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Original Research Article.

Male Fertility: Association with Ca^{2+} ATPase

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ABSTRACT

Background: Infertility is the inability to have pregnancy, it affects 10% to 15% of married couples worldwide. Almost 20% of infertility are attributed to male factors, and another 20% due to combined male and female factors, with almost 10-15% due to unknown cause. Investigations are usually concerned about anatomical and morphological characteristics. Few researches and clinical practices take advanced laboratory tests in account. The current study aim at elaborating the role of Ca^{+2} –ATPase specific activity in infertility that might help in diagnosis and treatment of infertility cases who had been previously categorized as being of unknown etiology.

Materials and Subjects: Through cross sectional study design we recruited 63 volunteer married adult Saudi males who were selected randomly from two main general hospitals in Jeddah, they were categorized according to fertility into primary fertility, secondary fertility, and apparently normal who served as a control group. The fresh semen from the three groups was investigated for Ca^{+2} –ATPase specific activity. The laboratory findings were analyzed by ANOVA test and post hoc Tukey test.

Results: The mean specific activity of Ca++ ATPase for healthy fertile males (25.1 ± 1.2 µmol Pi I min. mg protein) was significantly higher than males with primary infertility (23.4 ± 1.4 µmol Pi I min. mg protein) $p<0.05$, and although the healthy fertile group, which was also higher than that in males with secondary infertility (24.3 ± 1.3 µmol Pi I min. mg protein), however, this difference is not statistically significant $p>0.05$.

Conclusion: The findings of this study help in better understanding of the etiology of unknown cases of infertility, and could be used as a basis for diagnosis and treatment of this problem.

Keywords: Ca^{+2} –ATPase, Infertility.

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INTRODUCTION

Infertility is defined as inability of couples to have pregnancy after 12 months or more of unprotected intercourse.[1] The primary infertility means inability to achieve first pregnancy, while the secondary infertility is the inability to get pregnancy after prior pregnancy regardless of being a live birth.[2] It had been estimated that male factors account for almost 20% of the couples' infertility.[3] Infertility imposes negative public health consequences including perceived stigma[4], psychological distress[5] and later adult onset disease.[6]

For fertilization to occur, the sperm is needed to become mature and fertile, this process passes through two main events, first is capacitation or maturation of the sperm, and acrosome reaction.[7] Capacitation means endow of the sperm with fertilization capacity, it starts as early as in the male reproductive tract, particularly in the epididymis, and continue through its journey in the female reproductive tract.[8]

Initially, the inhibitory effect of cholesterol covering the sperm is washed in the vaginal secretions, which trigger active motility of the sperm in the cervix, which helps the sperm to reach the fallopian tube.[9,10] At molecular level, the active motility and hyper-activation of the spermatozoa is mediated by Ca^{2+} influx in the sperm.[11] On reaching the fallopian tube, the sperm is attracted by chemotactic reactions with ovarian secretions till it comes in contact with the secondary oocyst.[12] As contact occurs, the second main event is initiated where the acrosome reaction starts by hydrolysis of the point of contact with the corona radiate covering the ovum, which facilitates penetration of the spermatozoa through the zona pellucida to evacuate its genetic materials into cystoplasm.[13,14] The fusion of the genetic materials of the sperm with the that of the ovum creates the zygote.[15] Again, previous studies suggested that the acrosome reaction rely on the influx of extracellular Ca^{2+} in human sperm treated with human follicular fluid.[16]

In this respect, Ca^{2+} ATPase (PMCA) is the main transport protein which is responsible for regulating the amount and influx of Ca^{2+} within the cells.[17] Calcium ions (Ca2+) often act as a second messenger similar to cyclic adenosine monophosphate (cAMP), it transient increase triggers numerous cellular responses, including

muscle contraction, release of neurotransmitters, glycogen breakdown and activation of oxidative metabolism.[18] Despite of this apparent significant role of Ca^{2+} and Ca^{2+} dependent signals in sperm motility and fertilization, Shuh et al (2004) pointed that its pathways are still incompletely understood.[19]

The main objective of this research work is to test the validity of the following hypothesis: Do sperm Ca2+ -ATPase activities have significant contribution to male fertility or not?

MATERIALS AND METHODS

A randomly selected 63 persons were invited to be enrolled in the study from attendants of two main general hospitals in Jeddah city. After ensuring ethical considerations, they were asked to provide fresh semen through masturbation after 3 days abstinence. The semen was collected in clear, sterilized polypropyline containers, it was centrifuged at 3500 rpm for 10 mm; and seminal plasma was then separated and stored at -80°C until analyzed for biochemical parameters. To assess the Ca2+-ATPase Activity, the supernatant was taken, and the precipitant which contains the sperm (1 g) was homogenized with (5 ml) of sucrose solution (0.25 M). The homogeneous solution contains sperm Ca^{2+} ATPase was preserved in (-80°C) freezer. Aliquots of 50µl of sperm homogenate was add to ATPase buffer without EGTA and to the one with EGTA. Incubated for 5 min at 37°C, then mM ATP (20µ1 or 30µ1) was added and incubated further for 10 min. Adding 2 ml of ammonium molybdate solution to stop the reaction.[20] The ATPase activity was quantified by measuring the amount of Pi release from ATP hydrolysis. The absorbency of the color complex was measured at 750 nm [Table 1]. The collected data were entered into a personal computer and analyses of data were performed using SPSS statistical package. ANOVA and post hoc Tukey test were used for comparing means. P value were considered to be statistically significant if < 0.05.

Table 1: Assay Protocol for Ca^{++} -ATPase

Reagent	Blank	ATP	Ca^{2+} ATPase	EGTA (-)	EGTA (+)
ATPase Buffer µL	910	910	910	910	-
H_2O µL	90	70	40	20	-
ATPase Buffer with EGTA	-	-	-	-	930
Ca^{2+} - ATPase µL	-	-	50	50	50
Incubation time: 5 min at 37°C					
ATP µL	-	20	-	20	20
Incubation time: 10 min at 37°C					
Molybdic (acid (ml)	2	2	2	2	2
Ascrbic Acid µL	20	20	20	20	20

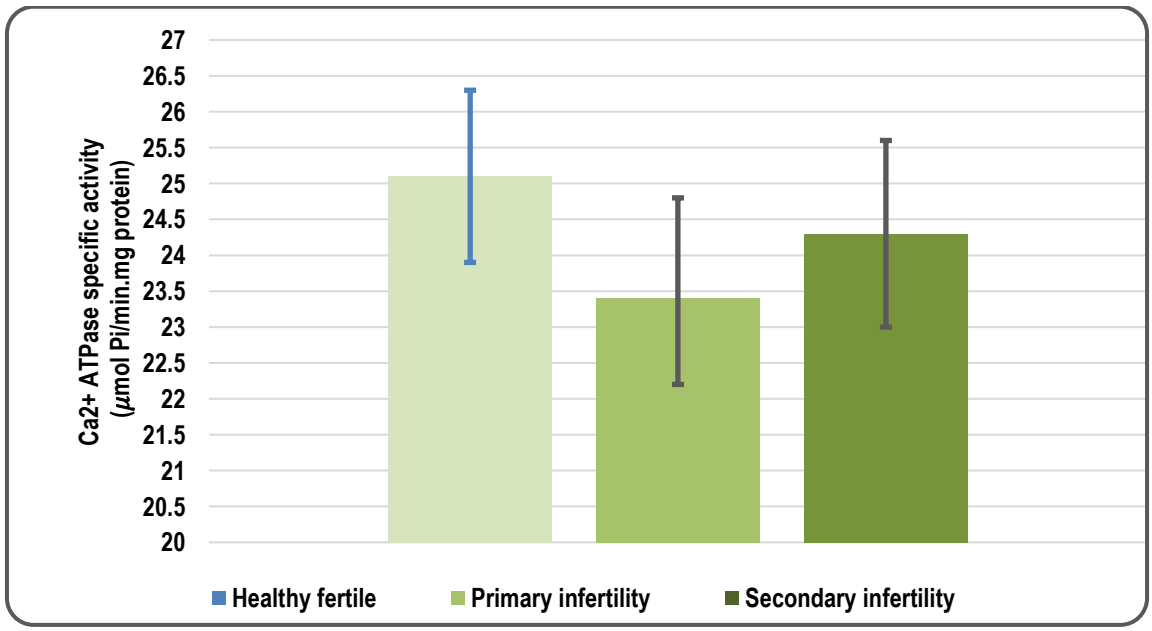

Figure 1: The mean values of Ca^{2+} ATPase specific activity of healthy fertile, primary infertility and secondary infertility groups

RESULTS

Sixty-three adult married Saudi males who were married for more than one year ,were involved in the study, their mean age accounted for 38.2±7.8 years, it ranged between 25-55 years, most of them (41, 65%) were employed in administrative jobs and (13, 25%) were working in technical jobs, while (9, 15%) were jobless. The study group were divided according to the fertility status into three groups. The first group, healthy fertile (5 subjects), primary infertility (43 subjects), and secondary infertility (15 subjects). Figure 1 shows the mean specific activity of Ca++

ATPase for healthy fertile, primary infertility and secondary infertility groups. Healthy fertile (25.1 ± 1.2 μmol Pi I min. mg protein) is the highest compared to primary infertility (23.4 ± 1.4 μmol Pi I min. mg protein), which is statistically significant (P = 0.03). Also, between the healthy fertile group, which is the higher compared to the secondary infertility (24.3 ± 1.3 μmol Pi I min. mg protein), however, this difference is not statistically significant p>0.05.

DISCUSSION

Male subfertility is acknowledged to contribute significantly to infertility problems experienced by couples. In some instances, morphological and or physiological defects known to interfere with normal sperm function can be identified. However, in others, no obvious cause of fertilization failure can be identified. The recent introduction of molecular methods has made it possible to diagnose more subtle defects that could affect the function of spermatozoa produced by some males. For others, though the problems may result from defects in the physiological mechanisms that need to be activated in spermatozoa so that they 'switch on' functionally following their release from the male reproductive tract. Capacitation, the term applied to this 'switching on', encompasses a number of changes that, collectively, confer fertilizing potential on sperm cells.[8-10]

Fertility is dependent on a complex set of event, involving both male and female components. Normal sperm function involves many processes, including motility, capacitation, acrosome reactivity and ultimately, fertilization of the oocyte. While male fertility is most often assessed by means of gross semen parameters, infertility may also be caused by abnormal sperm function, and only by performing evident, specific tests, which may be helpful, that include semen analysis, detailed sperm motility assessment, motility longevity, hypo-osmotic swelling test, mucus penetration assay and in-vitro fertilization.[21]

The findings of the current study showed that there is a significant association between primary infertility and relatively lower level of Ca2+ ATPase specific activity, which add a significant contribution to the pool of our knowledge about etiology of infertility. The role of both the Ca^{2+} stores and Ca2+ ATPase specific activity had been discussed in some researches long ago, for example Perry at all (1997) pointed that human sperm have an obligatory requirement for extracellular calcium during capacitation and the acrosome reaction, but may require either very little extracellular Ca^{2+} to maintain motility or possess internal Ca^{2+} stores sufficient for their requirements.[22] Because Ca^{2+} is argued to exert substantial role in most if not all the processes through which the sperm reach its full capacity and goal in merging its genetic contents with that in the secondary oocyst, However, although Ren et al (2001) stated that "gene ablation of the cation channel of sperm (CatSper) leads to impaired sperm motility and male infertility",[23] other authors reported that there is still little doubt about the importance of calcium homeostasis on motility and fertilization capacity of the sperm.[24,25] Moreover, Schuh et al (2004) stated that "the function of the plasma membrane Ca2+/calmodulin-dependent Ca2+ ATPase during this process remained enigmatic".[19] From these perspectives, we could place the findings of the current study, where it could help in clarifying these ambiguous arguments about the role of Ca2+ ATPase specific activity which is proven in our study.

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