Semen quality concentration for differentiation between fertile and infertile males

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Abstract

Background: semen is an organic fluid also known as seminal fluid that usually contains spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of male and can fertilize female ova. In human, seminal fluid contains several components besides spermatozoa. Photolytic and other enzymes as well as fructose are elements of seminal fluid which promote the survival of spermatozoa and provide a medium through which they can move or swim. The process that results in the discharge of semen is called ejaculation. Male infertility is defined by most authorities as the inability to achieve a pregnancy for at least one year. **Objectives:** The study used resazurin reduction test as additional, inexpensive, simple tool for diagnosis of male infertility . **Methods**: This study was carried on patients obtained from outpatient clinic of male infertility department at El Hussein Hospital of Cairo. The mean age of investigated patients was 27±7 years. Samples were collected by masturbation in wide sterile container after 3 days of abstinence. Samples were divided into two groups. Group one comprised (n=30) normal controls (normozoospermia) indicated by sperm count more than 20 millions/ml, motility more than 50% and normal morphology. Infertile group consisted of oligozoospermia (n=30) indicated by sperm count less than 20 millions/ml, athenozoospermia (n=30) indicated by motility less than 50% and azoospermia (n=30) indicated by the complete absence of spermatozoa. All samples were centrifuged at 4000 rpm for 15 minutes and seminal plasma was separated and kept until analyses. Seminal plasma was collected on sterile dry test tube to separate for the biochemical studies (semen quality concentration) by using resazurin reduction

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test (RRT). Results revealed that a highly significant decrease in semen quality was observed in male infertile (oligozoospermia, athenozoospermia and azoospermia) groups compared with findings of the control group. And significant decrease in semen quality was observed in azoospermia group and athenozoospermia group compared with findings of oligozoospermia group. Conclusion the semen quality concentration can be used as a new tool in the differentiation between the male infertile patients and normal cases, moreover it helps in differentiation between oligozoospermia, athenozoospermia and azoospermia.

Keywords: Seminal fluid, semen quality, male infertility, resazurin reduction test.

Introduction

Semen analysis is an integral part of the workup of couples consulting for infertility. The availability of semen renders possible direct examination of male germ cells,

giving precious data that are not accessible for female germ cells. The seminal fluid is a complex medium containing a great variety of molecules, mainly produced by sex accessory glands, and also cells other than spermatozoa (e.g. leucocytes). It is accepted that every male infertility work-up should start with the basics, namely, a thorough history, physical examination and at least two semen analyses (Zinaman et al. 2000).

This work aimed to put additional tools for diagnosis of male infertility by using new method as resazurin reduction test for semen quality.

Materials and Methods

This study was carried on patients obtained from outpatient clinic of male infertility department at El Hussein Hospital of Cairo; the mean age of investigated patients was 27±7 years. Samples were collected by masturbation in wide sterile container after 3 days of abstinence, shortly after collection semen samples were analyzed for liquefaction times, volume, leukocyte count, motility, morphology and count of spermatozoa according the World Health Organization (WHO 1993). Accordingly, samples were divided into two groups. Group I: Comprised normal controls (normozoospermia) (n=30) indicated by sperm count more than 20

millions/ml, motility more than 50% and normal morphology. Group II: Infertile group: Consisted of oligozoopermia (n=30) indicated by sperm count less than 20 millions/ml, azoospermia (n=30) indicated by the complete absence of spermatozoa and athenozoospermia(n=30) indicated by motility less than 50%.

All samples were centrifuged at 4000 rpm for 15 minutes and seminal plasma was separated and kept until analyses. Some of the seminal plasma was collected on sterile dry test tube to separate for the biochemical studies.

Determination of semen quality concentration by using resazurin reduction test:

The resazurin reduction test (RRT) depends on the ability of metabolically active spermatozoa to reduce the resazurin dye (blue) with maximum absorption at 615 nm, to resorufin (pink) with maximum absorption 580 nm. The ratio of the optical densities of reduced to oxidized form (i.e 580 to 615 nm) can be used to evaluate the various grades of semen sample. The highest correlation of absorption resazurin ratio (ARR) was observed with sperm motilty, count, morphology and viability (Reddy and Bordekar, 1999).

Reference value:

RRT Ratio = A580nm / A615nm

Azoospermic = 0.70 - 1.16

Athenozoospermic = 1.10 - 1.35

Oligozoospermic = 1.50 - 2.00

Normozoospermic = 2.25 - 6.00

Statistical analysis

The results were computerized. Statistical analysis was done through SPSS version 14. Quantitative data was expressed as mean and standard deviation (SD). Statistical comparison between the groups was done by Analysis of Variance

(ANOVA) and the post-hoc test least significant difference (LSD). The results were considered significant when P-value was equal or less than 0.05 ($P \leq 0.05$).

Results

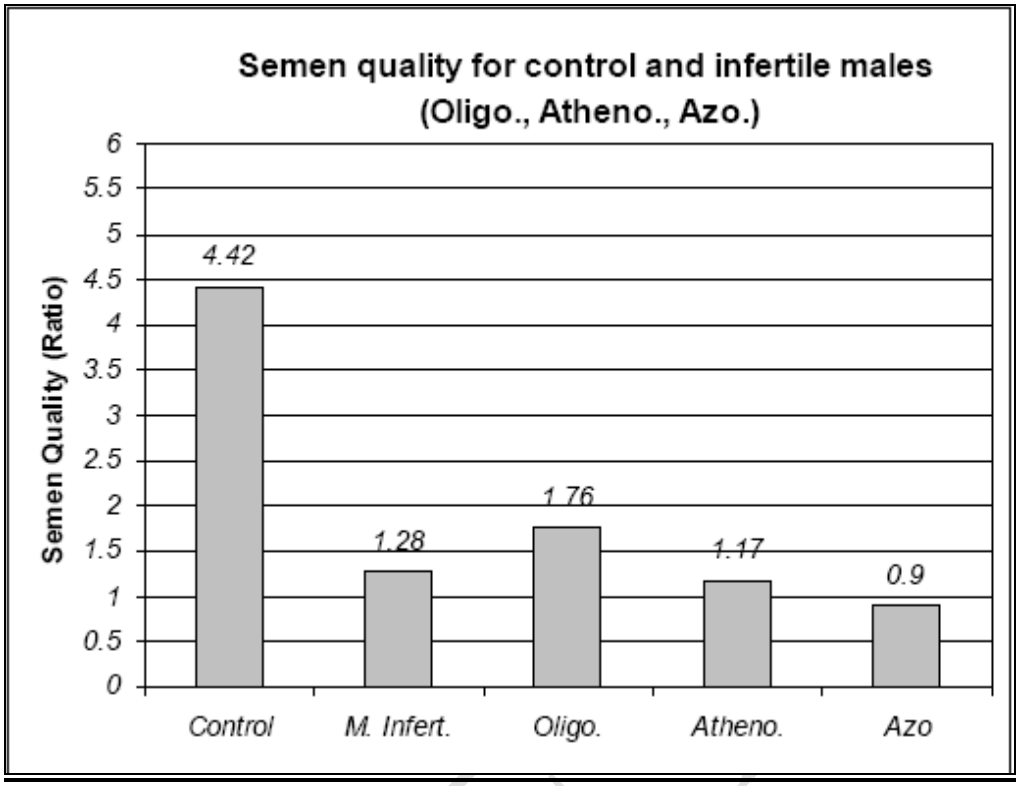
From the table and the figure, highly statistically significant decrease in semen quality was observed in infertile male (oligozoospermia, athenozoospermia and azoospermia) group as compared with findings of the control group, and significant decrease in semen quality observed in azoospermia group and athenozoospermia group as compared with findings of oligozoospermia group.

Table : The mean value and standard deviation of semen quality in control and infertile male (oligozoospermia, athenozoospermia and azoospermia) patients

Semen quality	Normozoospermic (30)		Oligozoospermic (30)		Athenozoospermic (30)		Azoospermic (30)		ANOVA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F-Ratio	P-value
	4.42	1.18	1.76	0.21	1.17	0.12	0.90	0.16	212	P< 0.0001
	(2,3,4)		(1,3,4)		(1,2)		(1,2)			

1 = Normozoospermic, 2 = Oligozoospermic, 3 = Athenozoospermic and 4 = Azoospermic

The mean values of the semen quality were shown in the figure to illusterate the comparison between the control and the infantile males (oligozoospermia, athenozoospermia and azoospermia).



Discussion

As regard to the results of semen quality, highly significant decrease in semen quality was observed in male infertility (oligozoospermia, athenozoospermia and azoospermia) groups as compared with findings of the control group. Significant decrease in semen quality was observed in azoospermia group and athenozoospermia group compared to findings of oligozoospermia group. These results are in agreement with (Cooper et al. 1992).

The expectancy of explanation that the result may be due to different factors such as the decrease in some trace elements in the male infertility (oligozoospermia, athenozoospermia and azoospermia) than the control group such as selenium (Se), Lithium (Li), Iron (Fe), calcium (Ca), magnesium (Mg), copper (Cu), and Zinc (Zn) which are very important in fertility and play very vital role in affecting various parameter of semen. Moreover, stabilizes the cell membrane and nuclear chromatin of spermatozoa (Levitas et al. 2007).

Poor sperm motility is a major abnormality and poor quality of seminal fluid is associated with an altered level of some of trace elements and enzymes excreted by

the prostate gland (Omu et al. 1995).On the other hand decrease concentration of fructose where fructose is the major carbohydrate source in seminal plasma and essential for normal sperm motility (Buckett and Lewis-Jones 2002). Moreover decrease antioxidant (vitamin c and coenzyme) which decrease semen quality where antioxidant stabilize the integrity of the sperm flagella (Cook et al. 2007).

Conclusion

The semen quality concentration is used as a new tool in differentiation between the male infertility patients (oligozoospermia, athenozoospermia and azoospermia) and normal cases.

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