Chapter VI

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Only during last 15-20 years, advances in understanding of sperm structure and function led to an increase in our knowledge of male infertility. Defective sperm structure and function is now known to be the most prevalent cause of infertility, yet the precise causes leading to impaired sperm function remain obscure.

The spermatozoon is certainly one of the most complex and highly specialized of all mammalian cells, yet there remains important gaps in understanding its biochemical and functional alterations which lead to its impaired fertilizing ability. Hence the evaluation of qualitative and functional aspects of semen is an essential pre-requisite in the differential diagnosis and treatment of infertile males.

Detailed investigation on the functional aspects of the spermatozoa in semen of cases of unexplained infertility from Ahmedabad and its vicinity was therefore carried out in two groups according to the age range of 20-30 (Group II) and 31-40 (Group III).

The physical properties of semen like colour, odour pH showed no significant alteration in all the groups of unexplained infertility as compared to control. This analysis of physical properties was however imperative to determine altered accessory gland function, which may influence sperm survival and function.
The semen quality, judged by ejaculate volume, sperm density, percent motility and morphology of spermatozoa was found to be altered in the cases investigated. The cases under study, were in fact, grouped as Group A and B, based on the sperm density, where Groups IIA and IIIA had counts comparable to the normospermic range, whereas Group IIB and IIB had counts in the oligozoospermic range.

Morphology of spermatozoa was altered in semen of the cases investigated in all the Groups. A higher percentage of abnormal forms, correlated with low motility and motility and loss of nuclear integrity, was scored in semen of infertile individuals, which probably resulted in poor penetrating ability of spermatozoa. Abnormal forms in human ejaculate are related to malformations of nucleus, acrosomal changes, defects in organelles during spermatogenesis and improper differentiation and maturation in epididymis and in corroboration with these results, significant alteration was also obtained in the sperm function tests for nuclear membrane integrity and sperm maturation. The frequency of abnormal forms was greater in oligospermic cases of unexplained infertility of our study.

Low sperm viability and altered sperm membrane function (HOS test) may contribute to loss of fertilizing potential in spermatozoa of infertile semen. Alteration in sperm viability, its membrane, acrosomal and nuclear integrity, sperm morphology probably contributes to lowered sperm penetrating and fertilizing ability and poor fertility in these cases. Impaired fertilization in these cases may therefore be due to loss of sperm viability and membrane integrity.
A lower percentage of green fluorescing spermatozoa (Acridine Orange Staining) were scored in the semen in infertile cases, reveals loss of native double-stranded structure of DNA and nuclear integrity.

This test did not show any correlation with sperm count and motility, but was related to the percent abnormal spermatozoa and was therefore a marker for functional properties of the sperm. The effective sperm count was consequently, significantly lower in the cases of unexplained infertility as compared to men of proven fertility (Control, Group I).

Biochemical evaluation was carried out for various metabolities and enzymes, which are specific markers of epididymal and accessory gland function and is necessary to elucidate metabolic changes in the spermatozoa. Evaluation of protein and fructose in seminal plasma provides data regarding metabolic and functional changes of the spermatozoa in infertile semen. High fructose concentration suggested reduced sperm metabolism and utilization of the metabolite in semen of nomospermia and moderately oligospermic cases of unexplained infertility. Protein levels were reduced in the infertile group, correlated with reduction of secretory activity of epididymis and accessory glands.

Studies have been demonstrating the role of cholesterol in control of sperm function. Our data revealed no significant increase in cholesterol in semen of cases of unexplained infertility. The role of cholesterol in semen and its correlation with metabolism and fertilizing properties of spermatozoa though not fully understood is evidently associated with the normal membrane structure and function.
Estimation of total lipid showed decline in seminal plasma of semen of unexplained infertility groups (Groups II and III) as compared to normal fertile groups (Group I). These alterations in cholesterol and Total lipids could be correlated with the increased lipid peroxidation and oxidative stress.

In present study, lipid peroxidation (LPO) has been used as a marker of oxidative damage to the sperm membrane lipid. One of the byproducts of LPO is malondialdehyde (MDA), which has been measured as the end product in biochemical assay to monitor the degree of peroxidation. Current data revealed significant increase in LPO. Determining the structural and functional changes in sperm membrane lipids during the process of peroxidations is useful in understanding the role of lipid metabolism in spermatozoa. This may help to develop the therapeutic strategies for male infertility. Future research should be directed towards understanding the role of particular components of sperm membrane. It is still not clear that sperm membrane peroxidated products are indispensable or detrimental in this process.

Free radical induced oxidative damage to spermatozoa is one such condition, which is recently gaining a considerable attention for its role in inducing poor sperm function and infertility. Understanding of how such conditions affects sperm functions will help to design new and effective treatment strategies.

Production of very low amounts of reactive oxygen species (ROS) in semen appears to play a physiological role in regulating normal sperm functions, whereas high levels of ROS endanger sperm function and viability.
Oxidative stress (OS) due to excessive production of ROS, impaired antioxidant defense mechanisms, or both precipitates a range of pathologies that are currently believed to negatively affect the male reproductive function. Oxidative stress-induced damage to sperm may be mediated by lipid peroxidation of the sperm plasma membrane, reduction of sperm motility, and damage to the DNA in the sperm nucleus. One important reason for the inability to utilize the OS test in clinical practice is related to the lack of a standard protocol for assessment of seminal OS. However, the present study indicates the vital significance of determining the effect of ROS and the antioxidant status in understanding functional changes in spermatozoa.

The present study has provided evidence that the enzyme system in human seminal plasma protects spermatozoa from peroxidative damage. The contribution of three antioxidant enzymes (superoxide dismutase, Catalase, and Glutathione peroxidase) is imperative in order to understand the alteration in sperm function.

Evaluation of superoxide dismutase (SOD) activity was carried out to understand the protective mechanism against the damaging effects of superoxide radical (O$_2^-$) and to determine whether this mechanism was operative or reduced in semen of individuals with unexplained infertility.

The Superoxide dismutase and Catalase activities in semen samples of cases of unexplained infertility were found lower than those of normal subjects. The decline in activity in SOD and Catalase indicated an inadequate protective enzymatic system against the action of the free radical. This led to
increased free radical attack on the sperm cell, which subsequently showed a decline in sperm function and alteration in sperm metabolism of sperm.

Glutathione (GSH) has a protective action against free radicals reactive oxygen species (ROS) like peroxidase and other toxic compounds. It has a central role in defence against oxidative damage. The present data showed significant decline in glutathione content of all the men investigated for unexplained infertility as compared to normal.

α-Tocopherol plays an important role in protection of cells against oxidative stress. The present data revealed a significant decrease in α-tocopherol level in samples taken from cases of unexplained infertility cases. Vitamin E is considered to be extremely important because of its association with cell membrane, for its free radical quenching properties and also for maintaining the normal immune system. The present results suggest an important role of sperm α-Tocopherol as the anti oxidant status of human spermatozoa and its possible clinical relevance. The present investigation revealed that the inadequate α-Tocopherol levels, along with the lowered protective enzyme machinery as revealed by the concurrent with the assessments of SOD and Catalase activities of spermatozoa led to a deficient combat against the content of ROS. This in turn resulted in poor sperm function.

Ascorbic acid (Vitamin C) is the most powerful donor and first plasma anti-oxidant to be utilized upon exposure to oxidative stress. The data obtained in this investigation indicated a decrease in ascorbic acid level in cases of unexplained infertility in all the groups studied.
Hence it is evident, that along with low $\alpha$-Tocopherol, low ascorbic acid levels causes poor antioxidant status of the semen, consequently spermatozoa's functional ability declines. It was observed from the parameters analyzed that samples from Group III individuals of the older age group, showed a highly significant alteration as compared to that of the younger age group cases of unexplained infertility (Group II).

In addition to evaluation of sperm function in cases of male unexplained infertility, a separate observation was undertaken in the present study to determine whether the individuals selected as the normal control group, comprising of men of proven fertility from a random cross-section of the population from Ahmedabad and its vicinity, showed any indication of a declining trend of sperm density over a period of 20 years.

In 1992 Carlsen et al. analysed data collected from 1938 to 1990 in which semen samples from presumably normal men were investigated. Data of Carlsen et al. (1992) showed highly significant trends towards lower sperm concentration along with reports from several other studies that indicated lowered sperm counts in the past fifty years. Comparing data obtained in our laboratory, over the last two decades, from 1985 to 2005, there appeared to be no significant decline in sperm density and motility of the normal population, from which the control samples have been analyzed.

Hence, in conclusion, it was evident from the results obtained that in the cases of Unexplained Infertility investigated, the failure of fertilization was due to altered sperm membrane and nuclear integrity, along with biochemical and functional alterations. These changes in sperm function was possibly the
outcome of free radical attack, which was enhanced in the absence of an adequate protective enzymatic machinery from SOD, catalase, as well as a poor antioxidant status, with low ascorbic acid and α-tocopherol levels.

This deficiency of the protective enzymes SOD and catalase, correlated with increased lipid peroxidation, is further substantiated with the loss of sperm membrane function, leading to impaired sperm fertilizing potential.