International Journal of Research in BioSciences
Volume 10 Issue 1, pp. (31-37), January 2021
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Cytogenetic effect of agro-pesticides on sperm shape abnormality in *Channa punctatus* in vivo

Sarangi Pradipta Kumar
Department of Zoology, Indira Memorial College, Chandiput, Gajapati, ODISHA-761017, INDIA

(Received September 29, 2020, Accepted December 24, 2020)

Abstract

The objective of this study was to determine the associations of cytogenotoxic effect of agro pesticides on sperm head morphology. The quality of sperm from *Channa punctatus* fish was assessed to determine as a suitable indicator of the effects of pollution in freshwater. The fish was exposed in vivo to three different concentrations (MC,MC/2&MC/5) of eight pesticides (Dimethoate, Dichlorovos, chlorpyriphos and Malathion, Methylparathion, Fenvalerate, Cypermethrin and Cabaryl) belongs to Organophosphates, pyrethroid and Carbamate group in different time periods (5,10,15,20,25days). Sperm head analysis and tail used as simple bioindicator for monitoring aquatic pollution in *Channa punctatus* subjected to breeding soundness evaluation were designated as unsatisfactory solely on the basis of sperm morphology highlights its importance. Though insufficient data refrain us from significant statistical analysis but will play significant role in further research of cytogenotoxic and teratogenic end point of aquatic pollutants on male reproductive physiology in this species.

Keywords: genotoxicicity, cytotoxicity, teratogenetic, germ Cells, Pesticides, Semen, Sperm head analysis; sperm quality.

Introduction

The increased use of pesticides is necessary for better crop production[1-3] since the Green Revolution of the 1960s, hence it introduced new hazards to human being and animal[4.] A broad spectrum of pesticides are extensively being used in agriculture to enhance production[5], minimize losses, protect food grains from fungal contamination, repel ecto-parasites, control vector borne diseases, repel household pests and as anti-helminthes[6] with limited guidelines and restrictions. More and more use of pesticides in agricultural practices has resulted in contamination of food and food resources. These toxic chemicals influence the physiology of the numerous non-target species including man[7]. A number of animal species included humans have accumulated traces of pesticides through food chain or by occupational exposure[8] Genetic abnormalities, including sperm abnormality, chromosome aneuploidy as well as structural aberrations, are one of the major causes of infertility. The effects of aquatic pollution on the reproductive endocrine system of fish are well documented[9], but there have been very few studies of its effects on gamete quality. Computer Assisted Sperm Analysis (CASA) has been used for some years to assess the motility of mammalian sperm and during the last few years has been applied to fish[10,11].

Methods such as the measurement of the concentration of spermatozoa in the milt[12]. Allow an estimation of the whole spermatozoa population without taking into account the individual spermatozoon status. Thus, techniques that focus on features of individual sperm cells, such as motility and morphology, should be fundamentally more discriminatory than bulk measurements of the whole milt. Teleost sperm is characterized by the absence of an acrosome, which contrasts with mammalian sperm, although an active acrosome is present in Acipenseri form fish[13], The shape of the head and the nucleus is highly variable between species[14-17] and the mid-piece containing a few mitochondria is either well developed (guppy) or reduced (salmonid, cyprinid)[18,19]. Some internally fertilizing fish like the ocean prout have biflagellate spermatozoa[20,21] while in general fish with external fertilization have a simple flagellum, although some biflagellate spermatozoa have been reported in channel catfish (*Ictalurus punctatus*)[22], So far there is no evidence that longer sperm achieve faster swimming velocities[23]. However, sperm deformities are associated with functional deficiencies and cause reduced motility and fertilization ability. As an example, fish sperm directly exposed to Hg2 + ions is characterised by broken tails[24] and reduced motility and fertilising capacity[25]. Exposure of zebra fish (*Danio rerio*) to tributyltin (TBT) during a critical period in early life resulted in the production of sperm lacking flagella at sexual maturity[26].

Now a days agro-pesticides are the main cause of cytotoxic, genotoxic and teratogenic[27-29,] enter into natural water destroying spawning ground, feeding area and reproductive capacity in fish. *Channa punctatus* is one of the most popular edible teleost fish in many parts of India and Bangaldesh[30] is now one of the most endangerd fish species too. Sperm morphology has been identified as a characteristic that can be used to predict a male's semen quality. The overall aim of the work is to provide a simple non-invasive assessment of the effects of aquatic pollution on fish fertility using sperm shape morphology of fresh water live fish *Channa punctatus*.

Materials and Methods

Table 1: List of pesticides used in the present study along with their LC50, MC, MC/2 and MC/5 concentrations

Pesticide (Trade name)	Manufacturer	LC50 (inµg/liter)	MC/2 (inµg/liter)	MC/2 (inµg/liter)	MC/5 (inµg/lite)
Dimethoate (Roger-30E)	Rallies India Ltd.,21,D,Sukhadev Marg,Mumbai-400001,India.	100	50	25	10
Dichlorovos (Nuvan)	Hinustan Ciba-Geigy Limited,14.J.Tata Road,Mumbai-400020,India.	500	250	125	50
Chlorpyriphos (Tafaban-20E)	Rallies India Ltd.,21,D,Sukhadev Marg,Mumbai-400001,India	10	5	2.5	1
Methyl Parathion (Metacid-50)	All India Medical Corporation, 185, Princess Street,P.B.No.239,Mumbai,India.	300	150	75	30
Malathion (Mal-Tox)	All India Medical Corporation, 8thRoad, Akhand Jyoti Building, SantaCruez East Mumbai-400020,India.	250	125	67.5	25
Fenvalerate (Sumicidin)	Rallies India Ltd.,21,D,Sukhadev Marg,Mumbai-400001,India	250	125	67.5	25
Cypermethin (Polytren-20E)	Solar FARMACHEM Ltd.Sorodhi, Valsad, Gujarat.	10	5	2.5	1
Carbaryle (Sevin)	Rallies India Ltd.,21,D,Sukhadev Marg,Mumbai-400001,India	250	125	67.5	25

Doses and route of exposure

From among the specimens acclimatized for at least a fortnight in the laboratory aquaria, only strong and active fishes were released into different aquaria containing pesticides correspond to LC50,MC, MC/2 and MC/s doses respectively as per table-1. MC represent the maximum tolerable concentration of the test compound at which no death of animal beyond 5% was observed during the period of treatment and was determined from preliminary experiments on groups of 20 specimens in aquaria containing 100 litre of water. The test lasted for 25 days with change of water, chemical and food every alternate day. The lowest concentration leading to 50 % death after the treatment was considered as LC50 and half of this corresponds of MC. MC/2 and MC/5 represent 1/2 and 1/5 of MC. The treated specimens received an intramuscular injection of 0.02% colchicine solution at the rate of

1ml per 100 mg body weight 2 h prior to their sacrifice on completion of 5,10,15,20 and 25 days of exposure to the test chemical.

Sperm Shape analysis

The best technique for cytological preparation of germ cells is those of [31], with few modifications the method. We used in this study, good sperm head preparations could only be obtained after teasing out the testis of sexually mature healthy specimens of *Channa punctatus* in normal saline(2.24% tri-sodium citrate)prepared the cell suspension and making a squash preparation, fixed the slides in 1:3 aceto-alcohol for one hour followed by staining in 2% Giemsa for 15 minutes. Wash in deionised water to remove excess stain, dry the slides and used for the examination of sperm head and tail abnormalities under Zeiss-Jana microscope in 40x lens and microphotographs taken under 100xlens by overhead camera. For each animal 500 hundred sperms were scored[32].

Results and Discussion

Ever since the development of technique for cytological preparation of germ cells of mouse by[31,] the sperm head abnormality as a mutagenecity testing protocol has received the attention of large number of workers to analyse the genotoxicity of a variety of toxicants. According to[33,34], the sperms are the important cells in the reproductive toxicology which could be used in assessing spermatogenic damage and fertility effect not only treated specimens but also in F_1 and F_2 progeny in view of this, efforts we made to develop sperm head abnormality protocol employing *Channa punctatus* as test model to evaluate the genotoxic potentiality of water-borne pollutants. The results obtained were encouraging and similar to those described by[35,] in fresh water Cichilied species of tilapia *Oreochromis mosambicus*. However technique used by[35] did not yield satisfactory results. Morphological analysis from control groups showed that sperms with normal histological structure(Figure 1.a)but in treated groups enlarged and multi nucleated head and biflagellated sperms were found (Fig.1.a,b,c)Sperm morphology is still a standard laboratory analysis in diagnosing infertility in men[36]. The germ cells determine the fertility potential of an individual because they form the spermatozoa. Failure of the germ cell to survive during the development may lead defective or no gamete production and hence can lead to infertility[37].

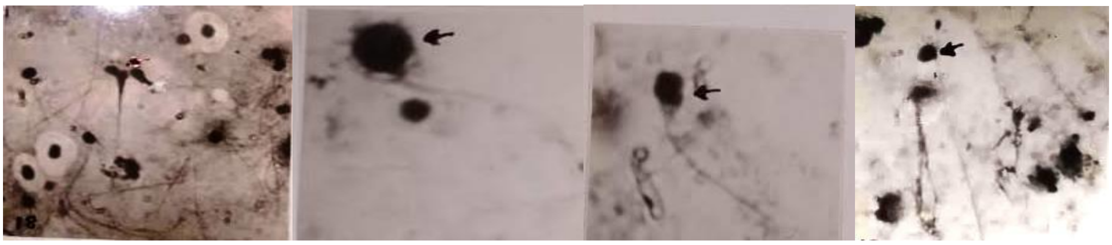

Figure 1: a. Normal spermatozoa b. Enlarged nucleated spermatozoa c. Multinuclear headed spermatozoa d. Biflagellated spermatozoa

The pycnotic nuclei, chromolysis, vacuoles of various shapes and sizes were observed in the cytoplasm of germ cells and somatic cells on exposure of Endosulphan[38]. Chlorpyrifos cause accumulation of exfoliated germ cells within the affected tubules and appearance of cytoplasmic vacuolation[39-41]. The Methyl parathion also caused a pronounced cytoplasmic vacuolization and pycnosis in germ cells[42-44,] demonstrated the adverse effects of dimethoate on the reproductive performance of male mice. The sperm viability, motility and density were reduced in dimethoate treated mice. Pyrethroid[45] Deformed and disordered arrangement of germ cells was observed after exposure of Cypermethrin[46] and Malathion[47,48] Sloughing off germ cells was recorded in many experiments after exposure of different pesticides. The exposure of Endosalphan, Methyl Parathion, Carbaryl, Malathion and Cypermethrin[54] resulted in the decrease in the numbers of germ cells in testicular tissue[48-54]. Spermatogenesis is the process by which mature spermatozoa is develop from germ cell inside seminiferous tubule. Damage to the spermatozoa or their precursors can result in reversible or irreversible impaired spermatogenesis, depending on the stage of differentiation affected by the chemical. Damage to spermatogonia causes impaired sperm production and decreased fertility because of changes in the cell number, structure, motility, or viability of spermatozoa[55].

Exposure to pesticides lowers sperm levels well below the limit for male fertility[56]. Fish sperm physiology is under control of various parameters of the external milieu[57,] latter sperm cells are subjected to changes due to the different environmental conditions[58], Pesticides may operate through hormonal or genotoxic pathways to effect male reproduction[59].The Semen quality is determined by the semen volume, sperm motility and by number of normal sperm. The good quality semen enhances the chances of fertilization and semen with depleted quality are generally fail to cause the fertilization[60].

Genetic studies define a relationship between sperm abnormalities and meiotic errors[61], These errors are associated with mutations in meiosis-specific genes, such as those involved in the processes of DNA recombination and repair[62], Environmental factors are also hypothesized to result in meiotic deficiencies[63], Abnormal meiotic recombination, which causes the production of aneuploid gametes, may also give rise to different degrees of meiotic arrest many times occurring at specific stages of meiosis[64,65]. Occupational exposure of male agricultural workers to organophosphate pesticides was associated with an increased frequency of sperm aneuploidy and sex chromosome disomy[66,67].

Conclusion

Toxic pesticides are known to cause Germ cells disintegration, alternation in spermatogenesis, depletion in semen quality, teratospermia and sperm motility. These data represents as pesticides are testicular toxicant in fish, human and other laboratory animals and it has been subjected to disturbed reproductive functions. The data obtained by us is insufficient enough for statistical analysis and hence we refrain ourselves presenting in a tabular form and statistical analysis. *Channa punctatus* may be used as test model for both as a basis of laboratory cytogenotoxicity test for monitoring the reproductive viability of wild populations exposed to water born pollutants.

Acknowledgement

The author is gratefully acknowledged to the Principal, Indira Memorial College, Chandiput Gajapati, Odisha for providing all types of infrastructural facilities during the time of work and encouragement.

References

1. Ragade V.R. Kengar A., Khade B.S., Shaikh J.D., Pradhan P.S., Effects of monochrotophos pesticide on liver, gill and kidney of fresh water fish *Channa punctatus.* Trends in Fisheries research, 4(1): 2319–4758 (**2015)**
2. Babazadeh M**.,** Najafi G**.**, Effect of chlorpyrifos on sperm characteristics and testicular tissue changes in adult male rats. Veterinary Research Forum : an International Quarterly Journal, 8(4):319-326 **(2017)**
3. Rahman M.Z., Hossain Z., Mollah, M.F.A., Ahmed G.U., Effect of Diazinon 60 EC on *Anabas testudineus, Channa punctatus* and *Barbodes gonionotus* Naga. The ICLARM Quarterly 25(2): 8-12 **(2002)**
4. Briggs J., Green revolution. Int. Encycl Hum Geography**,** 634-8: **(2009)**
5. Sharma R.K., Gulati A., Malathion induced changes in catalase and superoxide dismutase in testicular tissues of goat *in vitro*. IJPBS 3(4):193-7 **(2013)**
6. Sharma R.K., Chauhan P.K, Fulia, A. Endosulphan induced changes in fine morphology of goat spermatogonia *in vitro*. Res. J. Environ. Toxicol. 4(4): 214-222 **(2012)**
7. Sharma R.K. and Goyal A.K., Agro-pesticides and andrology. Int. J. Pharm PharmSci., 6(10) 1-8 **(2016)**
8. Fulia A., Chauhan P.K., Sharma R.K., Ameliorating effect of vitamine on testicular toxicity induced by endosulphan in *Capra hircus in vitro*. J PharmacoToxicol., 6(2):133-40 **(2011)**
9. Kime D.E., The effects of pollution on reproduction in fish. Rev. Fish. Biol. Fisheries, 5: 52-96 **(1995)**
10. Kime D.E., Ebrahimi M., Nysten K., Roelants I., Rurangwa E., Moore H.D.M. and Olivia F., Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on spe m quality of fish; application to the effects of heavy metals. Aquat. Toxicol. 36: 223-237 **(1996)**
11. Rurangwa E., Roelants I., Huyskens G., Ebrahimi M., Kime D.E. and Ollevia F., Aluminium effective spermatozoa to egg ratio for artificial insemination and the effects of heavy metal pollutants on sperm motility and fertilization ability in the African catfish (*Clarias gariepinus*, Burchell 1822); J. Fish Biol. (In Press). **(1998).**
12. McMaster M.E., Portt C.B., Munkittick K.R. and Dixon D.G., Milt characteristics, reproductive perforrnance, and larval survival and development of white sucker exposed to krafLrnill effluent. Ecotox. Environ. Saf: 23: 103-117**(1992)**

13. Linhart O., Kudo S., Surface ultrastructure of paddlefish eggs before and after fertilisation. J. Fish Biol., 51: 573–582**(1997).**
14. Billard R., Cosson J., Some problems related to the assessment of sperm motility in freshwater fish. J. Exp. Zool., 261: 122–131 **(1992)**
15. Billard R., Fostier A., Weil C., Breton B., Endocrine control of spermatogenesis in teleost fish. Can. J. Fish. Aquat. Sci., 39: 65–79 **(1982)**
16. Billard R., Spermatogenesis and spermatology of some teleost fish species. Reprod. Nutr. Dev., 26: 877–920 **(1986)**
17. Jamieson B.G.M., Fish Evolution and Systematics: Evidence from Spermatozoa. Cambridge Univ. Press, Cambridge **(1991)**
18. Billard R., Cosson J., Crim L.W., Suquet M., Sperm physiology and quality. In: Bromage, N., Roberts R. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell, Oxford, 25–52**(1995).**
19. Billard R., Cosson G., Perchec G., Linhart O., Biology of sperm and artificial reproduction in carp. Aquaculture, 129: 95– 112 **(1995)**
20. Mattei X., The flagellar apparatus of spermatozoa in fish. Ultrastructure and evolution. Biol. Cell 63: 151–158 **(1988)**
21. Yao Z., Emerson C.J., Crim L.W., Ultrastructure of the spermatozoa and eggs of the ocean pout (Macroozo arcesamericanus L.), an internally fertilizing marine fish. Mol. Reprod. Dev. 42: 58– 64, **(1995)**
22. Jaspers E.J., Avault Jr., J.W., Roussel J.D., Spermatozoal morphology and ultrastructure of channel catfish, Ictaluruspunctatus. Trans. Am. Fish. Soc., 33: 475– 480 **(1976)**
23. Gageeet M.J.G., Macfarlane C., Yeates S., Shackleton R., Parker G., A Relationships between spermmorphometry and sperm motility in the Atlantic salmon. J. Fish Biol., 61: 1528– 1539, **(2002)**
24. Van Look K.J.W., Kime D.E., Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm. J. Fish Biol., 63: 1020– 1033 **(2003)**
25. Rurangwa E., Roelants I., Huyskens G., Ebrahimi M., Kime D.E., Ollevier F., The minimum effective spermatozoa: egg ratio for artificial insemination and the effects of mercury on sperm motility and fertilisation ability in *Clarias gariepinus*. J. Fish Biol., 53: 402-413**(1998)**
26. McAllister B.G., Kime D.E., Early life exposure to environmental levels of the aromatase inhibitor tributylt in causes masculinisation and irreversible sperm damage in zebra fish (Daniorerio). Aquat. Toxicol., 65: 309–316 **(2003)**
27. Deka S., Mahanta R.A., Study on the Effect of Organophosphorus Pesticide Malathion on Hepato-Renal and Reproductive Organs of *Heteropneustes fossilis* (Bloch). The Science Probe, 1(1): 1-13 **(2012)**
28. Porichha S.K., Sarangi P.K. and Prasad R., Genotoxic effect of chlorpyriphusinchannaPuctatus. Perspectives in cytology and Genetics 1998. 9: 631-638. (Eds. G. K.Manna and S. C. Roy, AICCG Publi. Kalayani University**) (1998)**
29. Sarangi P.K., Patnaik R., Porichha S.K., Prasad R., Genotoxicity of malathion in *Channa punctatus* cultured in vivo Perspective in Cytology and Genetics, 2001, 10:835-844 (Editors G. K. Manna and S. C. Roy, AICCG-3Publication, Kalyani University). (**2001)**
30. Ashaduzzaman Huda M.D., Rahman M.A., Hossain M.A., Neela F.A., Sultana,S., Akhtar S. and Habiburahman M., Effect of Cabofran on haematological parameters of *Channa punctatus* (Bloch) Int. Jorn. of Pure and Applied Zoology, 4(3): 282-288**(2016)**
31. Evans E.P., Breckon G. and Ford, C. E. An air-drying method for meiotic preparations from mammalian testes. Cytogenetics, 3*:* 289 **(1964).**
32. Jeong S.H., Kim B.Y., Kang H.G., Ku H.O., Cho J.H., Effects of butylated hydroxyl-anisole onthe development and functions of reproductive system in rats. Toxicology, 208: 49-62 **(2005)**
33. Wyrobek A.J., Watchmaker G., Gordon L., An evaluation of sperm tests as indicators of germ-cell damage in men exposed to chemical or physical agents. Teratogen Carcinogen and Mutagen, 4(1): 83-107 **(1984)**
34. Rahiman M.A., Induction of microncles and sperm shape abnormality as indicator of mutagenicity of environmental chemicals. Proceedings of Environment and Pesticide Toxicity (Academy of Environmental Biology, India) 41-45 **(1987**)
35. Manna G.K. and Biswas S., Induction of abnormal sperm head in tilapia fish as a means of testing genotoxic potentiality of the bacterium, Pseudomonas aeruginosa. La Kromosomo II. 51-52: 1659-1664 **(1988)**
36. Syahputra M.F., Chairani R., Seniman, Rahmat R.F., Abdullah D., Napitupulu D., Setiawan M.I., Albra W., Erliana C.I., Andayani U., Identification Male Fertility Through Abnormalities Sperm

Based Morphology (Teratospermia) using Invariant Moment Method. Journal of Physics Conference Series, 978, 10.1088/1742-6596/978/1/012107**(2018)**
37. Sharma R.K., Bhat R.A., Goyal A.K., Germ cells apoptosis during spermatogenesis in mammals. Int. J. Pharm. PharmSci. In Press. **(2014)**
38. Sharma R.K, Fulia A., Chauhan P.K., Protective effect of ascorbic acid against Endosulphan induced testicular toxicity in goat *in vitro*. J. BiolSci., 10(7): 624-630 **(2010).**
39. Reham Z.H., Protective role of black berry juice against hepatotoxicity and reproductive toxicity of chlorpyrifos in male rats. Biosci Biotechnol Res. Asia., 10(2): 961-71 **(2013)**
40. Abdelaziz K.B., El Makawy A.I., Elsalam A.Z. E.-A. A., Darwish A.M., Genotoxicity of Chlorpyrifos and the Antimutagenic Role of Lettuce Leaves in Male Mice. Comunicata Scientiae, 1(2): 137-145 **(2010)**
41. Joshi S.C., Mathur R. and Gulati N., Testicular toxicity of chlorpyrifos (anorganophosphate pesticide) in albino rat. Toxicol. Ind. Health, 23: 439-444 **(2007)**
42. Sharma R.K., Goyal A.K., Thareja K., Bhat R.A., Effect of nano molar concentration of methyl parathion on goat testis. Int. J. Pharm. PharmSci., 6(2):200-202 **(2014)**
43. Sayým F., Histopathological effects of dimethoate on testes of rats. Bulletin of Environmental Contamination and Toxicology, 78: 479-484 **(2007)**
44. Farag A.T., Ahmed F., Aswad E. and Shaaban N.A., Assessment of reproductive toxicity of orally administered technical dimethoate in male mice. Reprod. Toxicol., 23: 232-238 **(2007)**
45. Yao K.W., Wang J.D., Progress in studies of the male reproductive toxicity of Pyrethroid insecticides. Zhoghua nan ke Xue. Ma, 14(3): 268-71 **(2008)**
46. Fang L.Y., Chen P., Xia H.J., Jing L., Chun X.L., Effects of cypermethrin on male reproductive system in adult rats. Biomed Environ Sci., 26(3): 201-8 **(2013)**
47. Eduardo B.O., Patricio G.H., Effect of a single dose of malathion on spermatogenesis in mice. Asian J Androl., 5:105-107 **(2003**)
48. Choudhary N., Goyal R., Joshi S.C., Reproductive toxicity of endosulfan in male albino rats. Environ. Contam. Toxicol*.,* 70: 285–289 **(2003)**
49. Mohamed S.A., El-Gerbed, Histopathological and ultrastructural effects of methyl parathion on rat testis and protection by selenium. J Appl Pharm Sci., 3(8):53-63 **(2013)**
50. Meltem U., Yusuf K., Kerem D., Suna K., Ayse O. and Fatma B., Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E. Pesticide Biochemistry and Physiology, 87:115-122 **(2007)**
51. Esmail F., Seyed G.A.J. and Mossa G., The effect of Carbaryl on the pituitary-gonad axis in male rats. Iran J Reprod Med., 10(5):419-24**(2012)**
52. Wyrobek A.J., Watchmaker G. and Gordon L., Sperm shape abnormalities in carbaryl- exposed employees. Environ. Health Perspect., 40: 255-265. (1981).
53. Rani A., Sahai A., Srivastava A.K., Rani A., Carbaryl induced histopathological changes in the testis of albino rats. Journal of the Anatomical Society of India, 56: 4-6**(2007)**
54. Eduardo B.O., Patricio G.H., Mice testicular damage elicited by Malathion. Int. J. Morphol., 21(2):155-9 **(2003)**
55. Giewereman A., Bonde J.P., Declining male fertility and environmental factors. Endocrinol Metabclin. North Am., 27(4): 807-30 **(1998)**
56. Mathur N., Pandey G., Jain G.C., Pesticides: A review of the male reproductive toxicity. Journal of Herbal Medicine and Toxicology, 4(1): 1-8 **(2010)**
57. Cosson J., Sperm Physiology: Structure, Factors Regulating Motility, and Motility Evaluation Recent Developments in Fish Biology Fish 1-27DOI: http://dx.doi.org/10.5772/intechopen.85139. **(2019)**
58. Fauvel C., Suquet M., Cosson J., Evaluation of fish sperm quality Journal of Applied Ichthyology. 26(5): 636–643**(2010)**
59. Perry M.J., Effects of environmental and occupational pesticide exposure on human sperm: a systematic review Human Reproduction Update, 14(3): 233–242 **(2008)**
60. Owen D.H., Katz D.F., A review of the physical and chemical properties of human semen and the formulation of a semen simulant. J Androl, 26(4):459–69 **(2005)**
61. Sarrate Z., Blanco J., Anton E., Egozcue S., Egozcue J., Vidal F., Fish studies of chromosome abnormalities in germ cells and its relevance in reproductive counseling. Asian J Androl., 7: 227–236 **(2005)**
62. Baarends W.M., van der Laan R., Grootegoed J.A., DNA repair mechanisms and gametogenesis. Reproduction, 121: 31–39 **(2001)**

63. Mroz K., Hassold T.J., Hunt P.A., Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors. Hum Reprod 14: 1151–1156**(1999)**
64. Martin R.H., Meiotic chromosome abnormalities in human spermatogenesis. ReprodToxicol, 22: 142–147**(2006)**
65. Egozcue J., Sarrate Z., Codina-Pascual M., Egozcue S., Oliver-Bonet M., Blanco J., Meiotic abnormalities in infertile males. Cytogenet Genome Res., 111: 337–342**(2005)**
66. Recio R., Robbins W.A., Borja-Aburto V., Moran-Martinez J., Froines J.R., Hernandez R.M., Organophosphorous pesticide exposure increases the frequency of sperm sex null aneuploidy. Environ. Health Prospect, 109: 1237–1240 **(2001)**
67. Padungtod C., Hassold T.J., Millie E., Ryan L.M., Savitz D.A., Christiani D.C., Sperm aneuploidy among Chinese pesticide factory workers: scoring by the Fish method. Am. J. Ind. Med., 36: 230–238 **(1999)**
68. Bustos-Obregon E., Ricardo H.B., Ecotoxicology and Testicular Damage (Environmental Chemical Pollution): A Review. Int. J. Morphol. 26(4): 833-840 **(2008)** http://dx.doi.org/10.4067/S0717-95022008000400009**.**
69. Obregn E.B., Hormazabal P.G., Effect of a single dose of malathion on spermatogenesis in mice. Asian J. Androl., 5: 105-7**(2003)**
70. Rurangwaa E., Kimeb D.E., Ollevier Nash J.P., The measurement of sperm motility and factors affecting sperm quality in cultured fish Aquaculture, 234: 1-28 **(2004)**