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Ultra Structural Alternations in Spermatozoa of Leiognathus equulus (Perciformes) from AL-Shabab Lagoon, in Jeddah Province

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Abstract: *The common ponyfish Leiognathus equulus was caught from the Red Sea and Al-Shabab lagoon. This study was carried out to investigate the ultra-structural alternations in L. equulus spermatozoa induced by the polluted water of Al-Shabab lagoon. The spermatozoa was of type I, which is typical of species of external fertilization. It appears that each spermatozoon possesses kidney shaped head, mitochondria and underdeveloped middle piece. However, fish caught from Al-shabab lagoon shows variable deformations in sperm including changes in head morphology, malformed mid piece, fusion of head sperm*.

Key words: *Common ponyfish., Histopathology., Red Sea., Al-Shabab lagoon., Sperm., Ultra structural abnormalities.*

I. Introduction

Leiognathids, commonly known as ponyfishes, are characterized by a strongly protrusible mouth. The family is distributed in coastal and estuarine waters of the Indo-West Pacific (Nelson, 2006) and is commercially important in Asian wild fisheries and aquaculture as a fresh or processed product, with wild catch rates in the west central Pacific of 120, 268 tons per annum in the mid 1990s (Woodland, *et al.*, 2001).

Water pollution affects the ecological conditions which usually affect the biological and physiological conditions of the fish, especially reproduction, which is reported to be affected seriously (Santiago, *et al.*, 1985).

Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment having led various deleterious effects on the aquatic organisms (McGlashan and Hughies, 2001). Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly via the food chain (Sasaki, *et al.*, 1997).

Ultra structural changes in cells, tissues or organs can afford good biomarkers of pollutant stress. Using of histopathological changes as a biomarker has the benefit of permitting researchers to examine specific target organs and cells as they are affected by exposure to environmental chemicals (Salamat and Zarie, 2012).

Water quality and pollution of aquatic body is likely to have adverse impact on fish sperm as revealed from some earlier studies. It is worthwhile to mention here that sperm motility in some fish was reported to be very low in acidic pH of water (Kime and Tvieten, 2002). Similarly, a recent study has reported a number of abnormalities in sperm surface ultrastructural morphology in some fish exposed to acidic pH of water (Dey, *et al.*, 2009). Fine structural abnormalities have also been reported in some fish species inhabiting a polluted lake in Egypt (Abdelmeguid, *et al.*, 2007).

It is to be noted in this context that despite a large number of published works in the existing literature about sperm ultra structure, very few reports exist on the same topic with reference to environmental stress and pollution (Dey, *et al.*, 2009 & Psenicka, *et al.*, 2008).

Deformation in sperm and Sertoli cells structure were detected of Japanese eel, Anguilla japonica (Miura, *et al.*, 2005); Yiannis, *et al.* (2000) reported that there were reduction in number of mature sperm cells and lobular fibrosis of testis of Japanese medaka, Oryzias latipes.

Saad and Fahmy (1994) recorded four main pollution sources at Jeddah coast: the untreated domestic sewage wastes, oil pollution from oil refinery of factory Petromin, fish wastes from the big fish market of Bankalah region and probably desalination plant effluents.

This study aimed to evaluate the occurrence of ultrastructure alternations in spermatozoa of *L. equulus*. It is expected that this study will contribute to our knowledge useful information for providing a basis on which future monitoring of the pollutants or assessment of the fate of environment in this area can based.

II. Materials And Methods:

Fish samples:

Fish samples in the present work were obtained from the Red Sea waters in front of Jeddah (178 fish) and from Al-Shabab lagoon in Jeddah (226 fish). The fish were transported to the laboratory in ice aquarium.

The fish were weighted to the nearest gram. Total, forked and standard length were measured to the nearest cm .

Directly after recording the length and weight, the fish were dissected to determine sex and maturity stage. The testes were removed, weighed and thoroughly examined.

Electron Microscopy Technique:

Immediately after dissection of the fish, the testes were cut several parts. Those assigned for the ultra structural studies were fixed in 2% gloter aldehyde in 0.2 M sodium cacodylate buffer pH 7.2.

1. Transmission Electron Microscopy (TEM):

1.1 Dehydration And Embedding:

The tissues were dehydrated in ascending series of ethanol and embedded in epoxy resins.

1.1 Sectioning And Staining:

For light microscopy, semithin sections (0.5-1 um) were cut on a JEOL JUM-7 ultra microtome with glass knifes and stained with 1% toluidine blue in 1% sodium borate.

Ultrathin sections (70 - 90 nm) were placed on 200- mesh copper grids. The sections were stained with uranyl acetate (saturated in 70% ethyl alcohol) followed by lead citrate and examined with PHILIPS CM 100 transmission electron microscope at 60 kv at King Fahd Medical Research Center (Electron Microscope Unit).

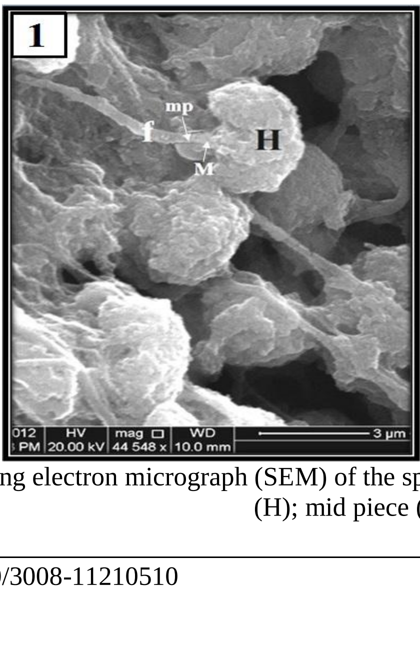
2. Scanning Electron Microscopy (SEM):

Samples were fixed in Trump's fixative from half to one hour, after which samples were rinsed three times in distilled water (10 min each), fixed in Osmium Tetra oxide (OsO_4) (1 hour) , rinsed twice in distilled water (1min each), 50 % Ethanol 1 min , 100 % Ethanol (10 min), ethanol : acetone (1:1) for 10 minute, pure acetone three changes (10 minutes each), Acetone: Hexamethyldisilazane (1:1) for one hour, pure Hexamethyldisilazane also one hour and remove the Hexamethyldisilazane and was put the sample under vacuum (10 min (H.P) or Overnight (L.P) then critical –point dried and gold coated. Preparation were viewed in a QUANTA FEG 450 at Faculty of Science Biology Department King Abdul-Aziz University (Electron Microscope laboratory).

III. Results

Ultra Structure Of Non-Polluted Spermatozoa:

The sperm of *L. equulus* is a typical anacrosomal aqua sperm, differentiated into head, a short mid piece, and cylindrical tail (Fig. 1). The head is ovoid with the lateral axis greater than longitudinal axis. The nucleus is kidney shaped in longitudinal section measuring 1.386 µm ± 0.064. It is also tilted relative to the axoneme. Chromatin is very coarsely granular and condensed. A cytoplasmic collar, which extends around the base of the flagellum, is present and it is separated from the flagellum by a periaxonemal space, the cytoplasmic canal. Two Spherical mitochondria (0.530 µm ± 0.070 in diameter) are present and they are situated in the cytoplasmic collar. The flagellum is parallel to the base of the nucleus and a depression is present at this point, the nuclear fossa. The mitochondrion contained an electrons–dense matrix and has irregularly arranged cristae; it is separated from by an invagination of the plasma membrane (Fig. 2). A cytoplasma membrane surrounding the whole structure is also present (Fig. 1 & 2). The proximal and distal centrioles formed the centriolar complex which is short and located inside the nuclear fossa (Fig. 1). The flagellum, which has a cylindrical shape throughout its length measures 2.725 µm and is inserted perpendicularly and eccentrically with



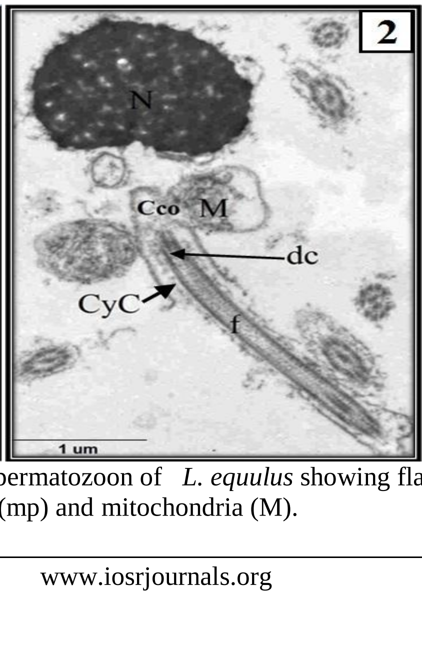


Figure 1: Scanning electron micrograph (SEM) of the spermatozoon of *L. equulus* showing flagellum (f); head (H); mid piece (mp) and mitochondria (M).

Figure 2: Transmission electron micrograph (TEM) of *L. equulus* sperm showing cytoplasmic canal (CyC); cytoplasmic collar (Cco); distal centriole (dc); flagellum (f); mitochondria (M) and nucleus (N).

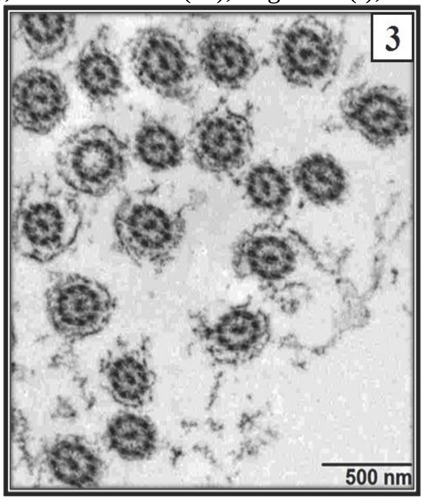


Figure 3: Transmission electron micrograph (TEM) of axoneme of non-polluted *L. equulus* sperm showing a typical eukaryotic organization, consisting of nine outer doublet microtubules and a central pair of doublet microtubules 9+2 pattern. respect to lateral and longitudinal axis of ovoid nucleus (Fig. 1 & 2). The axoneme showed a typical eukaryotic organization, consisting of nine outer doublet microtubules and a central pair of singlet microtubules 9+2 pattern (Fig. 3).

Ultra Structure Of Polluted Spermatozoa:

In fish collected from polluted water, the absence of differentiation of head with mid piece is evident (Fig. 4). Vacuolization is observed in sperm head (Fig. 4). Further, the size of sperm head (1.130 µm ± 0.096) is found to be less than that of non-polluted ones (1.386 µm ± 0.064) (Table 1). On the other hand, the most obvious alternations observed as complete absence of mid piece and mitochondria (Fig. 5). Longitudinal section of sperm tail reveals dilation in membrane and defected axoneme (Fig. 4). On the other hand, the sperm tail is shorter in length (2.053 µm) than that of non-polluted spermatozoa (2.725 µm).

As an expected result, the sperm of fish collected from Al-Shabab lagoon revealed variable degrees of deformation, including changes in head morphology. Also, the mid piece appeared highly deteriorated and its mitochondria disappeared.

Morphologically changed spermatozoa were observed in males. Sperms with forked nuclei and short tail with dilation of the membrane (Fig. 6).

A remarkable increase in sperm abnormalities in fish collected from Al-Shabab lagoon. Among the commonest head anomalies was the deformed head shape in which agglutination of sperm in head-to-head. Also, degenerated middle piece and the lateral lobes are irregular in shape and some of the mitochondria showed signs of distortion (Fig. 7).

Morphological assessment of sperm revealed abnormal head location and its nucleus located lateral to the flagellum resembling type II spermatozoa. Also, degenerated of proximal centeriol, undulating plasma membrane and deformed axoneme (Fig. 8).

Table (1) illustrates the measurement of both non-polluted and polluted spermatozoa of *L. equulus*. It is obvious that there were decreasing in the diameter of head, mitochondria and mid piece and length of tail of polluted spermatozoa (about 1.130 µm ± 0.096, 0.512 µm ± 0.072, 0.329 µm ± 0.083 and 2.053 µm respectively).

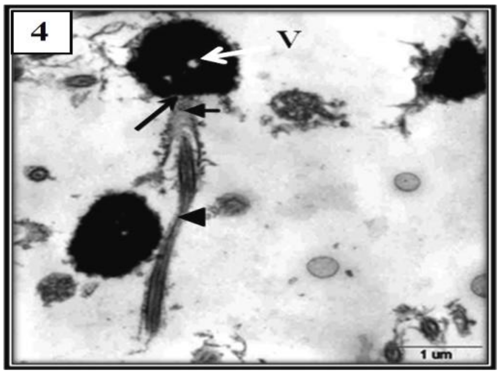


Figure 4: Transmission electron micrographs (TEM) of sperm of polluted *L. equulus* showing abnormal spermatozoa having elliptical head, absence of mitochondria (short arrow); adhesion of sperm head to cytoplasmic collar (long arrow); the middle piece is completely absent and axonemal defect (arrow head) and vacuoles (V).

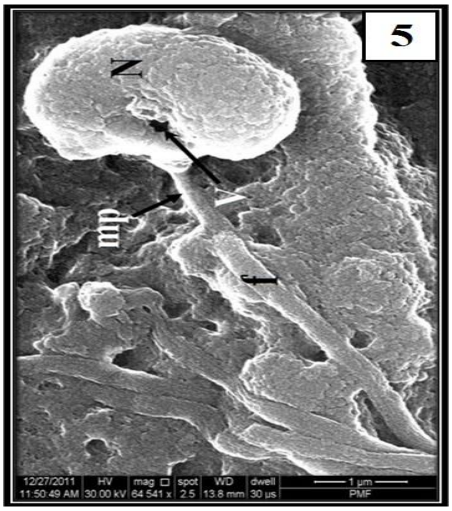


Figure 5 : Scanning electron micrograph (SEM) of sperm of polluted *L. equulus* showing abnormal granulated chromatins sperm head having vacuoles (V) and absence of mitochondria; flagellum (f); mid piece (mp) and nucleus (N).

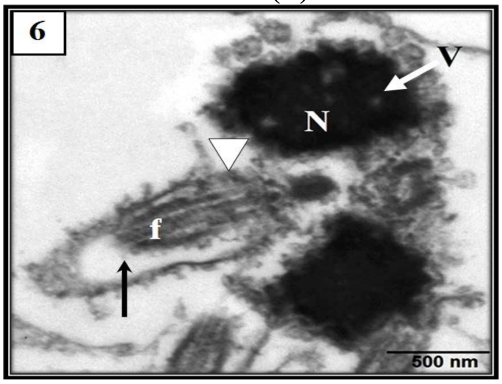


Figure 6: Transmission electron micrographs (TEM) of sperm of polluted *L. equulus* showing abnormal chromatin content and distribution of most sperm head, sperm exhibiting forked nucleus (N; arrow); neck with absence of mitochondria (arrow head); shortage of the tail and dilation of plasma membrane of the tail (f; black arrow) and vacuoles(V).

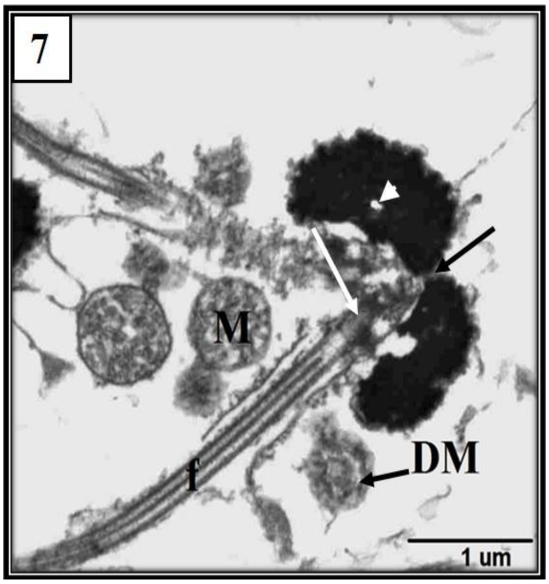


Figure 7: Transmission electron micrographs (TEM) of sperm of polluted *L. equulus* showing abnormal sperm with head-to-head agglutination (black arrow); degenerated mid piece separating from sperm head (white arrow); distorted mitochondria (DM); flagellum (f) and mitochondria (M).

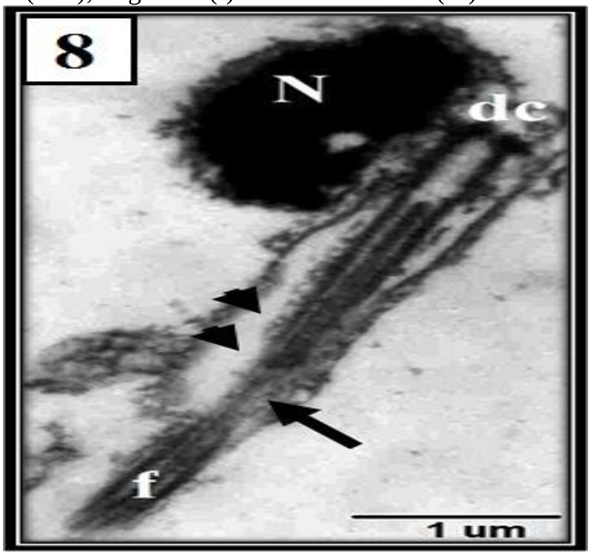


Figure 8: Transmission electron micrographs (TEM) of sperm of polluted *L. equulus* showing malformed sperm with a nucleus (N) located lateral to the flagellum (f) and distal centriole (dc) positioned outside the nucleus; axonemal defect (arrow) and absence of mitochondria; dilation of plasma membrane of the tail (arrows head).

Table 1: Spermatozoa measurements of non-polluted and polluted of *L. equulus*

Diameter of sperm organelle	Non-polluted		Polluted	
	Mean	**Standard deviation**	**Mean**	**Standard deviation**
Head diameter	1.3856 µm	± 0.0644	1.1304 µm	± 0.0957
Mitochondria diameter	0.5302 µm	± 0.0704	0.5123 µm	± 0.0719
Mid piece diameter	0.4277 µm	± 0.1898	0.3294 µm	± 0.0826
Tail length	2.7246 µm	---	2.0526 µm	---

While in non-polluted spermatozoa these organelle had a measurements of 1.386 µm ± 0.064, 0.530 µm ± 0.070, 0.428 µm ± 0.19 and 2.725 µm respectively.

IV. Discussion

The study area receives different pollutants from four sources: untreated domestic sewage wastes, oil from the oil refinery, fish wastes from the big fish market (El-Bangalah) and desalination plant effluents which contribute both organic and metallic contaminants (Behairy and Saad, 1984; Basaham, *et al.*, 1990 & Mohorjy and Khan, 2006).

The basic task of the spermatozoon head is to transfer genetic material localized in the nucleoplasma to the egg. Hence, an optimal shape and size of sperm head is a prerequisite for proper penetration of spermatozoon through the micro pyle of egg (Psenicka, *et al.*, 2007).

On that consideration, the dislocation of sperm head, and breakage of sperm tail at places as observed in the present study are likely to cause adverse effects on functioning of the sperm of the fish inhabiting the polluted lagoon.

Ultra structural study of the testis of *L. equulus* revealed that abnormality results in atrophy of nuclear material, degenerative changes in the tail with discontinue filament and appearing of empty mitochondria. Similar features were reported by Zutshi and Murthy (2001) in gobiid fish.

Poor differentiation of head and neck of the sperm, shortening of sperm tails and abnormalities in the shape of sperm head in some of the fish collected from polluted lagoon, as revealed in the current study suggests that the pollutants contaminating the lagoon has serious adverse effects on the male reproductive unit of the fish. Since the sperm tail is primarily responsible for motility of sperm, the present observation on shortening of the sperm tail as well as its abnormal structural features in some of the sperm appears to be relevant to the poor sperm motility and consequent failure in fertilization. These findings are similar to observation of Massar, *et al.*, (2011).

The distortion of mitochondria in the lateral lobes of neck in many of the sperm of fish inhabiting the polluted lagoon suggests disturbances in energy release required for sperm movement of the fish (Massar, *et al.*, 2011). In some affected specimens, the mitochondria showed signs of disintegration and disorganization. Similarly, Au, *et al.* (2000) indicated that exposure to toxicants changed the size and shape of the mid piece of spermatozoa of mussel and sea urchin, and this might affect the balance of spermatozoa in their swimming. They also reported disorganization of mitochondria supply for sperm movement.

The present observation thus suggests that the fine structural components of the sperm of *L. equulus* have been adversely affected due to the pollutants present in Al-Shabab lagoon. These fine structural abnormalities are bound to cause physiological and functional disturbances in the sperm, leading to reproductive inefficiency.

The present study demonstrated occasional occurrence of sperm agglutinations in the polluted groups. The spermatozoa were agglutinated in a head-to-head manner, which invariably makes them fail to fertilize the egg as reported by Mochida and Takahashi (1993) and Abdelmeguid, *et al.* (2007).

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