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Original Article

Evaluation of acrosome intactness status in male infertility in Mysore, South India

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Abstract

Background and Objective: The objective of this study is to determine the status of acrosome intactness in different infertile conditions among men who have attended the Mediwave Fertility Research Center, Mysore, South India. **Materials and Methods:** A total of 70 infertile and 20 control subjects were employed in the study. Infertile subjects were classified into different conditions according to the WHO protocol. The data obtained was statistically analyzed. **Results:** In the present study, seven different infertile conditions were reported. For the acrosome intactness test, except oligospermia, all other conditions recorded a statistically significant value ($P < 0.05$) compared with the control group. **Conclusions:** The present study shows the decreased acrosomal enzyme activity in infertile males compared with fertile males. If diagnosed and treated earlier, it may help in the success of the *in vitro* fertilization technique.

Key words: Acrosome test, infertility, spermatozoa

Introduction

Infertility is defined as failure to conceive after 12 months of unprotected sexual intercourse.[1] In the general population, approximately 15% of all human couples are infertile, and male factors contribute to 50% of this condition among infertile couples.[2] The shape of the sperm reflects normal development of sperm in the testicle, which indicates the normal spermatogenesis process. Men with defect in sperm maturation process have high levels of abnormal sperm morphology and associated risk for the failure of the sperm to fertilize the egg.[3] Low sperm acrosome integrity has been observed in several infertile conditions.[4] It has been suggested that the level of acrosin activity of the sperm may be a useful determinant of sperm fertility potential.[5] In most of the infertility centers, clinical diagnosis of male infertility involves physical and microscopic examination followed by hormonal analysis, whereas at some of the clinics Trans Rectal Ultrasound Scanning (TRUS) is also practiced. Accordingly, the treatment is provided for few months and, if found futile, assisted reproductive techniques like *in vitro* fertilization (IVF) are recommended and performed. Assessment of the functional status of the sperm along with the conventional and routine evaluation will enable to improve the success rate of IVF. Here, an attempt has been made to assess the quality of semen through the sperm function test named acrosome intactness test (AIT), in the view of studying the extent of damage in semen profile among different types of infertility diagnosed.

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Access this article online

Quick Response Code:

Website: www.ijabmr.org

DOI: 10.4103/2229-516X.96801

Materials and Methods

The present study was carried out in the Department of Studies in Zoology, Molecular Reproductive and Human Genetics Laboratory, University of Mysore, Mysore. Semen samples of 70 infertile subjects and 20 controls were collected from Mediwave IVF and Fertility Research Center, Mysore. On the basis of microscopic analysis of semen, these study subjects were classified into different infertile conditions. Institutional ethical clearance was obtained and

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informed consent was taken from the subjects as well as from the controls.

The semen samples were collected from the infertile subjects as well as from the control group through masturbation after 3–5 days of ejaculatory abstinence. The samples were collected in a sterile plastic container in a room specially provided for this purpose according to the WHO protocol.[6] The semen volume, pH, motility, morphology, vitality and count were analyzed and recorded in both infertile and control groups.

Acrosome intactness test

The AIT was carried out by the protocol described by Gopalkrishnan *et al.* (1995).[7] Semen samples were diluted with phosphate-buffered saline–d–glucose solution and left for incubation at 37°C. These diluted semen samples were gently smeared on gelatin-coated slides and Incubated room temperature for 5–15 min. The slide was then transferred to a moist chamber and incubated at 37°C for 2 h. The slides were examined under bright field microscopy using a 40X objective. Spermatozoa having intact acrosome responds with a halo, which indicates the acrosome reaction and acrosome intactness.

Statistical analysis

The data obtained was analyzed using Minitab (version 15) statistical software. Paired t-test was employed to find out the significant correlation.

Results

Table 1 shows the prevalence of different infertile conditions and the spermiogram. Oligoteratozoospermic condition shows decreased mean sperm viability (49.1 ± 21.7) than the other condition. The AIT shows a decrease in the mean value for all the infertile conditions compared with the control group.

Table 2 shows the statistical analysis of the AIT in different infertile conditions. Except oligospermia ($P = 0.767$), all other conditions recorded a significant ($P < 0.05$) variation in the sperm acrosome intactness compared with controls.

It was found that there was a significant decrease in the AIT in the infertile group compared with controls. It was 44.6 ± 10 in the infertile group, compared with a value of 63.7 ± 15 in the control group. Figure 1 shows the acrosome intactness status in the control and infertile subjects.

Discussion

The acrosome of the sperm is a specialized, cap-like structure covering the anterior portion of the sperm nucleus. Although the acrosome varies in size and shape from species to species, its basic structure remains the same in all mammals.[8] Acrosomal reaction (AR) assists in assessing the ability of sperm fertilization and its reaction with zona pellucida. The activity of the acrosomal enzymes indicates the intact status of the acrosome. Intact acrosome prevents loss of acrosomal enzymatic activity during acrosome reaction in the female genital tract prior to fertilization.[9]

The sperm cell membrane harbors molecules necessary for the recognition and binding of sperms to the zona pellicuda and has to retain the membrane throughout the process. The outer membrane must remain intact after the release of the acrosomal enzymes as the acrosome contains a variety of hydrolytic enzymes such as acrosin, which is a trypsin-like serine protease that hydrolyzes the egg membrane to enable the spermatozoa to navigate the interstices of the corona radiata.[10]

In the present study, we found reduced AR in all infertile

Table 2: Statistical analysis of the Acrosomal Intactness Test in different infertile conditions (compared with controls)

Condition	Paired t-test
Oligozoospermia	t = -0.31, P = 0.767, 95% CI = -17.23, 13.13
Teratozoospermia	t = -2.45, P = 0.029*, 95% CI = -38.17, -2.40
Astenozoospermia	t = -4.32, P = 0.003*, 95% CI = -45.46, -13.29
OAT	t = -4.78, P = 0.003*, 95% CI = -43.20, -13.94
Oligoteratozoospermia	t = -2.90, P = 0.034*, 95% CI = -54.7, -3.3
Oligoasthenospemia	t = -3.69, P = 0.005*, 95% CI = -51.38, -12.33
Asthenoteratozoospermia	t = -7.43, P = 0.018*, 95% CI = -27.37, -7.29

OAT: Oligoastenoteratozoospermia; Significant *$P < 0.05$

Table 1: Prevalence of different infertile conditions and spermeogram

Condition	n = 70 (%)	Sperm count	Vitality	Motility	AIT
Oligozoospermia	18 (26)	12.1 ± 5.2	58.4 ± 14	46.6 ± 26.4	59.6 ± 13
Teratozoospermia	13 (18)	49.7 ± 20	69.2 ± 15	58 ± 16.2	41.4 ± 21
Asthenozoospermia	9 (13)	47.3 ± 11	62.1 ± 13.7	22.4 ± 18	41.7 ± 16
OAT	8 (12)	6.9 ± 5.7	53 ± 21	17.23 ± 11	41.7 ± 15
Oligoteratozoospermia	7 (10)	11.8 ± 5.1	49.1 ± 21.7	42.5 ± 20.9	47.1 ± 22
Oligoasthenospemia	12 (17)	8.5 ± 5.19	53.7 ± 22	18.5 ± 13	35.4 ± 20
Asthenoteratozoospermia	3 (4)	27.6 ± 10	75 ± 10	31.6 ± 2.8	50.5 ± 0.7
Control	20	68.4 ± 44	74.5 ± 10	67.1 ± 8.8	63 ± 15

All values are in mean ± SD; AIT: Acrosome Intactness Test; OAT: oligoastenoteratozoospermia

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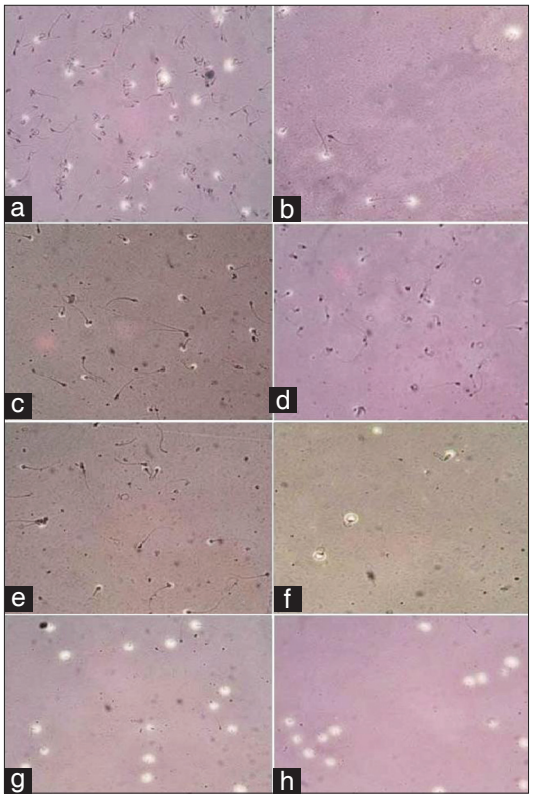

Figure 1: Response of acrosomal enzyme intactness and reaction in different infertile conditions. (Spermatozoa having intact acrosome will show a halo, which indicates the acrosome reaction, and acrosome intactness), (a and e) Response in teratozoospermia (b) Response in oligospermia, (c) Response in oligoasthenospemia, (d) Response in asthenoteratozoospermia, (f) Response in oligoteratozoospermia, (g and h) Response in the control subjects

conditions and hence our study accords the study done by Emokpae and Uadia.[11] The present study confirms a strong association between acrosome enzyme activity and fertility potential of spermatozoa. The acrosome reaction creates structural alteration required for the various constraints to be overcome. Suitable acrosome formation and the intracellular relocation of acrosome proteins during spermiogenesis are believed to be prerequisite modifications for eventually mature sperm to function properly as the fertilization process proceeds.[12] Hence, reduced acrosomal enzyme activity in the present study could be due to various conditions such as sperm malformation and local inflammation with leucocyte infiltration.

In the present study, acrosomal enzyme activity in infertile men was found to be significantly lower than that in fertile men. Hence, apart from the routine physical and microscopic examination, analysis of AIT plays a vital role to evaluate the complete sperm profile. Further, it will be a cost-effective and precise diagnostic tool to assess the sperm-fertilizing potential at the initial stage to evaluate male fertility rates. Effective and personalized treatments can be recommended to improve the quality of sperms if diagnosed earlier and if found to be of very poor quality. Thus, the chances of improving the fertility status of the individual are better instead of directly opting for assisted reproductive techniques.

Acknowledgment

The authors are thankful to Dr. Sharath Kumar C, Chief Surgeon, Mediwave Fertility Research Center, Mysore, for kindly referring the patients and timely inputs. Thanks are also due to Prof. M. M. Misro NIHFW, New Delhi, for his kind support. The authors would like to thank UGC-RFSMS for financial support.

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How to cite this article: Sreenivasa G, Vineeth VS, Kavitha P, Malini Sv. Evaluation of acrosome intactness status in male infertility in Mysore, South India. Int J App Basic Med Res 2012;2:31-3.

Source of Support: Nill. **Conflict of Interest:** None declared.