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RESEARCH ARTICLE

Effect of ATP on Asthinozoospermic Men In Vitro

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Abstract

The lack of proper mobility of sperm is the main cause of male infertility. One of the ways to deal with this problem is in vitro activation of the sperm by adding some materials.Objective of the present study is to investigate the effect of different concentrations of adenosine triphosphate (ATP) on the outcome of in vitro human sperm activation of Asthinozoospermic patients. Thirty samples of semen of infertile men was shared in this experimental laboratory study, during their attendance of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al- Nahrain University with mean age was (33.61 ± 5.23) year. Sperm concentration, morphology (%) and motility (progressive, non-progressive and immotile) (%) were analyzed according to WHO guidelines. Before using centrifugation swim-up technique, the washed samples were divided into 3 groups: control group (G1) without ATP. While, in G2, G3 doses of 1.25 mM, and 2.5 mM ATP was added respectively. Moreover, all groups enriched with SMART media. The sperm parameters were evaluated after the addition of ATP the results showed a significant increase ($P<0.05$) in the progressive sperm motility percentage as compared to the G1. While, in G3 with dose of the 2.5 mM ATP, progressive sperm motility percentage was significantly increased ($P<0.05$) as compared to G1and non-significant ($P>0.05$) when compared with G2. Based on the results of this study, it can be concluded that the addition of 2.5 mM ATP to washed sperms can improve sperm motility of Asthinozoospermic samples. Data were analyzed statistically using complete randomized design (CRD) (one way)..

Introduction:

Clinical definition of male infertility is the presence of abnormal semen parameters in the male partner of a couple who have been unable to conceive after 1 year of unprotected intercourse [1]. Infertility is a major problem in 15-20% of couples trying to conceive in the reproductive ages. On the other hand, male infertility may contribute in half of all couples who refer to infertility clinics [2]. Furthermore, infertility is classified into two types, primary infertility, the couples have never achieved a pregnancy at any time or secondary infertility denotes that a previous pregnancy was achieved, regardless of outcome [3]. The standard semen analysis is the first line and the most popular laboratory test in the diagnosis of male fertility. It evaluates sperm concentration, motility, morphology and vitality. However, it is well-known that normal results of semen analysis cannot exclude men from causes of couples infertility [4].

Adenosine 5-triphosphate (ATP) is a fundamental factor to maintain the life, by providing energy, and controlling the cell function and metabolism. In spermatozoa, ATP plays important roles for the movement to female reproductive tract, viability and penetration to fertilize with oocyte[5]. Human spermatozoa treated with extracellular ATP (ATPe) have an increased motility and fertilization rates, suggesting that ATPe may

be helpful to combat the male infertility [6].Therefor, the current study aims to study the effects of different concentrations of ATP treatment on sperm parametersof Asthinozoospermic Men during in vitro sperm activation

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Materials and methods:

All samples of semen were 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 obtained the sample of semen must be labeled with the information about name, age, abstinence period and time of sample collection. The specimens were placed in the cubator at 37ºC for 30 minutes to allow liquefaction [7]. The liquefied semen is then carefully mixed for few seconds, and then subjected to both macroscopic and microscopic examinations within one hour from collection according to [8]. The standard of [9] is used to record details of the semen analysis results.

.**Statistical analysis:**

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago). Sperm parameters pre- and post in vitro sperm activation and groups of infertile men were analyzed using complete randomized design (CRD) (one way ANOVA).

Results:

The results for asthinozoospermic semen samples in figures 1,2,3,4 and 5 revealed a significant decrease ($P < 0.05$) in sperm concentration.While, a significant increase ($P < 0.05$) in the percentage of sperm motility , non- progressive motility and normal sperm morphology as compared to pre- activation. Meanwhile, results of progressive motility (%) indicated significant increased ($P<0.05$) in the G2 and G3 groups only. The mean progressive motility (%) of the group G1 was significantly ($P < 0.05$) lower than the others groups post-activation and non-significant ($P>0.05$) with pre-activation . However, progressive motility (%) non-significant ($P>0.05$) in G3 group as compared with G2 group.

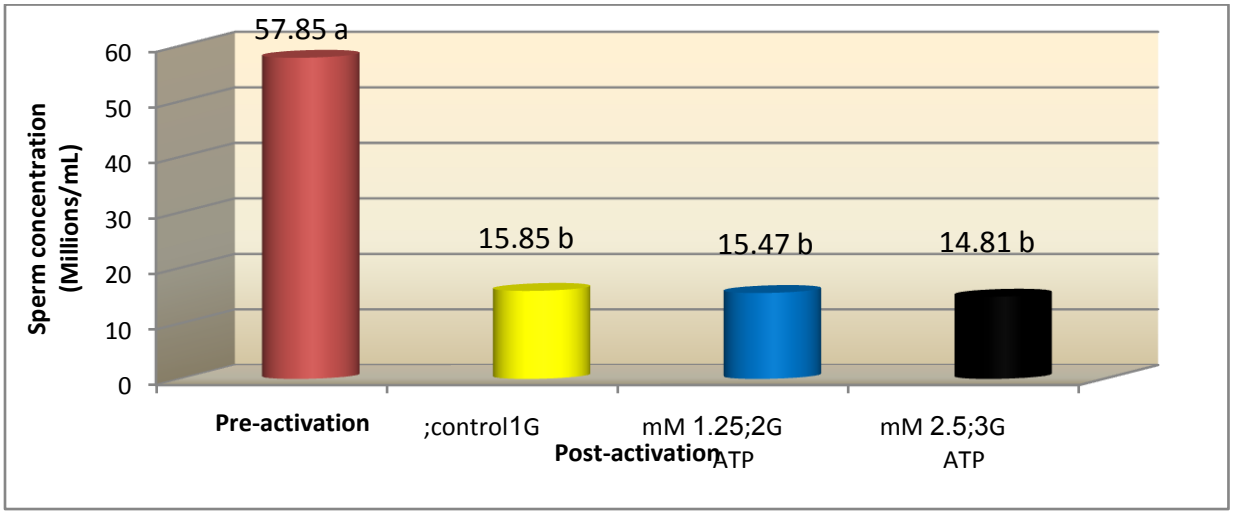

Figure (1): Sperm concentration for subjects involved in the present study using SMART medium enriched with two concentrations of ATP.

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

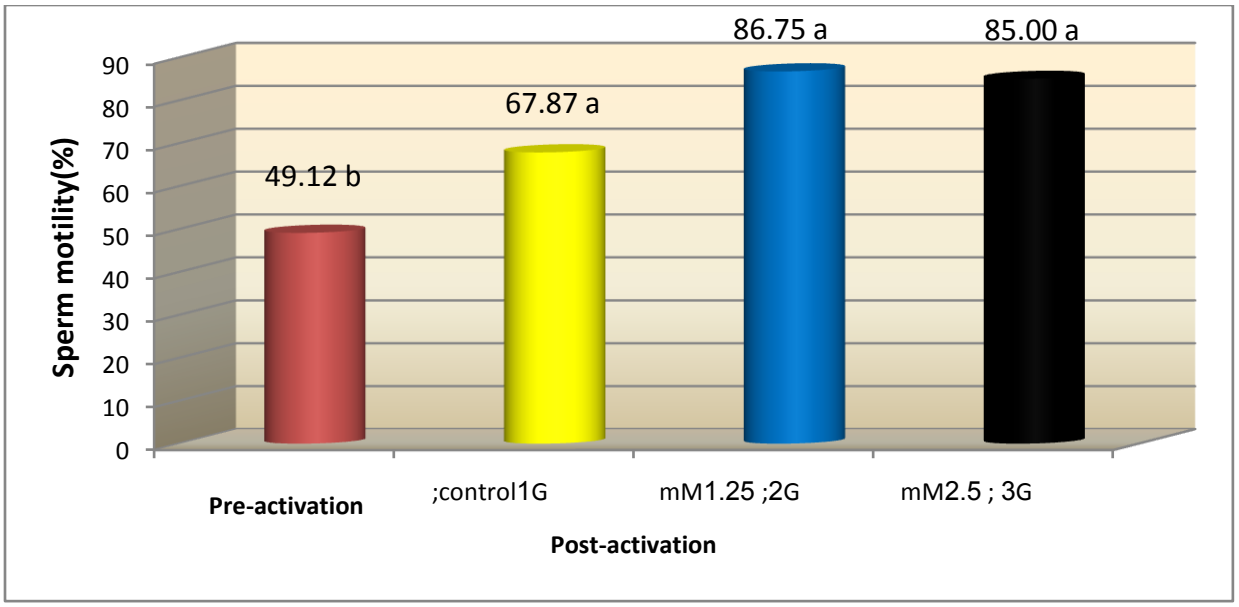

Figure (2): Sperm motility (%) for subjects involved in the present study using SMART medium enriched with two concentrations of ATP.

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

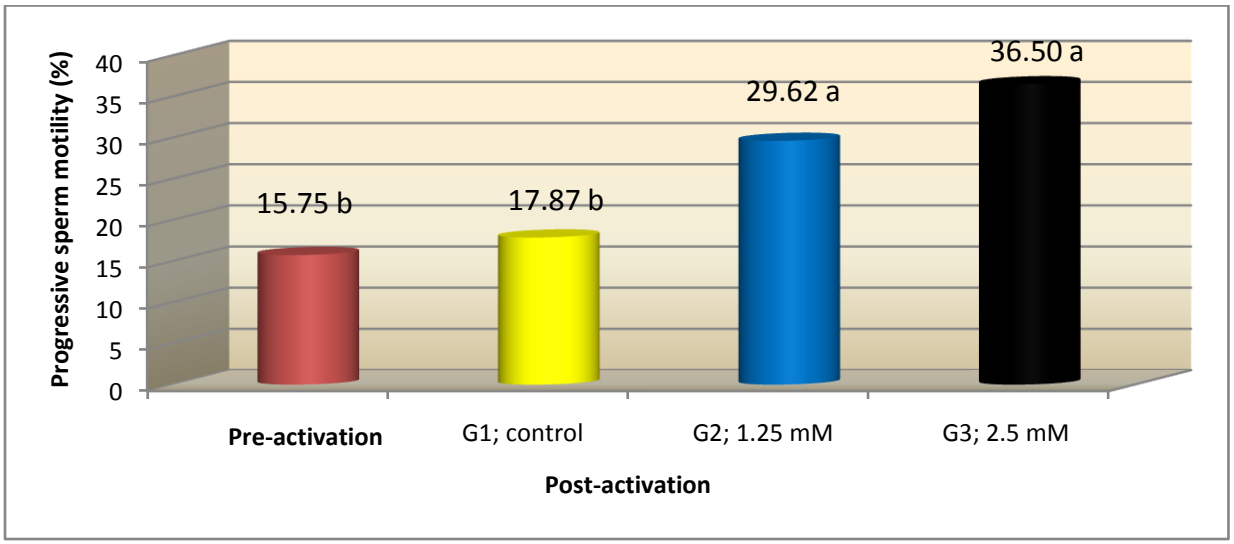

Figure (3): Progressive motility (%) for subjects involved in the present study using SMART medium enriched with two different concentrations of ATP.

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

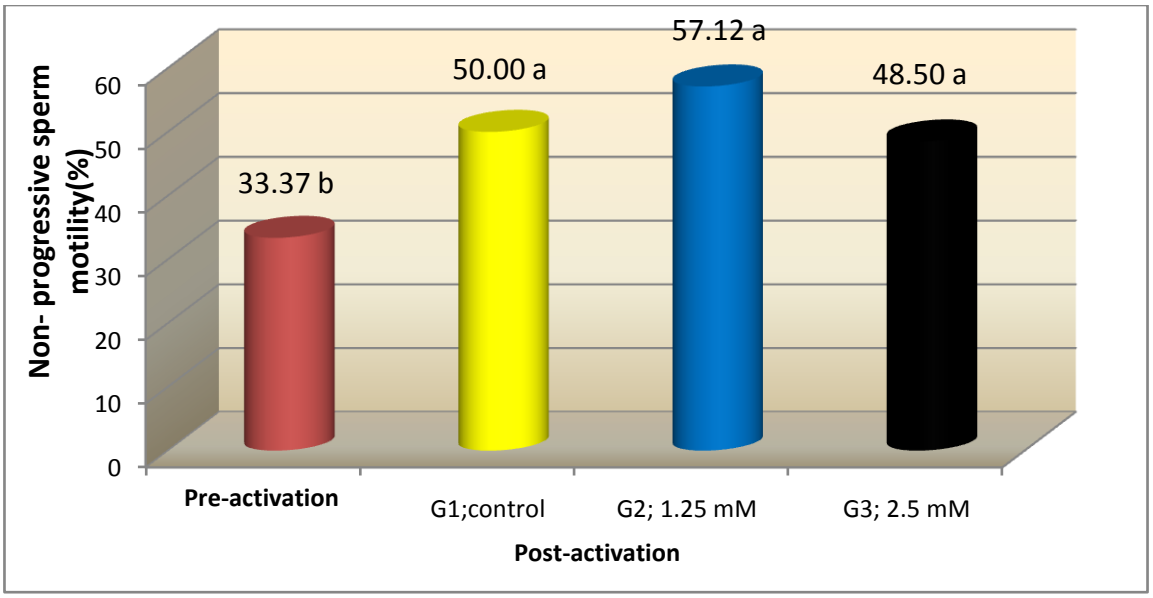

Figure (4): Non-Progressive motility (%) for subjects involved in the present study using SMART medium enriched with two concentrations of ATP.

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

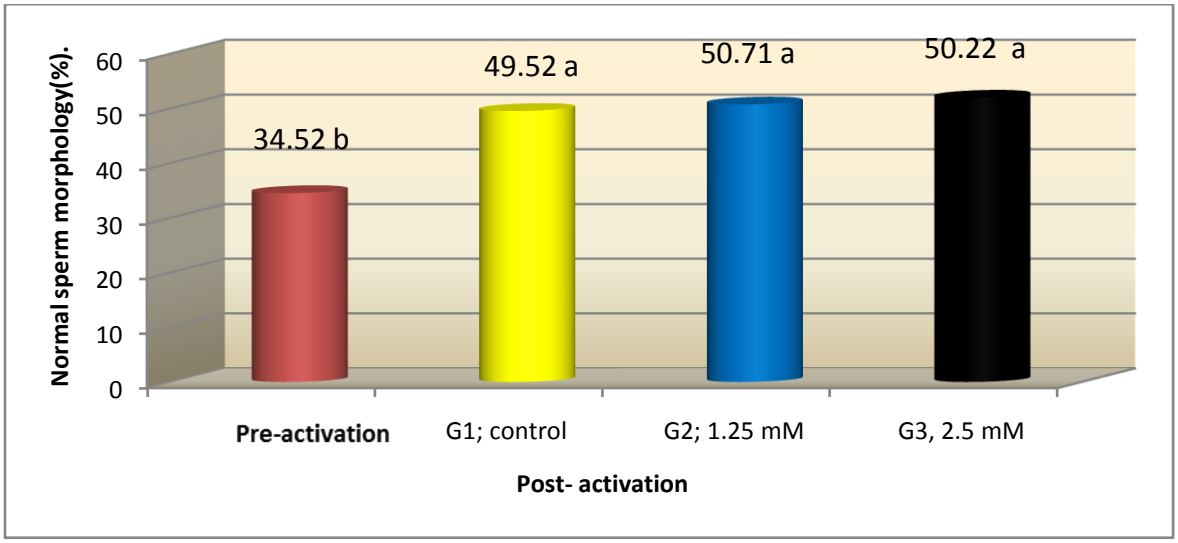

Figure (5): Normal sperm morphology (%) for subjects involved in the present study using SMART medium enriched with two different concentrations of ATP.

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

Discussion:

Sperm preparation for ART is required to select and maximize the superior quality spermatozoa [10]. The quality of semen samples, sperm preparation techniques and culture media can play a critical role in determining successful the outcomes of ART [11].

In regard to centrifugation swim-up technique that was used in this study, it is one of the common sperm preparation techniques for both experimental and practical programs[12,13], characterized with simplicity, rapid and cost effectiveness[14]. In the efficiency of this method for preparing infertile male specimens, centrifugation facilitates the removal seminal plasma and concentrates the spermatozoa to prepare the sperms for re- dilution [13], with respect to sperms isolated with the swim up are clean and motile.

Studies have shown that sperm preparation techniques which employ centrifugation lead to ROS production [15]. [10] were confirmed, where 10 and 30 minutes of centrifugation led to increased ROS production, ROS may play a beneficial role in normal physiological function [16], but once their presence is in excess they induce pathologies. Excessive ROS lead to lipid peroxidation of the sperm plasma membrane which results in a loss of membrane fluidity which is essential for sperm motility [17].

In the present study, the centrifugation force was 2500 rpm for 6 min to avoid oxidative damages of sperm plasma membrane via produce very high levels of ROS by pelleting of the semen with the impairment of sperm functions and decrease in the percentage of normally chromatin-condensed spermatozoa.

In vitro human sperm activation techniques using SMART medium in this study. The results showed a significant reduction ($P<0.05$) in the sperm concentration after ISA using centrifugation techniques for all infertile males as compared to pre-activation 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 [18].

Furthermore, 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 [19].

It is noticed in the current study that the use of in vitro culture media a significant increases ($P <0.05$) in sperm motility, as shown in G1 (control) compared to pre-activation. The reason is that the seminal fluid with high viscosity obstructs sperm progressive motility so that the uses of in vitro media with aqueous nature lead to decrease the viscosity of the seminal fluid and as a result sperms move more freely because of their aqueous nature with lower viscosity than of seminal plasma resulted in making spermatozoa move more freely [20].

ATP is a physiological compound present in all living cells, it has been demonstrated that it has a role in mediating important physiological functions in many cell types [21]. [22] found that ATPe is a non-toxic potent and rapid activator of human sperm fertilizing potential. [23] found that a substantial and continuing supply of ATP is necessary for the various events associated with mammalian sperm function. [24] they chosen of ATP to improve sperm fertility.

[25] revealed that the effect of adding different levels 5,10 and 15 mg of ATP to semen diluter of sperm of sheep that treatments have non-significant effect on spermatozoa motility. On the other hand, in mouse sperm, concentrations of ATPe as high as 2.5mM stimulated fertilizing ability, without any toxic or detrimental action on sperm motility or viability [26].

In this study, 2.5mM ATP was selected for several factors:

1- The DNA fragmentation was more sensitive to ATP concentration, being greatest at 0.5 mM ATP but then decreasing towards higher ATP concentrations [27].
2- [28] who deals with Sea-Urchin sperm flagella revealed that ATP concentration is a major factor determining the beat frequency of reactivated flagella; changes in beat frequency of two orders of magnitude can be observed over the ATP concentration range of 0-005 to 4 mM
3- The optimum ATP concentration for the dynein-ATPase activity was found to be between 1 and 2 mM [29]. [30] which concluded that semen samples with ATP concentrations of less than 40 pmol/l0^6 sperm had limited success in in vitro fertilization.

This study showed a significant improvement in the sperm motility (%) after addition of ATP to the SMART medium for involved subjects. Furthermore, G3 was slight better from G2, this result goes with the finding of [31], they revealed that activation, the change in motility parameters seems to be directly related

to ATP content, This finding also is compatible with the finding of [5] in which they demonstrated that fertilization rates obtained with the use of ATP-treated spermatozoa were significantly higher than those of a control group.

[32] in which they noticed that significantly increased the hyperactivation percentage of sperm with poor motility by the addition ATP, there was a significant increase in straight-line velocity (VSL), curvilinear velocity (VCL) and percentage of rapid which is similar to the current finding. The beneficial effect of ATP could have been due to alterations in sperm motility parameters, faster and straighter movement of sperm after the treatment [26].

[33] they concluded that the relationship between ATP concentration and fertilization rate is due to the fact that the flagellar beat frequency of spermatozoa depends on ATP concentration and dynein ATPase activity which hydrolyses ATP to initiate motility. In order to determine whether pathological forms of motility can be recognized through changes in the activity of dynein ATPase. ATP is the main energy source used by the sperm flagellum to initiate and propagate forward motility [34].

Several studies of human spermatozoa have not found a significant correlation between ATP content and fertility or IVF rate [35,36 ,37]. Meanwhile, [38] they concluded that ATP levels in semen cannot be directly related to motility. These findings apparently contradict those of some authors who have stated that ATP levels are positively related to total numbers of motile spermatozoa and total viable spermatozoa as well as fertilizing potential [39; 40, 24]. A decrease in ATP levels correlates to a decrease in motility and possibly fertilization capability [30, 41, 42].

These results disagree with other studies depending on the differences in the experimental methodology [43]. The conditions in reproductive laboratories, due to the limited number of IVF attempt [24], high doses of ATP [38, 23, 34].

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