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Contributory Effect of Adenosine Triphosphate (ATP) To Male Infertility

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ABSTRACT

Infertility comes at a cost to the couples/spouses as the associated trauma ranges from depression to rejection, emotional imbalance to mention a few. Adenosine triphosphate (ATP) plays very significant function in sperm function. Any disruption in ATP production or action contribute significantly to male infertility. The aim of this study was to determine the contributory effects of ATP on fertilization by the spermatozoa. This was a cross-sectional study that randomly selected 45 male partners of infertile couples as test participants and 45 male partners of fertile couples as controls all aged between 30 years and 55 years. Semen samples were received from the participants immediately after production through masturbation for semen analysis which was done on the day of ejaculation. ATP value in the semen samples were estimated using Enzyme Linked Immunosorbent Assay (ELISA) technique. Independent's t-test was used to determine the difference in ATP values between male partners of infertile couples and male partners of fertile couples as well ATP values in male partners of infertile couples with normal and abnormal sperm motility. ANOVA was used to determine the differences in ATP values among male partners of infertile couples with normal and abnormal sperm counts. P value less than 0.05 was considered significant. The mean ± SD ATP value in male partners of infertile couples (598.27 ± 67.90 nmol/L) was significantly lower than the mean ± SD ATP value in male partners of fertile couples (838.86± 74.77 nmol/L), $p<0.0001$). The mean ± SD ATP value in male partners of infertile couples with abnormal sperm motility (559.62 ± 57.38 nmol/L)) was significantly lower than the mean ± SD ATP value in male partners of fertile couples with normal motility (638.68 ± 53.52 nmol/L), $p<0.0001$). On ANOVA statistics, the mean ± SD ATP value in male partners of infertile couples with zero sperm count was significantly lower than mean ± SD ATP value in male partners of infertile couples with sperm count greater than 20 sperm cells/ml ($p = 0.043$). However, there were no significant differences in the mean values of ATP in male partners of infertile couples with sperm count less than 10 sperm cells/ml, less than 20 sperm cells per ml and sperm count greater than 20 sperm cells/ml ($p>0.05$). This study observed that adenosine triphosphate (ATP) concentration in male partners of fertile couples was higher than ATP concentration in male partners of infertile couples and male partners of infertile couples with abnormal motility of spermatozoa had lower concentration of ATP than those who had normal motility. Reduced levels of ATP in semen which may be caused by mitochondrial dysfunction, oxidative stress, or metabolic defects contributes largely to male infertility due to impairment of spermatozoa motility and capacitation incapability which hinders fertilization of the oocyte by the spermatozoa.

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Keywords: ATP, spermatozoa, infertility, acrosome reaction, sperm motility

INTRODUCTION

Infertility comes at a cost to the couples/spouses as the trauma associated with couples that are experiencing infertility is unimaginable. The psychological effect or response on its own is a disruptor of reproductive functions. This is much frustrating when there is no known cause and therefore no solution for the cause of the challenge. Several conditions or stressors have been implicated in cases of infertile couples in previous studies. Such stressors as hypothyroidism [1], academic examination [2], HIV/AIDS [3] and biochemical alterations [4] have been reported previously. There have been well documented reports on diseases and endocrine disruptors causing infertility in both male and female individuals but there has been less attention to possible contribution of lack of energy to infertility. However, a rare area that has not been populated with reports is the contribution of energy unavailability to the spermatozoa to facilitate fertilization. ATP plays very significant role in sperm function. Sperm motility is a critical factor in male fertility, as it ptovides evergy required for spermatozoa to migrate along the female reproductive tract towards the oocyte for fertilization. Given that sperm motility is an energy-intensive process, the sustained availability of ATP enhances the function of the spermatozoa [5]. The flagella of the spermatozoa generates its characteristic movement through the coordinated action of dynein, a motor protein that functions by hydrolyzing ATP to produce the mechanical force required for flagellar bending and progressive motility [6]. Therefore, any disruption in ATP production or action contribute significantly to male infertility. Just as all synthesis or catabolism in the human eco-system requires energy with lack or insufficient energy aborting any process, unavailability of ATP can significantly affect fertilization ability of the spermatozoa.

Aim

The aim of this study was to determine the contributory effects of ATP on fertilization by the spermatozoa.

Study design

This was a cross-sectional study that was done among male partners of infertile couples visiting Federal Medical Centre, Asaba, Delta State.

Methods

Ethical approval was obtained from the Ethics Committee of Federal Medical Centre, Asaba. Informed consent was obtained from all participants before recruiting them into the study. A total of 90 participants aged between 30 years and 55 year, 45 of who were male partners of infertile couples and 45 male partners of fertile coples were recruited into the study. Sociodemographic data was collected using a structured questionnaire that also captured information about their fertility history. Semen samples were received for the participants immediately after production through masturbation in universal bottles and an aliquot was transferred into plain sample bottle after mixing to homogenize the samples for ATP assay. All samples for ATP assay were stored at -40^{o} until day of analysis. All assays were done within 3 three weeks of sample collection. However, semen analysis was done on the day of ejaculation according to World Health Organisation protocol for semen analysis [7] indicating the period of abstinence (between 3 and 5 days), date and time of sample collection as well as ejaculate volume. Upon receipt, sample was place in the incubator at 37^{o}C for 30 minutes to allow for liquefaction. The volume of each sample was measured using 20 ml micro measuring cylinder. Macroscopic evaluation of the semen was done describing the homogenicity, viscosity, colour, as well as pH determination which was done by dropping the sample on a pH strip after which the colour was compared with pH calibration strip to determine the pH. The sample was well mixed by swirling for between 15 to 30 seconds before removing an aliquot of the sample. Microscopic examination was done by placing 10µl of well mixed semen sample onto to clean pre-warmed microscopic slide which was covered with a coverslip while avoiding formation and trapping of bubbles in-between the slide and coverslip. The initial microscopy was done using a x10 objective lens to have an overview observing the even spread of spermatozoa across the fields so as to ensure that to spermatozoa are evenly distributed in the preparation void of any visible mucus strands and sperm aggregation or agglutination. Microscopy was continues with higher magnification using a x40 objective lens to assess sperm motility, to

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determine sperm morphology and for the determination of the presence of other cells other than spermatozoa such as epithelial cells, red blood cells or pus cells. Commercially prepared semen diluting fluid containing 0.595M sodium bicarbonate and approximately 0.14M formalin was used to make 1 in 20 dilution of the well mixed semen sample by adding 50µl of sample to 950µl of semen diluting fluid and thoroughly mixed. The haemocytometer was loaded and left in a humid chamber to allow the spermatozoa to settle onto the bottom of the counting chamber. The number of spermatozoa in two large squares were promptly counted after removal from the humid chamber. The concentration of spermatozoa per ml was calculated.

ATP Estimation

This was estimated using Enzyme Linked Immunosorbent Assay (ELISA) kit manufacturer by Shangai Ideal Medical Technology Co., LTD, where an indirect assay method which is based on the principle of specific antigen-antibody interactions was used [8].

Statistical Analysis

Independent's t-test was used to determine the difference in ATP values in male partners of infertile couples and male partners of fertile couples as well ATP values in male partners of infertile couples with normal and abnormal sperm motility. ANOVA was used to determine the differences in ATP values among male partners of infertile couples with sperm count greater than or equal to 20million sperm cells/ml, male partners of infertile couples with zero sperm low sperm count of greater or equal to 10 million sperm cells/ml, male partners of infertile couples with less than 10 million sperm cells/ml and male partners of infertile couples with zero sperm cell. P value less than 0.05 was considered significant.

RESULT

The mean ± SD ATP value in male partners of infertile couples (598.27 ± 67.90 nmol/L) was significantly lower than the mean ± SD ATP value in male partners of fertile couples (838.86± 74.77 nmol/L, $p<0.0001$). The mean ± SD ATP value in male partners of infertile couples with abnormal sperm motility (559.62 ± 57.38 nmol/L) was significantly lower than the mean ± SD ATP value in male partners of fertile couples with normal motility (638.68 ± 53.52 nmol/L, $p<0.0001$). On ANOVA statistics, the mean ± SD ATP value in male partners of infertile couples with zero sperm count was significantly lower than mean ± SD ATP value in male partners of infertile couples with sperm count greater than 20 sperm cells/ml ($p = 0.043$). However, there were no significant differences in the mean values of ATP in male partners of infertile couples with sperm count less than 10 sperm cells/ml, less than 20 sperm cells per ml and sperm count greater than 20 sperm cells/ml ($p>0.05$).

DISCUSSION

This study observed that adenosine triphosphate (ATP) concentration in male partners of fertile couples was higher than ATP concentration in male partners of infertile couples. This study also observed that male partners of infertile couples with abnormal motility of spermatozoa had lower concentration of ATP than those who had normal motility. This study also examined the values of ATP in male partners on infertile males based on their sperm counts and observed that ATP values in the men azoospermia was very low compared with ATP values in men with sperm count greater than 20million cells/ml. However, ATP values in male partners of infertile couples were generally similar. ATP has been proven to play very important roles in fertilization ability by spermatozoa, particularly by supplying the required energy for the stages of fertilization including movement of the spermatozoa towards the oocyte, capacitation, acrosome reaction, and fusion with the oocyte. ATP is the primary source of energy required for spermatozoa to move within the female reproductive tract toward the oocyte. Also, ATP energizes the biochemical changes that occur during capacitation stage of fertilization. ATP increases intracellular calcium ion concentration in over 45% of individual spermatozoa, which in turn leads to a sperm-head volume increase, likely involving acrosomal swelling resulting in acrosome reaction [9][10][11]. The observation from this study is similar to the findings from another study [12] that reported that Patients with high ATP levels had better sperm morphology, higher concentration and motility and lower semen viscosity. This shows that ATP concentration in human spermatozoa may serve as a potential physiological biomarker in combination with classical sperm parameters. Also, an earlier study [13] published online in 2009, reported that

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ATP levels in human spermatozoa was reported to positively correlate with good motility. This has given rise to the impression that good ATP levels are related to good motility. Spermatozoa rely heavily on mitochondrial ATP for movement. This means that, the higher the ATP concentration, the better the motility which increases the fertilizing potential of the spermatozoa. In another research carried out to observe the contributory effect of ATP to fertilization, the result demonstrated that ATP induces a significant increase of sperm fertilizing potential, and suggested the administration of exogenous ATP during *in vitro* fertilization procedures can enhance fertilization thereby improving success rate[14].

Low sperm count with reduced ATP suggests mitochondrial dysfunction or oxidative stress. The Mitochondria has been termed the powerhouses of sperm cells because of its primary roles in the generation of ATP through oxidative phosphorylation. Also, Glycolytic defects as observed in hexokinase deficiency reduces ATP generation in the spermatozoa thereby causing an alteration in the cascade events in ATP generation. This was corroborated in separate studies [15][16] where they demonstrated that reduced sperm motility and decreased sperm fertilizing capacity are associated with impaired mitochondrial activity as shown by a decrease in semen ATP. This finding suggests that oxidative phosphorylation, the primary ATP production pathway within mitochondria, is essential for powering sperm motility.

CONCLUSION

Reduced levels of ATP in semen which may be caused by mitochondrial dysfunction, oxidative stress, or metabolic defects contributes largely to male infertility due to impairment of spermatozoa motility and capacitation incapability which hinders fertilization of the oocyte by the spermatozoa. Therefore, addition of ATP assays and possible inclusion of exogenous ATP as well as therapies that maintain ATP generation and distribution may improve fertility outcomes to a very reasonable extent.

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Table I Mean ± Sd Of Atp Values In Fertile Males And Infertile Males

Parameter	Fertile Males N = 45	Infertile Males N =45	t	P-value
ATP (nmol/L)	838.86± 74.77	598.27 ± 67.90	-15.846	0.000

Table Ii Mean ± Sd Of Atp Values In Infertile Males With Abnormal Motility And Normal Motility

Parameter	Abnormal Motility n = 23	Normal Motility n = 22	t	P-value
ATP (nmol/L)	559.62 ± 57.38	638.68 ± 53.52	-4.774	0.000

Table Iii Anova With Lsd Post Hoc Of Atp Values In Infertile Males Compared With Their Sperm Count Values

Group	n	ATP Value (nmol/L)
Zero sperm count (A)	6	556.38 ± 42.49
Sperm count less than 10cells/ml (B)	11	579.07± 50.91
Sperm count less than 20cells/ml (C)	16	607.04 ± 44.65

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Sperm count greater than 20cells/ml (D)	12	625.33 ± 101.50
P value		0.146
F value		1.895
A vs B		0.503
A vs C		0.113
A vs D		0.043
B vs C		0.281
B vs D		0.099
C vs D		0.472