SEMEN EXAMINATION IN INFERTILITY WITH SPECIAL REFERENCE TO BIOCHEMICAL PROFILE OF SEMINAL PLASMA

by

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Despite all the emphasis on population control, sterility is certainly an important subject in its own right.

For a long time semenalysis was the only consideration to evaluate the male factor. Later, the attention shifted to the change in the constitution of seminal plasma. Extensive analysis of seminal plasma has unfolded new vistas for biochemical approach to the subject of male infertility.

Through this paper, we are presenting the results of biochemical and electrophoretic analysis of seminal plasma performed in our laboratory.

Material and Methods

One hundred cases of exclusive male infertility constitute the material of this study. Following criteria were adopted for selection of these cases:

(i) Married for atleast 3 years; no use of contraception.

(ii) No conception since marriage.

(iii) No cause of infertility found in the female partner by basal body temperature, tubal insufflation test, hysterosalpingography and endometrial biopsy.

History of hereditary, systemic, viral or venereal disease was sought. General examination was done with particular emphasis on health, development of secondary sex characters and congenital anomalies. Local examination was conducted to find other causes of sterility, e.g., epispadias, hypospadias, hydrocele, varicocele and surgical operations.

Specimen of semen obtained by manipulation was collected for investigation. Sperm count was done with Neubauer's chamber, using Davidson's diluting fluid. The cases were grouped according to the criteria laid down by American Society Standard:

Normospermia (60-150 mill/ml).

Hypozoospermia (30-60 mill/ml).

Oligospermia (1-30 mill/ml).

Azoospermia: No sperms; presence of spermatocytogenetic cells.

Aspermia: No sperms; no spermatocytogenetic cells.

The sperm motility was graded according to the criteria defined by Hotchkiss (1941).

Biochemical analysis of seminal plasma

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was performed for estimation of fructose content (Mann, 1964) and acid phosphatase level (Lewis and Beer, 1973).

Filter paper electrophoresis of seminal plasma was done on Whatman No. 3 paper strips, using 0.3 M-barbitone buffer at pH 8.7. Graphs plotted from electrophorectograms were employed for relative quantitation of protein fractions.

For comparison, similar investigations were performed in 75 fertile males constituting the control group.

Observations

Age of 100 infertile males varied from 20 to 40 years (mean 31.0 ± 3.8). Age of control cases ranged from 20 to 37 years (mean 30.1 ± 4.1). Duration of infertility according to age groups is shown in Table I.

TABLE I
Duration of Infertility in Different Age Groups

Age group (Years)	Duration of infertility (Years)			
	3-5	6-10	11-15	16-20
20-25	24	—	—	—
26-30	26	17	—	—
31-35	3	16	3	2
36-40	—	1	5	3
Total	53	34	8	5

Evidence of local or systemic disease in relation to infertility was sought. Such evidence was found in 35 cases. The incidence of these ailments is given in Table II.

TABLE II
Incidence of Various Disorders in Relatios to Infertility

Disease	No. of cases
Trauma	10
Epididymo-orchitis	8
Chicken-pox	5
Mumps	2
Venereal disease	2
Liver cirrhosis	2
Cryptorchidism	1
Varicocele	1
Genital tuberculosis	1
Irradiation	1
Total	35

Some physical characteristics of semen are shown in Table III. All the 75 control cases had a normally viscid semen, while 44 of the 100 infertile cases ejaculated watery semen. As later observations revealed, 28 of these 44 patients with watery semen were suffering from azoospermia.

TABLE III
Some Physical Characteristics of Semen From Fertile and Infertile Individuals

Characteristics	Control cases		Infertile cases	
	Range	Mean	Range	Mean
Volume (ml)	1.8-5.6	3.5	0.5-5.0	2.1
Liquefaction time (min.)	18-26	24.3	14-29	24.4
pH	7.0-7.18	7.14	7.0-7.1	7.09

The sperm count in control cases ranged from 60-125 mill/ml (mean 88.2). Maximum count in infertile cases was 55 mill/ml. The grouping of cases according to sperm count is shown in Table IV. Number of cases showing different grades of motility is presented in Table V.

Mean fructose content of semen in normal controls was 172.0 mg/dL. Among the infertile cases, an inverse relationship was found between sperm count and fructose level. As shown in Table VI, the

TABLE IV
Grouping of Cases on the Basis of Sperm Count

Sperm count (mill/ml)	No. of cases	Grouping	Mean sperm count for the group
10	31	Oligospermia	12.5
10-20	6		
21-30	4		
31-40	6	Hypozoospermia	51.6
41-50	3		
51-60	3		
Nil	47	Azoospermia	—

rise of seminal fructose level in azoospermia, oligospermia and hypozoospermia was statistically significant in comparison to fructose level in normospermic fertile controls.

Acid phosphatase level of semen was variable, as evidenced by results shown in Table VII. The variations from control group were statistically insignificant.

Table VIII represents the results of relative quantitation of various electrophoretic fractions obtained from different types of cases.

TABLE V
Various Grades of Sperm Motility in Fertile and Infertile Cases

Grads of motility	Control		Infertile	
	No. of cases	Mean motility %	No. of cases	Mean motility %
0	—	—	47*	—
1	—	—	7	15.4
2	14	55.0	30	46.7
3	27	78.0	11	72.3
4	36	90.0	5	82.0

(* Cases of azoospermia).

TABLE VI
Analysis of Observations on Seminal Fructose Content

Type of cases	No. of cases	Seminal fructose (mg/dL)		
		Range	Mean ± S.D.	'p' value
Control	75	75-275	172.0 ± 87.5	—
Azoospermia	47	220-380	297.5 ± 142.6	<0.001
Oligospermia	41	132-320	232.8 ± 38.5	<0.001
Hypozoospermia	12	80-265	224.3 ± 32.4	<0.05

TABLE VII
Seminal Acid-Phosphatase Level in Different Groups of Cases

Type of cases	No. of cases	Seminal acid-phosphatase (i.u./ml)		'p' value
		Range	Mean ± S.D.	
Control	75	100-750	455.0 ± 201.6	—
Azoospermia	47	65-635	421.8 ± 162.2	>0.05
Oligospermia	41	150-615	387.4 ± 189.6	>0.05
Hypozoospermia	12	90-700	331.5 ± 224.7	>0.05

TABLE VIII

Relative Quantitation of Different Fractions of Seminal-Plasma Proteins

Type of cases	No. of cases	Alb. %	Globulins %			Electroph. immobile
			Alpha	Beta	Gamma	
Control	75	6.8	15.7	41.0	24.0	12.5
Azoospermia	47	4.6	15.1	51.3	18.0	11.0
Oligospermia	41	6.5	17.2	42.3	21.7	12.3
Hypozoospermia	12	6.0	16.5	40.5	24.0	13.0

Discussion

In the present study, 100 cases of male infertility were investigated. In 35 cases, evidence of antecedent disease was found, predominently, trauma, epididymo-orchitis and chicken pox. Subfertility due to trauma, scrotal disease and infective or hormonal disorders is well documented (Russell, 1954; Veenema and Latimer, 1957; Phadke *et al*, 1973).

Macleod (1950) observed that viscocity of semen does not affect motility of spermatozoa and sperm count. However, we noticed that quite a few cases with low sperm count and azoospermia were associted with watery semen.

Mean sperm counts in oligospermia and hypozoospermia were 12.5 mill/ml and 51.6 mill/ml respectively, as against 88 mill/ml in control cases. The reported mean sperm count of fertile males ranged from 79.7 mill/ml (Naghma-E-Rehan *et al*, 1975) to 107 mill/ml (Macleod, 1951). Eliasson (1971) has reported it to be 86 mill/ml, which is close to our figures.

Majority of our patients had grade-1 and grade-2 motility of spermatozoa, whereas over 50% of fertile controls showed grade-4 motility.

Seminal acid phosphatase level in infertile individuals appeared to be lower than that of fertile controls. This difference was, however, not statistically significant. Among infertile cases, no relationship was found between sperm count and acid phosphatase level. This is in agreement with the observations of Vaishwanar and Abhayankar (1971). However, some workers have found positive correlation between seminal acid phosphatase activity and the sperm count (Das *et al* 1975).

An inverse relationship between seminal fructose level and sperm count was observed by us. Similar findings have been reported by Schirren (1963) and Phadke *et al* (1973).

Fructose content of semen varies inversely with the germ-cell activity, and thus indirectly depends upon androgenic activity (Mann and Parsons, 1950). This finding would have much more endocrinological significance for evaluation of male infertility and subfertility when larger projects are undertaken for quantitative correlation of seminal fructose content and plasma testosterone level.

Various fractions of seminal plasma proteins in our normospermic subjects were very similar to those reported by Mann (1964). Infertile cases showed interesting variations; but we are not able to find other reports for comparison with our results.

In cases of azoospermia, albumin and gamma globulins were distinctly low and betaglobulins quite high. In oligospermia, there was slight rise of alpha- and beta-globulins and slight fall of gamma-globulins. Alpha-globulin and immobile fraction did not reveal significant variation from controls.

Summary

Semen examination has been done in 100 cases of male infertility and 75 fertile controls. Viscocity of semen and motility of spermatozoa were reduced in the infertile individuals. Seminal acid phosphatase level was not significantly altered, while rise of fructose level in the study group was significant. An inverse relationship between sperm count and seminal fructose level was observed. Observations of electrophoretic fractions of seminal-plasma proteins have also been recorded.

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