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### RESEARCH ARTICLE

#### A STIMULATORY APPROACH FOR *IN VITRO* ACTIVATION OF HUMAN SPERM MOTILITY BY USING THEOPHYLLINE.

Huda S. Abbas<sup>1</sup>, Hayder A. L. Mossa<sup>1</sup>, Lubna A. Al-Anbari<sup>1</sup>, Mohammad Oda Selman<sup>1</sup> and Balsam A. Al-Zahawi<sup>2</sup>.

1. High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University.
2. Kamal Al-Samarrai Hospital Fertility Center Infertility Treatment and IVF.

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#### Abstract

**Background:** In developed countries, about (2–4%) of births involve the using of assisted reproductive technologies (ART's). In these technologies before using semen samples for insemination firstly it must be processed and specifically sperm preparation methods and materials used to improve sperm functions especially motility in order to ensure increasing in the successful of pregnancy rates next ART's. Theophylline pharmacological agents recently commonly used in stimulating motility in human sperm thus by increasing intracellular levels of cyclic adenosine 3', 5'-monophosphate (cAMP), a molecule involved in formation of sperm energy, which is result of inhibitory properties on phosphodiesterase (PDE) function.

**Objective:** To evaluate whether *in vitro* sperm activation by Theophylline improves sperm functional parameters as compared with non-activated semen samples.

**Subjects, Materials and Methods:** Sixty male were participated in this study, twenty Normozoospermic subjects and forty Asthenozoospermic patients. Semen samples were collected and seminal fluid analysis was done according to (WHO 1999,2010) guidelines. Each sample was divided equally into three portions, centrifugation was done to remove seminal plasma from all, and then Swim-up technique was dependent for *in vitro* sperm activation by using FertiCult Flushing medium, Theophylline solution and SpermMobil dilution respectively. Sperm parameters were measured in each activated portion according to (WHO 1999) guidelines.

**Results:** Activation of semen sample groups by using of FertiCult Flushing medium, Theophylline solution and SpermMobil dilution in this study resulted in significant reduction in sperm concentration and highly significant improvement ( $P < 0.001$ ) in the percentage of sperm parameters in comparison with non-activated ones. However, SpermMobil dilution resulted in least reduction in sperm concentration and best result of sperm motility and morphologically normal sperm (MNS).

**Conclusion:** From all comparisons between activation media which have been used in this study with non-activated semen, whatever semen is activated, concentration of sperm will be decreased. Sperm motility

and MNS will be improved after activation by FertiCult medium, Theophylline solution and SpermMobil dilution, best results were in increases of sperm motility and MNS which was attributed to SpermMobil dilution.

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## **Introduction:-**

Infertility affecting about 15% of all reproductive age couples, can be defined as a failure of reproductive tract to achieve pregnancy after one year or more of regular unprotected sexual intercourse, and half of infertility cases traced back to male <sup>(1)</sup>.

Male factors which including decreased semen quality are responsible for 25% of these male infertility causes. Semen analysis can be done as basic diagnosis offer insight into cause of male infertility. Asthenozoospermia is important cause of male infertility and may influence on pregnancy occurring <sup>(2)</sup>. Nowadays many substances has been developed to be added to culture media (CM) and deliver spermatozoa with needs that maintain the optimal function in order to give good results during preparation; Methylxanthines supplement to CM are commonly used to improve sperm characteristics and among methylxanthine derivatives; Theophylline has been used for enhancement of human sperm motility by increasing of cAMP concentrations into sperm and that is essential for sperm functions <sup>(3)</sup>.

## **Subjects , Materials and Methods:-**

### **Subjects:-**

This research was carried out in the high Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University and Kamal Al-Samarrai Hospital Fertility Center Infertility treatment and IVF. From November 2016 till April 2017, sixty male were involved in this study, twenty normozoospermic subjects and forty asthenozoospermic patients.

### **Specimen Collection:-**

Semen samples were collected by masturbation into wide-mouth containers in a private room near laboratory, after a 3 -7 days of abstinence period, sample directly placed in an incubator at 37 C°for 30-60 minutes for complete liquefaction, macroscopically and microscopically examination were done according to( WHO 1999,2010) guidelines<sup>(1)</sup>.

### **Preparation of Theophylline Solution for in vitro sperm activation:-**

Theophylline solution was prepared by dissolving 0.036 mg from Theophylline powder in (10ml ) of FertiCult Flushing media and then stirred until getting dissolved<sup>(3)</sup>. The solution was prepared daily under a sterile conditions.

### **Preparation of SpermMobil dilution for in vitro sperm activation:-**

SpermMobil dilution was prepared from (GM501 SpermMobil, Gynémed, Lensahn, Germany) is ready-to-use product, used in current study as standardized source of Theophylline as final dilution 1:20 with FertiCult Flushing medium <sup>(4)</sup>.

### **In vitro sperm activation technique by using activation media:-**

After liquefaction, each semen sample was divided into three equal portions, processing by centrifugation of semen with FertiCult Flushing medium to wash it and then pellets of these portions were resuspended by FertiCult medium, Theophylline solution and SpermMobil dilution, respectively, and then incubated for 30 minute for first and second portion and 10 minute for SpermMobil dilution. Certain sperm parameters were assessed microscopically for each activated portion according to (WHO, 1999) guidelines <sup>(5)</sup>.

### **Statistical Analysis:-**

Data were collected, summarized, analyzed and presented using the three statistical software programs: statistical package for social science (SPSS version 22), Microsoft Office Excel 2013 and MedCalc 2014. Categorical variables were presented as number and percentage while numeric variables were presented as mean and standard deviation (SD). The comparison of mean values between any two groups was carried out using independent

samples-t test; comparison of mean values among more than two groups was carried out using one way ANOVA followed by post hoc LSD test. P-value was considered highly significant when it was equal to or less than 0.001<sup>(6)</sup>.

### Results:-

Results of most sperm parameters in healthy subjects and asthenozoospermic male patients were highly significant ( $P < 0.01$ ) improved after using FertiCult medium, Theophylline solution and SpermMobil dilution as compared with non-activation in current study. In the healthy subjects, after being activated with FertiCult Flushing medium resulted in: significant reduction in sperm concentration, significant increase in progressive sperm motility (grade A and A + B), insignificant change in sperm motility grade B and C, significant reduction in sperm motility grade D and significant increase in percentage of MNS as compared with non-activated ones, while in asthenozoospermic patients, after being activated with FertiCult Flushing medium resulted in; insignificant reduction in sperm concentration, significant increase in percent of progressive and non-progressive sperm motility ( grade A, B, A+B, C ) , significant reduction in sperm motility grade D and significant increase of MNS as a compared with non-activation as shown in Table(1).

In healthy group, Theophylline solution resulted in significant reduction in sperm concentration, significant increase of sperm motility grade (A ,B , A + B)%, non-significant change sperm motility grade C% , significant reduction in sperm motility grade D% and significant increase of MNS%; while in asthenozoospermic male group, Theophylline solution resulted in significant reduction in sperm concentration, significant increase of sperm motility grade ( A, B, A + B, C)%, significant reduction in D% and significant increase in MNS% as compared with without or non-activation Table(1).

In the healthy group, a comparison between sperm parameters of unactivated semen and after being activated by SpermMobil. SpermMobil dilution resulted in; significant reduction in sperm concentration, significant increase of sperm motility grade (A, B , A + B)%, insignificant change of sperm motility grade C%, significant reduction of grade D% and significant increase of MNS%, while, in asthenozoospermic male patients, SpermMobil dilution resulted in; significant reduction in sperm concentration, significant increase in percent of progressive and non-progressive sperm motility and MNS with significant reduction of sperm motility grade D% as shown below in Table 1.

**Table 1:-**Sperm parameters comparison between untreated semen and the semen after being activated by FertiCult Flushing medium, Theophylline solution and SpermMobil dilution in both male groups.

Groups	Sperm parameters	No activation	FertiCult medium	Theophylline solution	SpermMobil dilution	P-value
		M ± SD	M ± SD	M ± SD	M ± SD	
Normozoospermic male group n = 20	Sperm Concentration	47.00 ±10.66 A	30.55 ±6.50 C	31.20 ±7.46 C	33.00 ±5.81 B	<0.01
	Sperm motility Grade A %	3.60 ±4.50 A	9.10 ±5.42 D	10.70 ±5.86 C	15.25 ±9.13 B	<0.001
	Sperm motility Grade B %	36.30 ±7.99 C	39.25 ±6.73 B	39.95 ±8.44 B	44.35 ±6.90 A	<0.001
	Sperm motility Grade (A+B)%	39.90 ±5.19 D	48.35 ±8.15 C	50.65 ±8.31 B	59.60 ±9.17 A	<0.001
	Sperm motility Grade C %	29.95 ±6.82 A	27.60 ±7.34 A	28.10 ±7.87 A	25.90 ±9.51 A	0.151
	Immotile sperm Grade D %	30.15 ±7.29 A	24.05 ±5.84 B	21.25 ±6.78 C	14.50 ±6.83 D	<0.001
	MNS %	40.25 ±4.72 C	41.90 ±4.19 B	41.65 ±3.80 B	43.15 ±5.02 A	<0.001
Asthenozoospermic male group n = 40	Concentration	33.40 ±8.70 A	17.98 ±4.67 C	18.28 ±5.14 B	19.93 ±5.29 B	<0.001
	Sperm motility Grade A %	2.55 ±3.18 D	10.25 ±5.45 B	9.25 ±5.28 C	15.10 ±5.12 A	<0.001
	Sperm motility Grade B %	22.05 ±4.15 D	32.38 ±5.55 C	35.28 ±7.36 B	39.00 ±6.19 A	<0.001
	Sperm motility Grade A+B %	24.60 ±4.20 D	42.63 ±8.68 C	44.53 ±8.94 B	54.10 ±7.51 A	<0.001
	Sperm motility Grade C %	24.53 ±6.61 A	28.10 ±6.86 A	29.23 ±6.45 A	27.00 ±5.57 A	0.098
	Immotile sperm Grade D %	50.88 ±7.17 A	29.28 ±8.22 B	26.23 ±7.09 C	18.88 ±5.80 D	<0.001
	MNS%	35.38 ±3.08 D	36.50 ±3.08 C	37.20 ±3.28 B	38.55 ±3.45 A	<0.001

M±SD = Mean ± Standard Deviation

Different letters mean a significant

Same letters mean non-significant

### Discussion:-

The semen *in vitro* activation of healthy subjects and asthenozoospermic patients by FertiCult Flushing medium, Theophylline solution and SpermMobil dilution resulted in significant reduction in sperm concentration as compared with non-activated semen, in current study, percent of reduction resulted in SpermMobil dilution was least, because of the effectiveness of semen preparation technique which used in this work ,centrifugation and swim-up to reduce

low sperm motility and immotile sperm, only a good quality sperm were could swim up to upper and others dead and immotile sperm remain down<sup>(7,8)</sup>. SpermMobil dilution resulted in least reduction in sperm concentration that may be due to beneficial effect of its components and a short incubation time, ten minute required for SpermMobil action was useful for sperm yield<sup>(9)</sup>. *In vitro* sperm activation with FertiCult medium led to enhance of progressive sperm motility and MNS as compared with without activation, this due to their aqueous nature with lower viscosity than of seminal plasma resulted in making movement of sperm more freely in culture medium<sup>(10)</sup>, FertiCult Flushing medium contain many components needed in physiology and metabolism of sperm such as serum albumin which contains cysteine-34; may act to capture radicals, that is critical for maintaining MNS. Regarding improvement of sperm motility, in healthy subjects and asthenozoospermic patients study after being activated by Theophylline solution, these results were in an agreement with results of Loughlin *et al*<sup>(3)</sup>, Theophylline has ability on inhibition of PDE activity and thus increasing cAMP level which play a main role in glycolytic pathways of sperm, it can influence on energy generation for sperm motility. Motility is a critical sperm function to complete the reaching to oocyte and occurring fertilization (WHO, 2010). Theophylline (1,3-dimethylxanthine) is one of methylxanthine derivatives family which enhance many functions of sperm for asthenozoospermic male group, in this study the total sperm motility was 48% improved to become 73% after activation by Theophylline, about 25% percent more than of original sample, This result was agreed with result of Hong *et al*<sup>(11)</sup> who found the stimulating effect of dimethylxanthine can induce amplitude the motility of sperm more than 50% of control. After being activation of semen sample by SpermMobil dilution for both groups as a compared without activation, result in highly significant increase in progressive sperm motility and this result was in agreement with Ebner *et al*<sup>(12)</sup>, who reported the addition of (GM501 SpermMobil) led to increase in fast progressive sperm motility in minutes, best result of sperm motion was attributed to the useful components of SpermMobil dilution, a combination of (GM501 SpermMobil and FertiCult Medium). Mortimer<sup>(13)</sup>, Proved sperm hyperactivation *in vitro* is occurred when sperm suspended in a CM having bicarbonate, exogenous calcium ions, energy source like glucose and serum albumin; SpermMobil dilution contains all these components and Theophylline with essential and non-essential amino acid.

Theophylline enhances sperm motility by increasing cAMP level into sperm<sup>(3, 14)</sup>. In this study, the mean of progressive sperm motility (A+B) was below fifty percent for both of healthy subjects and asthenozoospermic male patients before activation (raw semen) increased to exceed 50% (within fertile values according to (WHO,1999)) just only after activation by SpermMobil dilution.

Finally, there was significant increase of MNS%, after activation by Theophylline solution and SpermMobil dilution for both groups as compared with non-activated semen, Theophylline has a protective effect on sperm membranes as it being oxygen radical scavengers and reducing the peroxidation of lipid and that prevents oxidative stress-induced DNA damage<sup>(15)</sup> also sperm preparation technique, Centrifugation and swim up technique used in this study was effective, had been developed to separate most of motile morphologically normal sperm<sup>(16,17)</sup>.

## Conclusion:-

Whatever the treatment or activation of semen is, concentration of sperm will be decreased and among all activation media used in this study, SpermMobil resulted in best results regarding sperm motility and MNS.

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