Medal East Fertility Society.1998;Vol.3,No.2.
11. Sushil Kumar, Hemangi AZRole of intrauterine insemination and sperm preparation techniques in treatment of unexplained Infertility and male infertility. MJAF. 2000;Vol.56,No.3.
12. Shekarriz M., Dewire DM., Thomas AJ. and Agarwal A. Amethod of human semen centrifugatttion to minimize the iatrogenic sperm injuries caused by reactive oxygen species. Eur.Urol. 1995; 28:31-35.
13. Jeremy T, Stewart I, Paul H, Norma F.Iatrogenic DNA damage induced in human spermatozoa During sperm preparation: protective significance of seminal plasma. Molecular Human Reproduction. 1998 .Vol.4. No.5 pp. 439-445.
14. Al-Dujaily, S.S., and Al-Shammary, R. N. Effect of modified Tris solution with Glycyrrchiza glabra extract on cryostorage of infertile semen several days after ejaculation. Proceeding of the 4th Sci. Conf.2008,81-89.
15. Arora,M., Carver-Ward, J.A.,Jaroudi, K.A. and Sieck, U.V.: Is Percoll safe for in vitro use? Ferti. Steril. 1994, 61: 979-981.
16. Al-Azzawi, K. S.,(1997). Activation of epididymal sperms in vitro in mice. Msc. Thesis, college of Science, Al-Mustansiriya University.