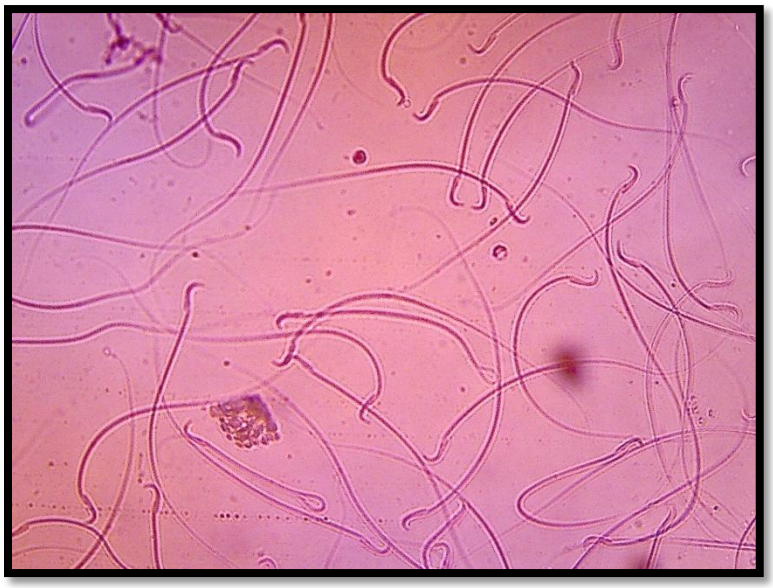
FINAL TECHNICAL REPORT

UGC Minor Research Project, WRO Pune

Effect of Vinca alkaloid Vincristine on sperm study in albino rat (*Rattus rattus*)



Submitted by

Dr. Rajeshwar M. Chaudhari

Principal Investigator

Department of Zoology

Shri. S.I.Patil Arts, G.B.Patel Science and S.T.S.K.V.S. Commerce College, Shahada, Dist. Nandurbar (M.S.) 425409

Email: chaudhari_rm@rediffmail.com

Acknowledgements

I am grateful to the University Grants Commission (UGC), Western Regional Office (WRO), Pune for providing the financial assistance for the implementation and completion of this research project. I am extremely thankful to **Mr. I.B. Chaudhari** (Principal, Shri. S. I.Patil, Arts, G. B. Patel Science &STKVS Commerce College, Shahada), **Hon'. Bapusaheb Deepak P. Patil, (**President,Pujya Sane Guruji Vidhya Prasarak Mandal, Shahada),**Dr. A.S. Patil,** Director of P.G and **Dr. S.S. Bhande** Assistant Professor, Department of Zoology, Shri. S.I. Patil Arts, G. B. Patel Science &STKVS Commerce College, Shahada) for their moral thrust and support.

I would like to thank **Dr. Y.H. Wasu,** Assistant Professor, Department of Zoology, **Mr.Govind Wankhede, Mr. M.S. Manik**(Ex. Laboratory Assistant), **Mr. S.V.Patil**(Ex. Laboratory Attendant) and **Mr. Gopal Sonar**(Assistant Clerk) for helping me in many ways during the tenure of project.

I am also thankful to my dear students **Mr. Ganesh Sonwane and Pitambar Padvi** for helping me for handling and transporting the experimental animals.

Lastly, I am grateful to my wife **Mrs. Anjali Chaudhari** and my son master **Yash,** without whose support I could not have completed this project.

Dr. Rajeshwar M. Chaudhari
(Principal investigator)

FINAL TECHNICAL REPORT

Contents **Page No.**

1. GENERAL INTRODUCTION

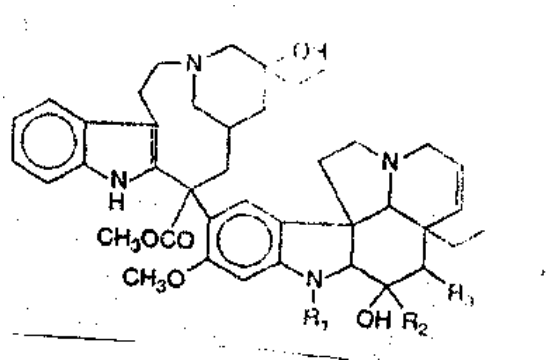
VINCRISTINE

O O O

| | | | | |

– CH – C – OCH_3 – O – C – CH_3

Molecular formula



Structural formula of Vincristine

Vincristine (C_{46} H_{54} N_4 O_{10}) is one of the most widely used effective curative agents for cancer. It is an indole-indolin alkaloid extracted from periwinkle plant *Vinca rosea*. It is an important clinical agent for treatment of leukemia's, lymphomas, and testicular cancer (Jorden *et al.,* 1985). The biological activities of this drug can be explained by their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. The cell division is arrested at metaphase is mainly due to disruption of the microtubules of the mitotic apparatus. However in the absence of an intact mitotic spindle, the chromosomes may disperse throughout the cytoplasm (exploded mitosis) or may clump in unusual groupings, such as balls or stars. Thus the chromosomes lose its ability to segregate correctly during mitosis presumably leads to cell death due to chromosomal mutation.

This drug is also known to arrest both mitosis and meiosis of male germ line results in the death of cells. Indeed, these cells die through apoptosis and are exfoliated into the lumen of seminiferous tubules (Averal *et al.,* 1995). Administration of

vincristine to adult male rat results in the arrest of spermatogenesis that leads it to azoospermia. It is reported that in the lumen of disorganized seminiferous tubules there are giant cells of different sizes are present. Similarly, the cells appear to originate from the spermatogonia due to endomitosis (Stanley and Akbarsha 1992a). Thus vincristine affects the mitosis and meiosis that results in impairment of spermatogenesis. However the causeof apoptosis may be directly correlated with sperm count, sperm morphology and its motility. Another cause for this reason can be due to the deprivation of blood testosterone level and hence the changes in sperm might be due to anti-androgenic and anti-spermatogenic effects of vincristine.

Vinca leaf extract affects by relative decrease in percentage of motile sperm and introduce the abnormality in the sperm including the categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation. It is also known to decrease significant relative percentage of live sperm. However sperm count is considered to be one of the important parameters which affect fertility.

Vinca rosea extract that generally lead to decrease in sperm count has opened up a promising avenue for anti-fertility methods and therefore toxic in effects. There is one view that sperm have two principal attributes, namely motility and fertilizing ability. Motility is an important pre-requisite for fertilization in the case of internally fertilizing organisms. Thus any negative impact on motility would seriously affect fertilizing ability. *Vinca rosea* appears to exhibit the deleterious effect by increasing the relative percentage of abnormal sperms and decreasing live sperm of cauda epididymis (Murugavel et al., 1989).

Present work is a little piece of effort in terms of effect of vincristine on sperm count, sperm morphology and sperm motility. It is point out that very little work has been done in this direction except few work of Gobello and Corrada, 2002 and Sarastis et al., 2000, but the detail study related to sperm is not available till now.

2. HISTORICAL REVIEWS

Vincristine (C_{46} H_{56} N_4 O_{10}) is one of the most widely used effective curatives for cancer. It is an indole-indolin alkaloid from periwinkle plant. The beneficial properties of the Madagascar periwinkle plant, *Catharanthus roseus* (formerly called *Vinca rosea*), a species of myrtle, have been described in medicinal folklore in various parts of the world. While exploring claims that extracts of the periwinkle might have beneficial effects in diabetes mellitus, Noble and coworkers (1958) observed granulocytopenia and bone marrow suppression in rats, effects that led to purification of an active alkaloid. Other investigations, by Johnson and associates (1963) demonstrated activity of certain alkaloidal fractions against an acute lymphocytic neoplasm in mice. Fractionation of these extracts yielded four active dimeric alkaloids; vinblastine, Vincristine, vinleurosine, and vinrosidine. Two of these, vinblastine and Vincristine, are important clinical agents for treatment of leukemias, lymphomas, and testicular cancer. Another agent, vinorelbine, has important activity against lung cancer and breast cancer (Jorden et al., 1985).

The vinca alkaloids are cell-cycle specific agents and, in common with other drugs such as colchicine, podophyllotoxin, and taxanes, block cells mitosis (George et al., 1965; Bensch and Malawista, 1968; Dustin, 1984). The biological activities of these drugs can be explained by their ability to bind specifically to tubulin and to block the ability of the protein to polymerize into microtubules. When cells are incubated with Vincristine, dissolution of the microtubules occurs, and highly regular crystals are formed that contain 1 mol. of Vincristine per mol of tubulin. Through disruption of the microtubules of the mitotic apparatus, cell division is arrested in metaphase. In the absence of an intact mitotic spindle, the chromosomes may disperse throughout the cytoplasm (exploded mitosis) or may clump in unusual groupings, such as balls or stars. The inability to segregate chromosomes correctly during mitosis presumably leads to cell death. Both normal and malignant cells exposed to vinca alkaloids undergo changes characteristic of apoptosis (Smets, 1994). The Vinca alkaloids are also known to arrest

the cell cycle at the stage of synthetic phase and to suppress nucleic acid synthesis (Creasy and Markiw; 1964; Cook et al., 1978).

Bensch and Malawista (1968) stated that the precise mechanism of action of vincristine in arresting cancer is not fully known. Still it can be suggested that tubulin left unpolymerised or produced due to microtubule disassembly is known to be acted upon by Vinca alkaloids and known as Vinca crystals.

Joshi and Ambaye (1968) administered a crude alkaloidal extract of Vinca to male rats and noticed marked changes in the testis. The testis appeared flaccid and seminiferous tubules showed reduction in size, the spermatogenesis was suppressed and possessed upto primary spermatocyte and autolysis of spermatogenic elements.

De Krester et al. (1972) stated that in adults and in children basal and / or stimulated FSH levels are frequently used to assess reproductive capacity. Azoospermia was documented confirming the close relationship between elevated FSH levels and germ cell damage.

Sherins and Vita (1973) observed marked atrophic changes in the testis with most seminiferous tubules consisting chiefly of Sertoli cells after vinca alkaloids administration thus showing azoospermic condition.

Sherins et al. (1978) reported gynaecomastia and germinal aplasia in the Hodgkins disease patient after vincristine treatment as well as lower testosterone levels.

Brooks (1981) investigated that the regressive and degenerative changes in the seminiferous tubules reflecting the antiandrogenic action because it has been reported that most stages of spermatogenesis, particularly meiosis.

Chapman et al. (1981) described 80-90% incidence of azoospermia with vincristine treatment, lower testosterone values but high LH levels indicative of compensated Leydig cell failure and poor sperm quality.

De Vita (1981) reported germ cell depletion in the testis with antitumor drug, vincristine and therefore the incidence of high rate (80 to 90%) of azoospermia. Also observed normal ranges of testosterone. According to them Leydig cell damage also occurs but the injury appears to be less extensive.

Hoffer et al. (1981) noticed a change in the location of the cytoplasmic droplet from the proximal to distal end of mid-piece in an increasing percentage of spermatozoa during their epididymal transit in pig tail monkey (*Macaca nemestrina).*

Mann and Lutwak Mann (1981) stated that suppression and degeneration of Leydig cell point to antiandrogenic action. The regressive and degenerative changes in seminifeorus tubules reflect the anti-androgenic action because it has been reported that androgen is essential for most stages of spermatogenesis, particularly for meiosis.

Russell et al. (1981) studied that (VCR) and (VLB) both these drugs are known to cause disruption of spermatogenesis by disrupting microtubule leading to pathological changes.

Wyllie (1981) opined that extensive vacuolation in the principle cells and apical cells reflect a direct action of VCR in causing mitochondrial swelling and the consequent hypoxia.

Wilson et al. (1982) demonstrated that vincristine causes depolymerization of the existing microtubules.

Chinoy and Geetha Ranga (1983) observed that *Vinca rosea* leaf extract (1 mg/0.2 ml/day/rat) treatment manifested antiandrogenic and antifertility effects in intact male albino rats. According to them the antifertility effects were attributed to reduction in sperm density, percent motility and alteration in morphology of cauda spermatozoa after treatment for 7 and 15 days. The sperms were found to be sluggishly motile and were unable to fertilize the normal cycling fertile females. They further noticed that Vinca alkaloid treatment manifested a strong anti-androgenic effect, thereby causing reduction of most of the androgenic parameters in androgen dependent target organs namely the organ weight, activities of acid phosphatase, levels of fructose in seminal vesicle besides bringing about a significant decrease in the body weight of the treated rats elucidating an androgen deprivation effect to the target organ. The authors also stated that the antiandrogenic effect of Vinca alkaloids are coupled with its anti-anabolic action, as is evident from the decrease in body weight and those of the reproductive organs as well as marked histological alteration in testis, caput and cauda epididymides. According to them mechanism of action of Vinca rosea leaf extract seems to be via causing androgen deprivation to the target organs which results in alteration in their histo-physiology.

Jordan et al. (1985) examined vincristine and its derivatives for their abilities to inhibit net tubulin addition at the assembly ends of bovine brain microtubules at steady state. Vincristine caused 25% inhibition.

According to **Chakraborti and Mukherji (1988)** vinca rosea (Linn) (*Catharauthus roseus*) of the family Apocynaeceae, with proven efficacy is anticarcinogenic, from which vincristine has been extracted which is antispermatogenic and antiandrogenic.

Rao et al. (1988) described gonadal dysfunction with vincristine.

Murugavel et al. (1989) stated that vinca leaf extract did not affect body weight, sperm count decreased significantly to 67%, relative percentage of motile sperm decreased by 44% on treatment, relative percentage of abnormal sperm, categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation were predominant, relative percentage of live sperm decreased significantly. Sperm count is considered to be one of the important parameters which affect fertility. Decrease in sperm count of *Vinca rosea* extract opens up a promising avenue for anti-fertility methods and therefore toxic effects. According to them sperm have two principal attributes, namely motility and fertilizing ability. Motility is an important pre-requisite for fertilization in the case of internally fertilizing organisms. Thus any negative impact on motility would seriously affect fertilizing ability. In increasing the relative percentage of abnormal sperms and decreasing the relative percentage of live sperm vinca rosea appears to be deleterious at the level of cauda epididymis.

Hansen et al. (1990) with vincristine and cisplatin treatment observed azoospermia. Regarding the Leydig cells, only compensatory functional insufficiency was notes.

Russell and Russell (1991) are of the opinion that there is general agreement that various anti-neoplastic drugs exert a prejudicial effect on spermatogonia provoking the death of these cells including Cisplatin, Vincristine etc. According to them microtubules constitute one of the important aspects of the cytoskeleton of the Sertoli cell, and play a critical role in determining the shape of the cell which are vulnerable to VCR treatment and thereby causes early exfoliation of germ cell.

According to **Stanley and Akbarsha (1992)** vincristine is one of the most widely used effective curatives for cancer. At present it is one of the drugs used in combination

chemotherapy regimens. It is an indole – indolin alkaloid obtained from the periwinkle, *Vinca rosea* or prepared from vinblastine another Vinca alkaloid. It is proposed that this drug prevents metastatic growth by preventing the formation of spindle fiber, thereby arresting mitosis, without affecting replication of DNA. They emphasized that when used as anticancer, this drug is known to cause several toxic side effects, yet its toxic effects on the male reproductive system have been only poorly studied.

Stanley and Akbarsha (1992a) stated that treatment of vincristine sulphate to adult male albino rats' results in arrest of spermatogenesis and in azoospermia, the highly disorganized seminiferous tubules contained giant cells of different sizes in the lumen, the cells appear to originate from the spermatogonia due to endomitosis.

Stanley and Akbarsha (1992b) reported the fate of giant cells formed in the seminiferous tubule lumen in the testis of albino rats after vincristine treatment, by probably affecting spermatogenic mitosis. These giant cells in the caput epididymis were spherical and ranged in diameter from 10 to 40μm. Each cell contained a single nucleus, the size of which differed among the cells. The chromatin appeared as clumps, dispersed inside the nucleus. These cells showed no trace of cytolysis or phagocytosis. These giant cells densely pack the cauda epididymal lumen and appeared fragmented due to cytolysis and phagocytosis by phagocytes as it happens in the case of dead sperm. The authors suggested that since these giant cells remain intact in the caput, these giant cells can be isolated free from contamination by flushing of the caput epididymal tubule, for studying the nucleo-cytoplasmic ratio, ploidy, DNA content, tubulin etc. these can be cultured for nuclear transplantation experiments involving polyploid nuclei,in elucidating nucleocytoplasmic interactions in spermatogenic cells. The giant spermatogonial cells would be of two advantages in this regard, namely (i) the large size, overcoming the technical difficulty of smallness of the nuclei of renal adenocarcinoma cells which are used to achieve successful transplantation, (ii) the giant polyploid cells are germinal rather than somatic. Giant Hela cells used in elucidating nucleo-cytoplasmic interactions are produced in vitro by subjecting Hela cells to X-irradiation, which treatment invariably results in the formation of nuclear fragments therefore these giant cells produced in vivo would overcome this problem.

Akbarsha et al. (1995) tested the effect of vincristine (VCR), currently in use as a mitotic spindle poison in combination chemotherapeutic regimens for cancer, on the Leydig cell and the accessory reproductive organs in the light of the reports that it affects spermatogenesis. They administered VCR to Wistar strain male albino rats at a daily dose of 20μg for 15days. According to them the seminal vesicle and ventral prostate were regressed; lumen of the caput epididymis lacked sperm but contained giant cells, in the cauda, giant cells appeared disintegrating. Secretory acini / follicles of the seminal vesicle / ventral prostate exhibited decreased secretory activity. Fructose content of the seminal vesicle also decreased. Cytoplasm of Leydig cell of treated rats appeared highly vacuolated and the nuclear chromatin-depleted. The authors interpreted that the regression and other derangements in the accessory reproductive organs appear to be manifestation of the toxic effect of the drug on Leydig cell.

Averal et al. (1996) administrated Wistar strain male albino rats with Vincristine (VCR) sulphate (10μg/day for 15 days); epithelial cell types of the caput (zone II) and cauda (zone V) were studied light microscopically adopting semi-thin sectioning. VCR caused conspicuous pathological changes in the principal and apical cells of the caput and the clear cells of the cauda. According to them the study points to toxic effect of VCR on these cell types, suggesting impairment of epididymal function, particularly concerning sperm maturation and endo-cytotic removal of the contents of the cytoplasmic droplets and dead sperm.

Bokemeyer et al. (1996) described azoospermia with vincristine treatment.

Robbins et al. (1997)studied the effects of chemotherapy in children suffering from Hodgkins disease. They concluded that the patients receiving chemotherapy induced disomies and diploidies due to chromosomal aneuploidy.

Foresta et al. (2000) stated that chemotherapy reflects the alteration of testicular structure and resulted in to severe oligozoospermia. The sex chromosome abnormality passes to the children.

Saratsis et al. (2000) administered vincristine intravenously at dose level 0.6 mg in dog and evaluated semen parameters like semen volume, sperm concentration, total spermatozoa per ejaculate, percentage of progressive motility, percentage of dead

spermatozoa and percentage of abnormal spermatozoa. He reported that semen quality transiently deteriorated during treatment.

Saba et al. (2009) injected ethanolic extract of the whole fruits of *Lagenaria breviflora*to the Wistar rats. The extract caused morphological alteration of sperm cells and resulted secondary abnormality in sperms. He further stated that the abnormality induced in sperm were bent mid piece, curved midpiece, bent tail, curved tail, normal tail without head , normal head without tail, looped tail and coiled tail. It was resulted due to interference with maturation stage of spermatogenesis. He further noted that the sperm count was lowered in comparison with control group.

Avadhani and Arunachalam (2014) administered Vincristine sulphate at different dose level to Swiss albino mice. They reported abnormal sperms from 0.74 to 11.16% in treated mice. The sperm shape reported were amorphous type predominantly, followed by hookless, banana shape head, folded and double headed or tail. In high dose drug induced nearly azoospermia.

Hasim et al. (2014) demonstrated that the natural incidence of abnormal spermatozoa was 13% after exposure to vincristine for 2 weeks, increased the number of morphological abnormal spermatozoa was four folds. It was occurred due to the damage of DNA and was detected by using FISH molecular test.

3. MATERIALS AND METHODS

About the drug

Vincristine is also known as vincristin sulphate and obtainedas cytocristin as its brand name. It is manufacture by Cipla Ltd. It is purchased from market and available in aqueous form as one mg one ml**.** Doses were decided from the previously carried out experiments.

Treatment of animals

In present study animals were collected from the animal house of R.C. Patel College of Pharmacy, Shirpur, Dist Dhule (M.S.). Animals brought with special care to the College departmental laboratory and acclimatized for a week. Even though the order rodentia is highly exploited for the experimental purposes are easily available and can undergo all tests. The six adult male rats weighing between 284 to 360 gms were selected for the laboratory tests without any difficulty, hence is suitable for experimental work.

Handling of animals

A special care was taken while injecting the drug. The animal was hold fast out of the cage by its leg and drug was injected intravenously with disposable insulin syringe (fig. 1).

Experimental protocol

In all three sets of experiments using high and low-doses of Vincristine (0.06 and 0.12 mg/kgBW/day) were performed for the present study for the duration of 15 days (Tables 1).Animals were sacrificed 24 hours after the last day of each experiment by exposing to chloroform. Immediately the organs were excised. Both the cauda epididymis was utilized for sperm analysis.

The spermatozoa present in the cauda epididymis were collected after mincing/slicing the tissue in a cavity block containing 1ml of physiological saline and centrifuged at 600rpm for 1 minute with a drop of 5% aqueous eosin (WHO, 1999).

Coslab digital microscope with Phase contrast adjustment was used to observe the sperms. All evaluations were done at 25X, 45X and 100X.

Table 1: Experimental Design for Vincristine Treatment

No of animals and sex	Treatment	Dose (mg/Kg BW)	Route	Duration
6 males (Experimental)	Vincristine	0.06 mg daily	I.V.	15 days
6 males (Experimental)	Vincristine	0.12 mg daily	I.V.	15 days
6 males (Control)	Saline	E.V.	I.V.	15 days

Abbreviations:- E.V. = Equal Volume, I.V. = Intra Venous, BW=Body weight.

Classification of sperm morphology

Normal spermatozoa:

Head Region	-	Curved
Acrosomal Region	-	Well defined, occupying 40-70% of the head area
Mid piece Region	-	Slender, about one and half times the length of the head and attached axially to the head.
Tail Region	-	Straight, uniform, thinner than the middle piece, Uncoiled.

Abnormal spermatozoa

Head Defects

Namely large, hook less, banana shaped head, flattened, pin head, amorphous, vacuolated head (>20% of the head area occupied by unstained vacuolar areas) heads with small acrosomal area (<40% of head area) and headless.

Neck and Midpiece defects

Namely 'bent' neck (the neck and tail form an angle greater than 90% to the long axis of the head) asymmetrical (the neck may be absent or bifurcated or swollen or insertion of the midpiece into the head, thick or irregular mid piece, bent mid-piece, abnormally thin midpiece (i.e. no mitochondrial sheath) or any combination of these.

Tail defects

Namely short, multiple, hairpin, broken tails, bent tails (>90%) tails of irregular width, coiled tails, looped tail, double tails, curve tail, rudimentary or absent or any combination of these.

4. OBSERVATIONS

In the present study the Vincristine (VCR, Cytocristin) drug was used to find out the changes in the sperm of albino rat *Rattus rattus*. They includes:

4.1.Vehicle Treated Control

Sperm Count

Sperms from cauda epididymis were counted in Neubaure's slide. As the animal was in breeding stage the epididymis of vehicle treated group showed swarms of spermatozoa (11.8×10^6) and hence a condition of normospermia existed.

Sperm motility

We have recorded sperm motility by capturing the video but poor quality of it could not succeed to keep the record. However, rapidly fast moving sperms were observed. The types of motility were rapid progressive motility, slow or sluggish progressive motility, non-progressive motility, immobility and rotational motility. The numbers of sperms showing sluggish progressive motility, Non-progressive motility, and Immobility and Rotational motility were few in quantity

Sperm morphology

The head was hooked; acrosomal region was well defined, occupying 40-70% of the head area. Midpiece region was slender, about one and half times the length of the head and attached axially to the head (figs. 2a-b). Some sperms were bent and had curved mid-pice (figs.8&9). The tail region was straight, uniform, thinner than the midpiece and uncoiled. Very few sperms were had bent tail, curve tail, coiled tail, and looped tail (figs.11, 12, 13&14a-b).

4.2. Low Dose Treatment

Sperm count

Sperms from cauda epididymis were counted. Reduction in the number of sperms was observed in the aqueous saline solution (Table-2 & bar diagram). Thus a significant condition of oligospermia was observed ($P < 0.001$).

Sperm motility

Some sperms showed ‘progressive movement’, however, most were immotile, some with ‘non-progressive movement’ and largely remaining sperms were showing “rotational movement”. We were not able to keep a record of motility because of poor video quality.

Sperm morphology

Several observation of cauda epididymis with measured quantity of saline in the cavity slide showed following types of sperm abnormalities:

1 Head defects:-

i Hook less head (fig.3).

ii Banana shape head (fig.4).

iii Amorphous head (fig.5).

iv Pin head (fig.6a-b).

v Tailless head (fig.7).

2 Mid-piece defect:-

i.Bent mid- piece (fig.8).

ii Curved mid-piece (fig.9).

3 Tail defects:-

i Headless tail (fig.10).

ii Bent tail (fig.11)

iii Curved tail (fig.12)

iv Coiled tail (fig.13)

v Looped tail (fig.14a-b)

vi Double tails (figs.15).

4.3. High Dose Treatment

Sperm count

Sperms from cauda epididymis were counted showed reduction in their number of (Table-2 &bar diagram). Thus a significant condition of oligospermia was observed ($P < 0.001$).

Sperm motility

Some sperms showed “progressive movement”, however, most were immotile, and some were showing “non-progressive movement”. At the same time largely some remaining sperms were showing “rotational movement”. Of course due to poor video quality it was not possible to keep the record.

Sperm morphology

Several observations pertaining to cauda epididymis showed following sperms abnormalities:

a) Head defects:-

i. Hookless head (figs.3).
ii. Banana shape head (figs.4).
iii. Amorphous head (figs.5).
iv. Pin head (fig.6a-b)
v. Tailless head (fig.7)

b) Mid-piece defect:-

i. Bent mid- piece (fig.8).
ii. Curved mid-piece (fig.9).

c) Tail defects:-

i. Headless tail (figs. 10).
ii. Bent tail (fig.11)
iii. Curved tail (fig.12)
iv. Coiled tail (fig.13)
v. Looped tail (fig.14a-b)
vi. Double tails (figs.15).

5. DISCUSSION

The sperm count, sperm motility and sperm morphology were used in this study to evaluate the effects of Vincristine by using albino rat model. The drug can affect on reproductive system resulting in the sperm production. Abnormal forms of spermatozoa occurred in all mammals (Man& Man, 1981).Vinca alkaloid is an anti-neoplastic and anti-carcinogenic drug. The drug arrest cell growth through its effects on cytoskeletal elements and inhibits spindle formation essential for normal cell division. Vincristine disrupts ciliary action and cheated motility of spermatozoa.

Vincristine acts as a cytotoxic agent to differencing spermatogonia (Lu& Meistrich, 1979). Vincristine works as ancolytic agent, preferentially kill cells of specific stages of the spermatogonic pathway at doses with clinical range for human.

One of the most prominent effects of vincristine instead of tubulin inhibitor was to damage the DNA of germinal epithelium and it was detected by FISH molecular test (Hasim *et al.,* 2014). The chromosomal abnormality resulted due to Vincristine induced sperm abnormalities and the possible caused was hyper-ploidy (Brandrift *et al.,* 1994).The abnormality of sperm was affected by the administration of Vincristine. This drug mostly acts as a chemical mutagen and affect on sperm head abnormality (Topham, 1980). It was also noticed that the head parameters found main in the cytotoxicity of Vincristine (Ettin *et al.,* 1984).

Administration of cytotoxic drug in male rat resulted the different types of sperm abnormality includes bent mid piece, curved mid piece, bent tail, curved tail, normal tail without head and normal head without tail. All these abnormalities came under secondary types (Saba *etal.,*2009). Our results were accordance to it but we reported primary abnormality includes rudimentary tail in high dose treatment. Vincristine also resulted into induced deterioration of semen parameters like semen volume, sperm concentration, and total spermatozoa per ejaculate, percentage of progressive motility and percentage of dead spermatozoa (Saratsis *et al.,* 2000). Our results were also accordance to it and decrease sperm count, sperm motility and also resulted into different types of head, mid piece and tail abnormalities.

In present study we have reported that, the motility of sperm decreased in low dose and high dose as compare to control. The motility observed were of progressive type, very few sperms showed rotational motion. Our results were accordance to Soratsis *et al.,* (2000). We have also reported dead sperms and it was due to impairment of epididymal function (Averal *et al.,* 1996).

Abnormalities induced due to Vincristine in present study included primary and secondary abnormalities. I have reported both the types, primary types reported in our results were amorphous head, hookless head, tailless head, pin headed and headless tail. Secondary types of abnormality obtained in present study were banana shape head, bent tail, curve tail, bent mid piece, coiled tail, looped tail and curved midpiece. Our results were in accordance to Saba*et al.,* 2009. But I got some additional types of abnormalities like pin head and the sperm with two heads and two tails with single mid piece. In these both head and tail jointed by common mid piece.

Mine result of the sperm counts were reduced in low dose and high dose as compared to the control. But in the present study I have reported the oligospermia in low dose and high dose. These observations agreed with Choudhary *et al*., (2002) and Dobzynska *et al.*, (2005).

Thus in the present study Vincristine at dose level 0.06 mg and 0.12 mg resulted in decrease in sperm count and motile sperms with abnormal morphology. The results were more predominant in high dose as compared to control and low dose treatments. In high dose treatment the sperm count was very low but it did not showed the severeness of morphological defects. It might be due to the early death of germ cell during the process of spermatogenesis. It is suggested that the Vincristine treatment induce structural chromosomal aberration in spermatocytes, this attributed to interfering with DNA replication by preventing the cell from entering G_1 phase cause an arrest of mitotic and meiotic division to metaphase followed by cell death, so result in sperm defect or no sperm formation (Diab *et al.,*2011)

The effect of vincristine on sperm abnormalities and their causative mechanism are elaborated here. Vincristine is an anti-proliferative, radiomimetic, anti-carcinogenic

drug. This drug arrests cell growth through their effect on cyto-skeletal elements and tubulin formation. As a result there is no formation of spindle which is essential for normal cell division. Acrosomal head shape sperms is disrupted from the normal by affecting the tubulin polymerization in the microtubule and by inhibiting axoplasmic flow (Avadhani & Kumar, 1994). Vincristine disrupts ciliary action and check motility of spermatozoa. Vincristine induced all the wide range of abnormalities depending upon dose level, most prominent were amorphous head followed by coiled tail and Hookless head.

6. SUMMARY AND CONCLUSION

In present work Vincristine induced toxicities in sperm maturation and also affect on count and motility. Vincristine is a powerful alkaloid used in chemotherapy to cure the cancer. The mechanism of action of vincristine discussed by different workers proved that it inhibit the spindle formation by inhibition of tubulin synthesis. It results chromosomal mutation due to non disjunction. Thus from the present work it is concluded that:

1. Administration of Vincristine in low dose and high dose regiment significantly decreased in sperm count. But it never resulted azoospermia; in low dose it is oligospermia and in high dose oligozoospermia.

2. Vincristine also affect on the motility of sperms, in vehicle treated control the sperms with progressive motility were more. It decreases in low dose to high dose. Non-motile sperms were found more in low dose and high dose as compared to vehicle treated control.

3. After administration of Vincristine sperm morphology was adversely affected. They showed twelve different types of sperm abnormalities. These abnormalities categorized in to primary and secondary.

4. The sperm abnormalities induced due to administration of Vincristine resulted in to head, middle piece and tail.

5. All these parameters viz., sperm count, sperm motility and sperm morphology directly affects on fertility ultimately on progeny out come.

Thus Vincristine is a powerful drug which induced different anomalies in sperms at different dose level in albino rat *Rattus rattus.*

7. REFERENCES

Akbarsha MA, Stanley A, Averal HI (1995) Effect of vincristine of Leydig cell and accessory reproduction organs. Curr. Sci. **68**: 1053-1056.

Averal HI, Stanley A, Akbarsha MA (1995) Apoptotic death of male germinal line cells of rat caused by Vincristine: light and electron-microscopic study. Biomed. Lett. **52**: 171-180.

Averal HI, Stanley A, Murugaian P, Palanisamy M, Akbarsha MA (1996) Specific effect of vincristine on epididymis. Indian J. Exp. Biol. **34** : 53-56.

Avadhani R, Kumar A (1994) Sperm shape abnormalities in mice treated with Vincristine sulphate. J.Int.Med.Sci. Acad **7**(1): 9-13.

Avadhani R, Arunachalam K (2014) Vincristine sulphate induced sperm shape abnormalities in mice. Int. J. Curr. Res. **6**(9): 8829-8833.

Bokemeyer C, Berger CC, Kuczyk MA, Schmoll HJ (1996) Evaluation of long-term toxicity after chemotherapy for testicular. Cancer J. Clin. Oncol. **14** : 2923-32.

Bensch KG, Malawista (1968) Nature. **218** : 1176-1177.

Brandriff BF, Meistrich ML, Gordon LA, Carrano AV, Liang JC (1994) Chromosomal damage in sperm of patients surviving Hodgkin's disease following MOPP therapy with and without radiotherapy. Hum Genet. **93**(3): 295-299.

Brooks DE (1981). Physiol. Rev. **61**: 515.

Choudhary RC, Das B, Mishra S, Jagdale MB (2002) Spermatogonial cytogenetic toxicity of vincristine and its transmission in the germline cells of Swiss mice. J Environ Pathol Toxicol Oncol **21**(3): 249-257.

Chapman RM, Sutcliffe SB, Malpas JS (1981) Male gonadal dysfunction in Hodgkin's disease. A prospective study. JAMA : **245** : 1323-1328.

Chinoy NJ, Geetha Ranga (1983) Antiandrogenic and Antifertility effects of Vinca rosea leaf extract on male albino rat. Comp. Physiol. Ecol. **8** (1) : 41-52.

Chakraborti SK, Mukherjee SB (1988) J. Res Indian Med. **3**: 70.

Cooke RA, Nikles A, Roeser HP (1978) A comparison of the antifertility effects of alkylating agents and Vinca alkaloids in male rats. Br. J. Pharmac. **63**: 677-681.

Creasy WA, Markiw ME (1964) Biochemical effects of the vinca alkaloids II. A comparison of the effects of colchicine, vinblastine and vincristine on the synthesis of ribonucleic acids in Ehrlich ascites carcinoma cells. Biochem. biophys. Acta, **87**: 601-609.

De Vita VT Jr. (1981) The consequences of the chemotherapy of Hodgkin's disease. The 10th David A. Karnofsky Memorial Lecture. Cancer. **47**: 1-13.

DeKrester DM, Burger HG, Fortune D et al. (1972) Hormonal histological and chromosomal studies in adult males with testicular disorders. J. Clin. Endocrinol. Metab. **35**: 392-401.

Dustin P (1984) 'Microtubules', Springer-Verlag, Newyork/Tokyo.

Dobrzynska M, Czajka MU, Slowikowska MG (2005) Reproductive effect after exposure of male mice to vincristine and to a combination of X-ray and vincristine. CSIRO PUBLISHING, Repro.Ferti. Dev. **17**:759-767.

Diab, Sayed AA, Elshafey, Hassan ZM (2011) Assesment of the extract Vincristine induced genotoxicity in mice. Gen. cyto.Dept. Nat. Res.Cen. Dokki Egypt Comm.Sci. **2**(3):126-134.

Foresta C, Bettella A, Marin P, Galeazzi C, Merico M, Scandellari C (2000) Analysis of sperm aneuploidy in infertile subjects after chemotherapy treatment. Ann Ital Med Int. **15**(3): 189-94.

George P, Journey LJ, Goldstein MN (1965) J. Natl. Cancer Inst. **35** : 355-375.

Gobello C, Corrada Y (2002) Effects of Vincristine treatment on semen quality in a dog with a transmissible venereal tumour. J Small Anim Pract. **43**(9): 416-7.

Hoffer AP, Shalev M, Frisch DH (1981) Ultrastructure and maturational changes in spermatozoa in the pig tail monkey *Macaca nemestrina*. J. Androl. **2**:140.

Hashim MS, Khalil H, Jeboori A, Ban AM (2014) Molecular- Pathological study in the effects of Vincristine sulfate drug on DNA of sperm and spermatogenesis in mice. G.J.B.B. **3**(1):41-45.

Jordon A, Himes RH, Wilson L (1985) Comparison of the effects of vinblastine, vincristine, vindesine and vinepidine on microtubule dynamics and cell proliferation in vitro. Cancer Res. **45** : 2741-2747.

Johnson IS, Armstrong JG, Gorman M, Burnett JP Jr (1963) The Vinca alkaloids: a new class of oncolytic agents. Cancer Res. **23**: 1390-1427.

Joshi MS, Ambaye RY (1968) Indian J. Exp. Biol. **6**: 256-257.

Hansen SW, Berthelsen JG, Von der Maase H (1990) Long-term fertility and Leydig cell function in patients treated for germ cell cancer with Cisplatin, Vinblastine and Bleomycin versus surveillance. J. Clin. Oncol. **8** : 1695-1698.

Lu CC, Meistrich ML (1979) Cytotoxic effects of chemotherapeutic drugs on mouse testis cells. Cancer Res. **39** : 3575-3582.

Mann T, Lutwak-Mann (1981) Secretory function of male accessory organs of reproduction in mammals. Physiol. Rev. **3**: 27.

Murugavel T, Ruknudin A, Thangavelu S, Akbarsha MA (1989) Antifertility effect *Vinca rosea* (Linn.) leaf extract on male albino mice – A sperm parametric study. Curr. Sci. **58**(19) 1102-1103.

Noble RL, Beer CT, Cutts JH (1958) Further biological activities of vincaleukoblastine-an alkaloid isolated from vinca rosea (L). Biochem. Pharmacol. **1**: 347-348.

Russel LD, Russell JA (1991) Short term morphological response of the rat testis to administration of five chemotherapeutic agents. Am. J. Anat. **192**(2): 142-163.

Russel LD, Malone JP, MacCurdy DS (1981) Effect of the microtubule disrupting agents, colchicine and Vinblastine on seminiferous tubule structure in the rat. Tissue cell. **13**(2): 349-67.

Rao SV, Murugesan K, Vij U, Sarin R, Kapur BM (1988) Effect of cancer polychemotherapy on gonadal function in Hodgkin's disease in adult men. Ind. J. Med. Res. **87**: 37-41.

Smets LA (1994) Programmed cell death (apoptosis) and response to anti-cancer drugs. Anticancer Drugs. **5**: 3-9.

Stanley A, Akbarshah MA (1992) Male reproductive toxicity of the anticancer drug vincristine (a review). In Recent Advances in life sciences. pp 122-136. Edited by A.K. Saxena, R. Rammurthi, G. Sreerama Reddy and V.L. Saxena. Indian Society of Life Sciences, Manu Publications, Kanpur, India.

Stanley A, Akbarshah MA (1992a) Vincristine can cause giant cell formation in rat testis. Curr. Sci. **62**: 535-537.

Stanley A, Akbarshah MA (1992b) Giant spermatogonial cells generated by vincristine and their uses. Curr. Sci. **63**: 144-147.

Saratsis P, Ypsilantis P, Tselkas P (2000) Semen quality during vincristine treatment in dogs with transmissible venereal tumor. Theriogenology. **53**(5): 1185-1192.

Sherins RJ, Devita JT (1973) Effect of drug treatment of lymphoma on male reproductive capacity. Studies of men in remission after therapy. Ann intern. Med. **79**: 216-220.

Sherins RJ, Olweny CLM, Ziegler JL (1978) Gynecomastia and gonadal dysfunction in adolescent boys treated with combination chemotherapy for Hodgkin's disease. N. Engl. J. Med. **299**: 12-16.

Saba AB, Olayinka AO, Matthew OO, Oluwaseun DO (2009) Spermatozoa morphology and characteristics of male wistar rats administered with ethanolic extract of *Lagenaria Breviflora* Roberts. Afri. J. Biotech. **8**(7):1170-1175.

Topham JC (1980) The detection of carcinogen induced sperm head abnormalities in mice. Mut. Res.**69**(1):149-155.

Wilson L, Jordan MM, Morse A, Margolis RL (1982) Interaction of Vinblastine with steady-state microtubules in-vitro. J. Molec. Biol. **159**: 125-149.

Wyllie AH (1981) Cell death: a new classification separating apoptosis from necrosis. In **Cell Death in Biology and Pathology**.pp. 9-34. Edited by I.D. Bowen and R.A. Lockshin. Chapman and Hall, London.

Fig.1: Illustrating how the animal was hold fast by its leg for injecting drug intravenously on the thigh with disposable insulin syringe.

Table-2Effect of 0.06 mg and 0.12 mg VCR / day for 15 days on Cauda epididymal sperm count. The number of sperm was observed in 5 different microscopic fields (values are mean ± SE).

Parameter	Control	0.06mg / kg BW/ day for 15 days	0.012 mg / kg BW / day for 15 days
Sperm count (10^6/ml)	11.80 ± 0.170	5.25 ± 0.047	3.50 ± 0.095

P value - P < 0.001

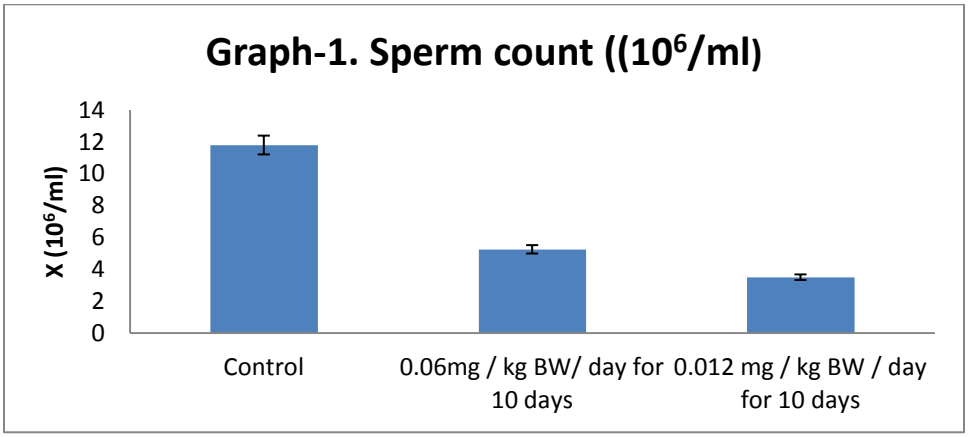
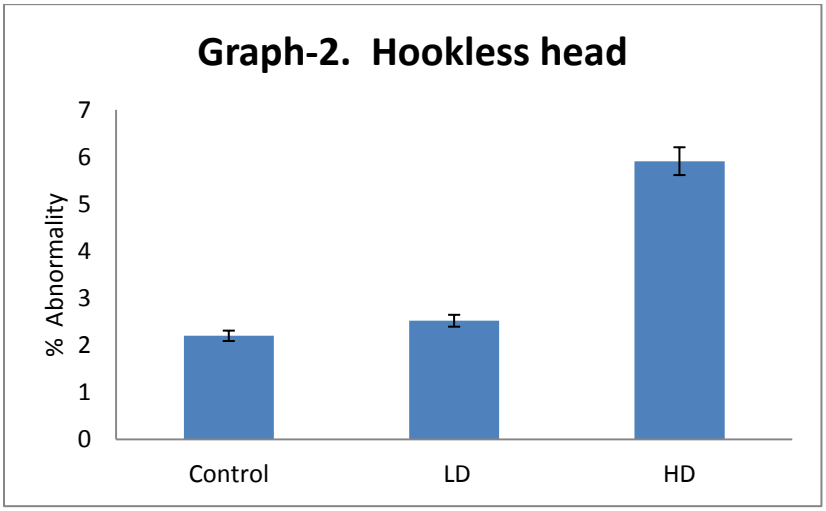
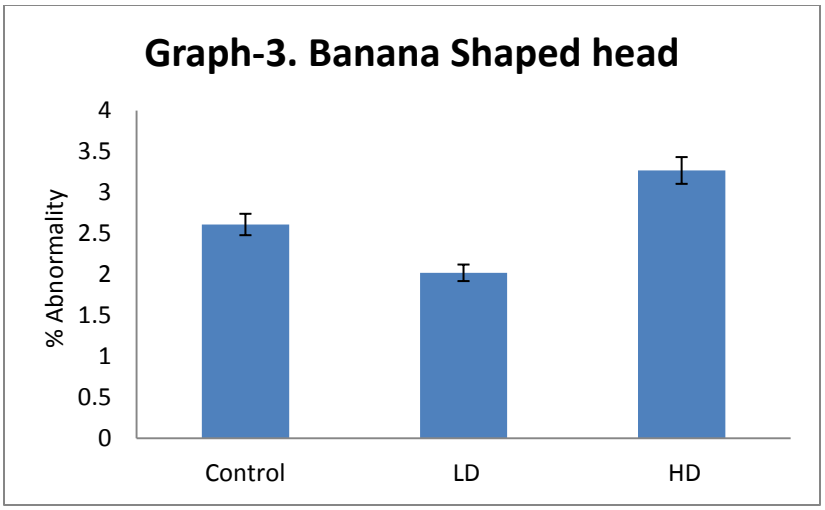


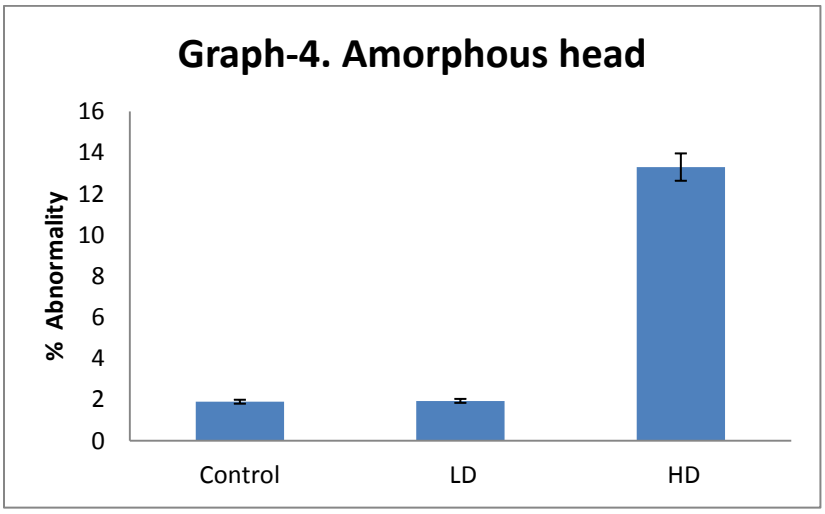
Table-3Effect of 0.06 mg and 0.12 mg VCR / day for 15 days on sperm morphology and percentage occurred of different sperm abnormalities (values are mean ± SE).

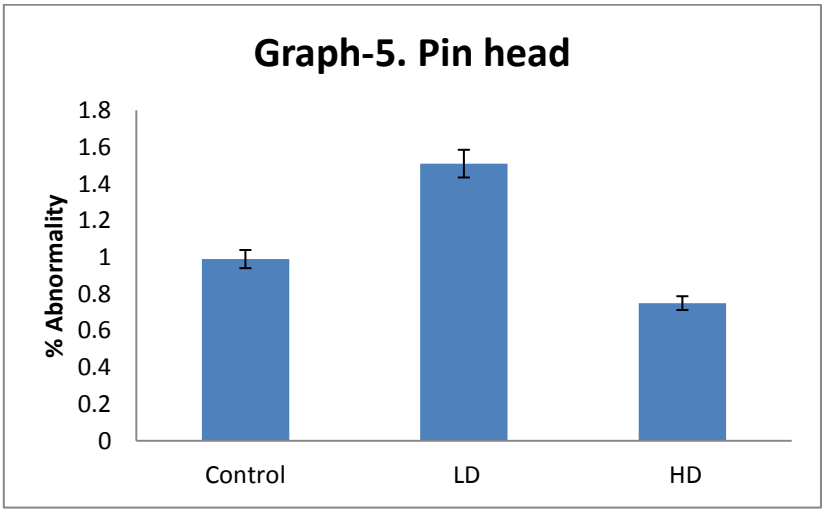
Sr. No.	Mean Sperms Abnormality(%)	Control	0.06mg / kg BW/ day for 10 days	0.012 mg / kg BW / day for 10 days
1	Hook less head	2.20±0.27	2.52±0.16#	5.91±0.15###
2	Banana shape head	2.61±0.29	2.02±0.18#	3.27±0.16##
3	Amorphous head	1.90±0.28	1.94±0.21#	13.30±1.44###
4	Pin head	0.99±0.11	1.51±0.11###	0.75±0.13#
5	Tailless head	0.43±0.19	2.53±0.17##	0.65±0.07#
6	Bent mid piece	3.80±0.17	3.73±0.23*	3.76±0.22*
7	Curved mid piece	2.71±0.23	1.53±0.26##	3.56±0.13##
8	Headless tail	1.26±0.21	1.35±0.25*	1.35±0.08*
9	Bent tail	3.10±0.12	4.06±0.72#	2.46±0.34#
10	Curved tail	0.95±0.10	4.27±0.18###	5.70±0.31###
11	Coiled tail	2.48±0.35	6.46±0.35##	4.44±0.16##
12	Looped tail	3.32±0.19	2.50±0.30#	2.43±0.14##
	Total mean sperm abnormality (%)	**25.75±0.35**	**34.42±0.36**###	**47.58±0.62**###

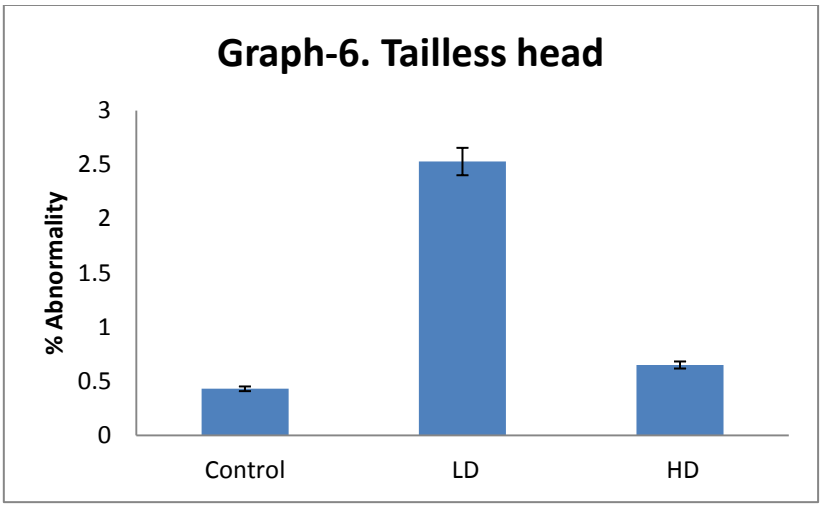
#p<0.05 , ##p<0.01, ###p<0.001 and *Insignificant

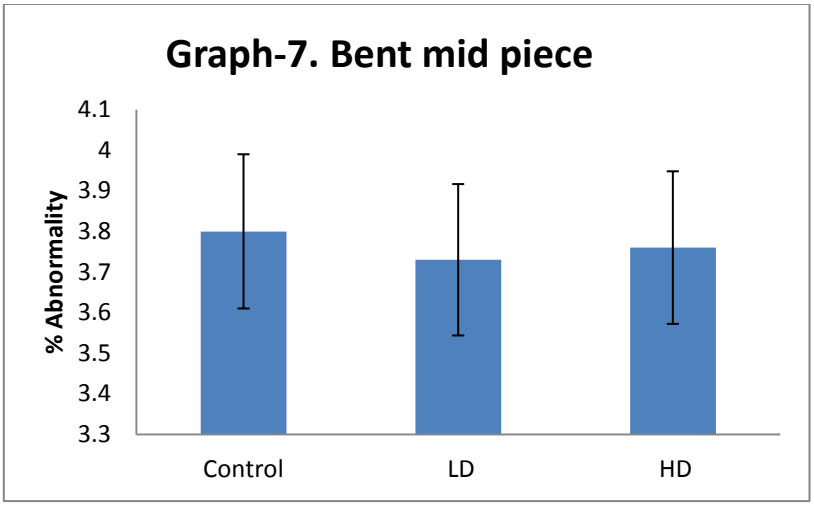


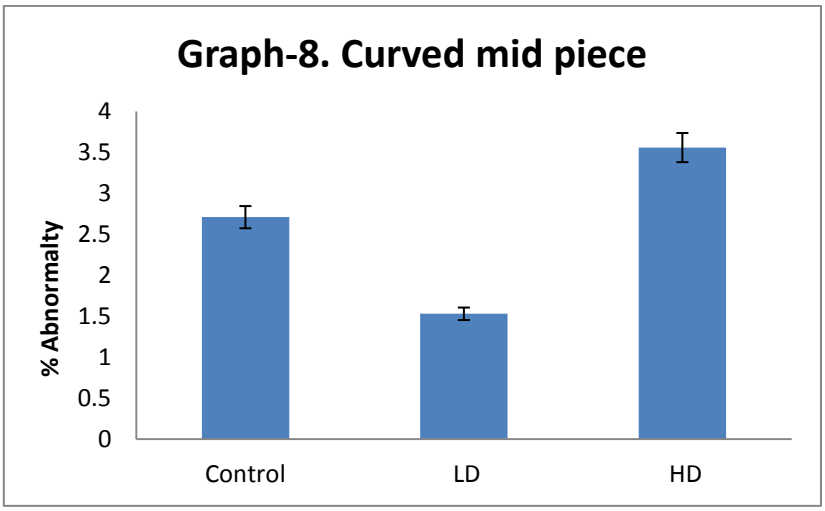

Graph-3. Banana Shaped head
% Abnormality
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
LD
HD

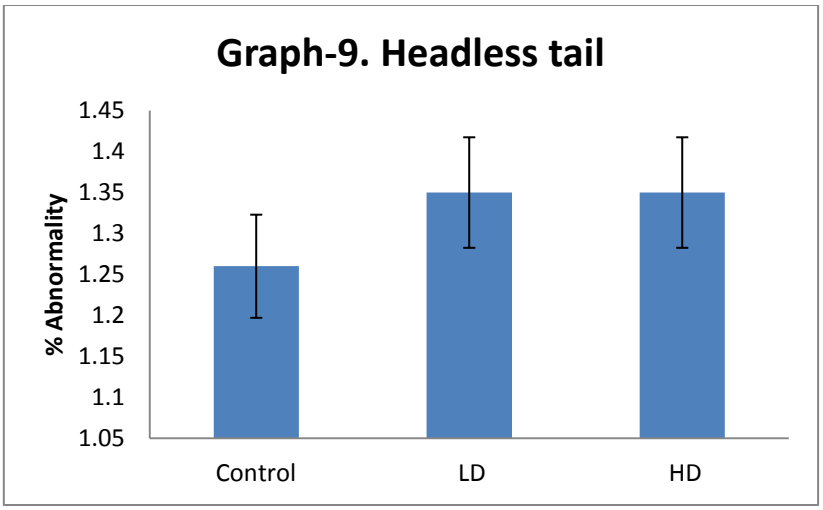

Graph-4. Amorphous head
% Abnormality
16
14
12
10
8
6
4
2
0
Control
LD
HD

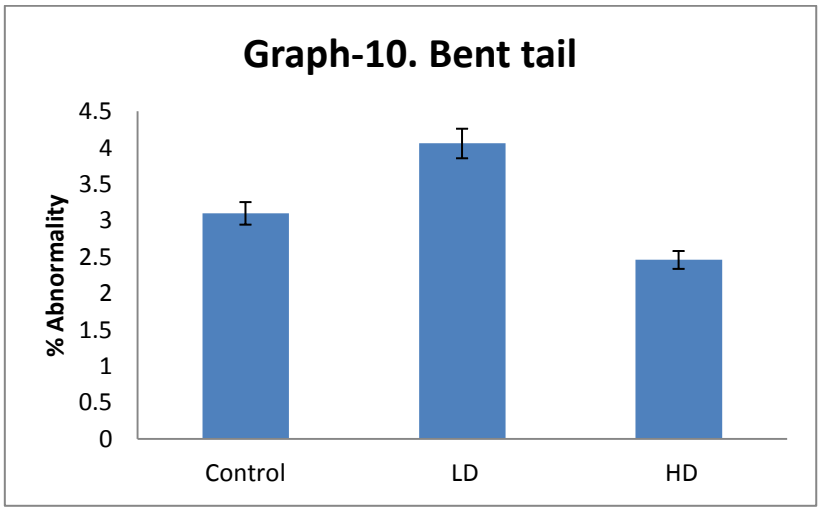

Graph-5. Pin head
% Abnormality
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
LD
HD

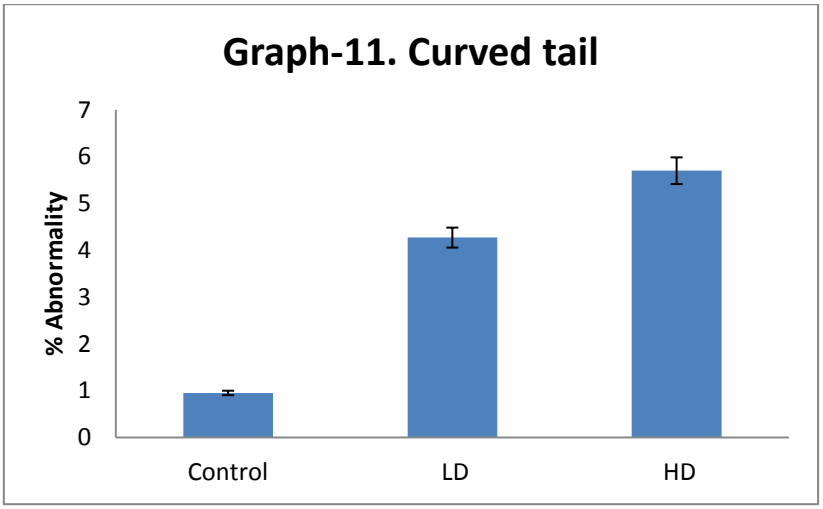

Graph-6. Tailless head
% Abnormality
3
2.5
2
1.5
1
0.5
0
Control
LD
HD

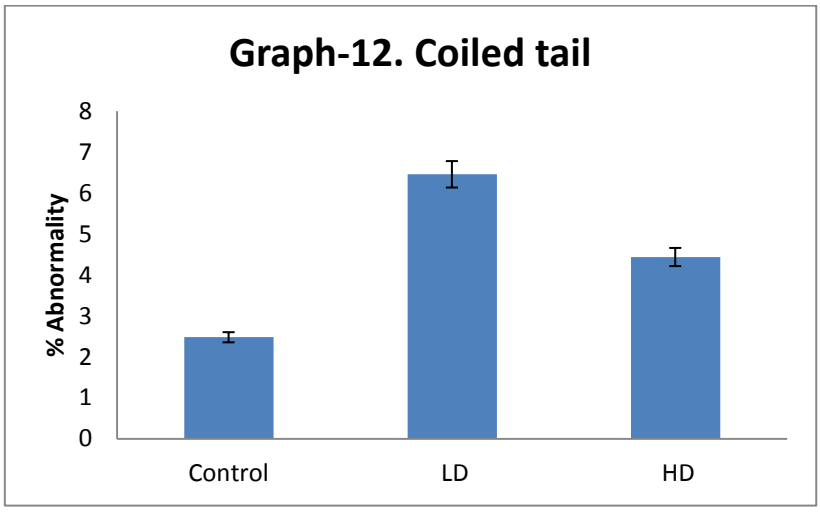

Graph-7. Bent mid piece
% Abnormality
4.1
4
3.9
3.8
3.7
3.6
3.5
3.4
3.3
Control
LD
HD

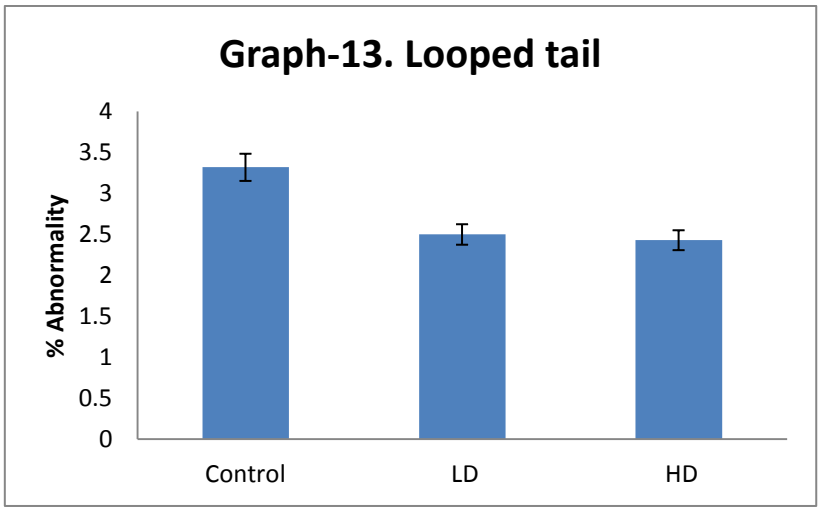

Graph-8. Curved mid piece
% Abnormalty
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
LD
HD


Graph-9. Headless tail
% Abnormality
1.45
1.4
1.35
1.3
1.25
1.2
1.15
1.1
1.05
Control
LD
HD


Graph-10. Bent tail
% Abnormality
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
LD
HD


Graph-11. Curved tail
% Abnormality
7
6
5
4
3
2
1
0
Control
LD
HD


Graph-12. Coiled tail
% Abnormality
8
7
6
5
4
3
2
1
0
Control
LD
HD


Graph-13. Looped tail
% Abnormality
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
LD
HD

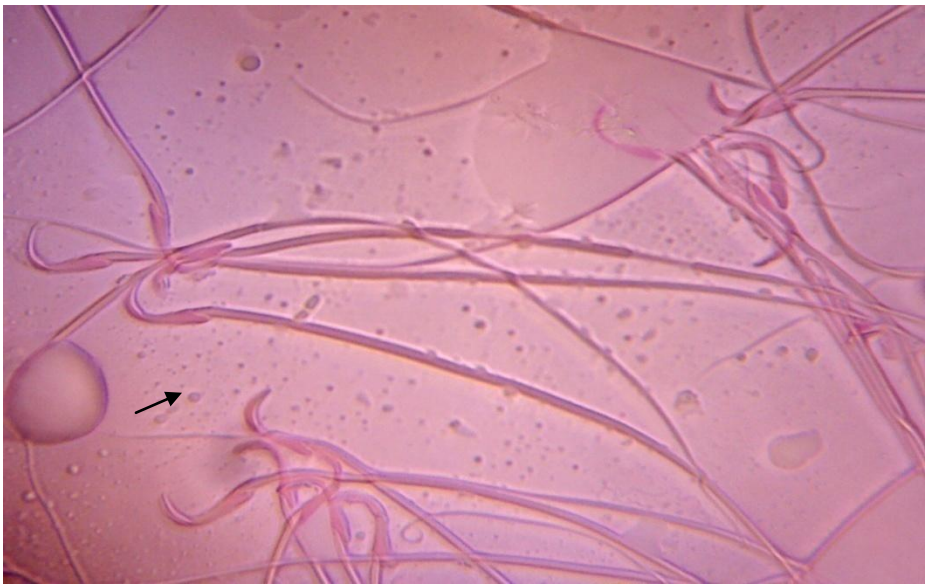


Fig.2a: Microphotograph showing swarms of normal sperms (arrow) X 1000.

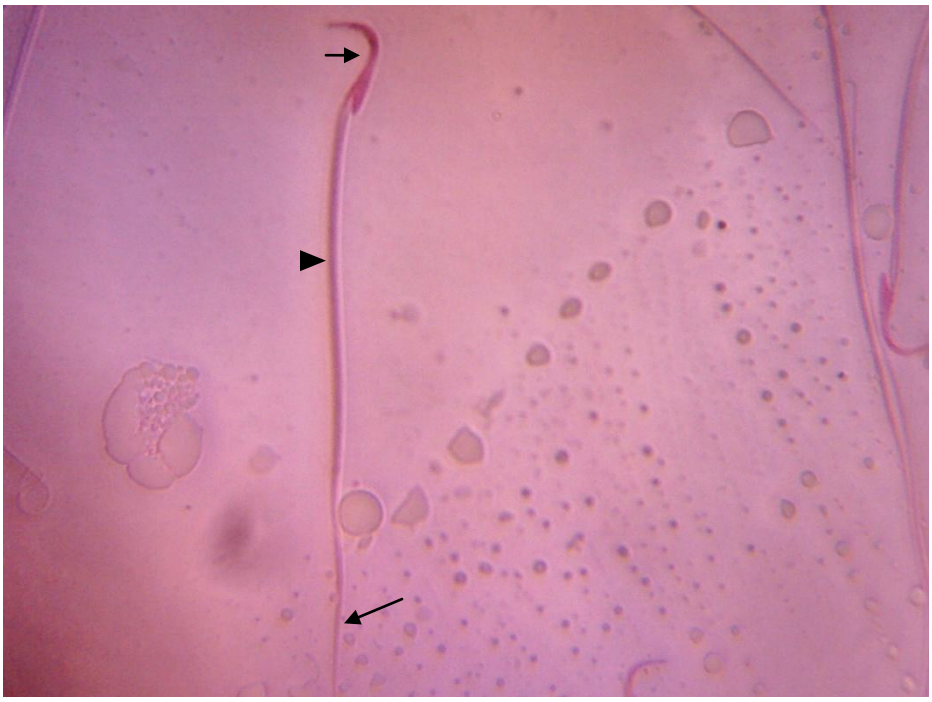


Fig.2b:Photograph showing single sperm with normal head (arrow), mid piece (arrow head) and tail (long arrow) X1000.

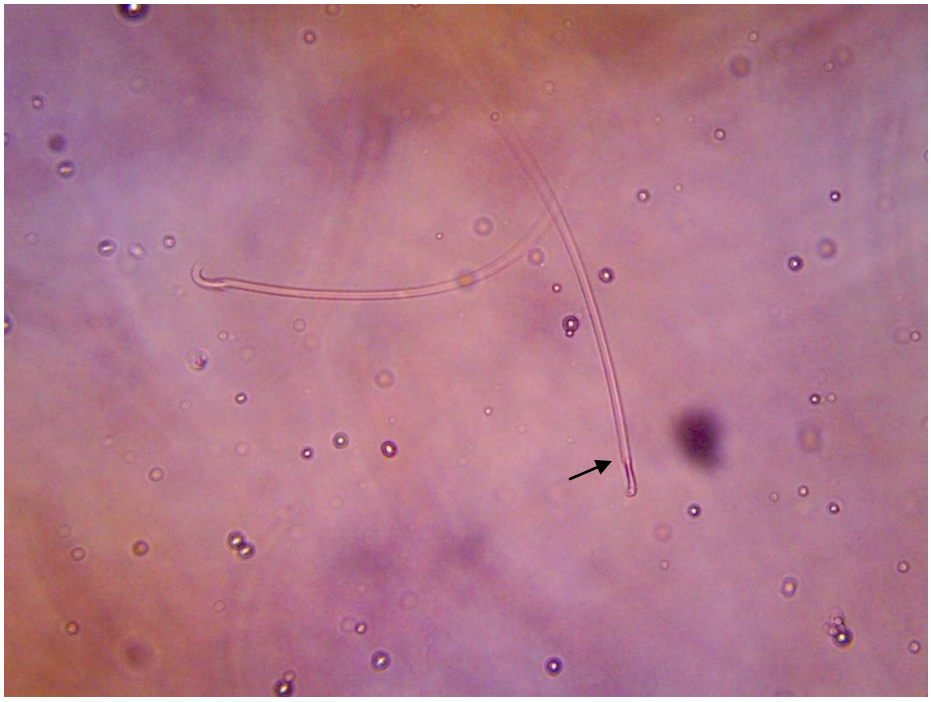


Fig.3: Microphotograph of sperm with hook less head (arrow) X 400.

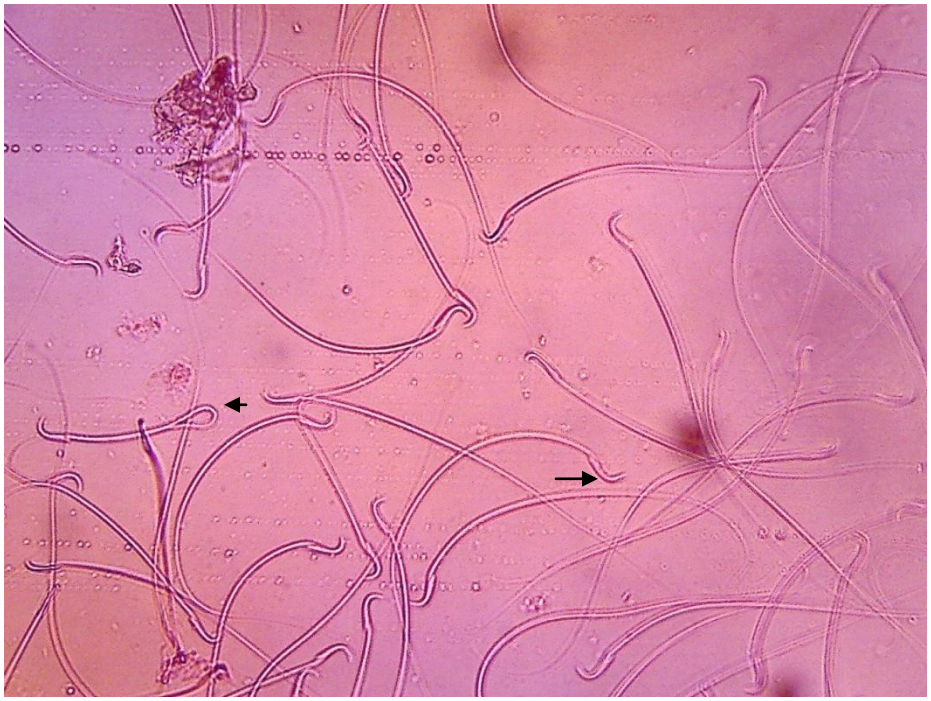


Fig. 4: Photograph of few sperms showing banana shape head (arrow) and looped tail (arrow head) X400.

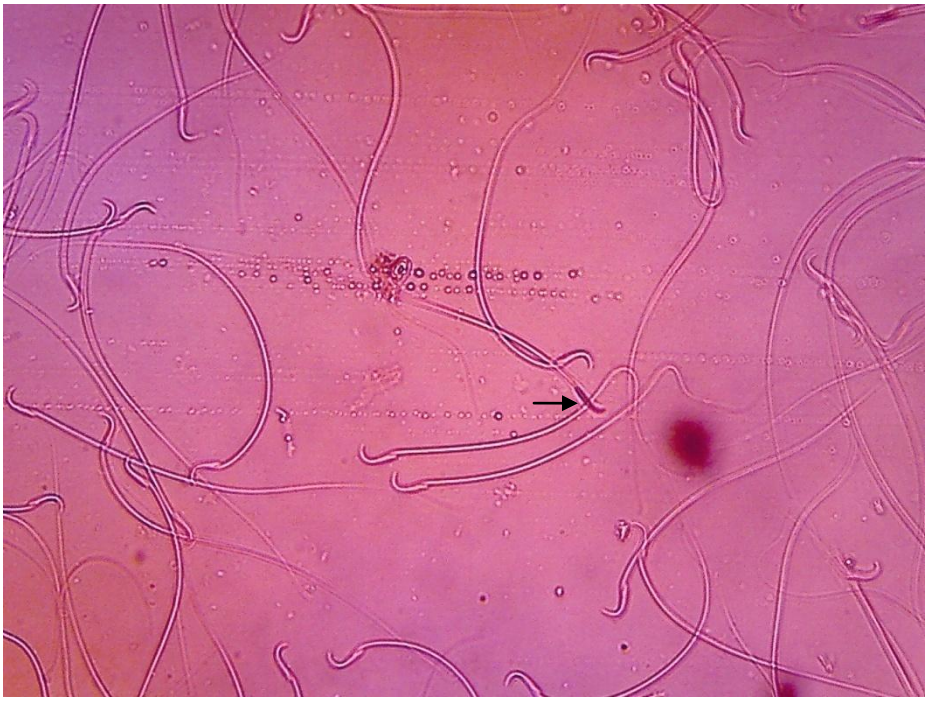


Fig. 5: Photograph of sperm showing amorphous head (arrow) X 400.

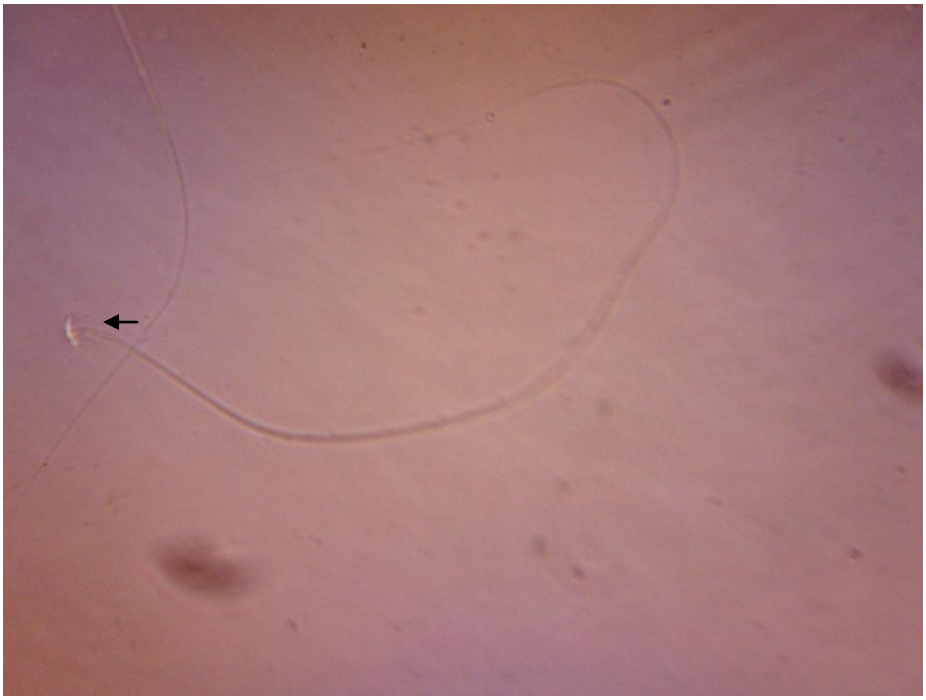


Fig. 6a: Photograph with pin headed (arrow) X 400.

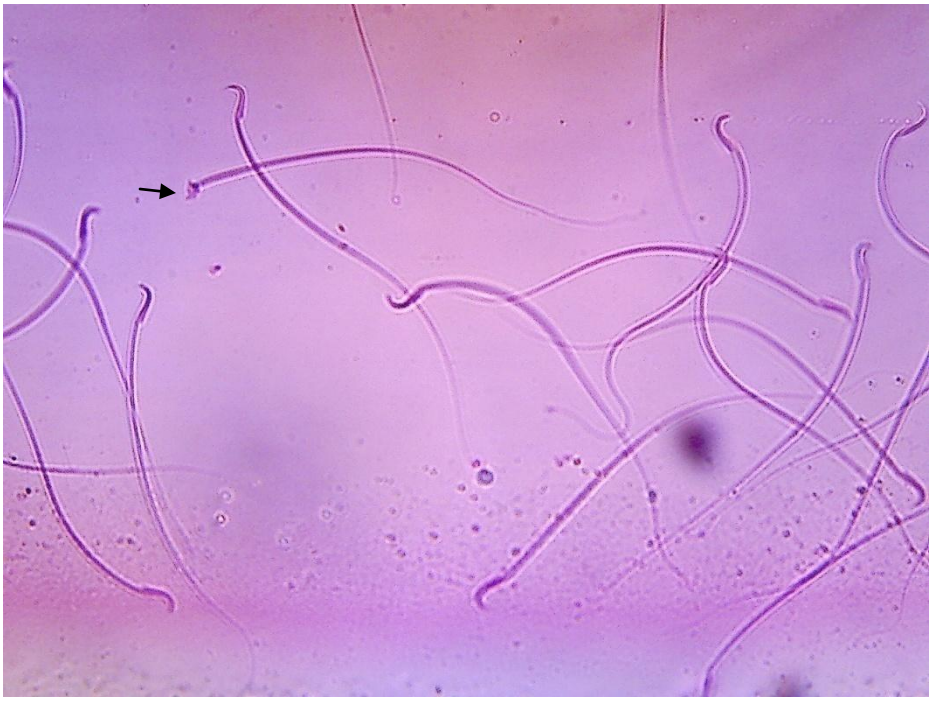


Fig. 6b Photograph of sperm with pinheaded (arrow) X 400.

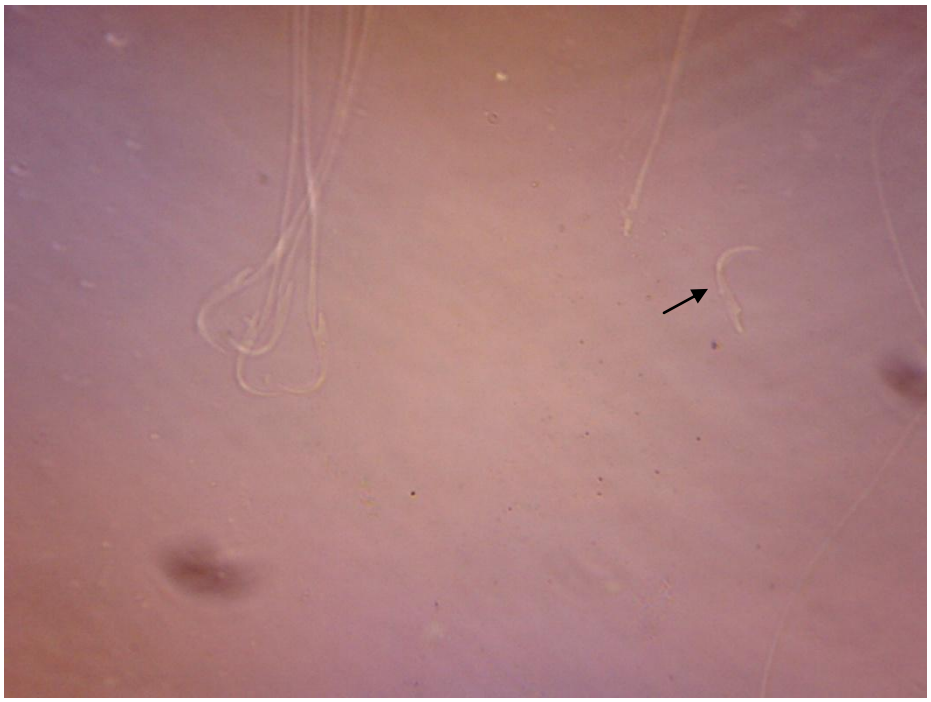


Fig.7 Photograph of sperm showing tailless head (arrow) X 400.

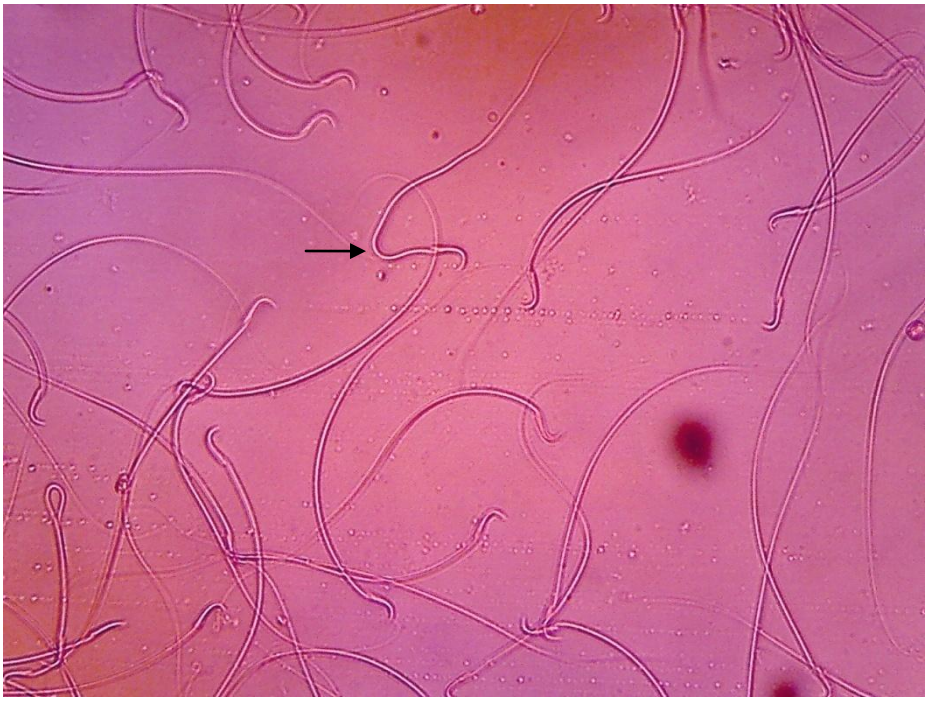


Fig.8Microphotograph showing sperm with Bent mid piece (arrow) X 400.

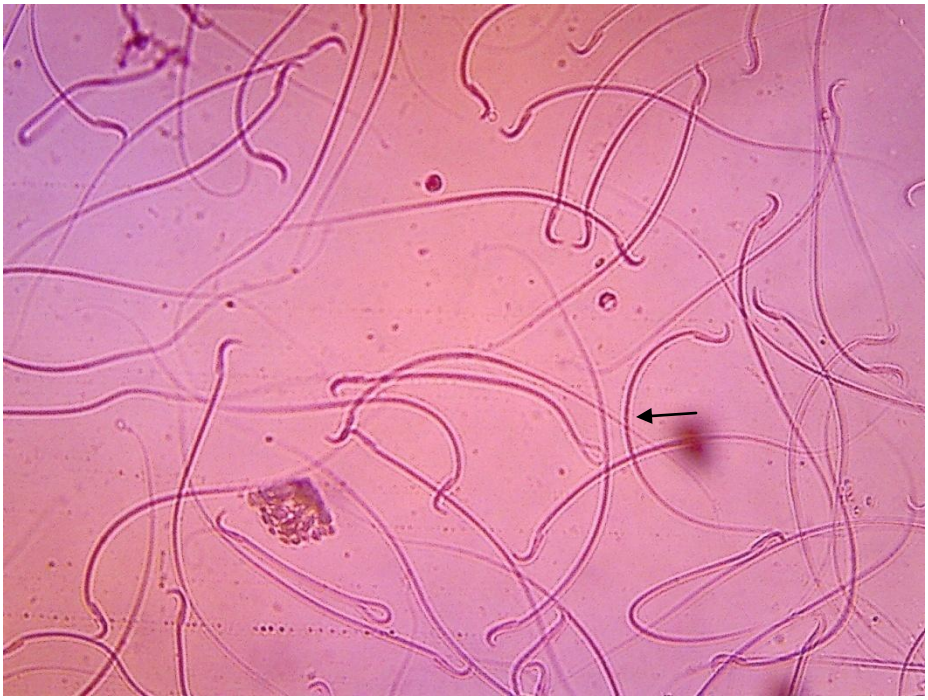


Fig.9 Photograph of sperm with curved midpiece (arrow) X 400.

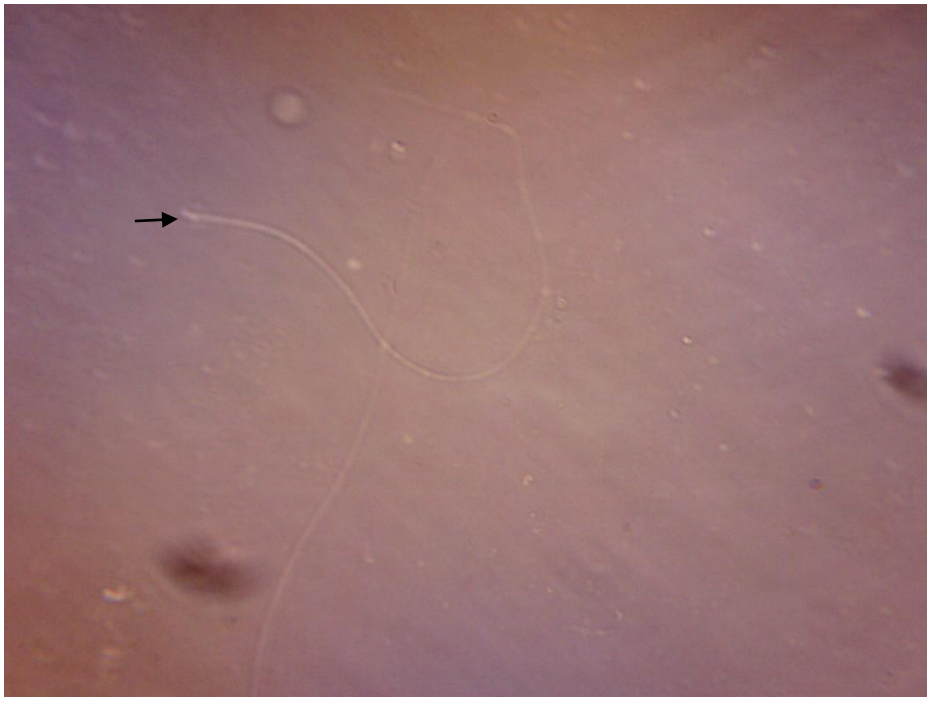


Fig.10 Photograph of sperm with headless tail (arrow) X 400.

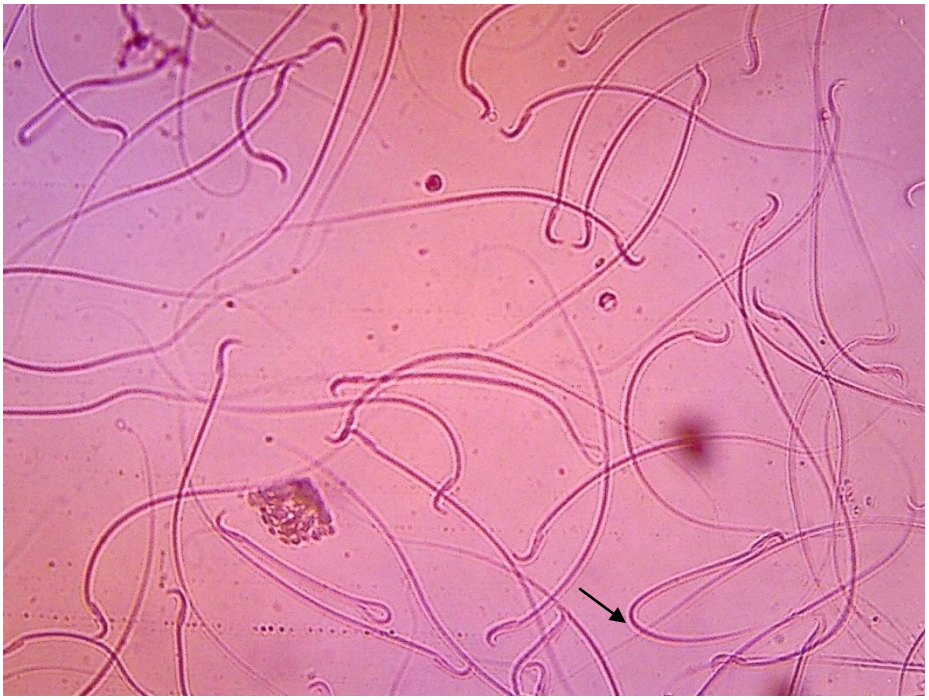


Fig.11 Photograph of sperm with bent tail (arrow) X 400.

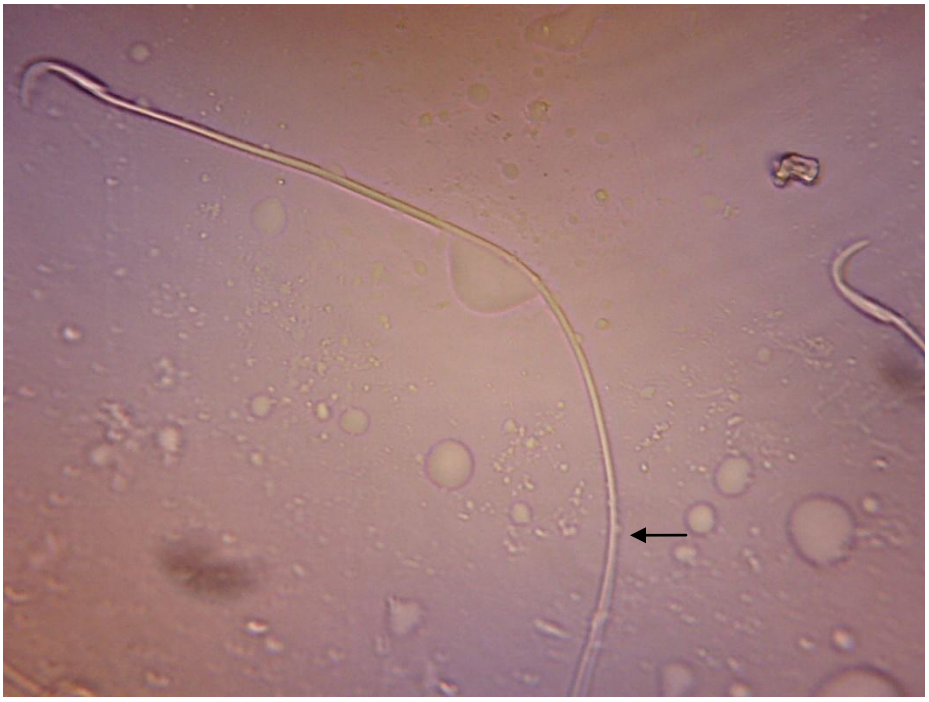


Fig.12 Photograph of sperm with curved tail (arrow) X 1000.

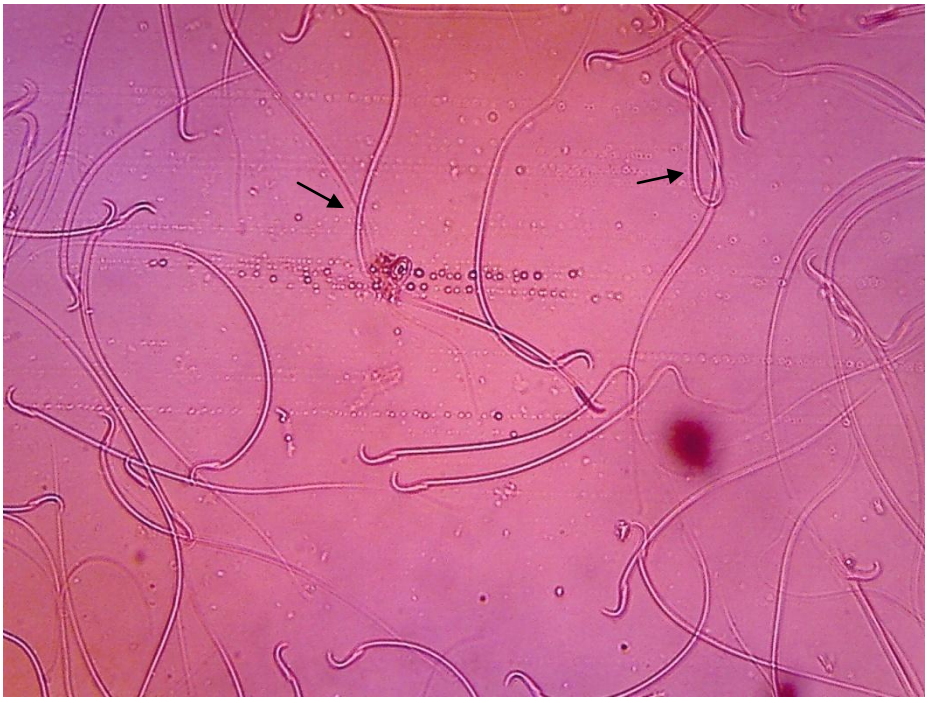


Fig. 13 Microphotograph showing sperm with coiled tail (arrow) X 400.

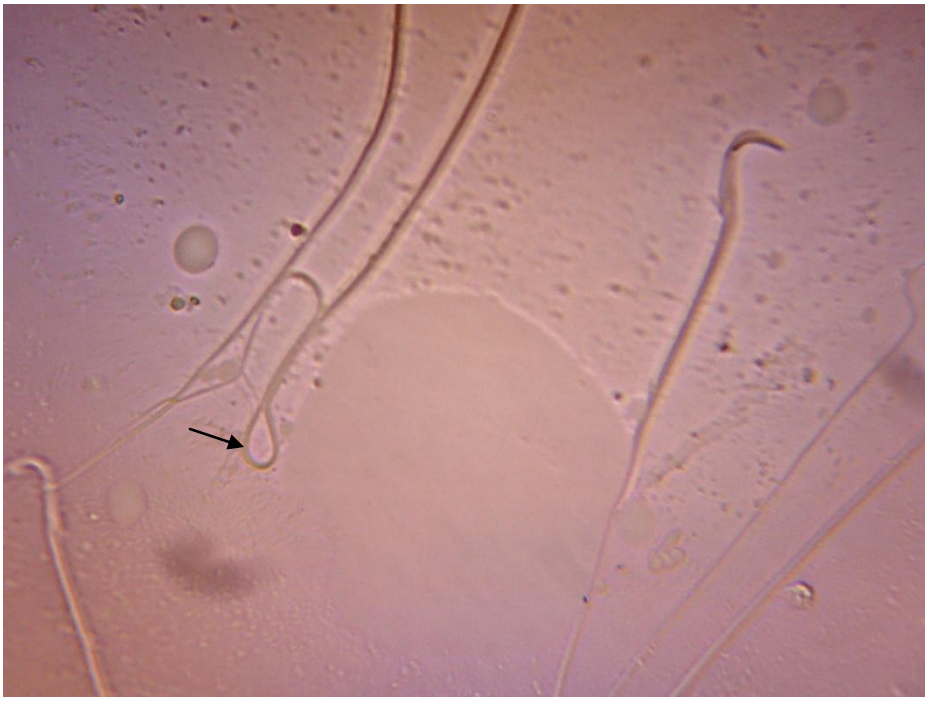


Fig.14aPhotograph of sperm with looped tail (arrow) X 1000.

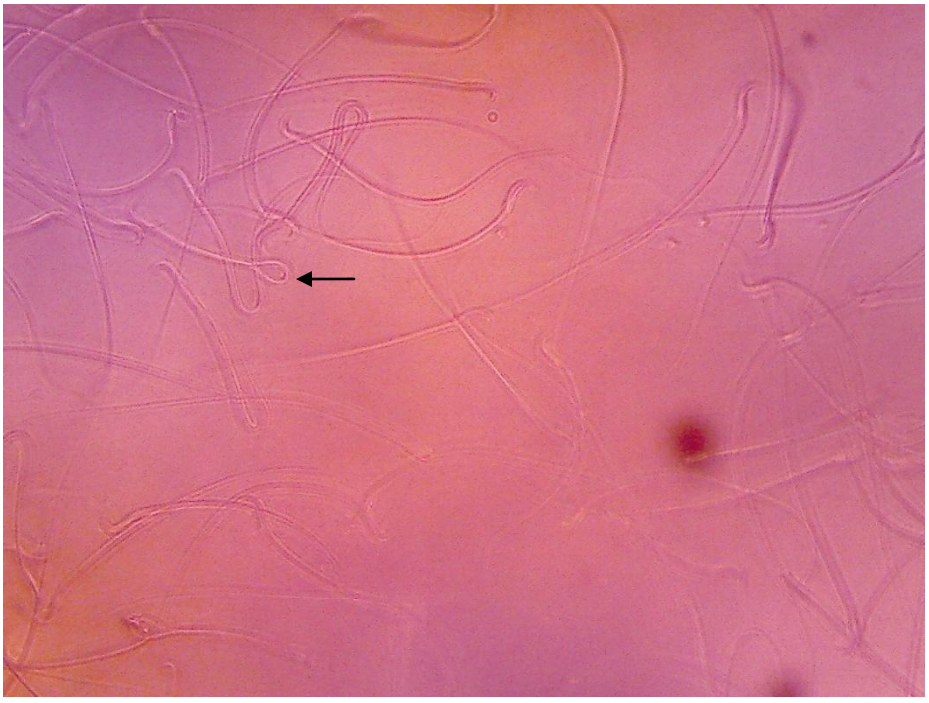


Fig.14b Photograph of sperm with looped tail (arrow) X 400.

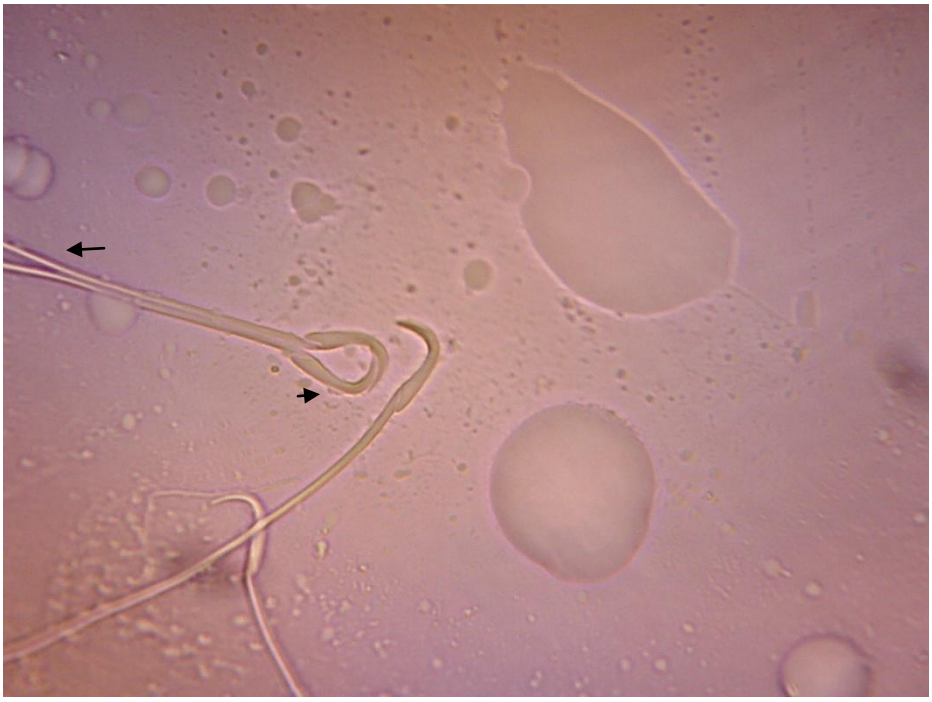


Fig.15 Photograph of sperm with double tail (arrow) and head (arrow head) X 400.