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Biochemical markers in semen and their correlation with fertility and semen quality among infertile men

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ABSTRACT *Male infertility is generally diagnosed in terms of the concentration of motility and morphology of spermatozoa. Often, these parameters do not accurately reflect the fertilizing capacity of the samples. For instance, some samples exhibit normal spermiograms are often seen to be infertile. Therefore, there have been increasing efforts to devise suitable biochemical parameters, which could serve as effective markers for fertility.Conventisonal semen analyses are used to evaluate male factor fertility/infertility in humans and other animals. However, their clinical value remains controversial. Therefore, assessing the male fertility based on sperm function and fertilization mechanism are of interest worldwide. While protein and fructose markers in spermatozoa that might help differentiate fertile and infertile sperm have been investigated. The present study evaluates the semen analyses of unexplained infertility cases in the two age group and is compared with normal fertile cases. Fructose and protein is measured in semen sample and we found that 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 normospermia and moderately oligospermic cases of unexplained infertility. Protein levels were reduced in the infertile group compared to the normal fertile group.*

INTRODUCTION

The goal of evaluating a man's infertility potential has been of great interest to researchers .Infertility is a common problem affecting young couples and is clear that, it results in considerable distress for the couples affected. Recent European data suggest that as many as one in four couples may experience difficulties in conceiving. An important cause of male infertility is poor structural and functional status of the sperm, as well as poor semen quality. Therefore biochemical analysis constitutes an important part of semen examination and has been reported to be of prognostic value. Of these biochemical parameters, fructose and protein content has been reportedly emphasized, as these parameters are vital markers of accessary gland function.

The seminal fructose is secreted by the seminal vesicles under the stimulus of testosterone (Mann, 1964)[10].One of the most striking features of seminal plasma is the high concentration of free fructose (3.5 to 5.8 mg/ml). Fructose has been extensively investigated, for the chemical marker of the seminal vesicle secretion and is the major source of glycolytic energy available to Spermatozoa. The presence of fructose in semen as a vesicular marker was first noticed by Mann (1945)[11]. Later, a large number of workers have been involved in the study of its origin, concentration, and utility in metabolism interactions and in infertility cases. Split ejaculate technique proved the origin of this chemical substance as seminal vesicle (Lundquist, 1949; Eliasson, 1968)[8 ,2].The ampulla of vas deferens also has the capacity of secretion of fructose (Mann, 1964)[10]. Many laboratories routinely estimate the concentration of fructose in the ejaculate to evaluate the function of seminal vesicle. Semen contains both albumin (a protein structure) and free amino acids.

The proteins come from the prostate, whereas the amino acids come from the seminal vesicles. Human seminal plasma is very rich in enzymes. It possesses considerable proteolytic activity, which is implicated in the degradation of seminal proteins to proteases and free amino acids. It has been reported (Mann and Lutwak-Mann, 1981; Lundquist et al., 1955)[12,9] that several proteolytic agents secreted by the male accessory glands also participate in two important process which spontaneously occur in the ejaculated semen viz. Semen coagulation and liquefaction. A close relation exists between the enzymatic equipment of Spermatozoa and their fertilizing capacity. Seminal fructose is often routinely measured in the assessment of seminal vesicle function and male factor infertility. We present the results of a prospective study of seminal fructose and protein in patients referred for routine semen analysis prior to infertility treatment Considerable information is available on the quantitative aspects of seminal fluids. A few biochemical markers have been employed in the present study.

MATERIAL AND METHOD

Semen samples were analyzed from selected cases referred with complaints of unexplained infertility and failure of sperm fertilizing ability, to evaluate for various structural and functional parameters of human spermatozoa, related to impaired fertilizing potential. The cases were divided into two age group ranges. Group II with age range from 20-30 years and Group III withage range 31-40 years. Normal volunteers of proven fertility, with no clinical history of infections or related disorders, were selected as controls for Group I, in an age matched manner viz., Group I of age range 20-30 years and Group IA of age range 31-40 years, as shown below: Group I Normal men of proven fertility. Age range 20-30 years (n=40).Group IA. Normal men of proven fertility, Age range 31-40 years (n=40)Group IIA Men with history of unexplained infertility having sperm counts . In the normozoospermic range, Age range 20-30 years (n=62).Group IIB Men with history of unexplained infertility, having sperm counts . In the oligozoospermic range <40 million (n=46) Age range 20-30 years.Group IIIA Men with history of unexplained infertility having sperm counts . In the Normozoospermic range, Age range 31-40 years (n=53).Group IIIB Men with history of unexplained infertility, having sperm counts. In the oligozoospermic range <40 million (n=59) Age range 31-40 years . Semen samples were collected into sterile

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containers. After liquefaction, samples were centrifuged to separate seminal plasma which was analyzed for volume and sperm count, sperm motility and morphology microscopically according to WHO guidelines [11]. Then biochemical parameter i.e fructose and protein was evaluated. The fructose level in semen was estimated by the modified method of Foreman et al. (1973)[3],The modified method is specific for fructose as it gives a red cooler complex with resorcinol and protein level semen sample were estimated by the method of Lowry et. al.(1951)[7].Fructolytic Index:The Fructolytic index of each sample was determined as the difference in fructose content between the fructose estimated initially at time 0, and that estimated after a duration of 60 minutes incubation at 37 degree C. The difference is a measure of fructose utilized in metabolism by the sperm.

RESULT

Semen analysis was carried out on freshly collected semen samples of men of proven fertility (Group I, normal - control) and various groups of men with clinical history of unexplained infertility (Group IIA and Group IIB of age range 20-30 years) and (Group III A and III B of age range 31-40 years). The analysis was aimed at determining functional alteration in the spermatozoa in order to evaluate and determine the semen quality.

The protein levels were insignificantly ($p<0.01$) lower in the unexplained infertility cases with normospermia (Group II A) as compared to the normal males of Group I. In Group B, protein level was highly significantly ($p<0.001$) lowered as compared to control (Table 13). The results obtained, as shown in table-14, indicated an insignificant decline in protein levels in semen samples from the cases of Group IIIA whereas a significant decline ($p<0.001$) was recorded in Group IIIB samples as compared to the control group (Group IA).

Fructose : A significant increase ($p<0.001$) was observed in the Semen fructose levels of all the unexplained infertility groups (Group II A, IIB, III A and III B) as compared to normal groups I and IA (Table 13 and Table 14).

Fructolyticindex : The Fructolytic index was significantly ($p<0.001$) lowered in the semen sample from individuals of Group I I A, II B, III A and III B as compared to fertile individuals. The data obtained in the present study have revealed a highly significant increase in fructose concentration in the samples analyzed from cases of Group II A, II B, III A and III B of unexplained infertility, as compared to control group. This probably results from decreased utilization of fructose, which in turn can be correlated to the poor functional status of the Spermatozoa.

TABLE I: Showing Protein,fructose And Fructolytic Index In Semen Of Group Ii Males (causes Of Unexplained Infertility Of Age Range 20-30 Years)

Group	Parameter		
	Protein(mg/ml)	Fructose (mg/ml)	Fructolytic index
Group I Normal n=40	11.3±0.21	6.3±0.8	1.51±0.5
Group II-A (Normospermia) (n=62)	10.8±0.04*	11.4±0.24**	0.67±0.032*
Group II –B (moderate oligozoospermia) n=46	5.2±0.03**	11.7±0.4**	0.34±0.07**

Values are Mean ±S.E.
+Not Significant
*p<0.01
**p<0.001

TABLE II: Showing Protein,fructose And Fructolytic Index In Semen Of Group Iii Males (causes Of Unexplained Infertility Of Age Range 31-40 Years)

Group	Parameter		
	Protein(mg/ml)	Fructose (mg/ml)	Fructolytic index
Group I Normal n=40	12.8±0.8	7.2±0.4	1.81±0.7
Group II-A (Normospermia) (n=53)	9.8±0.5*	11.7±0.6**	0.59±0.015**
Group III–B (moderate oligozoospermia) n=59	5.5±0.04**	14.9±0.3**	0.44±0.09**

Values are Mean ±S.E.
*p<0.01
**p<0.001

DISCUSSION

Fructose has received large attention, being the major source of glycolytic energy available to spermatozoa. Mann (1964) was the first to show the important role played by fructose, particularly as a marker of vesicular secretion. Normal levels of fructose in an ejaculate indicate that the seminal vesicles are actively secreting fluids. Case reports document the absence of fructose in semen samples from patients diagnosed as having congenital bilateral absence of the was deferens and Seminal vesicles or obstruction of the ejaculatory ducts.Several researchers (Phadke et al., 1974; Hafez, 1976;Spring Mills and Hafez, 1980)[13,4,&16] have reported that fructose is the most important metabolite for the spermatozoa and is the primary source of energy in the anaerobic environment.

The data obtained in the present study have revealed a highly significant increase in fructose concentration in the samples analyzed from cases of Group II A, II B, III A and III B of unexplained infertility, as compared to control group. This probably results from decreased utilization of fructose, which in turn can be correlated to the poor functional status of the spermatozoa, Schirren et al. (1979)[14] obtained an association between higher sperm density and lower fructose level and a positive correlation between fructose and percent non-motile spermatozoa. This is similar to the data obtained in this study of sperm density, motility and level of fructose.

Although semen volume and pH, in this study showed insignificant alteration, which reflects normal seminal vesicular, activity, fructose level showed insignificant increase. Secretion of the metabolite and the significant reduction in its utilization and metabolism, probably led to higher levels of fructose in semen, in cases of unexplained infertility as compared to that of control. Huang and Johnson (1975)[5] as well as Crabo and Hunter (1975)[1] have shown that spermatozoa acquire their protein content during their sojourn through the epididymis. An insignificant alteration was observed in Group Ain level of protein as compared to normal, while a significant decrease was observed in the other three groups of unexplained infertility (Group II B, III Aand III B). This possibly indicates reduced epididymal secretory function, leading to the decline in protein content. Lindholmer et al. (1974)[6] have shown that total

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protein concentration is negatively correlated with progressive motility, while Singer and his group(1976)[15] have correlated protein content of seminal plasma to sperm counts. These observations however, were not confirmed in the estimation of protein in the semen of individuals of unexplained infertility; however the decreased protein content reflects altered sperm metabolism and function.

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