International Journal of GEOMATE, Dec., 2016, Vol. 11, Issue 28, pp.2857-2861
Special Issue on Science, Engineering and Environment, ISSN: 2186-2990, Japan

COMPARATIVE STUDY OF MORPHO-HISTOLOGY OF OVARIES OF WT (HDRR) AND P53 (-/-) OF MEDAKA (*ORYZIAS LATIPES*)

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ABSTRACT: In the present paper comparative study of morpho-histology of ovaries of both WT (HdrR) and P53 (-/-) of medaka (*Oryzias latipes*) has been done. The ovaries of adult are paired and oval-rounded in shape and are situated ventrolaterally. Histologically the ovaries consist of follicles with various stages of development, such as primodial follicle, large follicle and growing follicle. Also, the ovaries can be differentiated into outer functional regions and inner non-functional regions. The histological structure of the ovary in both strains fishes represents the generalized teleostean structure with some differences in follicles count and distribution. We conclude that, the difference recorded in the present study has an impact on the maturation inside and eggs release from the ovaries.

Keywords: Compartive, Ovary, HdrR, P53 (-/-), Oryzias latipes

1. INTRODUCTION

Although, medaka is an important fish in japan and recently used as animal model in research, little is known about the histology structure of the different strains. From this start point, we begins to do an investigation for all of the body organs in details with highlights on the difference between the two main strains HdrR and P53 (-/-) of *Oryzias latipes*. Shima and Mitani [21] have been reported that many reports attest to the power and promise of the medaka for embryological studies. It is exciting that the medaka has also come of age as a fully developed model system for evolutionary studies, combining rapidly increasing genomic information with valuable inbred lines and highly divergent wild populations [21].

Recently, many published articles were focused on medaka as a developmental and genetic model system [9, 26]. Also, Shima et al., [20] has been described the current state of medaka genomic analysis [20] and medaka genetic mapping was studied [16]. The popularity of medaka among developmental biologists has led to important insights into vertebrate development [26].

It has been well established that inbred medaka strains display differences in numerous quantitative traits, such as body shape, brain morphology, behavior, reproductive rate, life span and susceptibility to chemical or radiation-induced tumorigenesis [9, 7, 8]. Therefore, inbred strains provide a powerful tool for genomic analyses since haplotype polymorphisms underlying complex polygenic traits can be identified that would be impossible to recognize in non-inbred wild populations [21].

Histology offers a powerful tool in the study of reproductive health of fishes. It is routinely used for sex verification, identifying stage of development, documenting presence of intersex, tumors, parasites and other abnormalities and quantifying atresia. One important way histology is commonly used in fisheries is the study of fish reproductive tissue. Although macroscopic evaluation of gonads can provide important information, the ability to examine reproductive tissue at the microscopic, or cellular, level gives biologists a powerful tool to understand the details of fish reproduction. Histology of ovarian tissue is commonly used to better understand: (1) size/age at maturity; (2) daily and seasonal pattern of spawning; (3) spawning location; and, (4) fecundity. On the other hand, gonadal histology, in conjunction with hormone and vitellogenin measurements, morphological and fecundity studies, can provide insights into the effects of various environmental stressors on reproductive health. However, much research, both field and laboratory, is needed to understand cause and effect for observed changes and to understand the meaning of many of the histological observations made in field studies, in terms of reproductive success of fish populations [3]. The usefulness and importance of histological techniques in reproductive studies have been widely discussed for gonochoristic fish species [1].

The medaka (*Oryzias latipes*), a small aquarium fish that is used widely in ecotoxicological studies, is a unique vertebrate model for investigating the effects of IR but there is a few articles about the structure and description of the different stages of the ovary in both WT and P53, so that, we will investigates and examine the structure and different developmental stages in comparative study.

2. MATERUALA AND METHODS

2.1. Fish

Sexually mature WT (Hd-rR) and p53 (-/-) adult female (3-4 cm) medaka (*O. letipes*) were studied during October 2011. The fish were kept at 20 -21 º C under 14 h light: 10 h dark cycle. They were fed a powdered diet (Tetra-min, Tetra Werke Co., Mells, Germany) and brine shrimp (*Artemia franciscana*) three times a day.

2.2. Specimen collection

Since most fishes used in this study are sexually mature, they are often large and hence have large gonads. One aspect of reproductive studies that needs standardization is the number, size and location of the pieces fixed for histology. The fish (6 fish per strain) were dissected and the whole ovary was isolated and used in following process for histological sections preparations.

2.3. Fixation

Ovaries were fixed in Davidson's solution overnight then the fixed gonads were extracted, dehydrated, embedded in paraffin, serial sectioned at 7 µm from anterior to posterior direction (Oviduct connection) as shown in fig (1), and stained with hematoxylin and eosin for microscopic analysis with a digital camera on digital microscope (Olympus, Japan).

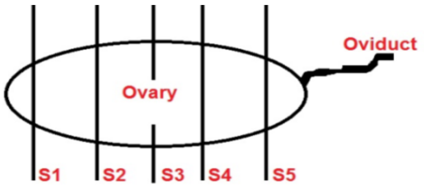

Fig. 1. Digram of the ovary showing the sectioning direction.

2.4. Developmental stages counting

Slides were selected on the basis of staining quality, coded, randomized and scored blindly. In each specimen, serial sections were used for counting under a 40 x objective and 10 × eyepiece to identify different developmental stages in separate slides. The established criteria for identifying of follicles (oocyte developmental stages) were according to [12] to ensure authentic scoring.

2.5. Statistical analysis

The means, standard divisions and ranges were estimated. One-way analysis of variance was used to analyze the data using SPSS software [22] at the 0.05 significance level.

2.6. Ethics statement

All experiments were performed in accordance with the Japanese laws and university guidelines for the care of experimental animals according to The University of Tokyo Animal Experiment Enforcement Rule.

3. RESULTS

3.1. Morphology of the Ovary

The histological structure of the ovary in the examined fishes represents the generalized teleostean structure. The morpho-histology of the ovary of *Oryzias latipes* as described before [23, 12] is a median and unpaired sac-like organ and fills up most of the body cavity behind the posterior edges of the liver. It contains a large number of egg follicles in different developmental stages during October 2011 when these experiments were carried out. The follicles are attached to the thin but strong muscular ovarian wall (epithelium). The developing oocytes are similar to those of teleostean fishes [19, 14] except in size. A small oocyte (primodial follicle) becomes transformed into a large egg containing a mass of yolk (growing follicle). During development the ova are surrounded by a thick chorion and follicular epithelium which form a protective covering. The oocytes at the youngest stage are characterized by a round nucleus which contains several deeply staining nucleoli (Fig.2). At this stage the oocyte has a large nucleus and a small amount of cytoplasm which is deeply basophilic. In the outer surface of the cytoplasm there is found Balbiani's vitelline body [17], in the form of a deeply staining oval body (Fig.2). With the growth of the oocytes the cytoplasm increases in relative volume and nucleus enlarges. As the oocyte grows, yolk vesicles appear, the as the formation of the yolk-vesicle proceeds, the oocytes become markedly enlarged and are provided with a thick follicular layer (large follicle). In subsequent development, the nucleus shifts to one side and the fully ripened egg is completely filled with yolk (growing follicle). Atretie oocytes have been observed quite frequently between the developing oocytes (Fig.2).

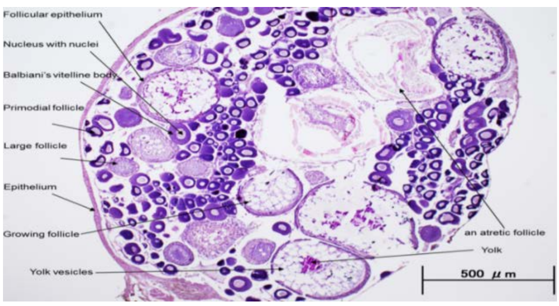

Fig. 2. T.S. of the ovary of *Oryzias latipes* showing the moropho-histology. H&E stain.

3.2. Difference between the Wt (Hd-rR) and p53 (-/-) mutant of the ovary:-

3.2.1. Ovary color, size and shape:-

The color and the size of the ovary is differs as it appears white and small in Wt while yellow-white in P53t. The size was indicated by number of sections obtained at 7 µ, where the ovary of Wt give about 17-18 sections while 22-34 Sections from P53t ovary. The shape of both strains was rounded and the P53 (-/-) ovary showed less thick ovary wall (ovary epithelium) in comparison with Wt ovary (Fig. 3). Also, the interstial tissue in P53t between follicles is more than that of Wt (Fig. 3). No ova-testis in both Wt and P53t was observed.

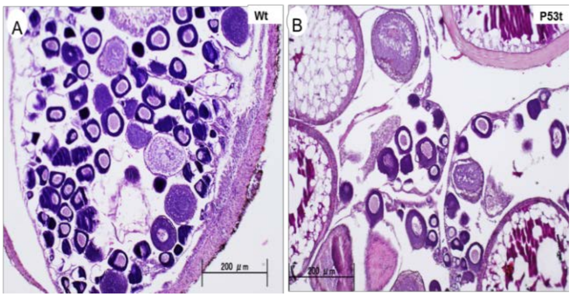

Fig. 3. T.S. of the ovary of *Oryzias latipes* showing large number of primary (primodial) follicles in (A) Wt (Hd-rR) compared with (B) p53 (-/-) mutant. H&E stain.

3.2.2. Average number and frequency of oogonial stages:

Chart 1 and Fig (4 &5) summarizes the frequency of ovarian follicles in both Wt and P53. The percentages of total follicles in P53 was little than in Wt (Chart 1 & Fig. 2). Statistical analysis showed small number of the primodial follicles in P53 (-/-) compared with the number in Wt (Fig. 3).The distribution of the follicles in P53t is regular distributed in whole the cavity of the ovary while in case of Wt, it is look like aggregations of follicles (Fig. 4). Although the results showed large total number of follicles in Wt, the growing follicles and large follicles recorded high number in P53 (-/-) than Wt (Chart 2& Fig. 5).

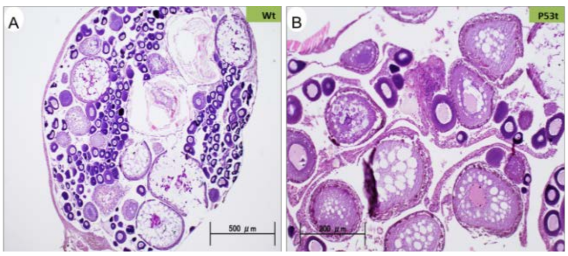

Fig. 4. T.S. of the ovary of *Oryzias latipes* showing clumps distribution of follicles in (A) Wt (Hd-rR) compared with regular distribution in (B) p53 (-/-) mutant. H&E stain.

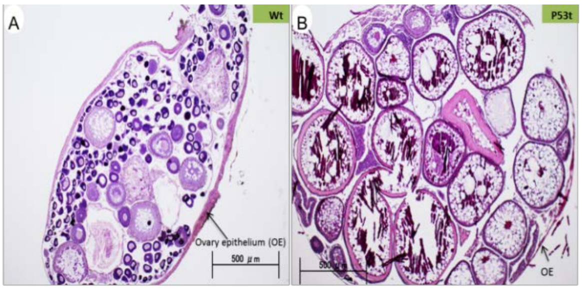

Fig. 5. T.S. of the ovary of *Oryzias latipes* showing small number of growing follicles in (A) Wt (Hd-rR) compared with (B) p53 (-/-) mutant. H&E stain.

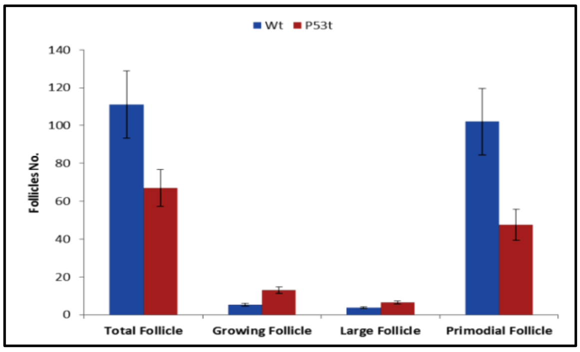

Chart 1. Quantification of ovarian follicles frequency of the ovary of *Oryzias latipes* showing the difference between Wt (Hd-rR) compared with p53 (-/-) mutant.

4. DISCUSSION AND CONCLUSION

The purpose of this present study was to use a histological approach to establish the difference between two strains from WT (HdrR) and P53 (-/-) of medaka (*Oryzias latipes*) in the organization of the gonad histology. Wide studies about structural changes in ovarian morphology in fish tissue have done by different researchers [10, 3, 1]. Those studies including seasonal changes can be the study ovarian tissue red mullet *Mulets surmuletus* southern coast of the UK [15], reproductive cycle and time of the annual reproductive Sea bass on ovarian morphology and histology [6], Histology study and development stages fish ovarian bester sturgeon [2], macroscopic and microscopic stages of ovarian development in white sturgeon *Acipenser transmuntanus* [4], effects of ionizing radiation on the ovaries of Japanese medaka [23], comparative study on ovarian structures in scorpaenids [11] ovarian cycles of Hungarian riverine fish species [13], and on *sparus aurata* ovary [5], ovarian development and sexual maturity of kutum [18] and ovarian development of female white bream [10], study the reproductive biology of hermaphrodite fishes [1] and for assessment of gonadal tissue in wild fishes [3]. Historically, much less attention has been focused on gonadal histology of fishes [3]. In the present study, differences in the ovaries structure in of ovaries of Wt (HdrR) and P53 (-/-) of medaka (*O. latipes*) were reported.

The present study results of histological observation of the ovarian structure and developmental stages quantification are summarized in (Fig. 2-5 & chart 1). The histological structure of the ovary in medaka has been described before morphologically but not numerically, although a lot of studies have been done as comparative study for different fish species with different reproductive mode indicated many changes inside the ovary [11]. On the other hand, El -Sayed et al., [5] reported variations between sea fishes and farm in different points, including pattern of sex inversion, gonadosomatic indices and oogenesis by assessment either histological structure or by scanning and transmission electron microscope.

Our results showed normal development of the ovarian structure and stages of medaka in both WT (HdrR) and P53 (-/-) of medaka (*O. latipes*) compared with other teloests fishes. The histological structure of ovarian follicles in WT (HdrR) obtained in this investigation, similar to P53 (-/-) fish. During ovarian development of teleosts, oocyte undergoes vitellogenisis which induced by estradiol in the liver, then transported vitellogenin via blood to the ovary for starting another process called oogenesis [25, 5]. Previous studies have been reported seasonal variations in gonadosomatic indices in fishes [5], While other studies recorded histological structure changes due to different reproductive modes of the scorpaenidae [11]. From the present study, histological variations of female in the medaka fish, *Oryzias latipes* inhabiting both WT (HdrR) and P53 (-/-) revealed high developmental stage frequency changes. There was a considerable change in the oocyte maturation stages. It has been reported changes in the building level morphology and structure of ovarian oocyte referrals can be index and good in different stages of maturity in fish species and other valuable fish species [24].

Oogenesis and development as indicated from the histological investigations and assessment of frequency of different developmental stages appeared being much more in number in WT (HdrR) than in P53 (-/-). The developmental structure of ovaries in female white bream has been studies indicated the different developmental stages inside the ovary [10].

Our findings can be concluded as follows (1) The histological structure of the ovary in the examined fishes represents the generalized teleostean structure (2) The color and the size of the ovary is differs in both WT strains and p53 -mutants (3) No ova-testis in both Wt and P53t was observed (4) Total follicles in P53 was little than in Wt while small number of the primodial follicles in P53 (-/-) compared with the number in Wt (5) The distribution of the follicles in P53t is regular distributed in whole the cavity of the ovary while in case of Wt, it is look like aggregations of follicles (6) Although the results showed large total number of follicles in Wt, the growing follicles and large follicles recorded high number in P53 (-/-) than Wt.

5. ACKNOWLEDGMENTS

This research was supported partly by a Grant-in-Aid for Scientific Research to H. Mitani (21221003) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

6. REFERENCES

[1] Alonso-Fernández A, Alós J, Grau A, Domínguez-Petit R and Saborido-Rey F The Use of Histological Techniques to Study the Reproductive Biology of the Hermaphroditic Mediterranean Fishes Coris julis, Serranus scriba, and Diplodus annularis. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 3, 2011, 145–159.

[2] Amiri M, Maebayashi M, Hara A, Adachi S and Yamauchi K Ovarian development and serum sex steroid and vitellogenin profiles in the female cultured sturgeon hybrid the Bester. J. Fish Biol. 48, 1996, 1164-1178.

[3] Blazer VS Histopathological assessment of gonadal tissue in wild fishes. Fish Physiology and Biochemistry 26, 2002, 85–101.

[4] Doroshov SI, Moberg GP and Venennam JP.Observation on the reproduction cycle of culturedwhite sturgeon, Acipenser transmuntanus. Envir. Biol. of Fishes 48, 1997, 265-278.

[5] El -Sayyad HI, Elsayyad FI, El -Shershaby EM, Amer MA and El -Gammal IMM Comparative studies on sparus aurata ovary from fish farm and sea. Egyptian Society of Experimental Biology (Zoology) 9, 2013, 181 – 188.

[6] Guiguen Y, Cauty C, Fostier A, Fuchs J and Jalabert B Reproductive cycle and sex inversion of the Sea Bass, Lates calcalifer, reared in sea cage French Polynesia, histological and morphometric description. Envir. Biol. Of Fishes 39, 1993 231-247.

[7] Hyodo-Taguchi Y and Etoh H (1985) Tritium effects on the gonads of the aquarium fish, Oryzias latipes. 1. Fecundity and fertility. In Tritium Radiobiology and Health Phys. (Edited by H. Matsudaira, T. Yamaguchi and H. Etoh), pp. 207–220. National Institute of Radiological Sciences, Chiba.

[8] Hyodo-Taguchi Y and Matsudaira H Higher susceptibility to N-methyl-N'-nitro-N-nitrosoguanidine-induced tumorigenesis in an interstrain hybrid of the fish, Oryzias latipes (medaka). Jpn J Cancer Res. 78(5), 1987, 487-93.

[9] Ishikawa Y Medakafish as a model system for vertebrate developmental genetics. Bioessays 22, 2000, 487-95.

[10] Kopiejewska W and Kozłowski J Development structure of ovaries in female white bream,

Abramis bjoerkna from Lake Kortowskie in North-Eastern Poland. Folia Zool. 56, 2007, 90–96.

[11] Koya Y and Muñoz M Comparative study on ovarian structures in scorpaenids: Possible evolutional process of reproductive mode. Ichthyological research 54, 2007, 221–230.

[12] Kurokawa H, Saito D, Nakamura S, Katoh-Fukui Y, Ohta K, Baba T, Morohashi K and Tanaka M Germ cells are essential for sexual dimorphism in the medaka gonad. Proceedings of the National Academy of Sciences of the United States of America 104, 2007, 16958–16963.

[13] Lefler KK, Hegyi Á, Baska F, Gál J, Horváth Á, Urbányi B and Szabó T Comparison of ovarian cycles of Hungarian riverine fish species representing different spawning strategies. Czech J. Anim. Sci. 53, 2008, 441–452.

[14] Marza VD, Marza EV and Guthrie MJ Histochemistry of the ovary of Fundulus heteroclitus with special reference to the differentiating oocytes. Biol. Bull. 73, 1937, 67-92.

[15] N'Da K and Deniel C Sexual cycle and seasonal changes in the ovary of the red mullet, Mullus surmuletus, from the southern coast of Brittany. J. Fish Biol. 43, 1993, 229-244.

[16] Naruse K, Tanaka M, Mita K, Shima A, Postlethwait J and Mitani H A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. Genome Res. 14, 2004, 820-828.

[17] Raven CP (1961) Oogenesis: the storage of developmental information. Pergamon Press, New York.

[18] Saeed SS, Reza IM, Bagher AF and Saeed G Histological Study of Ovarian Development and Sexual Maturity of Kutum (Rutilus frisii kutum Kamenskii, 1901). World Applied Sciences Journal 8, 2010, 1343-1350.

[19] Sayed AE, Mekkawy IA and Mahmoud UM (2012) Histopathological Alterations in some Body Organs of Adult Clarias gariepinus (Burchell, 1822) Exposed to 4-Nonylphenol. In Zoology. (Edited by M. Garcia), pp. 163-184. Janeza Trdine 9, 51000 Rijeka, Croatia.

[20] Shima A, Himmelbauer H, Mitani H, Furutani-Seiki M, Wittbrodt J and Schartl M Fish genomes flying. Symposium on Medaka Genomics. EMBO Rep. 4, 2003, 121-125.

[21] Shima A and Mitani H Medaka as a research organism: past, present and future. Mech Dev 121, 2004, 599–604.

[22] SPSS (1998) SPSS for Windows. SPSS Inc, Headquarters, Chicago.

[23] Srivastava PN Effect of ionizing radiation on the ovaries of Japanese medaka, Oryzias latipes (t. et s.). Acta Anat (Basel) 63, 1966, 434-444.

[24] Tyler CR and Sumpter JP Oocyte growth and development in teleost. Rev Fish Biol Fisheries 6, 1996, 287–318.

[25] Wallace RA and Selman K Oogenesis in Fundulus heteroclitus II. The transition from vitellogenesis into maturation. Gen.Comp. Endocrinol. 42, 1980, 345–354.

[26] Wittbrodt J, Shima A and Schartl M Medaka—a model organism from the far East. Nat Rev Genet 3, 2002, 53–64.

International Journal of GEOMATE, Dec., 2016, Vol. 11, Issue 28, pp. 2857-2861.
MS No. 1387 received on Aug. 18, 2015 and reviewed under GEOMATE publication policies.

Pertinent discussion including authors' closure, if any, will be published in Dec. 2017 if the discussion is received by June 2017.
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