# RESULTS

## Results

#### **Control Group:**

The head of normal sperm shows a rounded contour in the post-acrosomal region and gradually flattened towards the tip. The karyoplasm is condensed and there are light areas, called the vacuole that contain only finely granular material of low density. The acrosome in the form of cap cover 1/2 to1/3 of the nucleus.

The middle piece about 5  $\mu$ m, contains the mitochondrial sheath in the form of 10-12 mitochondrial windings around the axoneme. The axoneme that originate within mitochondrial Sheath and extends the length of sperm tail formed of outer nine doublets microtubules that surround a single pair of microtubules and ensheated by fibrous Sheath, fig.(1).

#### **Patients group**

#### Light microscopic study

Semen samples were seen at level of light microscope showed various anomalies in the majority of cases. However, speciemens were considered abnormal only if more than 30% of spermatozoon presented with anomalies of head and tail or both.

According to vital staining we divided the patients into two groups. Group -A were the viable cells are more predominant than dead cells, and group -B were the dead cells are more predominant than viable cells table (5).

#### Transmission electron microscopic study.

Transmission electron microscopic (TEM) examination of these abnormal spermatozoa revealed much greater details of the structural defects.

### Group (A) (Viable spermatozoa)

The commonest head anomalies in this group are deformed head in four cases (80%), cephalic cytoplasm in one case (20%) and myelin figure like membrane in two cases (40%), together with separated acrosome in two case (40%), hypoplasia in two cases (40%) and vaculated acrosome in three cases (60%), all these anomalies are listed in table (6), fig. (3,4,5,6,7).

#### Group (B) (Dead spermatozoa)

The commonest head anomalies in this group included deformed head in three cases (60%), Myelin figure like membrane in three cases (60%), cephalic cytoplasm in one case (20%), separated head in three cases (60%), together with thick acrosome in two cases (40%), hypoplasia and degenerated acrosome in four cases (80%), all these anomalies are listed in table (7), fig. (13, 14, 15, 16, 17, 18, 19).

Sperm tail cross section taken from the ten infertile patients with immotile spermatozoa revealed a variety of fine structural abnormalities listed in table (8,9).

#### These are:

Degenerated tail, fig. (20, 22, 23) as evidenced by fragmentation of plasma membrane and necrosis of the microtubules, were consider to be indicative of necrospermia.

This was the main factor in group -B dead spermatozoa which had been seen in all cases (100%) table (9), and was the least common (partial

degeneration) in group -A viable spermatozoa which represented only (40%) of all defects was observed. Confuse arrangement of microtubules which was the major feature in both groups, four cases in group -A (80%), three cases in group -B (60%), table (8,9), fig. (9,12,21,22).

On the other hand missing of dynein arm was seen in one case in group -A, table (8), fig. (11).

Missing of central microtubules in the form of (9+0) was seen in another one patient in group -A, table (8), fig. (10). And missing of peripheral microtubules in the form of (6+2), (5+2) was seen in one patient in group -A, table (8), fig. (12).

Midpiece abnormalities in the form of disorganization, degeneration partial missing of mitochondrial sheath and empty mitochondria were seen more in group - B, table (9), fig. (24).

Hypertrophied, degenerated and malformed fibrous sheath were the commonest features in both group (A&B), table (8&9), fig. (10, 21, 20).

Nine patients have been classified as having pattern of multiple abnormalities when single defect only was seen in one case in group -A table(8).

Table (5): Results of Semen Analysis (Acording to (Hargreave and Nilsson, 1983 and Glover et al., 1990)

Cas	Volum Sperm/c (ml) 10 <sup>6</sup> ml		Total motility	Abnor. form	Liq. Time/	Visc.	Pus cell H.P.F.	Vital s	taine %
		10 1111	%	%	min			Viable	Dead
1	2.2	23.2	0	35	15 />	+	1-2	70	30
2	3.4	85	0	35	51	-	1-2	90	10
3	1.5	20	0	30	15	+	4-5	85	15
4	4	9.6	0	60	10	_	1-2	60	40
5	1.5	23	0	60	15	-	1-2	55	45
6	4	61.5	0	40	15	+	15-20	-	100
7	2	33	0	32	40	++	1-2	-	100
8	2.5	63	0	50	20	+	1-2	2	98
9	3.5	20.5	0	30	<b>_21</b> )	+	30-35	5	95
10	3.5	28	0	40	10	-	0-1	5	95

From 1-5 Immotile Sperm (Viable).

From 6-10 Necrospermia (Dead ).

Cas. = Case.

Abnor. = Abnormal

Liq. = Liquefaction

Visc. = Viscosity

min. = minute

H.P.F. = High power field

**Table (6)** Ultrastructure Head Anomalies Group -A- Cases No (1,2,3,4,5) Immotile (viable)

	Acrosome														
Anomalies	1	2	3	4	5	No.	%	Anomalies	1	2	3	4	5	No.	%
Deformed  Cephalic cytoplasm  Myelin figure-like membrane		+	+ +	+	+	1 2	80 20 40	Hypoplasia Separated Vaculated		+	+	+	+	2 2 3	40 40 60

Table (7) Ultrastructural Head Anomalies Group -B- Cases No (6,7,8,9,10) Necrospermia (Dead)

	Head									Acrosome						
Anomalies	6	7	8	9	10	No.	%	Anomalies	6	7	8	9	10	No.	%	
Deformed	+	+			+	3	60	Thick	+			+		2	40	
Cephalic cytoplasm	+					1	20	Hypoplasia	+		+			2	40	
Separated		+		+	+	3	60	Degenerated		+	+	+	+	4	80	
Multiple vacuoles					+	1	20									
Myelin figure-like membrane	+		+		+	3	60									

Table (8) Ultrastructural Anomalies of Locomotor System Group (A) Immotile(Viable) Cases No. (1,2,3,4,5)

	1	2	3	4	5	No.	%
Mid Piece							
- disorganization of			·	+	+	2	40
mitochondria bundle							
- Missing mitochondria							
sheath							
Fibrous Sheath							
- Hypertrophied	+		+			2	40
- Malformed			+	+	+	3	60
Axoneme							
- Absence Dynein arm	+					1	20
- Confuse arrangement of			+	+	+	3	60
microtubules							
- Missing peripheral		+				1	20
microtubules							
- Missing Central				+		1	20
microtubules.							
- Degeneration.			+	+		2	40

Table (9) Ultrastructural Anomalies of LocomotorSsystem Group (B) Necrospermia (Dead) Cases No. (6,7,8,9,10)

	6	7	8	9	10	No.	%
Connecting Piece							:
- Degeneration		+				1	20
Mid Piece							
		+	+	+		3	60
-Degeneration			+	+	+	3	60
- Disorganization of			]				
mitochondria bundle						2	40
- Missing mitochondria		+			+	2	40
sheath							
Fibrous Sheath							<u>.</u>
- Hypertrophied		+				1	10
- Malformed	+		+	+		3	60
- Degeneration	+	+	+	+	+	5	100
Axoneme							
- Confuse arrangement of			+	+	+	3	60
microtubules							
- Degeneration	+	+	+	+	+	5	100

Table (10) Ultrastructure of Locomotor System
Comparative Study between Iommotile (viable) and Necrospermia (Dead)

Tail defect	Group (A) (Viable)	No.	%	Group (B) (Dead)	No.	%
Absence dynein arm	+	1	20	-	-	-
Confuse arrangement of microtubules	++++	4	80	+++	3	60
Missing Peripheral microtubule	+	1	20	_	-	-
Missing Central microtubules	+	1	20	-	-	-
Degenerated axonemal structures.	++	2	40	+++++	5	100

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Fig (1):

Electromicrograph of semen sample from normal volaunteer showing the normal arrangement of axoneal structures hort thick (arrow (x 60000).



Fig (2):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing corfuse arrangement of microtebules long thick (arrow) (x22,000).

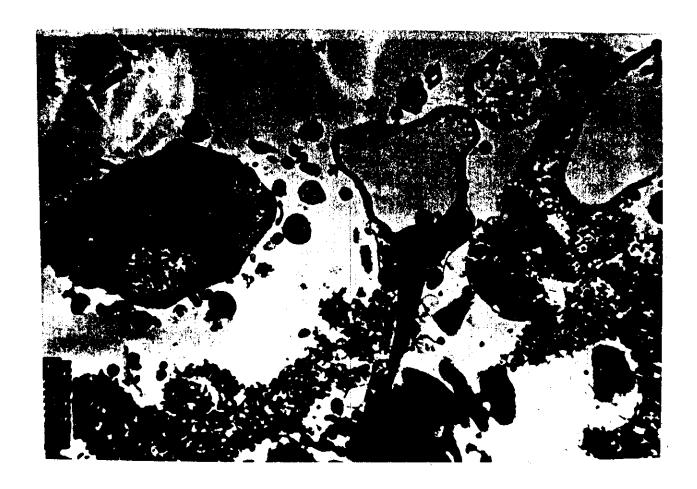


Fig (3):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing deformed head short thick (arrow)(x-10.000).



Fig (4):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing deformed head short thick (arrow). not also partial separation of acrosome and vacuolar formation long thin (arrow) (x-4.600).



Fig (5):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing deformed head short thick (arrow), rounded separated head long thick (arrow). note the presence of macrophages and granulocyte engulfing part of spermatozoa is a characteristic feature of the sample short thin (arrow)(x-3.600).



Fig (6):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing myelin figure-like membrane of acrosomal cap long thick (arrow) cephalic cytoplasm long thin (arrow) and malformed mid piece short thick (arrow)(x-13.000).



Fig (7):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing partial separation of acrosome with real vacuoles inside long thick (arrow)(x-10.000).

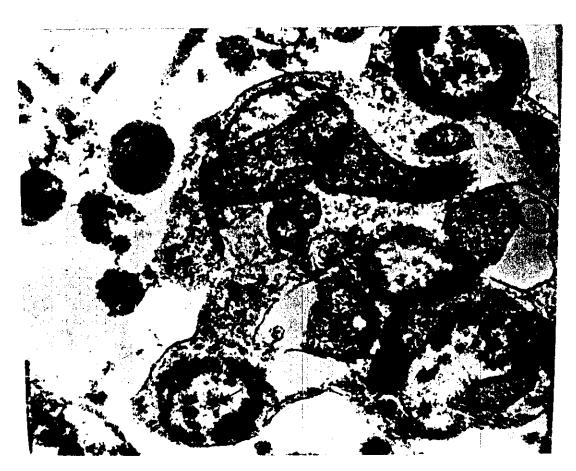


Fig (8):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing a partial axonemal degeneration long thick (arrow), confuse arrangement of microtubules short thick (arrow) and degenerated fibrous sheath long thin (arrow)(x-10.000).



Fig (9):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing confuse arrangement of microtubules short thick (arrow) (x-36.000).

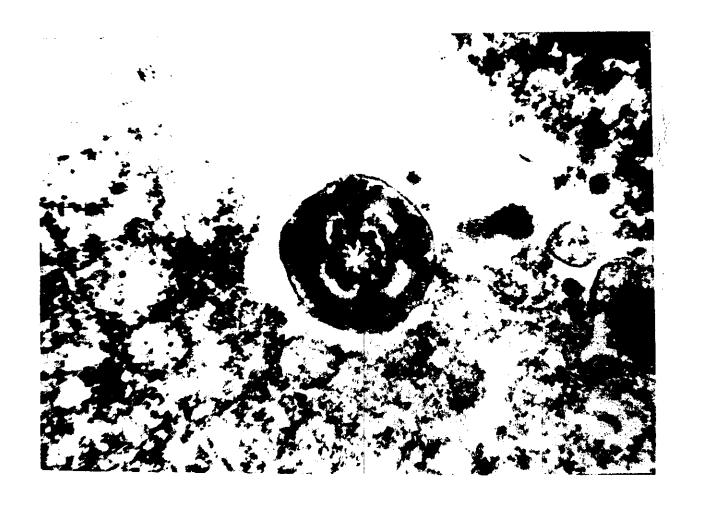


Fig (10):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing a missing of both central microtubules long thin (arrow) and malformed fibrous sheath short thick (arrow) (x-22.000).



Fig (11):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing a missing of dynein arms long thin (arrow) (x-60.000).

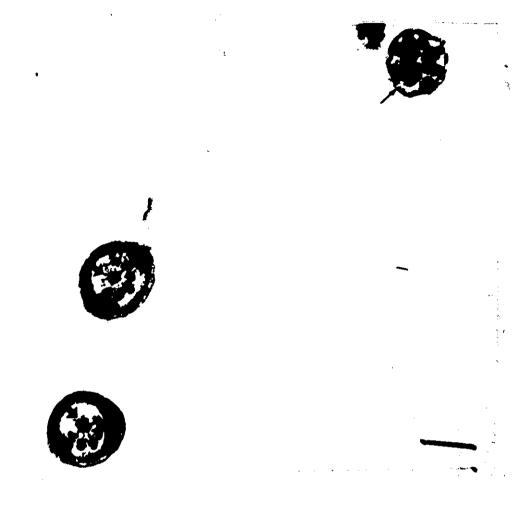


Fig (12):

Electromicrograph of semen sample from infertile patient with immotile (viable) spermatozoa showing a missing of peripheral microtubules (5+2), (6+2) short thick (arrow) and confuse arrangement of microtubules long thin (arrow) (x-20.000).



Fig (13):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing multiple vacuoles inside the head the short thick (arrow) and thick acrosome long thin (arrow). (x-17.000).



Fig (14):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing myelin figure-like membrane long thick (arrow), deformed head short thin (arrow) and thick acrosome long thin (arrow) (x-8.000).

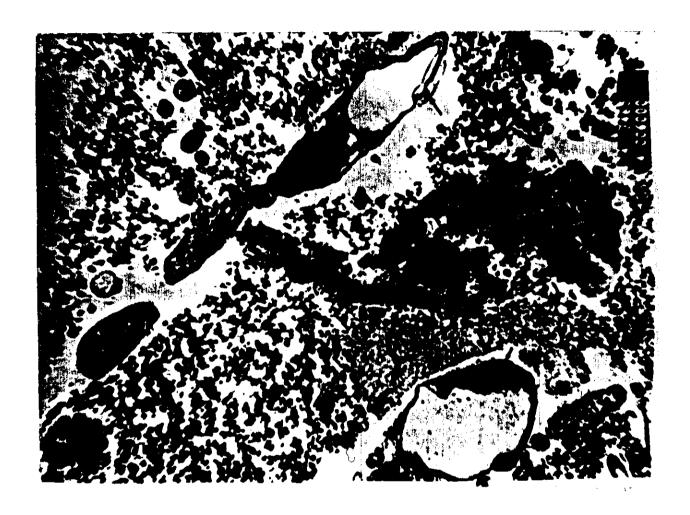


Fig (15):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing deformed head short thick (arrow) and cephalic cytoplasm long thin (arrow) (x-6.000).



Fig (16):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing myelin figure-like of acrosomal cap short thick (arrow) (x-13.000).



Fig (17):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing acrosomal hypoplasia short thick (arrow) (x-4.600).



Fig (18):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing complete separation of the head long thin (arrow) not also isolated round head short thin (arrow) (x-4.000).

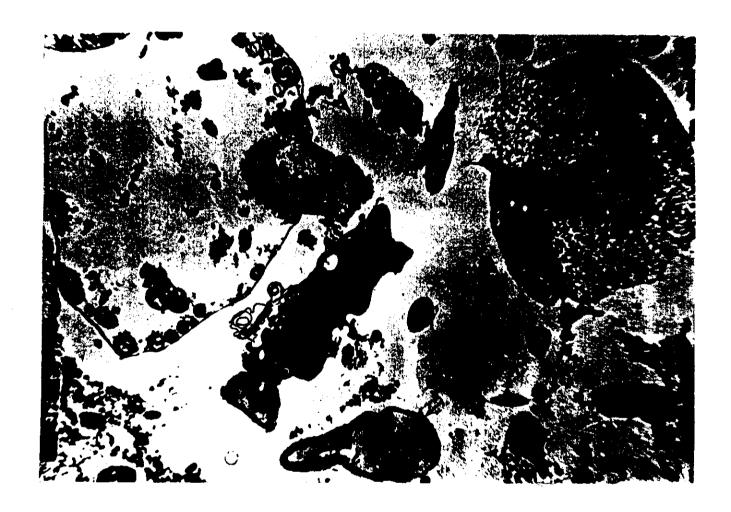


Fig (19):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing degenerated acrosome short thick (arrow) note also separated head long thin (arrow) (x -6.000).



Fig (20):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing confuse arrangement of microtubules long thin (arrow), parietal degeneration of axonemal structures short thick (arrow) and hypertrophied fibrous sheath long thin (arrow) (x - 36.000).



Fig (21):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing confuse arrangement of microtubules short thick (arrow), malformed fibrous sheet long thin (arrow) and partial degeneration of fibrous sheath long thick (arrow) (x-28.000).



Fig (22):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing complete degeneration of axonemal structures involving fibrous sheath short thick (arrow) and confuse arrangement of microtubules long thin (arrow) (x-22.000).

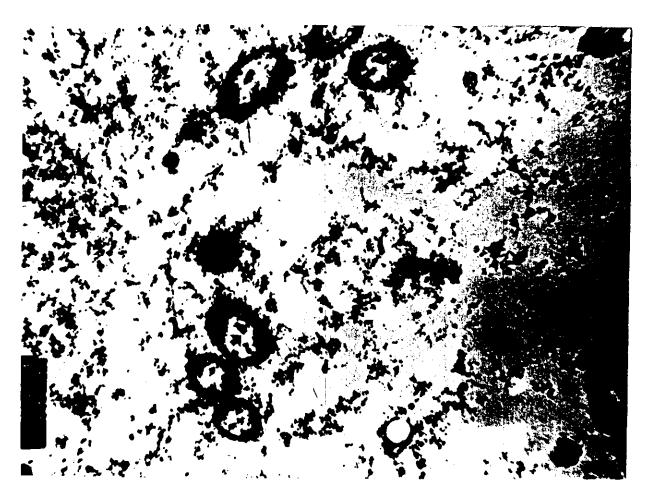


Fig (23):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing degenerated axonemal structures short thick (arrow) and malformed fibrous sheath long thin (arrow) (x - 10.000).



Fig (24):

Electromicrograph of semen sample from infertile patient with immotile (dead) spermatozoa showing part of mid-piece with partial missed mitochondrial sheath and empty mitochondria thick (arrow) (x-10.000).