RESULTS

During this work 10 fertile men and 61 infertile patients were studied.

The 10 fertile men were included in group I. The 61 infertile patients were grouped into:

group II : 14 patients with oligoasthenozoospermia.

group III: 13 patients with sperm agglutination.

group IV : 13 patients with asthenozoospermia.

group V : 9 patients with oligo-terato-asthenozoospermia.

group VI : 9 patients were studied to show the effect of CaCL on acrosin activity.

group VII: 3 patients were studied by electron microscopy.

All of the ten fertile men were selected so that their wives were pregnant at the time of the study. The ages of this group of men ranged between 22-45 years with a mean age of 34 years. The duration of their marriages ranged from 1-20 years with a mean of 12 years. Eight out of these ten fertile men had fathered children other than the present pregnancy, but the wives of the other two fertile men were pregnant for the first time. In all members of these fertile group there was no past history suggestive of venereal infection, orchitis of mumps or of any other urogenital disease and there were also no history of occupational or accidental injuries. No one of these fertile men had a complaint of libido deficiency or erctile or

ejaculatory disorders and all of them performed sexual intercourses with their wives at a rate of 2-3 times weekly. On clinical examination all of these fertile men were clinically free.

Regarding the infertile patients, their ages ranged from 24-48 years with a mean of 36 years and their marital periods ranged from 2-22 years with a mean of 14 years. All of these patients with exception of fourteen presented with primary infertility where their wives never got pregnant. The other fourteen patients presented with secondary infertility where their wives got pregnant before.

Three out of these 61 infertile patients gave past history suggestive of venereal infections with uretheral discharge. Another three patients were operated upon for left sided varicocele through suprapubic incision. No one of these patients had a complaint regarding the sexual desire, erection or ejaculation. All of them performed sexual intercourses at a regular rate of 2-3 times weekly.

All of these 61 infertile patients performed semen analysis for several times before being included in the present study. Most of these patients were treated previously for their infertility problem. The treatment was either treatment of the suspected obvious cause of infertility e.g. varicocele using naphtazone "Mediaven" and surgical varicocelectomy or emperical

treatment in cases of idiopathic infertility with either one or more of these drugs: androgen, chrionic gonadotropins, clomiphene citrate or tmoxifen.

On clinical examination most of these 61 infertile patients were clinically free and only 20 patients showed clinical abnormalities. Three out of these 20 patients showed only scars of previous operation which were discussed previously. Another seventeen from these 20 patients with clinical abnormalities, were varicocele patients and 9 of them presented by stress or OTA (oligo-terato-asthenozoospermia) pattern spermograms, five of them presented by sperm agglutination with head to head agglutination and three of them presented with oligo-asthenozoospermia pattern spermograms.

The collecting data of the study are presented in tables.

Group (I) Control Group.

In evaluating the results of semen analysis of the ten fertile men, all of them showed no abnormalities regarding the volumes of their semen, their colour, their odours, formations of seminal coagulums and their liquifaction within accepted time, viscosity and their pH value. Also none of the cases showed pyospermia. Regarding count of spermatozoa/ml, it ranged from 49 millions/ml to 104 millions/ml (mean 72.04 millions/ml $\pm$ SE 5.47 millions/ml).

Regarding the percentage of active motile sperms after liquifaction, it ranged from 70% to 86% (mean 76.6% $\pm$ SE 1.85%).

The percentage of abnormal forms ranged from 6% to 20% (mean 12.2% $\pm$ SE 1.39%).

In regard to the percentage of swollen sperm in the hypo-osmotic solution, it ranged from 62% to 77% (mean 70.1% $\pm$ SE 1.63).

Regarding the acrosin activity of the spermatozoa of these fertile men, it ranged from 0.85 - 0.96 mU/10^6 spermatozoa with a mean ($\bar{X}$) of 0.90 mU/10^6 spermatozoa $\pm$ standard error (SE) of 0.01 mU/10^6 spermatozoa as shown in table (1).

Table (1)
Spermagram of fertile control group, swelling test & acrosin activity.

Patient No.	count / ml	% of active motile Sperm after liquifaction	% of abnormal forms	% of Swollen sperms	Acrosin activity 10^6 sperms
1	104,000,000	85	11	74	0.93
2	98,200,000	86	6	75	0.91
3	65,000,000	75	8	72	0.89
4	72,200,000	73	17	70	0.89
5	80,000,000	80	15	77	0.94
6	65,000,000	80	9	73	0.96
7	49,000,000	70	11	64	0.88
8	65,000,000	70	15	62	0.85
9	60,000,000	72	20	64	0.88
10	62,000,000	75	10	70	0.90
X	72,040,000	76.60	12.20	70.10	0.90
S.E	5,471,607	1.85	1.39	1.63	0.01

Table (2)
Swelling test and semen parameters in control group.

Swelling test	r	P
acrosin activity	0.82	< 0.05
count	0.68	< 0.05
motility	0.85	< 0.01
abnormal forms	-0.47	> 0.05

Table (2) shows that there is a positive significant association between the swelling test ($P < 0.05$) and the acrosin activity, percentage of active motile sperm and the count of sperm. The association between the swelling test and percentage of abnormal forms was in significant.

Table (3)
Acrosin activity and semen parameters in control group.

Acrosin activity	r	P
count	0.43	> 0.05
motility	0.75	< 0.05
abnormal forms	-0.35	> 0.05

Table (3) shows a positive significant correlation between acrosin activity and percentage motility. No significant correlation existed between acrosin activity and the count of sperm nor the abnormal forms percentage.

Group II

Semen analysis of the 61 infertile patients, showed no abnormalities regarding volume, colour, odour, viscosity,

coagulation and liquifaction time.

Fourteen patients out of these 61 infertile patients showed decrease in sperm count/ml less than 20 millions/ml and decrease in percentage of active motile sperm less than 50%, had oligo-asthenozoospermia as their sperm counts ranged from 0.8 to 18 millions/mL with a mean ($\bar{X}$) 8.56 millions/mL $\pm$ 1.64 millions SE.

The percentage of active motile sperm ranged from 6 to 45% (mean = 29% $\pm$ SE 3.37).

The percentage of abnormal forms ranged from 10-35% (mean 24.36% $\pm$ SE 2.07). The percentage of swollen sperm in the hypo-osmotic swelling test ranged from 2-43% (mean 22.07% $\pm$ SE 3.32).

The acrosin activity per 10^6 spermatozoa from these infertile semen samples with oligo-asthenozoospermia ranged from 0.95-2.23 mU/10^6 sperm (mean 1.45 $\pm$ 0.12 SE). These data are shown in table (4).

The percentage of swollen sperm in this oligo-asthenozoospermia group was studied statistically in comparison to that of fertile control group, t-test with unequal numbers of data in compared groups was used for this study. As shown in table (5).

Table (4)
Spermogram, swelling test and acrosin activity of 14 infertile men with oligoasthenozoospermia.

Patient No.	cont of sperms/ ml	% of activity motile Sperm after liquifaction	% of abnormal forms	% of Swollen sperms	Acrosin activity 6 / 10 sperms
1	12,000,000	20	25	20	1.10
2	9,000,000	20	30	10	1.09
3	2,000,000	10	20	10	1.20
4	4,200,000	6	35	2	1.18
5	800,000	25	30	8	1.89
6	10,000,000	40	30	34	1.81
7	4,400,000	40	35	28	2.00
8	12,000,000	20	10	16	1.20
9	2,000,000	30	20	20	1.99
10	12,400,000	30	12	18	1.40
11	14,000,000	45	29	32	1.27
12	18,000,000	40	20	39	0.97
13	1,000,000	35	20	29	2.23
14	18,000,000	45	25	43	0.95
X	8,557,142	29	24.36	22.07	1.45
S.E	1,637,899	3.37	2.07	3.32	0.12

Table (5)
Swelling test of control and infertile oligo-asthenozoospermia group.

Group		% of swollen sperm
infertile group	$\bar{X}$	22.07
	SE	3.32
fertile group	$\bar{X}$	70.1
	SE	1.63
	t	13
	P	< 0.001

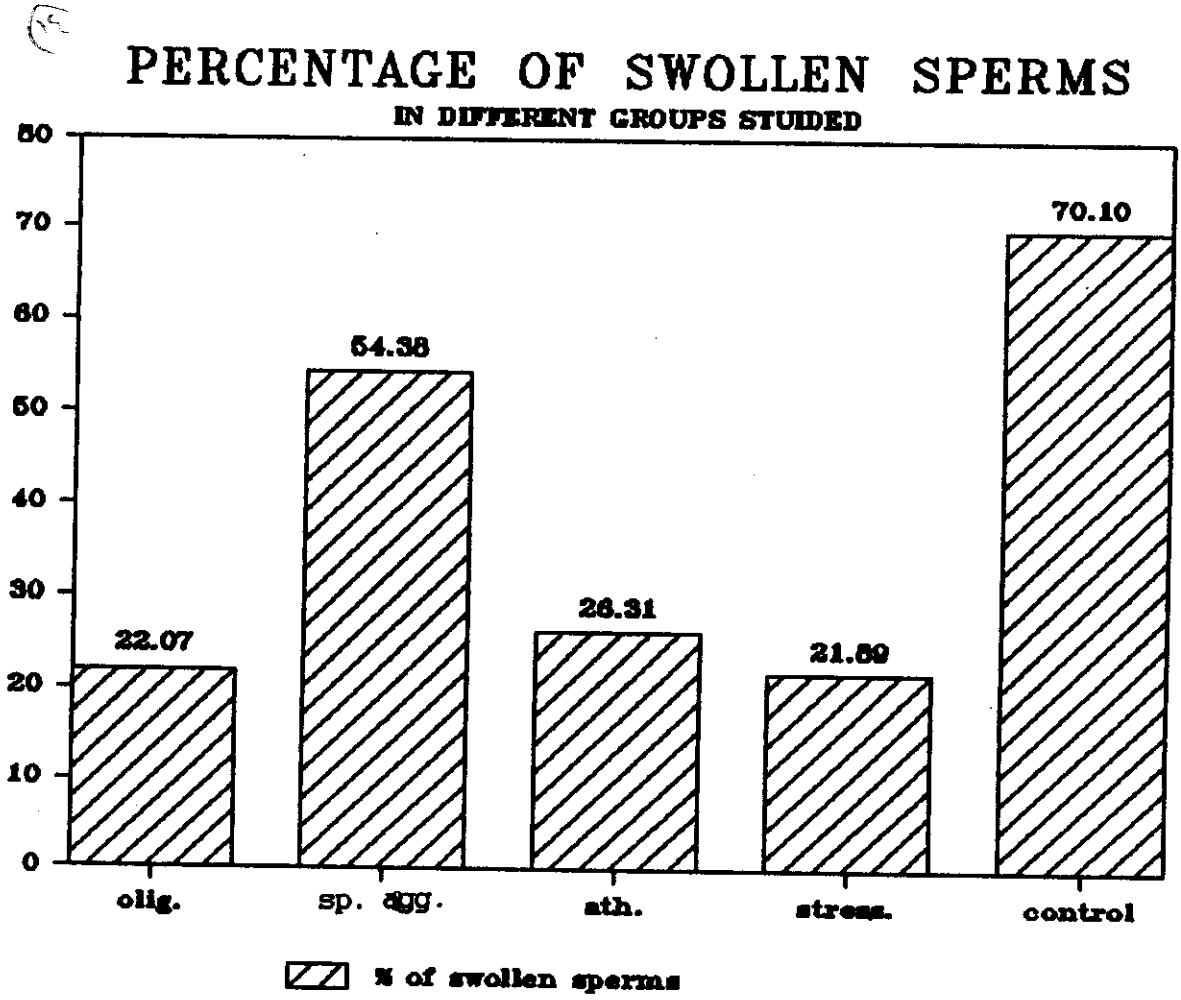
Table (5) shows that there is a highly significant increase in the percentage of swollen sperm in the fertile control group than in the oligo-asthenozoospermia group ($t = 13$ and $P < 0.001$).

The acrosin activity per 10^6 sperm from these infertile semen samples was studied statistically in comparison to that of fertile control group using t-test. As shown in table (6)

Table (6)
Acrosin activity of control and infertile oligo-asthenozoospermia group.

Group		Acrosin activity
infertile group	$\bar{X}$	1.45
	SE	0.12
control group	$\bar{X}$	0.90
	SE	0.01
	t	4.65
	P	< 0.001

Table (6) shows that there is a highly significant increase in acrosin activity of the infertile group than in the fertile


PERCENTAGE OF SWOLLEN SPERMS
IN DIFFERENT GROUPS STUDIED
80
70
60
50
40
30
20
10
0
22.07
54.38
26.31
21.89
70.10
olig.
sp. agg.
ath.
stress.
control
% of swollen sperms

control group (t = 4.65 and P < 0.001).

Table (7)
Swelling test and semen parameters in the oligo-asthenozoospermia group.

Swelling test	r	P
count	0.60	< 0.05
motility	0.90	< 0.01
abnormal forms	-0.06	> 0.05
acrosin activity	0.01	> 0.05

Table (7) shows that there is a non significant association (P > 0.05) between swelling test and acrosin activity nor percentage of abnormal forms.

While there is a positive significant association (P < 0.05) between swelling test in one hand and the percentage of active motile sperm and count of sperm/mL in the other hand.

Table (8)
Acrosin activity and semen parameters in the oligo-asthenozoospermia group.

Acrosin activity	r	P
count	-0.7	< 0.05
motility	0.23	> 0.05
abnormal forms	0.13	> 0.05

Table (8) shows that there is a negative significant correlation between acrosin activity and count of sperm. No significant correlation between acrosin activity and percentage of motile sperm nor percentage of abnormal forms.

Table (10)
Swelling test in the control and sperm agglutinates group.

Group		% of swollen sperms
infertile group	$\bar{X}$	54.38
	SE	2.33
fertile control group	$\bar{X}$	70.1
	SE	1.63
	t	5.52
	P	< 0.001

Table (10) shows that there is a highly significant increase in the percentage of swollen sperm in the fertile control group ($t = 5.52$ and $P < 0.001$) than the infertile group with sperm agglutination.

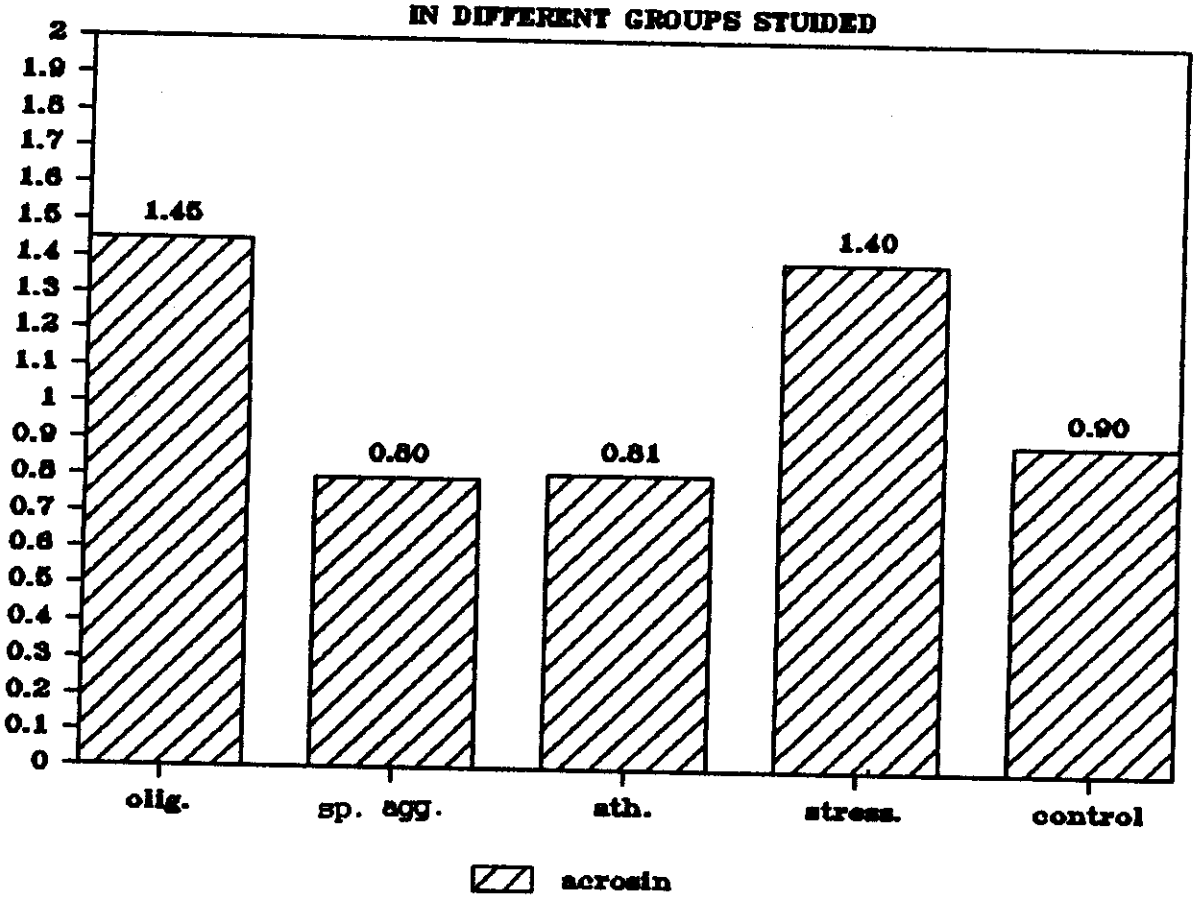
Also the acrosin activity/10^6 sperm in this group was studied statistically in comparison to that of the fertile control group. This is shown in table (11).

Table (11)
Acrosin activity in the control and sperm agglutinates group.

Group		Acrosin activity/10^6 sperms
infertile group	$\bar{X}$	0.80
	SE	0.02
fertile control group	$\bar{X}$	0.90
	SE	0.01
	t	5.60
	P	< 0.001

Table (11) shows that there is a highly significant increase

LEVELS OF ACROSIN ACTIVETY



in the acrosin activity/10^6 sperm in the fertile control group ($t = 5.6$ and $P < 0.001$) than the infertile group with sperm agglutination.

Table (12)
Swelling test and semen parameters in the infertile group with sperm agglutination.

Swelling test	r	P
acrosin activity	0.77	< 0.05
% motility	0.68	< 0.05
count	0.37	> 0.05
abnormal forms	-0.23	> 0.05

Table (12) shows that there is a positive significant correlation between swelling test and acrosin activity and percentage of active motile sperm ($P < 0.05$). Positive but non significant association between the percentage of swollen sperm and the count of sperm/mL ($P > 0.05$). Negative non significant correlation between the percentage of swollen sperm and the percentage of abnormal form.

Table (13)
Acrosin activity and semen parameters in the infertile group with sperm agglutination.

Acrosin activity	r	P
count	0.50	> 0.05
% motility	0.90	< 0.01
abnormal forms	-0.25	> 0.05

Table (13) shows that there is a positive highly significant correlation between acrosin activity and percentage of motile sperm. But no significant correlation exists between acrosin

activity and count of sperm or abnormal form percentage.

Group IV

Among the 61 infertile semen samples studied there were 13 samples that showed one abnormal parameter which was the decrease in percentage of active motile spem than 60%. This was the astheno-zoospermia group. The pattern of spermograms in this infertile group, their swelling test and their spermatozoal acrosin activity/10 sperms are shown in table (14).

The sperm count/mL in this group ranged from 30-128 millions/mL with mean ($\bar{X}$ = 53.55 $\pm$ 7.93 SE).

The percentage of active motile sperm in this group ranged from 15-50 with a mean ($\bar{X}$ = 35.23 $\pm$ 3.41 SE).

The percentage of abnormal forms in this group ranged from 10-38 with a mean ($\bar{X}$ = 20.92 $\pm$ 2.06).

The percentage of swollen sperm in this group ranged from 12-41 with a mean ($\bar{X}$ = 26.31 $\pm$ 2.73 SE).

The acrosin activity/10^6 sperms in this group ranged from 0.64-1.05 mU/10^6 sperms with a mean ($\bar{X}$ = 0.81 $\pm$ 0.04 SE).

The percentage of swollen sperm in this group was studied statistically in comparison to that of the fertile control group using t-test with unequal numbers of data in compared groups. This is shown in tabel (15).

Table (14)
Spermogram, swelling test and acrosin activity of 13 infertile patients with asthenozoospermia.

Patient No.	count / ml	% of active motile Sperm after liquifaction	% of abnormal forms	% of Swollen sperms	Acrosin activity 6 / 10 sperms
1	40,000,000	50	16	30	0.72
2	43,000,000	40	15	25	0.69
3	60,000,000	15	10	24	0.68
4	55,000,000	40	15	30	0.68
5	128,000,000	40	25	24	0.70
6	30,000,000	40	30	35	1.02
7	61,200,000	20	38	14	0.64
8	32,000,000	30	20	16	1.05
9	35,000,000	25	25	16	0.99
10	30,000,000	20	15	12	0.95
11	62,000,000	40	25	35	0.67
12	90,200,000	50	20	41	0.79
13	30,000,000	50	20	40	0.94
X	53,553,840	35.23	20.92	26.31	0.81
S.E	7,930,892	3.41	2.06	2.73	0.04

Table (15)
Swelling test of the control and asthenozoospermia group.

Group		% of swollen sperm
infertile group	$\bar{X}$	26.31
	SE	2.73
fertile control group	$\bar{X}$	70.1
	SE	1.63
	t	13.78
	P	< 0.001

Table (15) shows that there is a highly significant increase in the percentage of swollen sperm ($t = 13.78$ and $P < 0.001$) in the fertile control group than the infertile astheno-zoospermic group.

Also the acrosin activity/10^6 sperm in this group was studied statistically in comparison to that of the fertile control group. This is shown in table (16).

Table (16)
Acrosin activity of the control and asthenozoospermia group.

Group		Acrosin activity
infertile group	$\bar{X}$	0.81
	SE	0.04
control group	$\bar{X}$	0.90
	SE	0.01
	t	2.12
	P	< 0.05

Table (16) shows that thers is low significant increase in the acrosin activity/10^6 sperm in the fertile control group

(P < 0.05) than the infertile astheno-zoospermia group.

Table (17)
Swelling test and semen parameters in the infertile asthenozoospermia group.

Swelling test	r	P
acrosin activity	-0.12	> 0.05
% motility	0.82	< 0.01
count	0.15	> 0.05
abnormal forms	-0.09	> 0.05

Table (17) shows that there is a positive significant association between the swelling test and the percentage of active motile sperm P < 0.01. While there is a positive but non significant association between swelling test and count of sperm/mL.

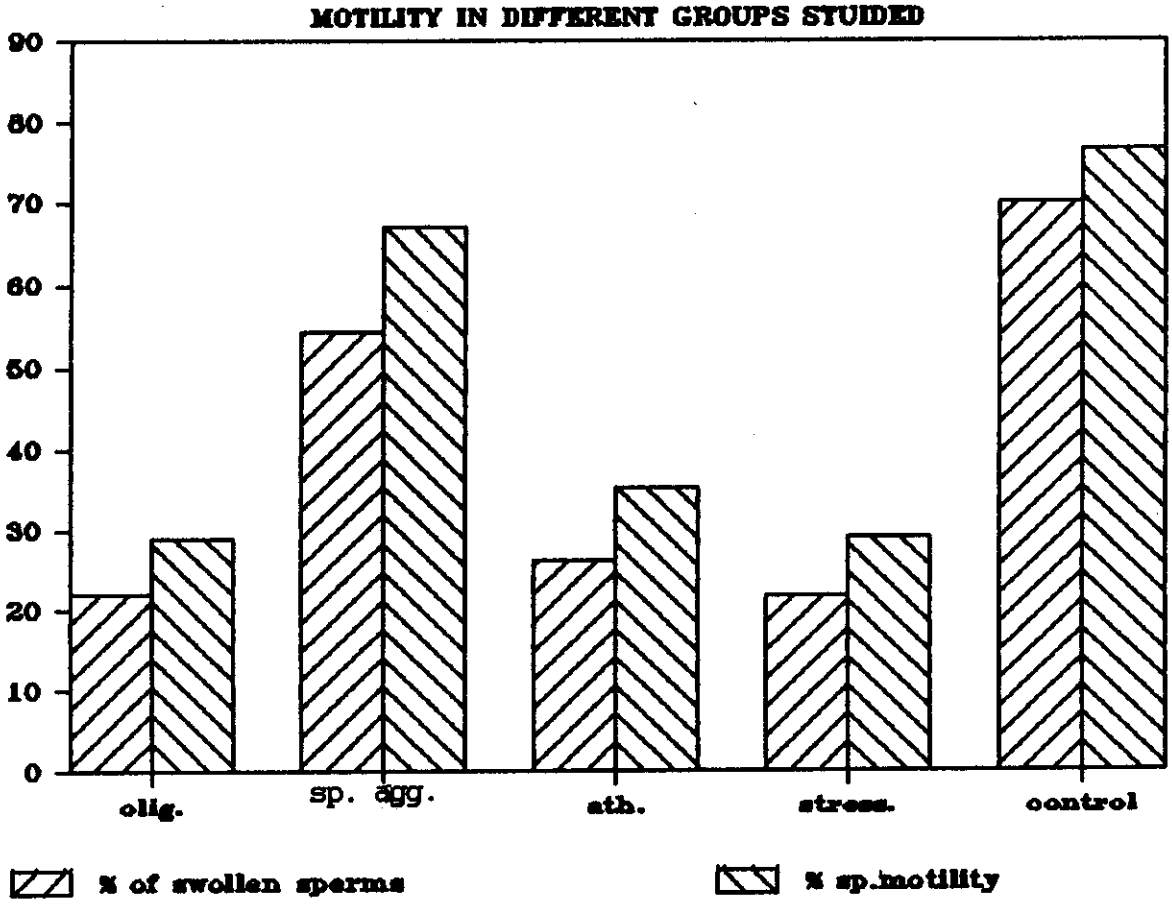
There is a negative but non significant association between the swelling test and acrosin activity or percentage of abnormal forms.

Table (18)
Acrosin activity and semen parameters in the infertile asthenozoospermia group.

Acrosin activity	r	P
count	-0.55	> 0.05
motility	-0.06	> 0.05
abnormal forms	0.08	> 0.05

Table (18) shows that there is no significant correlation between acrosin activity (P > 0.05) and count of sperm, motility or abnormal forms.

PERCENTAGE OF SWOLLEN SPERMS & SPERM



Group V

Among the 61 infertile semen samples studied there were 9 samples showing stress patterns spermograms OTA (oligoterato-asthenozoospermia). The patterns of spermograms in this infertile group, their swelling test and their spermatozoal acrosin activity/10^6 sperms are shown in table (19).

The sperm count/mL in this group ranged from 0.6-13.4 millions/mL with mean ($\bar{X}$ 7.8 $\pm$ 1.68 SE).

The percentage of active motile sperm in this group ranged from 5-50 with a mean of $\bar{X}$ 29.22 $\pm$ 4.16 SE.

The percentage of abnormal forms in this group ranged from 40-80 with a mean of $\bar{X}$ 50.56 $\pm$ 4.12.

The percentage of swollen sperm in this group ranged from 5-38 with a mean of $\bar{X}$ 21.89 $\pm$ 3.43 SE.

The acrosin activity/10^6 sperm in this group ranged from 0.93-2.20 with a mean of $\bar{X}$ 1.4 $\pm$ 0.16 SE.

The percentage of swollen sperm in this group was studied statistically in comparison to that of the fertile control group using t-test. This is shown in table (20).

Table (19)
Spermogram, swelling test and acrosin activity of 9 patients with stress pattern.

Patient No.	count / ml	% of active motile Sperm after liquifaction	% of abnormal forms	% of Swollen sperms	Acrosin activity 6 / 10 sperms
1	600,000	5	50	5	1.08
2	2,000,000	30	40	22	1.95
3	3,300,000	25	55	20	1.45
4	10,600,000	50	40	38	1.85
5	12,200,000	23	80	18	0.98
6	11,400,000	35	50	20	1.20
7	4,800,000	25	40	12	2.20
8	12,000,000	40	50	34	0.95
9	13,400,000	30	50	28	0.93
X	7,811,111	29.22	50.56	21.89	1.40
S.E	1.682,792	4.16	4.12	3.43	0.16

Table (20)
Swelling test of the control and stress pattern group.

Group		% of swollen sperms
infertile group	$\bar{X}$	21.89
	SE	3.43
fertile control group	$\bar{X}$	70.1
	SE	1.63
	t	12.69
	p	< 0.001

Table (20) shows that there is a highly significant increase in the percentage of swollen sperm in the fertile control group $p < 0.001$ than the infertile group with stress pattern spermogram.

Also the acrosin activity/10^6 sperms in this group was studied statistically in comparison to that of the fertile control group. This is shown in table (21).

Table (21)
Acrosin activity of the control and stress pattern group.

Group		Acrosin activity/10^6 sperms
infertile group	$\bar{X}$	1.40
	SE	0.16
fertile control group	$\bar{X}$	0.90
	SE	0.01
	t	3.05
	p	< 0.01

Table (21) shows that there is a significant increase in the

acrosin activity/10 sperm in the infertile group with stress pattern spermogram than the fertile control group.

Table (22)
Swelling test and semen parameters of the stress pattern group.

Swelling test	r	P
acrosin activity	-0.04	> 0.05
% motility	0.92	< 0.01
count	0.64	< 0.05
abnormal forms	-0.19	> 0.05

Table (22) shows that there is a positive significant association between swelling test ($P < 0.05$) and the percentage of active motile sperm and the count of sperm.

Also there is a negative but non significant association between the swelling test and the percentage abnormal forms or the acrosin activity.

Table (23)
Acrosin activity and semen parameters of the stress pattern group.

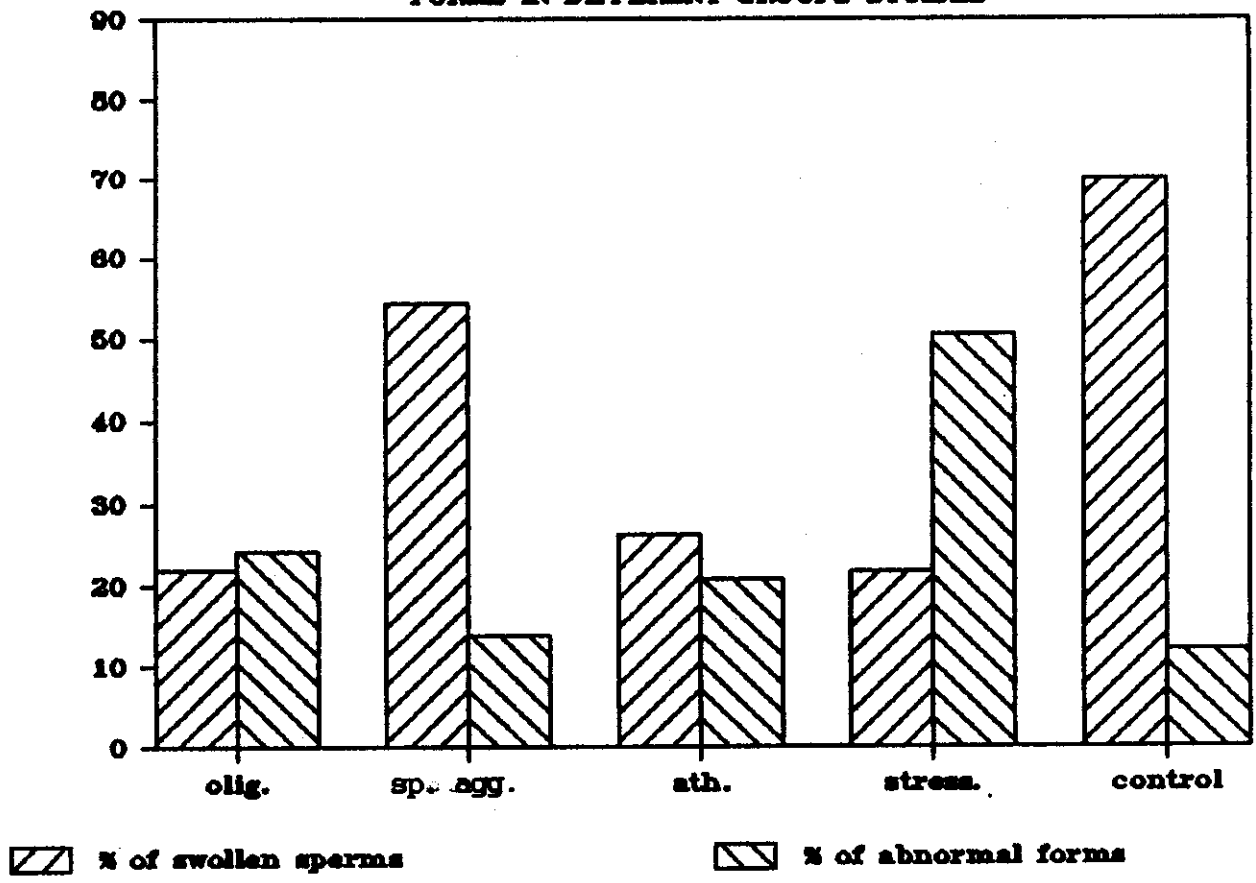
Acrosin activity	r	P
count	-0.47	> 0.05
motility	0.21	> 0.05
abnormal forms	-0.63	> 0.05

Table (23) shows that there is no significant correlation between acrosin activity and counts of sperm, percentage motility or abnormal form.

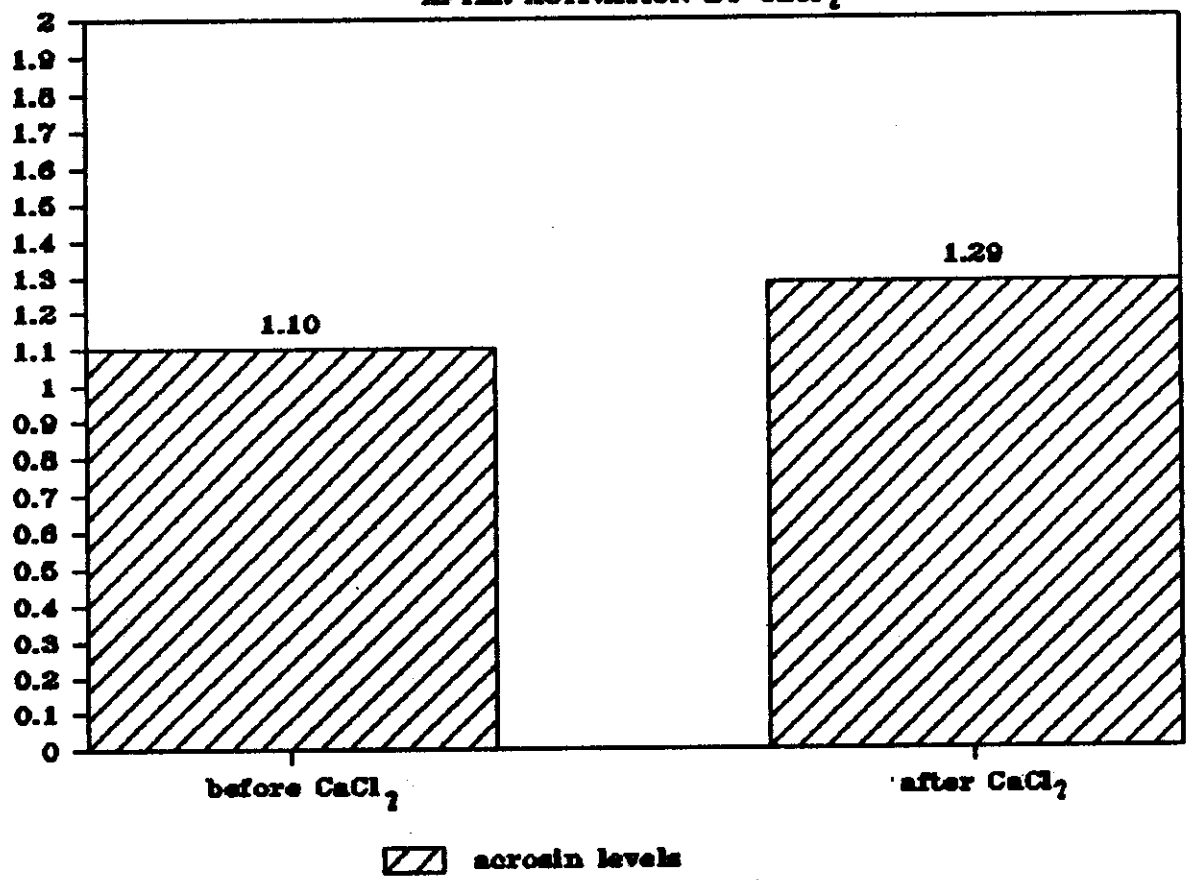
(२२

PERCENTAGE OF SWOLLEN SPERMS & ABNORMAL

FORMS IN DIFFERENT GROUPS STUDIED



LEVELS OF ACROSIN ACTIVITY BEFORE & AFTER ACTIVATION BY $CaCl_2$



seen.

Many malformed immature germ cells, mainly spermatids, of different developmental stages are present in the sample (Figures 5-11).

Electron microscopic study of the second sample showed the following:-

The semen sample examined under electron microscope showed the presence of abnormal spermatozoa, immature germ cells, mainly abnormal spermatids and spermatophages.

The most frequent pathology of spermatids and spermatozoa is acrosomal malformations such as abnormal acrosomal shape, enlargement of the acrosome, displacement or separation of abnormal acrosome from the nucleus. In the nucleus big vacuole with granular and/or membranous content can be seen. In some spermatids abnormally large endoplasmic reticulum vesicle is located just below the nucleus. Some spermatids are double headed.

The midpiece is usually defective. Mitochondrial sheath is incomplete or absent, mitochondria are degenerated.

The principal piece exibits disorganisation of the axoneme and disintegration of fibrous sheath. In the cross section of

axonemes numerical aberrations (in defect) of outer dense fibers and peripheral microtubules are seen. Sometimes dynein arms are partialy defective (Fig. 12-19).

Electron microscopy of the third sample showed the following:

The sample contains many spermatozoa, many immature germ cells, mainly spermatids and spermatophages.

The head of spermatozoa shows the following ultrastructural defects: acrosome has irregular shape, often there is a space between the acrosome and the nucleus, sometimes the acrosome is incomplete. Some spermatozoa are doubleheaded.

In the midpiece the presence of excessive membranous structure and vacules in the cytoplasmic droplet, disarrangement of mitochondrial sheath are seen.

Axonemal structure is within normal.

Immature germ cells present in the sample shows signs of vacuolar degeneration.

In spermatophages different stages of spermatozoal parts digestion can be seen (Fig. 20-24).

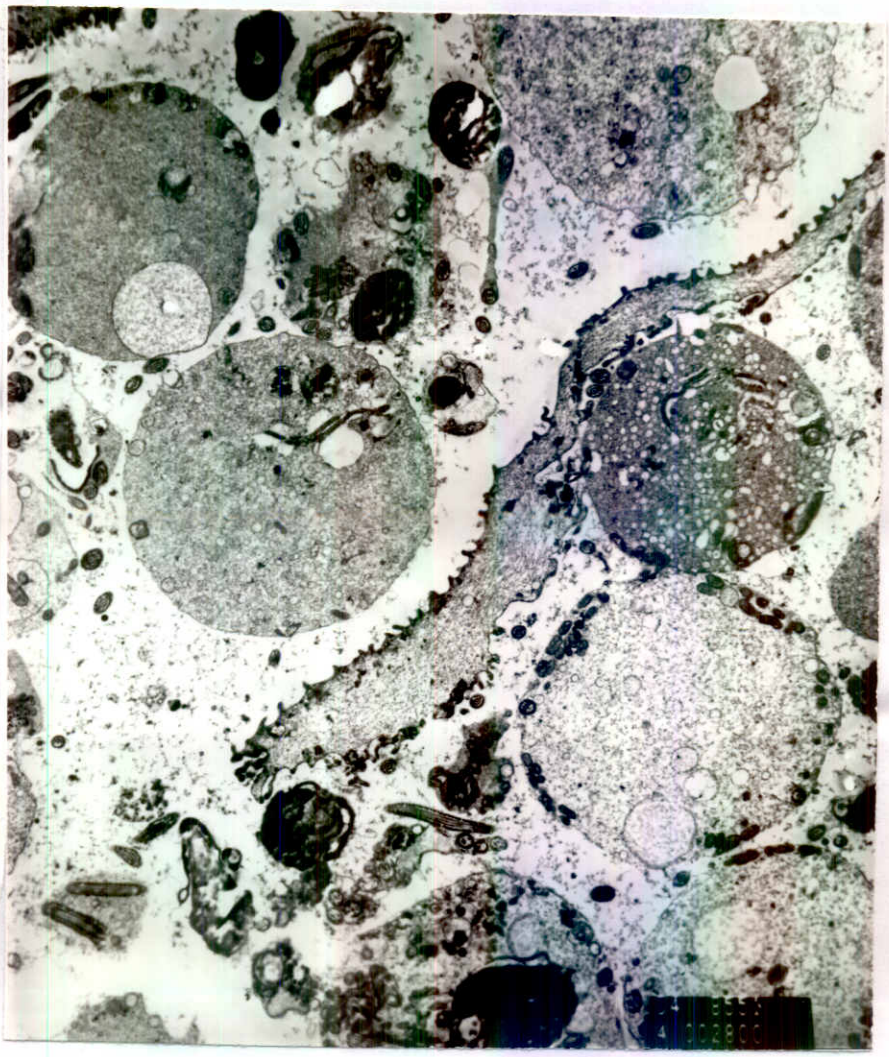


Fig. (5)
Electron microscopic picture of an area of a pellet of ejaculate from infertile man, showing few undifferentiated immature germ cells and several sections of sperm tails and late spermatid.

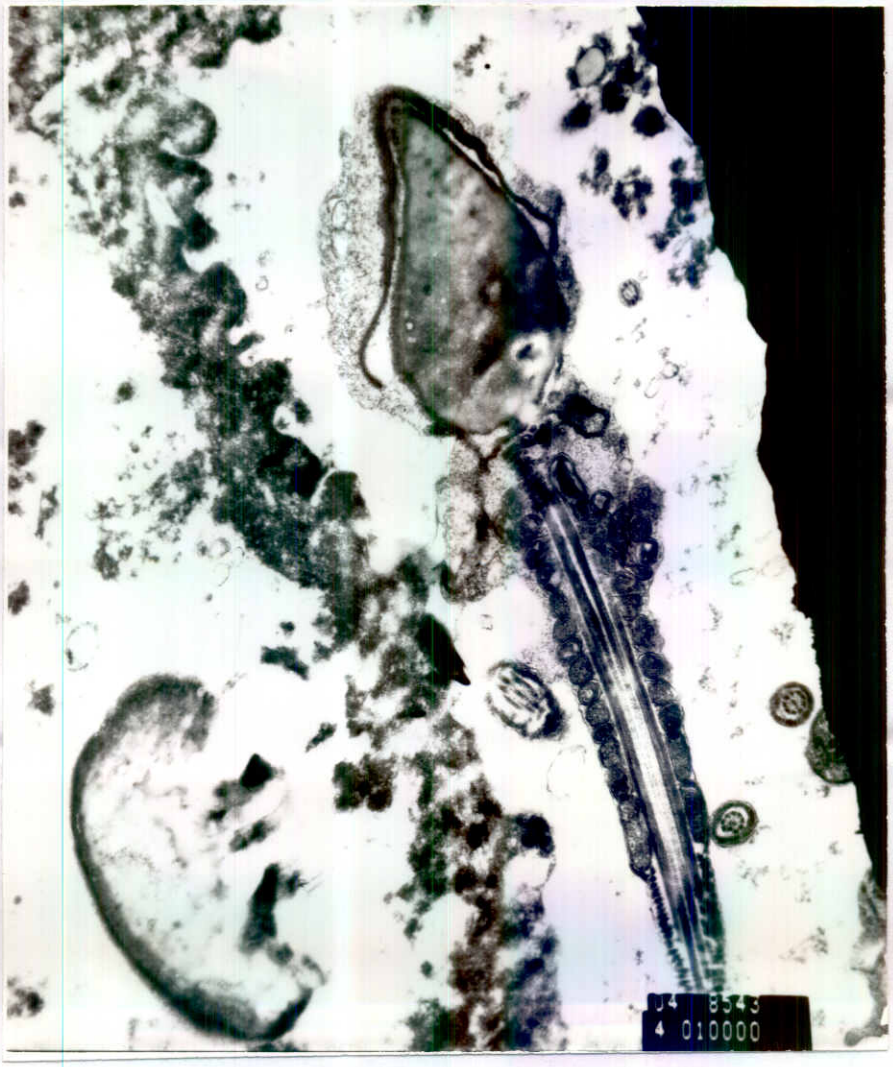


Fig. (6)

Electron microscopic picture of a longitudinal section of complete spermatozoon. The head looks normal in shape. Acrosome is seen covering anterior 2/3 of the head and abnormally separated from the neucleus. A cytoplasmic remnant is seen around the head and mid-piece. Mid piece shows normal mitochondrial sheath. Annulus is present. Axonemal structure is not seen through the whole length because of the plane of section.

Fig. (7)
Electron microscopic picture of a degenerated spermatozoon In the head, the acrosome looks rarified and fragemented. No basal plate is seen. The neck region is disorganized and small vacule is seen attached to the basal part of the nucleus. Mid piece shows only short mitochondrial sheath. Annulus is not seen. Principal piece shows two rows of ribs of fibrous sheath and the axoneme is unidentified clearly.

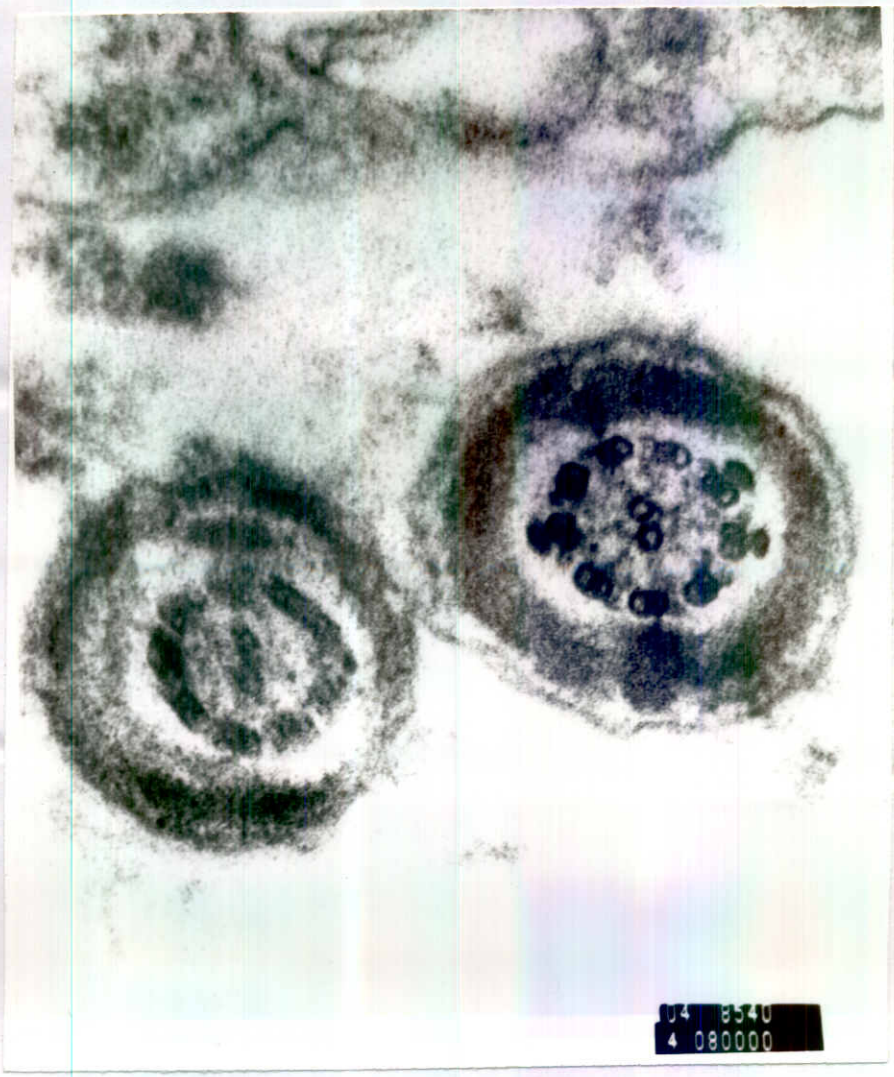


Fig. (8)
Electron microscopic picture of a T.S. of the sperm at the level of principal piece that show defective number of outer dense fibres (only 1, 2 and 6 are present). The longitudinal columns are not normally situated at position 3 and 8. The 9+2 pattern of the axoneme looks normal.

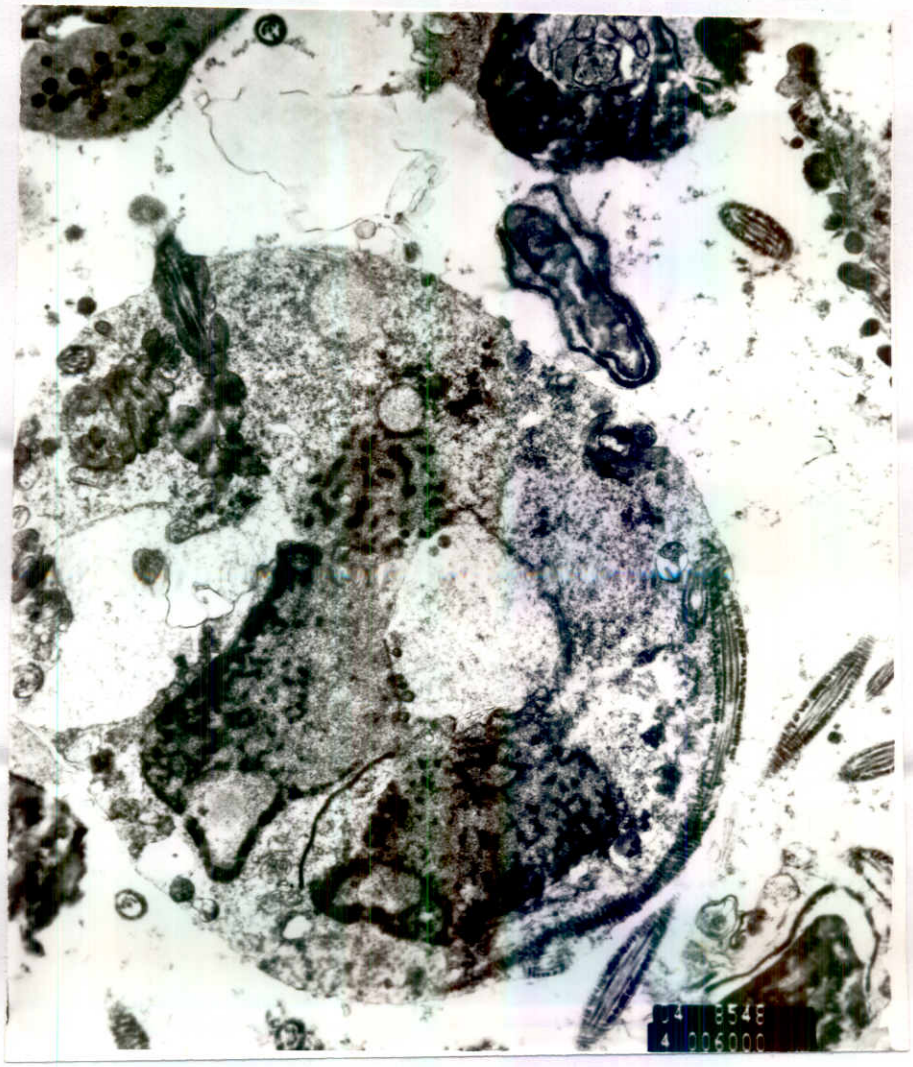


Fig. (9)
Electron microscopic picture of double-head spermatid showing abnormal head shape, abnormal acrosome and 2 big vacules. The mitchondria are disarranged and there are scattered reminant of fibrous sheath. Also L.S. of free head is seen in this figure with abnormally separated acrosome from the nuclear membrane, and the nucleus shows many vesicles.

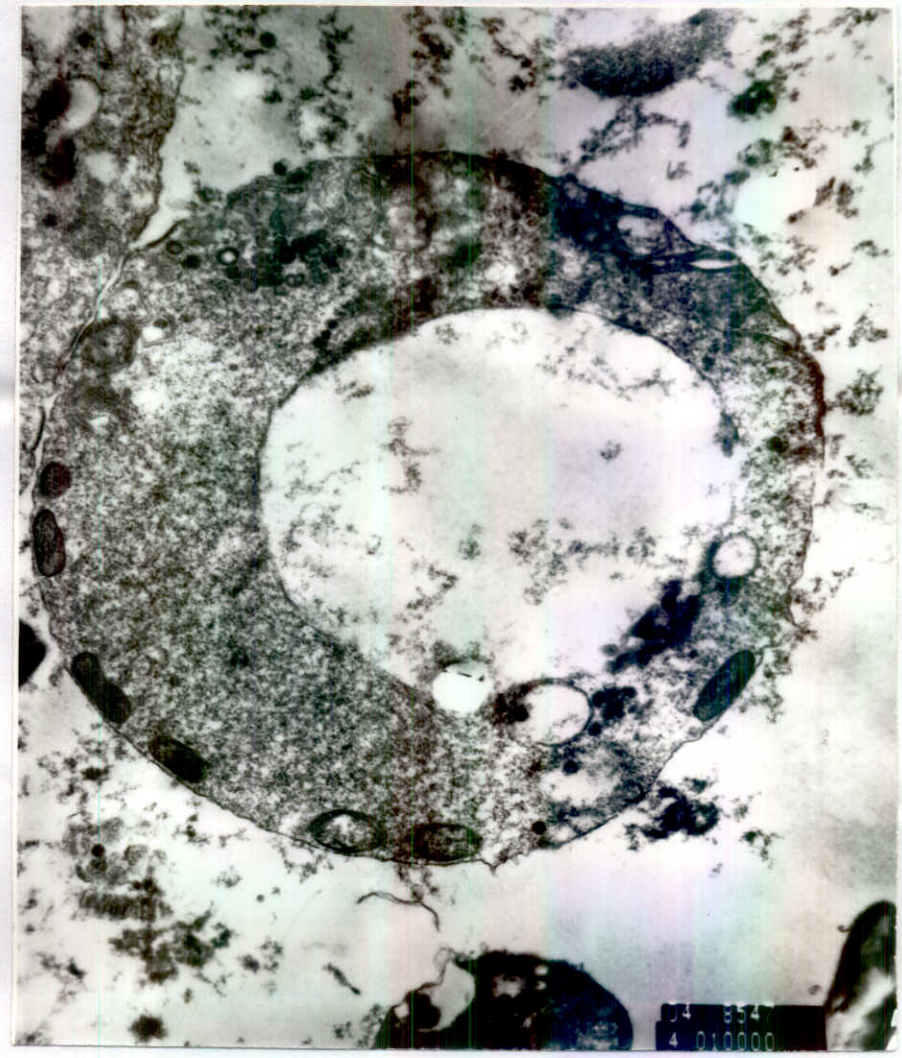


Fig. (10)
Electron microscopic picture of undifferentiated immature germ cell.

Fig. (11)
Electron microscopic picture of a macrophage igulfing parts of spermatozoon i.e.

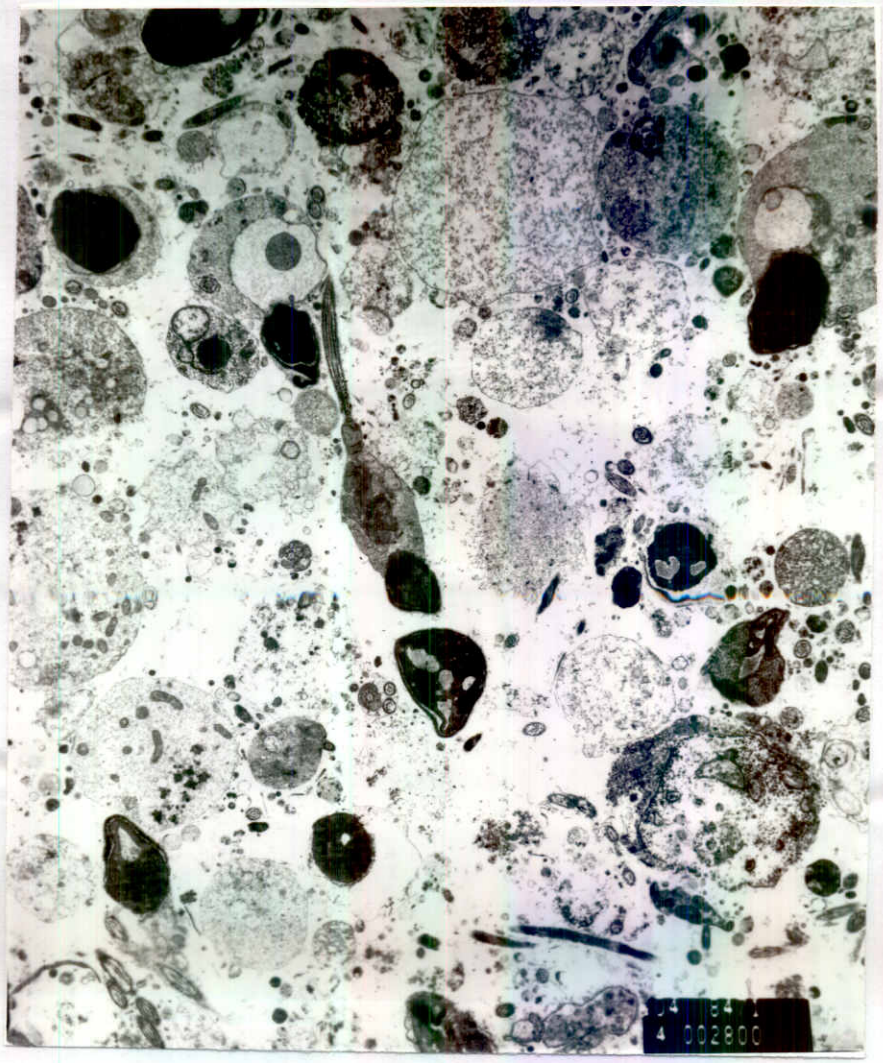


Fig. (12)
Electron microscopic picture of an area of a pellet of the ejaculate with teratozoospermia. The figure shows many sections of variably malformed spermatozoa and spermatids.

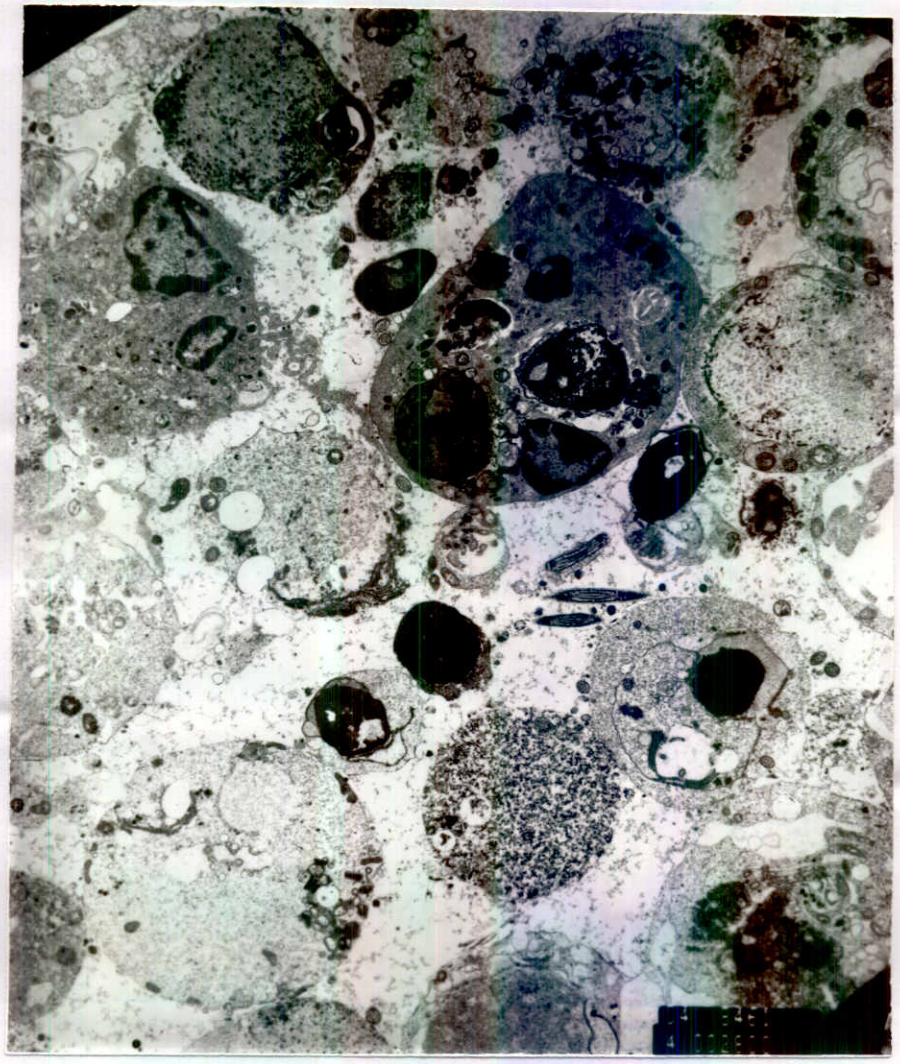


Fig. (13)
Electron microscopic picture of an area of pellet of the ejaculate contains immature germ cells, inflamatory cell and spermatophage in which fragments of spermatozoa are seen.

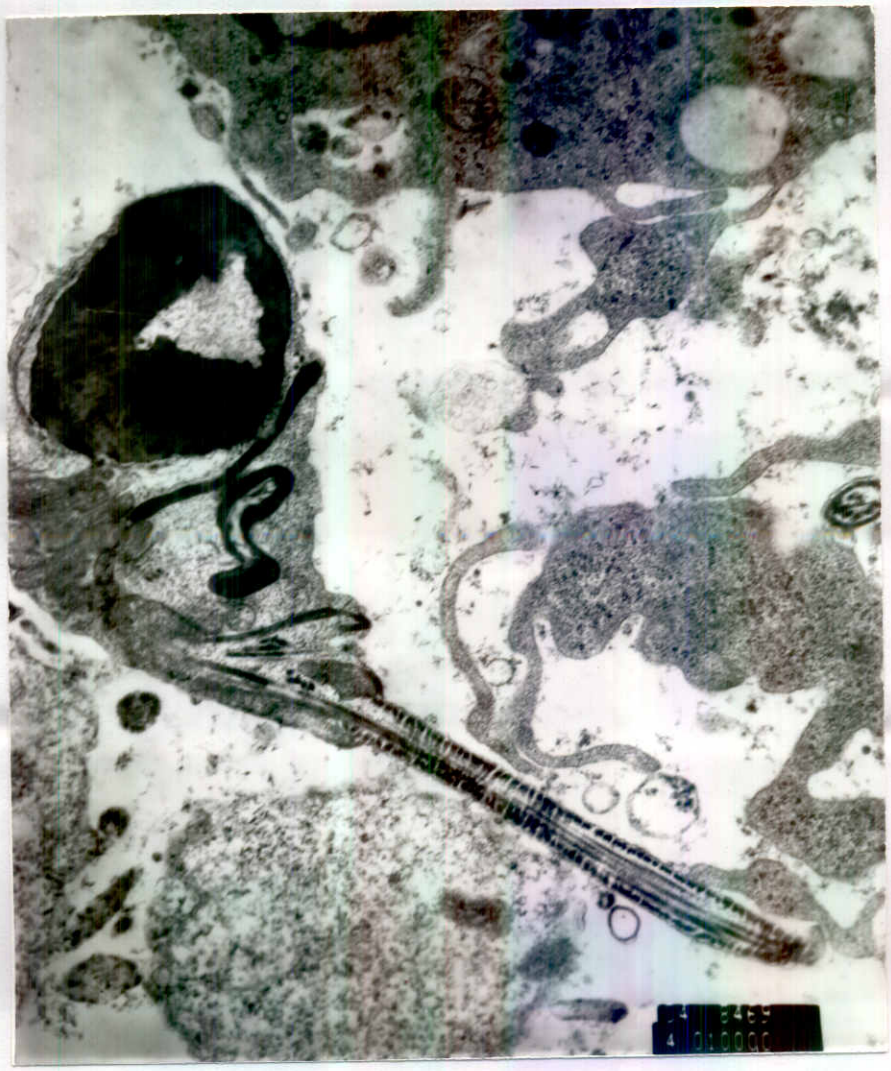


Fig. (14)
Electron microscopic picture of a spermatozoon with malformed acrosome and defective midpiece.

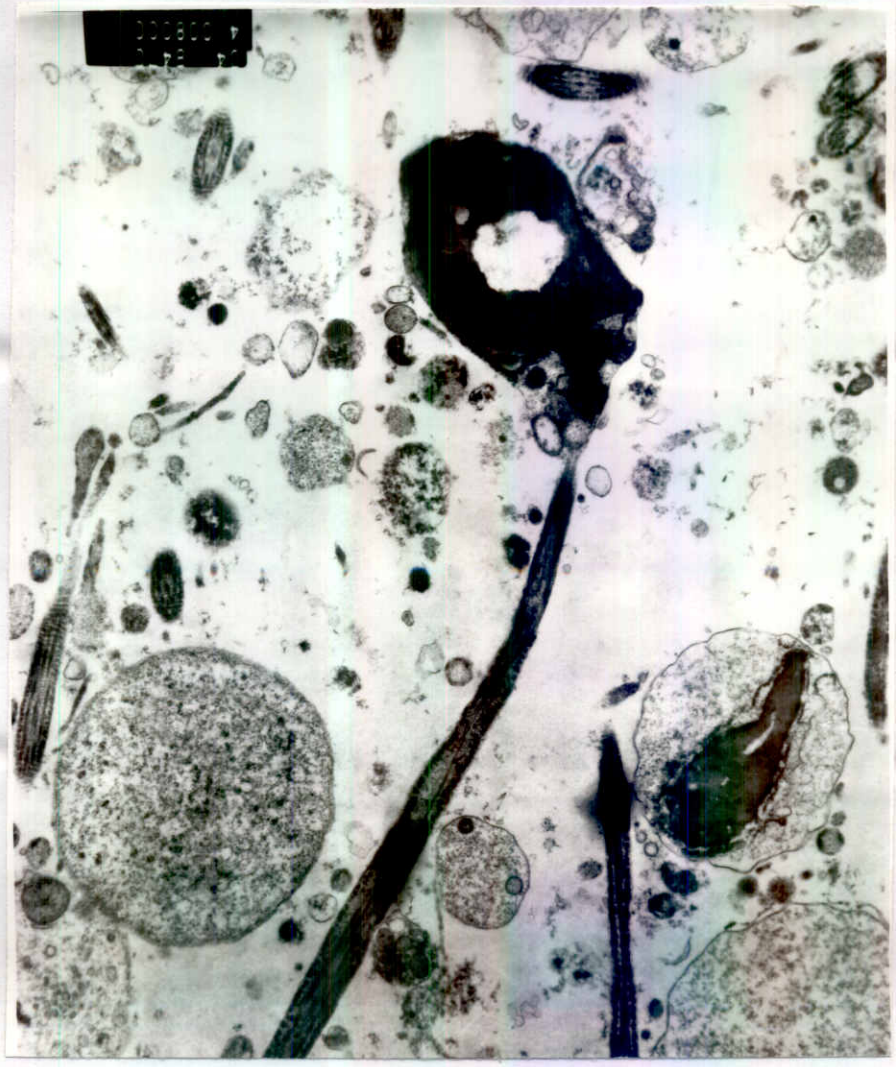


Fig. (15)
Electron microscopic picture of a degenerated spermatozoon. The head has no acrosome, there is a big vacuole in the nucleus, the mid piece is absent, the tail is abnormal.

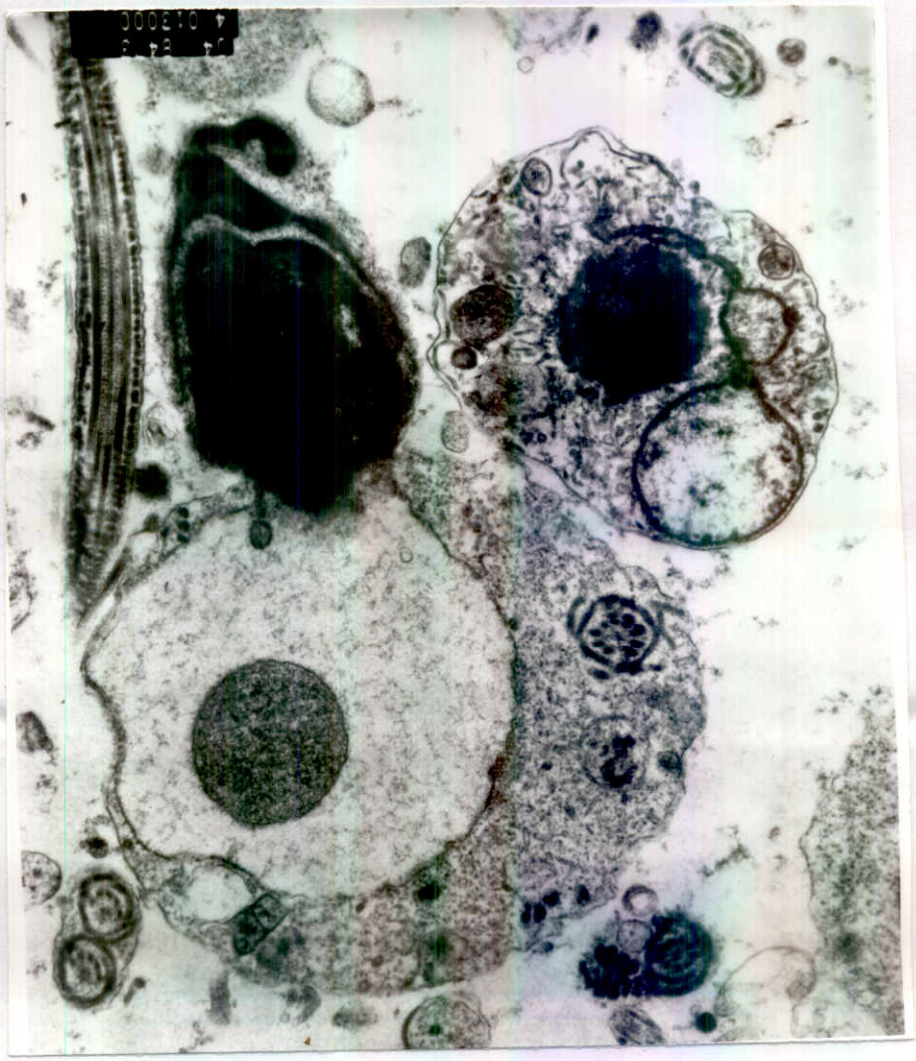


Fig. (16)
Electron microscopic picture of a spermatid with enlarged abnormally shaped acrosome. A large endoplasmic reticulum vesicle is located below the nucleus. Cross-sections of tail show numerical aberration of outer dense fibres.

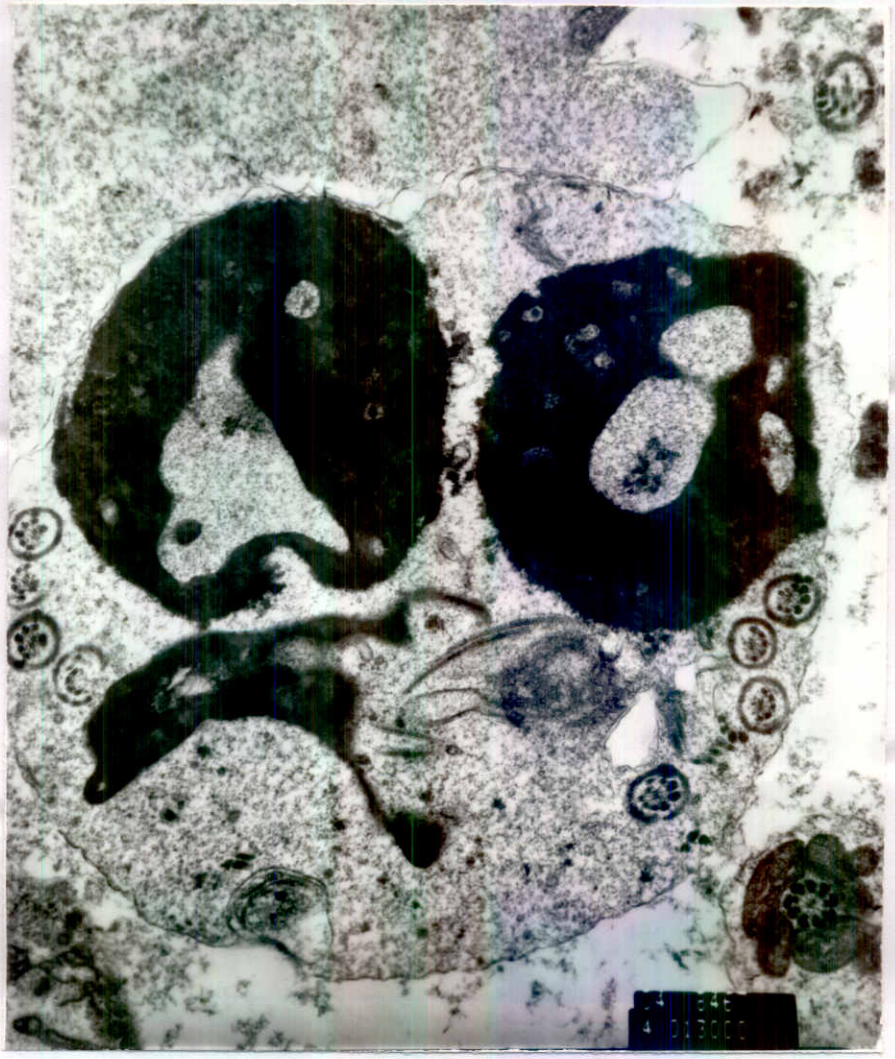


Fig. (17)
Electron microscopic picture of a spermatid contains 2 abnormal nuclei with big vacules, one enlarged separated acrosome and several cross sections of tails with disorganised axonemal structure.

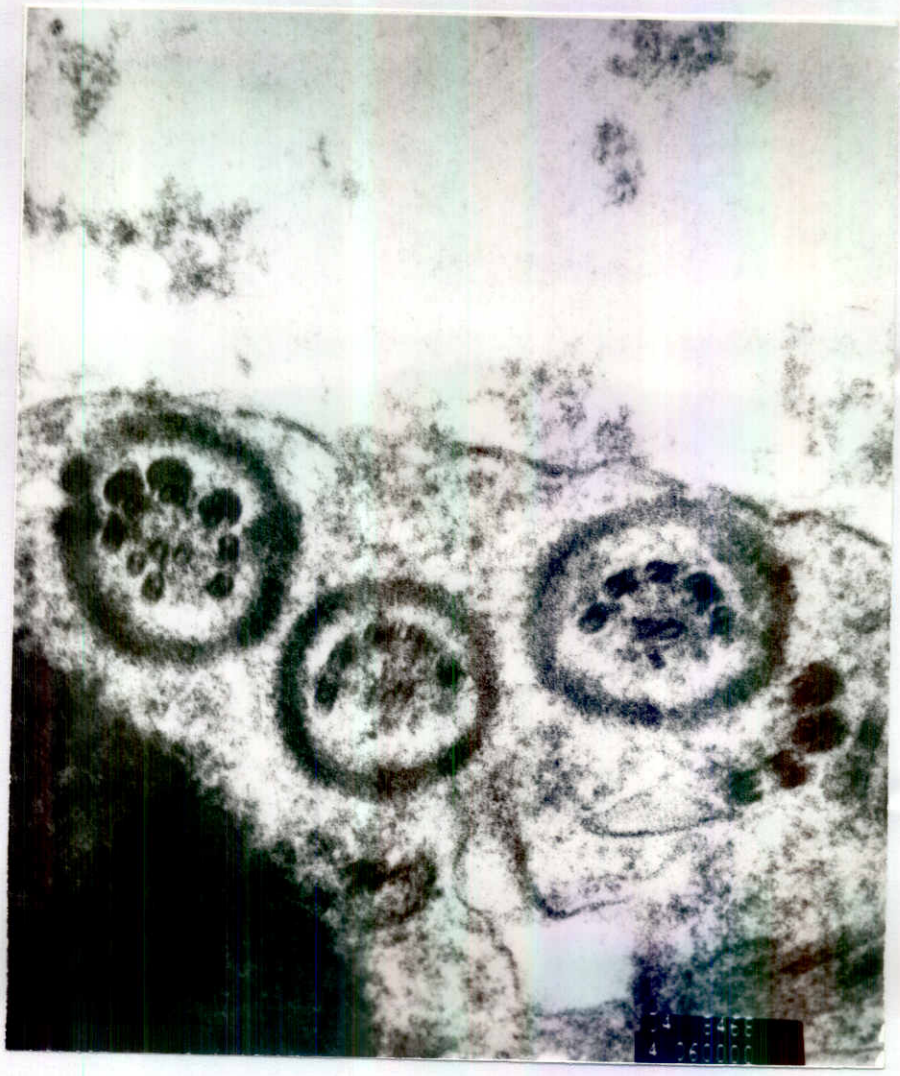


Fig. (18)
Electron microscopic picture of a T.S. of axonemes show deletion of peripheral microtubules and outer dense fibres.

ناقصة

٥٤

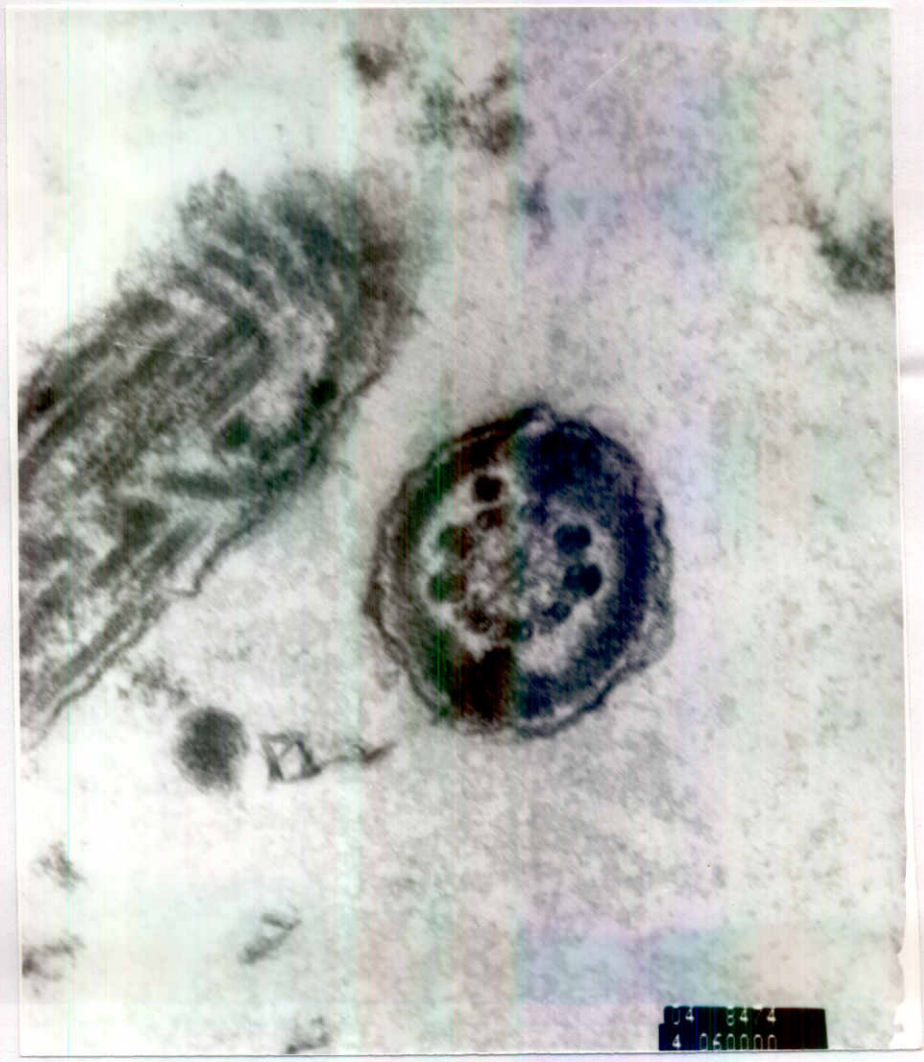


Fig. (19)
Electron microscopic picture of a cross section through the principal piece, showing numerical aberration of outer dense fibres.

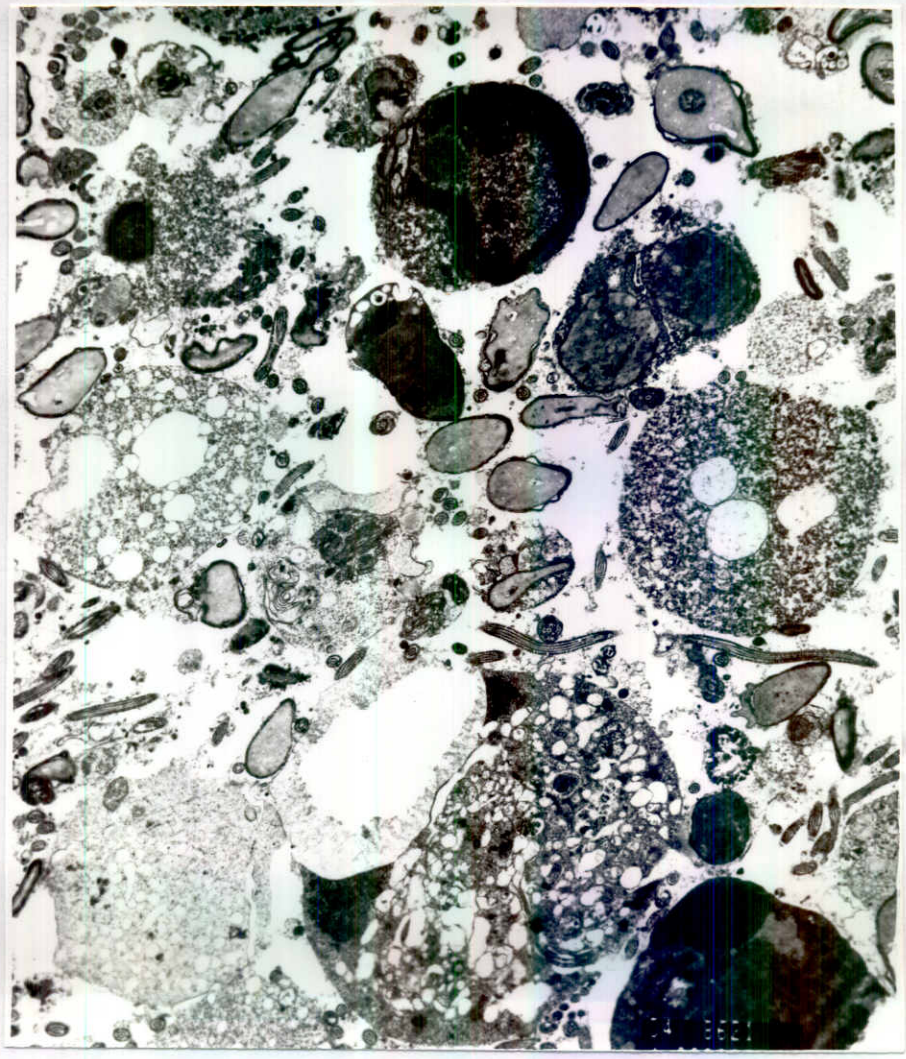


Fig. (20)
Electron microscopic picture of an area of a pellet of ejaculate of infertile man with teratozoospermia, acrosomal hypoplasia with few separation of acrosome from nuclear membrane. There are few immature germ cells and several sperm tail section.

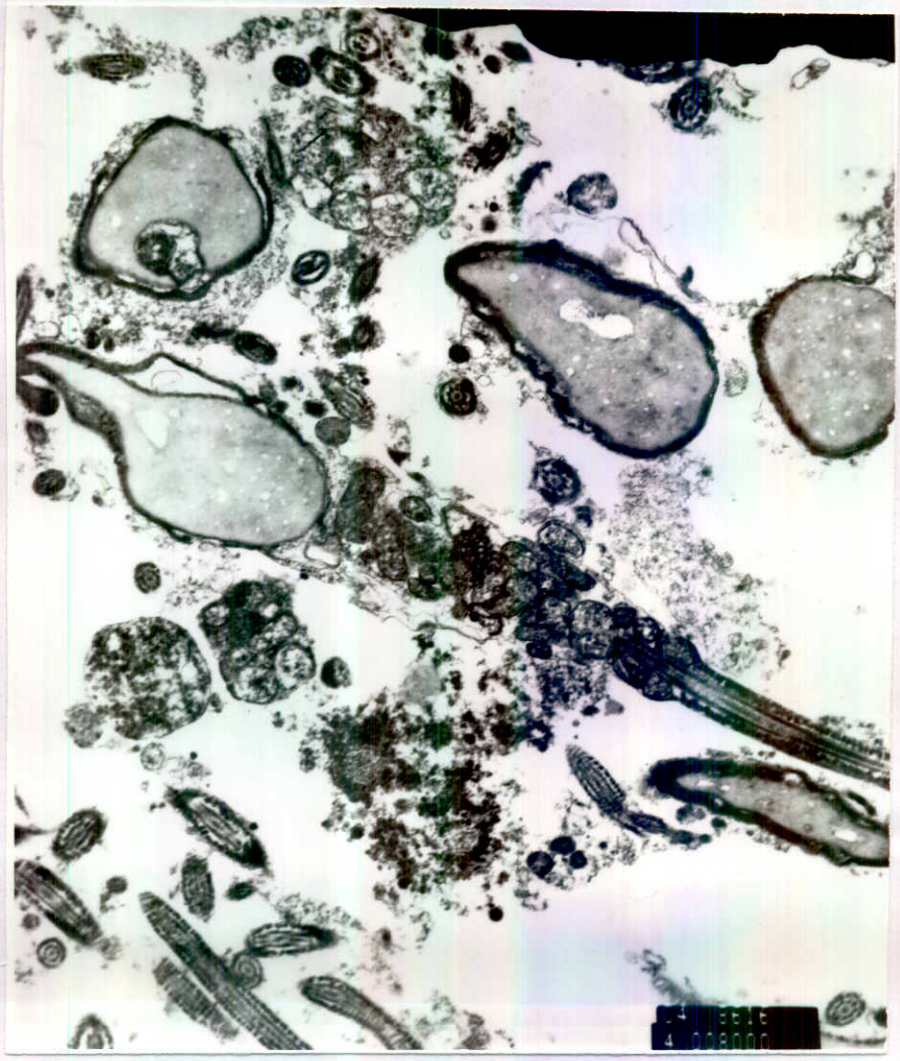


Fig. (21)
Electron microscopic picture shows one complete spermatozoon, 4 free heads and several sections of sperm tails. The spermatozoa have abnormal shape. Generalized acrosomal separation from the nuclei. Presence of a lot of vesicles or big nuclear vacules with membranous content are seen. In the complete spermatozoon, the neck and mid piece are disorganized, however mitochondria show their normal ultrastructure. Annulus is present and the structure of the principal piece is within normal.

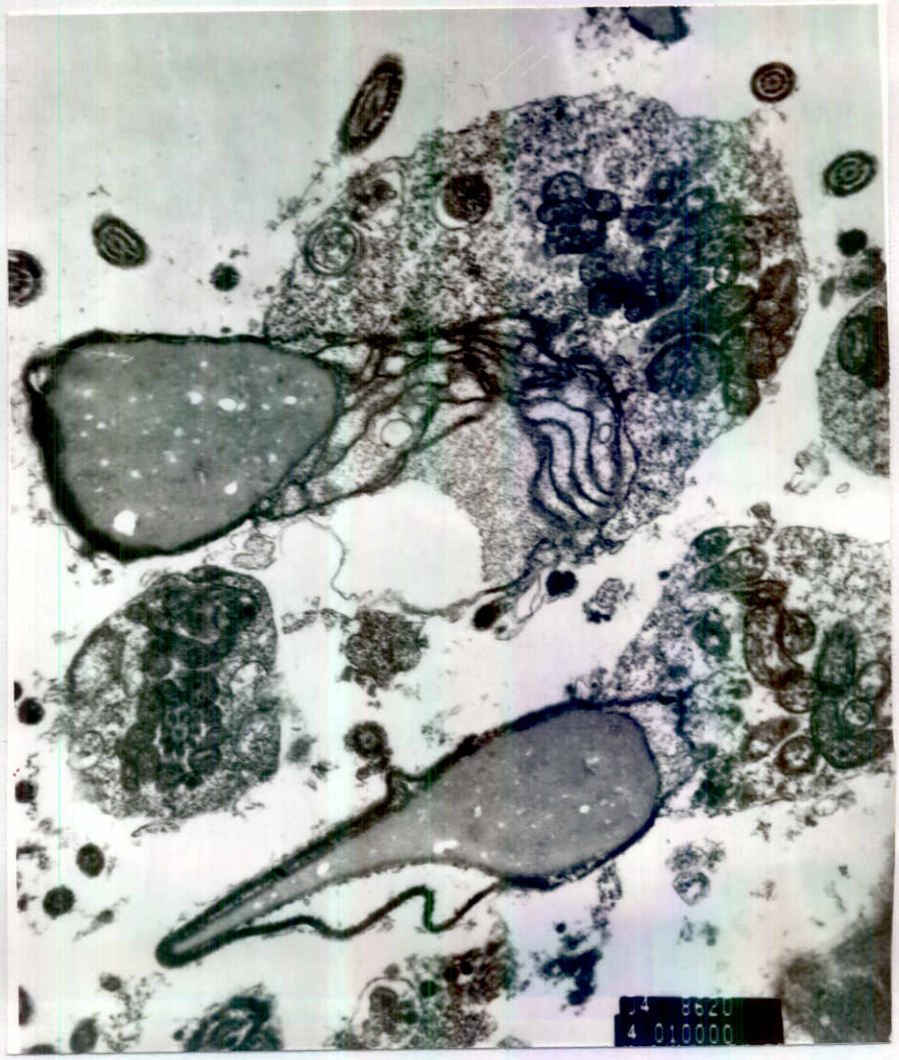


Fig. (22)

Electron microscopic picture shows two late spermatids with heads and cytoplasmic droplets. The two spermatids show abnormal heads shape and separation of acrosome from the nuclear membrane. Also the nucleus in each one show multiple vesicles. One spermatid shows multiple layers of redundant nuclear membrane in the cytoplasmic droplet. Mitochondria are of normal structure but are disarranged.

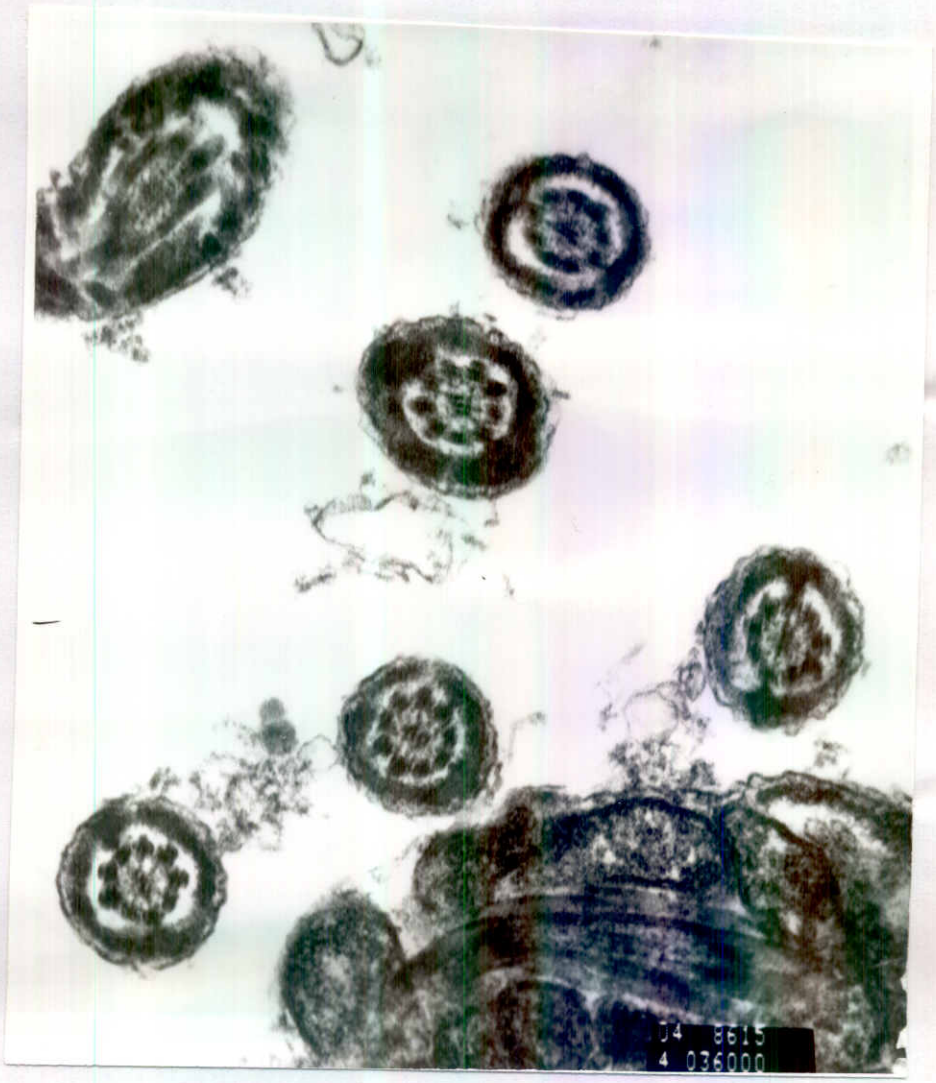


Fig. (23)
Electron microscopic picture of T.S. of sperms at the level of principal piece that show defective number of outer dense fibres. The longitudinal columns are not normally situated at position 3 and 8. The 9+2 pattern of the axonemes. look normal.

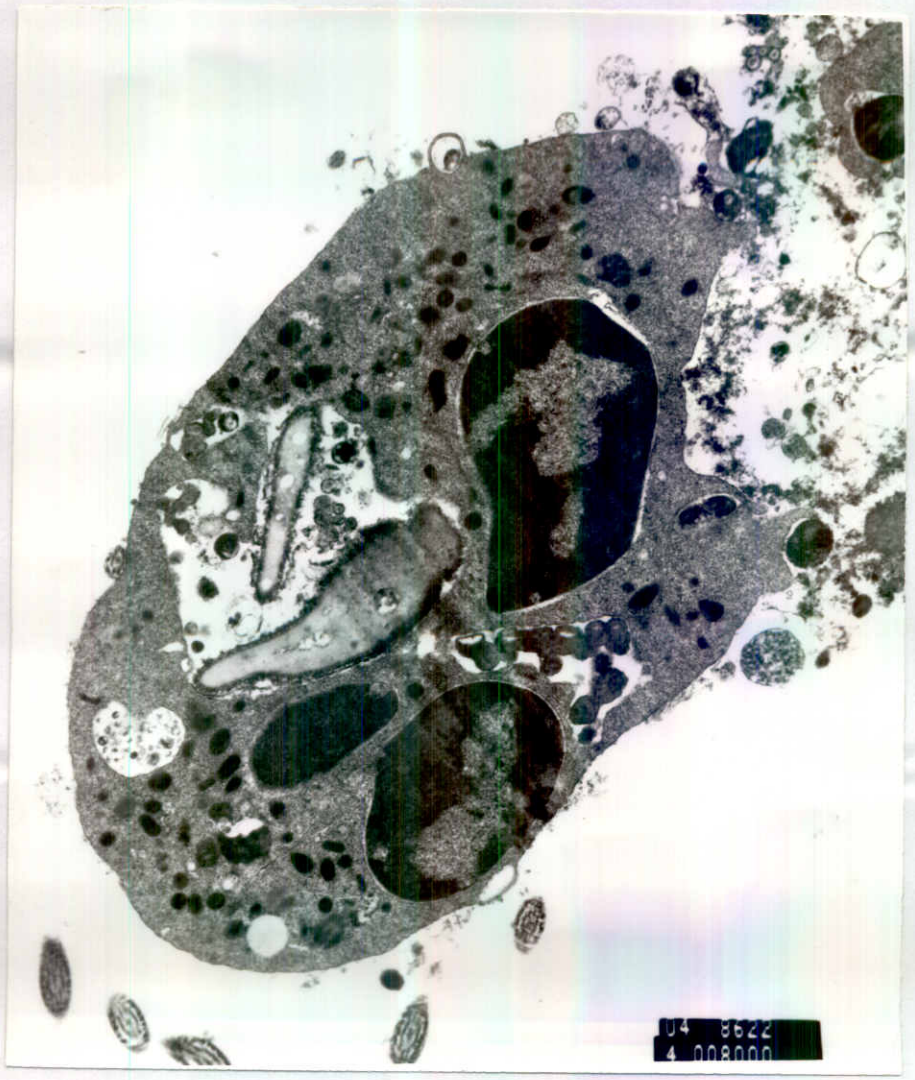


Fig. (24)
Electron microscopic picture of a macrophage ingulfing parts of spermatozoa i.e. spermatophage.

et al., 1984).

The hypo-osmotic swelling test (HOS-test) introduced by Jeyendran et al., (1984), seems to be a good indicator for the fertilizing ability of spermatozoa and also of prognostic value to predict the survival of sperm after cryopreservation.

For evaluation of the hypo-osmotic swelling test in relation to zona-free hamster ovum penetration test, a good correlation ($r = 0.9$, $P < 0.001$) existed between the percentage of spermatozoa that showed swelling under hypo-osmotic conditions in a semen specimen and the ability of spermatozoa from that sample to undergo capacitation and penetration of denuded hamster oocytes (Jeyendran et al., 1984). In a series of large number (270) of semen samples Chan et al., (1985), observed that their results could not demonstrate any significant correlation between the ability of sperm to swell in the hypo-osmotic condition and the ability of sperm to penetrate zona-free hamster ova in vitro. Chan et al., (1987) and Chan et al., (1988) supported this view.

For these controversies in the correlation between the hypo-osmotic swelling test and the zona-free hamster ova penetration test, and since Gould et al., (1983) concluded that denuded hamster egg test does not always reflect the ability or inability of spermatozoa to fertilize intact oocytes. Yet an alternative, simpler, less time-consuming and less expensive

procedure for studing the patient fertilizing capacity of spermatozoa is the assay of enzymes involved in the process of fertilization and one of the most important of these enzymes is acrosin. Many studies that were performed on the role of this enzyme in the process of fertilization suggested that acrosin enables sperm penetration through the zona pellucida of the oocyte (Zaneveld et al., 1972; Gould, 1973; Urch et al., 1985a; Urch et al., 1985b and Srivastava et al., 1986).

For all these reasons, the assay of acrosin activity was chosen as a substitute to zona-free hamster ova penetration test in evaluation of the hypo-osmotic swelling test introduced by Jeyendran et al., (1984).

The normal value of acrosin activity of the spermatozoa in fertile semen samples is still a matter of controversy in the available literature. Also, there was a real difference in the value of this enzyme activity depending on the method used in its determination. There are three major methods for determination of acrosin activity, namely spectrophotometric enzyme assay, radioimmunoassay and flurometeric enzyme method.

Spectrophotometric determination of acrosin activity was performed by many authors. Some of these reports are the studies of Schill (1974), Tobias and Schumacher (1977), Polakoski et al., (1977); Goodpasture et al., (1982) and Schill and Feifel (1984).

The first group of the present study included 10 fertile men as a control group. A correlation test was done between the result of swelling test in one hand and acrosin activity as well as other semen parameters in the other hand. It was found that there is a positive significant correlation between the swelling test and acrosin activity / 10^6 sperm ($r = 0.82$ & $P < 0.05$). In this group the mean percentage of swollen sperms in the fertile men (70.1 $\pm$ 1.63 SE) and this runs with the results of Jeyendran et al., (1984) who demonstrated that only ejaculates with more than 60% swollen spermatozoa can penetrate denuded hamster oocyte by a rate more than 55% and also with Van Der Van et al., (1985) who considered that 60% swelling is the cutoff point for the bottom of the normal range of semen samples in the hypo-osmotic swelling test.

In this study the mean acrosin activity of the spermatozoa of the 10 fertile control ejaculates studied was 0.90 $\pm$ 0.01 mU/10^6 spermatozoa. This runs with the results obtained by Schill (1974) and Tobias & Schumacher (1977).

Polakoski et al., (1977) and Goodpasture et al., (1982) studied the acrosin activity of the spermatozoa in ejaculates obtained from men who were semen donors for artificial insemination. Polakoski et al., (1977) reported that the mean acrosin activity of these spermatozoa was 6.5 $\pm$ 1.5 mU/10^6 spermatozoa. But the reading of Goodpasture et al., (1982) was 10.6$\pm$ 1.5 mU/10^6 spermatozoa. There are two factors explaining

the higher figures of these two reports than the results obtained during this work. These are: (1) These two groups of investigators obtained their ejaculates from men who were known semen donors for artificial insemination and so their spermatozoa were suspected to have an excellent fertilizing capacity while ejaculates of the present study were obtained from randomly collected fertile volunteers with different fertilizing capacity of their spermatozoa, (2) in both of these 2 reports the investigators added to the assay cuvettes 50 mU calcium chloride and $CaCL_2$ has been known to increase proacrosin autoactivation in the sperm acid extracts and it also increases the activity of acrosin itself as will be discussed later.

Schill and Feifel in their reports in (1984) stated that the acrosin activity of their normozoospermic ejaculates was between 0.29 - 13.7 $mU/10^6$ spermatozoa with a mean of 5.14 $mU/10^6$ spermatozoa. These authors did not depend only on acetic acid extraction of acrosin (as has been done in this thesis) but they also treated their spermatozoa by different physicochemical methods before acid extraction to achieve complete extraction and activation of acrosin and this may be rsponsible for their somewhat higher values of acrosin activity in comparison to that of the present work.

A good positive significant correlation ($r = 85$, $P < 0.01$) existed between the percentage of spermatozoa that showed swelling under hypo-osmotic conditions in this fertile control

group and the percentage of active motile spermatozoa and this runs parallel with the results of Jeyendran et al., (1984) who found a significant high correlation between the percentage of motile and swollen spermatozoa ($r = 0.61$, $P < 0.01$). They explained that the spermatozoa to be motile they probably require a biochemically intact plasma membrane. Lack of sperm motility in a semen sample, however, may be due to a number of other factors beside a lack of membrane integrity. Some of these factors can be reversed while spermatozoa are in the act of entering and migrating through the female genital tract or under in vitro incubation conditions. This may be the reason that samples with a large number of abnormally motile spermatozoa can fertilize oocytes both in vivo and in vitro.

A negative non significant correlation was found between the percentage of swollen sperm and percentage of abnormal forms in this study ($r = -0.47$, $P > 0.05$) in the fertile control group. This means that abnormal forms do not necessarily have an abnormal membrane integrity but some have an intact membrane that permits swelling in the hypo-osmotic swelling solution. As was shown in the study of Van Der Van et al., (1986) the hypo-osmotic swelling test only correlated with sperm motility.

In evaluating the results of group II which included 14 infertile men with oligo-asthenozoospermia in comparison to that of the normal fertile group we found that the percentage of swollen spermatozoa in the fertile control group was

significantly higher than that of the infertile group ($t = 13$, $P < 0.001$), and there is a positive highly significant correlation between the swelling test and the percentage of active motile spermatozoa in this infertile group ($r = 0.90$, $P < 0.01$) as was discussed by Jeyendrant et al., (1984).

Jeyendrant et al., (1984) found that the correlation between the percentage of swollen spermatozoa and motile spermatozoa was greater at a lower percentage of sperm swelling (< 50%) than at a higher percentage of swelling (> 50%) and this was shown in the present study since the result of swelling test in this infertile oligo-asthenozoospermia group ranged from 2-43% with a mean of 22.07% ($\pm$ 3.32 SE). The explanation of this phenomenon as was discussed by Jeyendrant et al., (1984) was that sperm swelling is only indicative of normal membrane integrity and function, whereas sperm motility depends not only on membrane transport but also on a large number of other biochemical functions such as sperm metabolism and the microtubular action of the tail fibres. This may explain at least in part, why the correlation between sperm swelling and motility was greater when the percentage of sperm swelling was < 50%. At low percentage swelling, sperm motility certainly be impaired but at a high percentage swelling, sperm motility may or may not be normal.

As discussed in the fertile control group, also in this infertile group a negative non significant correlation existed between the swelling test and the percentage of abnormal forms

($r = -0.06$, $P > 0.05$).

Regarding the acrosin activity of this oligo-asthenozoospermia group, it ranged from 0.59-2.23 mU/10^6 spermatozoa with a mean of 1.45 ($\pm$ 0.12 SE). There was statistically highly significant increase ($t = 4.65$, $P < 0.001$) in the acrosin activity of these oligo-asthenozoospermic ejaculates compared to that of normal fertile group.

There are two contrasting reports in the available literature, regarding the acrosin activity of oligozoospermic ejaculates. Schill (1974) by spectrophotometric enzyme assay of acrosin activity found a statistically highly significant increase in the mean acrosin activity ($P < 0.0005$) between ejaculates with oligozoospermia and those with normozoospermia. Furthermore he found statistically significant increase of acrosin activity with decrease in the count of the spermatozoa within this group of oligozoospermic ejaculates. In 1982 Mohsenian et al., by a more accurate radioimmunoassay of acrosin activity, attained more comperhensive results regarding the acrosin activity of the oligozoospermic ejaculates. These authors found that the mean acrosin activity of the oligozoospermic ejaculates was statistically significantly lower ($P < 0.01$) than that of normal fertile ejaculates. But they attributed these results to the higher proportion of morphologically abnormal sperm especially roundheaded sperm in these oligozoospermic ejaculates.

In this study, no significant correlation existed between the swelling test and acrosin activity in this infertile oligo-asthenozoospermia group ($r = 0.01$, $P > 0.05$). Also there was no significant correlation between acrosin activity in one hand and percentage motility and abnormal forms in the other hand.

With exception of Schill (1974), there was no reports in the available literature discussing the relation between spermatozoal motility and their acrosin activity. This author found that there was no statistical correlation between acrosin activity and spermatozoal motility in the asthenozoospermic ejaculates.

Also Schill (1974) studied the relation between spermatozoal morphology and their acrosin activity. He stated that with exception of round-headed spermatozoa there was no statistically significant difference between the mean acrosin activity in teratozoospermic ejaculates and those with normozoospermia. Regarding ejaculates with 100% round-headed spermatozoa no acrosin activity could be determined by Schill (1974).

In discussing the results of group III, it included 13 patients with normozoospermia spermogram i.e. the counts, percentage motility and abnormal forms are within the normal ranges, the only abnormality detected was that obivious agglutination of some of their spermatozoa with head to head and tail to tail agglutination. The mean percentage swollen

spermatozoa was highly significantly lower than that of the normal fertile control group ($t = 5.52$, $P < 0.001$). This is because the mean percentage motile spermatozoa is significantly higher in the normal fertile group than that of normozoopspermia group with sperm agglutination. A positive significant correlation existed between the percentage of swollen spermatozoa and percentage of motile spermatozoa in this normozoospermia group with sperm agglutination ($r = 0.68$, $P < 0.05$) and also a negative non significant correlation ($r - 0.23$, $P > 0.05$) was found between the percentage swollen spermatozoa and the abnormal forms, as was shown in the previous two groups and as was discussed by Jeyendran et al., (1984).

Regarding the acrosin activity in this infertile group with normozoospermia there is a highly significant decrease of acrosin activity in the infertile group than those of the fertile control group ($t = 5.60$, $P < 0.001$). The results of this work go in line with those of Mohsenian et al., (1982). These authors by two different methods of measuring acrosin activity, radioimmunoassay and flurometric enzyme method, found that there was statistically significant decrease of acrosin activity ($P < 0.01$) of ejaculates obtained from patients with unexplained infertility in comparison to those obtained from fertile men. Considering their results and the result of the present study low acrosin activity may be a cause of infertility in patients presented with normal spermogram and in turn patients with unexplained infertility.

There are no reports in the available literature discussing the relation between swelling test and acrosin activity. In this study a positive significant correlation ($r = 0.77$, $P < 0.05$) was found between the percentage swollen sperms and the acrosin activity of this infertile normospermic ejaculates. This also was found in the normal fertile control group (group I). Also a positive highly significant correlation was found between acrosin activity ($r = 0.9$, $P < 0.01$) and percentage motility of this infertile normozoospermic group as was found in the fertile control group.

In discussing the results of group IV, thirteen ejaculates with asthenozoospermia were present in this study. The sperm count and spermatozoal morphology in these ejaculates were within normal limits. Regarding the percentage swollen sperm, there is a highly significant decrease ($t = 13.78$, $P < 0.001$) in the infertile group with asthenozoospermia than the fertile control group. Also a positive significant correlation was found between the percentage of motile sperm and the percentage of swollen sperm ($r = 0.82$, $P < 0.01$) while a negative non significant correlation existed between the percentage of swollen sperm and abnormal forms as shown in the previous three groups and was discussed by Jeyendran et al., (1984).

Regarding the acrosin activity of this infertile group, a low significant decrease was found ($t = 2.12$, $P < 0.05$) in this group in comparison to the fertile control group. Schill (1974)

found that there was no statistically significant difference between the mean acrosin activity in ejaculates with asthenozoospermia and ejaculates with normozoospermia. Also no significant correlation was found in this infertile group with asthenozoospermia ($P > 0.05$) in one hand and swelling test, percentage motile sperm or abnormal forms in the other hand.

During this study 17 patients with varicocele with different grads (diagnosed on clinical bases only using diagnostic criteria of Steeno et al., 1976), were included. Nine ejaculates out of these 17 presented by stress or OTA (oligo-terato-asthenozoospermia) pattern spermograms. In this group there is a statistically highly significant decrease in the percentage of swollen sperm ($t = 12.29$, $P < 0.001$) in comparison to the fertile control group. Also there was a positive significant correlation between the percentage of swollen sperm and percentage of motile sperm in this infertile group ($r = 0.92$, $P < 0.05$) while there was negative non significant correlation between swollen sperm and abnormal forms ($r = -0.19$, $P > 0.05$). This also was shown in the previous groups and discussed by Jeyendran et al., (1984).

The acrosin activity of this infertile group with stress pattern spermogram was significantly high ($t = 3.05$, $P < 0.01$) in comparison to that of normal fertile group. Also there was no significant correlation between acrosin activity in one hand and swelling test, count of sperm, percentage of motile sperm or

percentage abnormal forms ($P > 0.05$ in all).

In the available literature there was no report discussing the acrosin activity in ejaculates with stress pattern spermograms.

In the available literature there are few reports discussing the effect of calcium on acrosin activity.

In 1975 Meizel and Mukerji in their study of acrosin/proacrosin system in hamster illustrated that proacrosin autoactivation and acrosin activity were stimulated by Ca^{+2}.

Schill et al., (1982) found that pretreatment of human spermatozoa with 0.5 mol/L $CaCl_2$ increased the acrosin activity by 35% when compared by acid extraction only. These authors concluded that Ca^{+2} increased acetic acid extraction of human sperm acrosin.

Later on Mukerji (1984) measured the increase in rabbit acrosin activity after addition of different molar concentrations of $CaCL_2$ to a purified acrosin solution. He found that acrosin was activated about 45% by 10 mM $CaCL_2$. Higher concentrations of Ca^{+2} had some inhibitory effect. So $CaCL_2$ at that molar concentration gave the highest increase of acrosin activity of purified enzyme solution.

Considering the results of these 3 reports one can conclude that $CaCL_2$ could increase the acrosin activity by 3 different mechanisms:

1- It can increase the extraction of acrosin from the spermatozoa,

2- It can increase proacrosin autoactivation to acrosin and since more than 90% of acrosin in ejaculated human spermatozoa is present in the form of proacrosin (Polakoski et al., 1977), this mechanism appeared to be the most important one by which Ca^{+2} increases the acrosin activity,

3- It can increase the activity of acrosin itself.

During this study the molar concentration of $CaCL_2$ which gave the highest increase of rabbit acrosin activity in the work of Mukerji (1984) was used. This molar concentration (10 mM) was added to the sperm acid extracts of the spermatozoa obtained from 9 infertile ejaculates. Statistically highly significant increase in the acrosin activity of this infertile group after addition of $CaCL_2$ ($Z = 2.66$, $P < 0.01$).

In discussing the results obtained by the electron microscope, it becomes clear that motility of the spermatozoa does not only depend on its membrane integrity but many defects that were shown in the mitochondrial sheath of the midpiece and the microtubular structures of the principal piece may be responsible for this defective motility. Also this three samples showed defect in the acrosome in many of their

spermatozoa and this may explain the decrease in their fertilizing capacity.

So the positive significant correlation between percentage of swollen sperm and percentage of motility may be disrupted especially if the swelling result is high than 50% i.e. swelling test may be normally above 50% while motility is impaired by many other defects especially in the mitochondrial sheath and microtubutes. But this disruption in the correlation between swelling test and motility is absent if swelling test is below 50%. In this case the motility is impaired and must be below 50% either due to defective membrane integrity or any other abnormality in the microstructure.

So swelling test is of value in detecting infertility & this value is diminished in detecting that the person is fertile.

SUMMARY AND CONCLUSIONS

In the last few years, extensive investigations have been conducted on the value of sperm hypo-osmotic swelling test as a measure of the fertilizing capacity of the spermatozoon.

Evaluation of the hypo-osmotic swelling test was done by many investigators in comparison to zona-free hamster ovum penetration test. Some of them found a good correlation between these two tests but others could not demonstrate any significant correlation between the ability of sperm to swell in the hypo-osmotic condition and the ability of sperm to penetrate zona-free hamster ova in vitro.

In this study acrosin activity assay of the spermatozoa was chosen as a substitute to zona-free hamster ova penetration test in evaluation of the hypo-osmotic swelling test. Why did we choose the acrosin activity?. This is for many reasons:-

1- Many studies that were performed on the role of this enzyme in the process of fertilization suggested that acrosin is the enzyme responsible for penetration of the sperm through the zona pellucida of the oocyte.

2- The denuded hamster egg test does not always reflect the ability or inability of spermatozoa to fertiliza intact human oocyte.

3- The assay of acrosin activity is a simpler less time consuming and less expensive procedure for studying the potential fertilization capacity of spermatozoa. On the other hand zona-free hamster ova penetration test is more expensive time consuming and requires a great experience.

This study has been carried out on 61 infertile patients and 10 normal fertile men. They were subjected to the following:

1- Selected case history taking.

2- Thorough clinical examination.

3- Routine semen analysis.

4- The hypo-osmotic swelling test.

5- Spectrophotometrical determination of acrosin activity in their spermatozoal acid extracts.

In addition sperm acid extracts from 9 infertile patients were reassayed for acrosin activity after adding to the assay cuvette 0.05 ml 10 mM CaCL2 to show the effect of CaCL2 on convertion of proacrosin to active acrosin.

Also, three cases out of these infertile men were studied by electron microscopy; they showed decreased percentage motile sperm than normal inspite of high percentage of swollen sperm i.e. more than 50%.

The results obtained were tabulated and discussed and they

can be summarised in the following observations:

(1) The sperm swelling test is not a replacement for semen analysis. In fact the two tests are complementary as the swelling test is a new parameter of the fertility status which can not be measured by semen analysis.

(2) The sperm swelling test is an economic and easy one. It can readily be performed in any clinical setting.

(3) There is a positive significant correlation between the swelling test and acrosin activity in the fertile group and also in the infertile group with normozoospermia but this significant correlation is absent in the other infertile groups.

(4) The result of swelling test is significantly higher in the fertile control group than the infertile groups, while there is a controversy in the result of acrosin activity. It is higher in the fertile group than the infertile group with normozoospermia and asthenozoospermia but not in the other infertile groups.

(5) Although the assay of acrosin activity is simpler, less time consuming and less expensive than the hamster ova penetration test, both are still showing a controversy in their relation with the hypo-osmotic swelling test. This indicates that all of them are complementary to the standard semen

analysis and each of them measures different entities. The hypo-osmotic swelling test measures the functional integrity of the membrane of human sperm, while the acrosin activity assay measures the capacity of the sperm to penetrate the zona pellucida of human ovum and the hamster ovum penetration test measures the capacity of the sperm to penetrate zona-free ovum.

(6) There is a significant positive correlation between the swelling test result and the percentage of active motile sperm in all groups studied. This means that the spermatozoon to be motile it probably requires a biochemically intact plasma membrane.

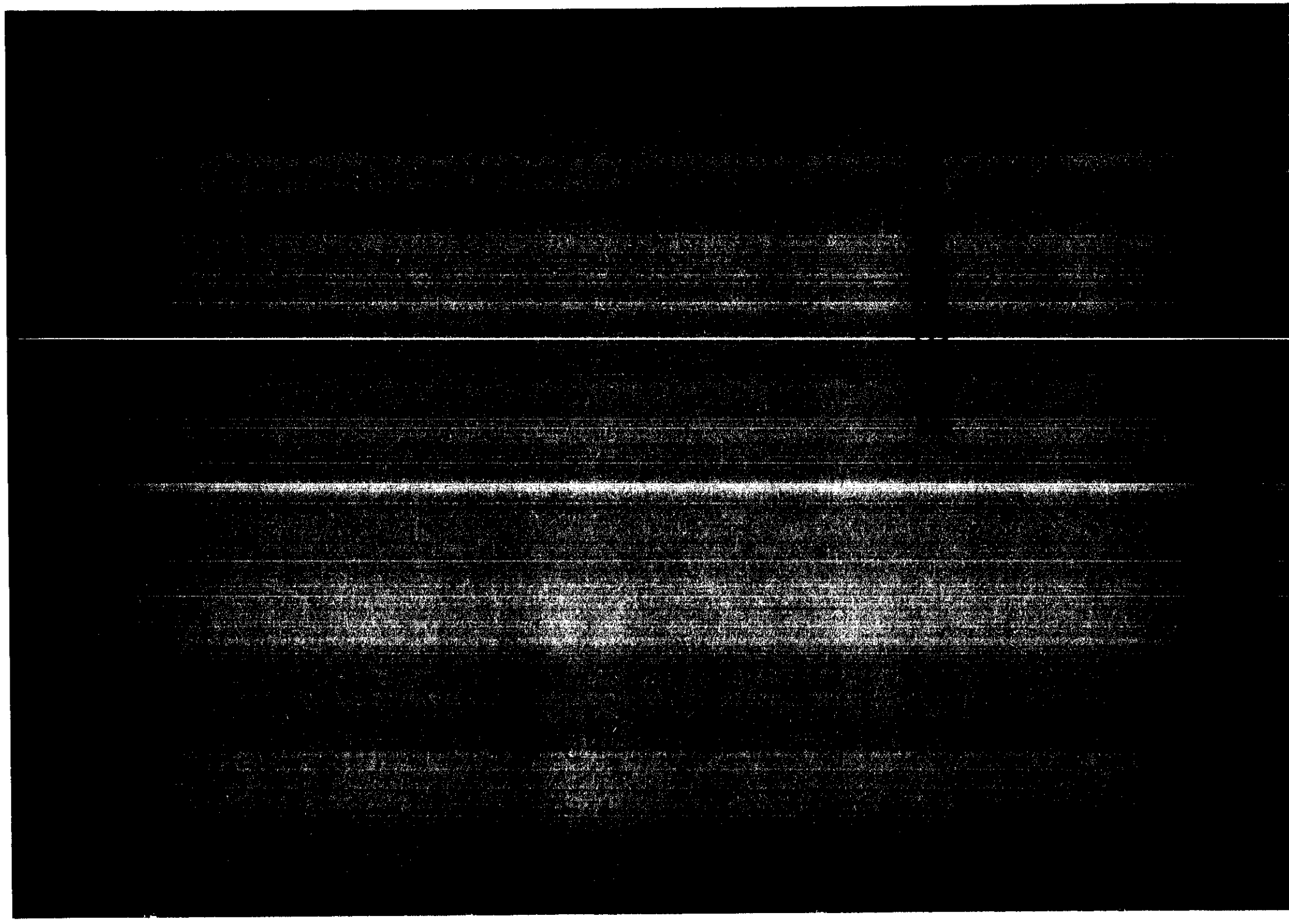
(7) The electron microscopic study indicates that motility of the spermatozoon does not only depend on its membrane integrity but many other defects may be responsible for the decreased motility.

(8) The correlation between sperm swelling and motility was greater when the percentage of sperm swelling was < 50%. At low percentage swelling, sperm motility would certainly be impaired but at a high percentage swelling, sperm motility may be good or impaired by other defects as was shown by electron microscope. So swelling test can diagnose infertility but can not ensure that the man is fertile.

(9) A negative but non significant correlation was found

between the percentage of swollen sperm and percentage of abnormal forms in this study. This means that abnormal forms do not necessarily have an abnormal membrane integrity but some have an intact membrane that permits swelling in the hypo-osmotic swelling solution.

(10) When CaCL2 in a molar concentration was added to sperm acid extracts of 9 infertile ejaculates, there was statistically significant increase in acrosin activity. This means that most of acrosin is present in the inactive proacrosin form.



REFERENCES

Ahlgern, M. (1975):
Sperm transport and survival in the human fallopian tube.
Gynecol. Obstet. Invest., 6: 206.

Aitken, R.J.; Best, F.S.; Richardson, D.W; Djahanbakhch, D. and Less, M.M. (1982):
The correlates of the fertilizing capacity in normal fertile men.
Fertil. Steril., 38: 68.

Alexander, N.J. (1981):
Evaluation of male infertility with an invitro cervical mucus penetration test.
Fertil. Steril. 36: 201.

Allison, A.C. and Hartree, E.F. (1970):
Lysosomal enzymes in the acrosome and their possible role in fertilization.
J. Reprod. Fertil. 21: 501.

Amelar, R.D.; Dubin, L. and Schoenfeld, C. (1980):
Sperm motility.
Fertil. Steril. 34: 197.

Ansbacher, R. (1973):
Vasectomy: sperm antibodies.
Fertil. Steril. 24: 788.

Austin, C.R. (1951):
Observations on the penetration of the sperm into the mammalian egg.
Aust. J. Sci. Res., 4: 481.

Baccetti, B. and Afzelius, B.A. (1976):
The biology of the sperm cell, Basal, Skarger P. 93.
Quoted by Amelar, R.D.; Dubin, L. and Schoenfeld (1980):
Sperm motility.
Fertil. Steril. 34: 197.

Bane, G. & Nicander H. (1966):
Lysosomal enzymes in the acrosome.
J. Reprod. Fertil. 12: 270.
Cited by: Allison A.G. & Hartree E.F. (1970).

Barros, C.; Bedford, J.M.; Eranklin, L.E. & Austin, C.R. (1967):
Membrane vesiculation as a feature of the mammalian acrosome reaction.
J. Cell Biol. 34: 313.

Bhattacharrya A.K. and Zaneveld, L. J.D. (1978):
Release of acrosin and acrosin inhibitor from human spermatozoa.
Fertile. Steril. 30: 70.

Binor, Z.; Sokoloski, J.E. and Wolf, D.P. (1980):
Penetration of zona-free hamster eggs by human sperm.
Fertil. Steril. 33: 321.

Blasco, L. (1984):
Clinical tests of sperm fertilizing ability.
Fertil. Steril., 41: 177.

Botella-Liusia, J. and Ruiz Velasco, V. (1960):
Postcoital test in vitro.
Int. J. Fertil. 5:301.

Bradford, M.M.; Dudkiewicz, A.B.; Penny, G.S.; Dyckes, D.F.; Burleigh, B.D.; Wooley, R.E. & Me Rore, R.A. (1981):
Localization of proacrosin on the inner acrosomal membrane of spermatozoa in rabbits and hamsters.
Am. J. Vet. Res. 42: 1082.

Bril-Peterson, E. and Westenbrinik, G.G.K. (1963):
A structural basic protein as a counterpart of deoxyribonucleic acid in mammalian spermatozoa.
Biochim. Biophys. Acta 70: 152.

Brown, C.R. & Hartree, E.F. (1974):
Distribution of a trypsin-like proteinase in the ram spermatozoa.
J. Reprod. Fertil. 36: 195.

Chan, S.Y.W.; Tang, L.C.H. and Ma, H.K. (1983):
Stimulation of the zona-free hamster ova penetration efficiency by human spermatozoa after 17B-estradiol treatment.
Fertil. Steril. 39: 80.

Chan, S.Y.W.; Fox, E.J.; Chan, M.M.; Tsoi, W., Wang, C.; Tang, L.C.; Tong, G.W. and Ho, P. (1985):
The relationship between the human sperm hypo-osmotic swelling test, routine semen analysis and the human sperm zona-free hamster ovum penetration assay.
Fertil. Steril. 44: 668.

Chan, S.Y.W.; Li, S.Q. and Wang, C. (1987):
Test-eggyolk buffer storage increases the capacity of human sperm to penetrate hamster eggs in vitro.
Int. J. Androl., 10: 507.

Chan, S.Y.W., Wang, C.; Ng, M.; So, W.W.K. and Ho, P.C. (1988):
Multivariate discriminat analysis of the relationship between the hypo-osmotic swelling test and the in-vitro fertilizing capacity of human sperm.
Int. J. Androl., 11: 369.

Chang, M.C. (1951):
Fertilizing capacity of spermatozoa deposited in the fallopian tube.
Nature, 168: 697.

Chang, M.C. (1957):
A detrimental effect of rabbit seminal plasma on the fertilizing capacity of sperm.
Nature, 179: 258.

Chang, M.C. (1969):
Hormone regulation of sperm capacitation. In Advances In Bioscience. Raspe, G. (ed). Pergaman Press., New York, P. 36.

Chen, C. and Jones, W.R. (1981):
Application of a sperm micro-immobilization test to cervical mucus in the investigation of immunologic infertility.
Fertil. Steril. 39: 542.

Clermont, Y. (1972):
Kinetic of spermatogenesis in mammals seminiferous epithelium cycle and spermatogonial renewal.
Physiol. Rev., 52: 198.

Coelingh, J.P., Rozijn, T.H. & Monfoort, C.H. (1969):
Isolation and partial characterization of a basic protein from bovine sperm heads.
Biochim. Biophys. Acta, 188: 353.

Cohen, J.; Fetten, P. & Zeilmaker, G.H. (1981):
In vitro fertilizing capacity of fresh and cryopreserved human spermatozoa: a comparative study of freezing and thawing procedure.
Fertil. Steril. 36: 356.

Comhaire, F.H. (1989):
Evaluation of male infertility. In: Perspectives in Andrology, Mario Serio (ed.), serona symposia pub. From Raven Press, New York, Vol. 53 P. 45.

Corchan, W.C. and Cox, G.H. (1969):
"Expermintal Designs" 2nd ed., Comstock, Publishing Association Ithaco, New York, P. 85.

Dahelberg, B. (1976):
A symptomatic bacteriospermia cause of infertility in men.
Urology 8: 563.

David, M.A.; Fraschini, F. and Martini, L. (1966):
Control of L.H. secretion: role of a "short" feed-back mechanism.
Endocrinol., 78: 55.

David, M.P.; Amit, A.; Bergman, A.; Yedwab, G.; Pas, G.F. and Homonnai, Z.T. (1979):
Sperm penetration in vitro: correlation between parameters of sperm quality and the penetration capacity.
Fertil. Steril. 32: 676.

De Jonge, C.J.; Rowlins, R.G. and Zaneveld, L.J. (1988):
Induction of the human sperm acrosome reaction by human oocytes.
Fertil. Steril., 50: 949.

Dravland, J.E.; Llanos, M.N.; Munn, R.J. & Meizel, S. (1984):
Evidence for involvement of a sperm trypsin-like enzyme in the membrane events of the hamster sperm acrosome reaction.
J. Exp. Zool. 232: 117.

Dudkiewicz, A.B. ((1983):
Inhibition of fertilization in the rabbit by antiacrosome antibodies.
Gam. Res. 8: 183.

Dukelow, W.R. (1971):
Bioassay techniques related to sperm capacitation.
Acta Endocrinol. 66: 503.

Dukelow, W.R. and Williams, W.L. (1988):
Capacitation of sperm. In: Progress In Infertility. Behrman, S.J.; Kistner, R.W. and Patton, G.W. (eds.). Little, Brown Co., Boston, P. 673.

Edwards, R.G.; Bavister, B.D. and Steptoe, P.C. (1969):
Early stages of fertilization in vitro of human oocytes matured in vitro.
Nature, 221: 632.

Elce, J.S.; Graham, E.J.; Zborii, G.; Leyton, L.; Perez, E.; Crozatto, H.G. & De Ioanes, A. (1986):
Monoclonal antibodies to bovine and human acrosin
Biochem. Cell Biol. 64: 1242.