CYTOCHEMISTRY OF SPERMATOZOA

by

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Introduction

Since the last three decades, lot of work has been contributed to the causation of male infertility. Charney (1940) and later on Hotchkiss (1948) studied the testicular biopsy in detail for histopathological changes. Since then, many workers (Engle and Ling, 1952; Nelson and Heller 1951; Jirasek and Jon Raboch 1963; Franklin and Dukes 1964, 1964 a,; Bo Fjallbraut 1965, 1968, Girgis *et al* 1969, Malhotra and Dev 1969, Wong *et al* 1973 and Vyas and Kalra 1978) extended this work in more detail using histopathological and immunological techniques. But very scant literature is available regarding the cytochemical changes in spermatozoa in male infertility. The object of our project was to study the morphology and cytochemistry of normal and abnormal sperms.

Material and Methods

The material for the present study was obtained from 100 patients of male infertility attending the Department of Obstetrics and Gynaecology and attached Infertility clinics of P.B.M. Group of Hospitals, Medical College, Bikaner. Each patient was subjected to two detailed semen examinations. All the semen specimens were examined cytochemically. On the basis of clinical and morphologic criteria, the semen samples were classified as shown in Table I.

TABLE I

Classification of Serum Samples

Groups	No. of semen samples out of 100	Cell count (million/ml)	Normal sperm (%)	Motile sperm (%)	Undifferentiated germ cell element (%)
Normospermic	49	60—120	80—90	75—85	1—2
Oligospermic	42	10—20	60—70	40—65	2—6
Azoospermic	9	—	—	—	Only present

Smears from each semen sample were prepared and were fixed in methyl alcohol and ether-alcohol. The following staining procedures were used; hematoxylin and eosine (by standard technique),

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Feulgen's method for demonstration of deoxyriboneucleinic acid (DNA) (Feulgen and Rosenbeck, 1924), methyl green pyronin method for ribonucleinic acid (RNA) (Brachet, 1940), periodic acid Schiff's reaction (PAS) for Glycogen and other carbohydrate with diastase treatment (Macmanus, 1948) and alkaline phosphatase demonstration lay (Burstone 1960).

Observations

The staining intensity of different stains were graded arbitrarily as —, +, ++, +++ and ++++ as shown in Table II.

The morphologic and histochemical observations in these groups were as follows:

(i) *Normospermic*: The H&E stain revealed about 80-90% of spermatozoa with normal morphology. A few abnormal forms of spermatozoa (oval and tapering or pear shaped heads and splitting and shortening of tail) were seen. There was a homogenous positive Feulgen reaction at the posterior part of head of spermatozoa. Feulgen negative vacuoles were frequently seen in anterior part of head (acrosome). RNA positive sites were variable in the body of spermatozoa, in an occasional spermatozoa RNA was seen surrounding the head region. PAS positive material was seen in head specially at anterior pole in semilunar forms. Some of the spermatozoa showed diastase resistant. PAS reaction at post pole of head. Alkaline phosphatase reaction was found to be slightly positive in mostly the matured spermatozoa as shown in Table II.

(ii) *Oligospermic*: The H&E stained semen smear revealed an increased proportion of spermatozoa of irregular structure and of undifferentiated germ cell elements. Abnormal forms were with oval shaped, tapering ends, pear shaped head and splitting, short or elongated tail were also noted in these forms.

Oligospermic semen showed spermatozoa with intense Feulgen staining of the head portion and in irregular form even diminished staining intesity very intense staining for RNA was noted in the body of spermatozoa. PAS positive substances were found in anterior parts of head in half-moon shaped spermatozoa. The staining intensity was relatively more intense than normospermic semen. Diastase treatment did not alter staining intensity.

Alkaline phosphatase activity was usually noted in most of spermatozoa upto grade I. The average alkaline phosphatase activity was graded from 0 to 1 as shown in Table II.

(iii) *Azoospermic group*: In this group the centrifuged and stained palperations of semen smears revealed faintly basophilic to light eosinophilic irregular masses of undifferentiated germinal elements. These were found Feulgen positive and methyl green pyronin positive for DNA and RNA. No significant amounts of PAS positive substances were seen in these elements as shown in Table II. The leukocytes and epithelial cells were also seen.

Discussion

In the present study histochemical alterations in the spermatozoa of normospermic, oligospermic and azoospermic groups have been recorded according to their staining intensity. These indices, however, do not mean to imply any quantitative alterations but reflect the state of aggregation or dispersion in the structural components of spermatozoa.

The intensity of Feulgen positive material was on the whole more intense as compared to oligospermic group, this is

TABLE II

Histochemical Reactions in Spermatozoa of Different Groups

Group	PAS			D. PAS			DNA			RNA			Alk. Phos.		
	H	B	T	H	B	T	H	B	T	H	B	T	H	B	T
Normo-spermic	++ to +	—	—	Resistant			+++ to ++	—	—	+ to ++	+++ to ++	—	± to —	± to —	—
Oligo-spermic	++ to +	—	—	Resistant			++ to +	—	—	+ to ±	+++ to ++	—	+ to ±	±	—
Azoo-spermic	—	—	—	—			Positive fragments			Positive fragments			—		

Index:— H — Head of spermatozoa
B — Body of spermatozoa
T — Tail of spermatozoa

— — No reaction
± — Faint reaction
+ — Mild reaction
++ — Moderate reaction
+++ — Strong reaction

possibly due to immature and abnormal forms of spermatozoa in the semen of these individuals. Similar observation has also been reported by Sroka (1965). On the other hand, RNA positive material was higher in the body of spermatozoa in oligospermic group. Brachet reaction has revealed that in normo- and oligospermic groups in some spermatozoan head is stained with methol green and in some spermatozoa with pyronin, thereby showing some spermatozoa of mature nature and others of younger generation. It was observed with PAS reaction that the anterior part of the head (aerosome) contained PAS positive diastase resistant material. It may be mucopolysaccharide in nature and probably originate from golgi apparatus as suggested by Sroka (1965).

Alkaline phosphatase was found to be decreased or absent in mature spermatozoa of normo- and oligospermic groups and relatively increased in immature spermatozoa of oligospermic semen. This indicates that together with the findings of RNA, DNA and PAS alongwith maturation and differentiation, various biochemical alterations do take place in the maturing germ cells and that their energy requirements for metaliolic activity are progressively reduced.

Summary

The histochemical study of semen samples derived from normospermic, oligospermic and azoospermic individuals has indicated a poor PAS reaction and alkaline phosphatase activity in normospermic, whereas Feulgen reaction and methyl green pyronin reaction were positive at head and body sites respectively in normospermic. Oligospermics showed relatively increased intensity of PAS (diastase resistant), RNA and alkaline phosphatase activity while azoospermic semen showed only loose fragments without any specific morphology stained with feulgen and methyl green pyronin stains, probably of germ cell origin.

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