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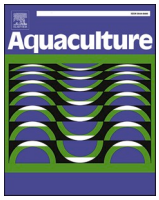

Molecular characterization of diploid YY sperm related to its developmental advantages

Rong Zhou [a,b], Ying Chen [a,b], Shudong Yang [a,b], Kaikun Luo [a,b], Conghui Yang [a,b], Rurong Zhao [a,b], Qinbo Qin [a,b], Shaojun Liu [a,b,*]

[a] State Key Laboratory of Developmental Biology of Freshwater Fish, Hunan Normal University, Changsha 410081, China
[b] College of Life Sciences, Hunan Normal University, Changsha 410081, China

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ABSTRACT

Previously, we have reported diploid YY male fish obtained by the combination of androgenetic technology and distant hybridization and described its special reproductive characteristics producing unreduced and diploid homogametes. The above diploid sperm displayed developmental advantages, forming all-male offspring, and directly developing into individuals without chromosome doubling during androgenesis. However, the related molecular advantages of YY fish sperm have not been reported. In this study, we firstly compared the semen of two kinds of male fish (XY and YY), both obtained by androgenetic technology with the autotetraploid fish sperm. Morphological features exhibited by electron microscope revealed that YY fish sperm is normal, while partial XY fish sperm has defects with uncompacted nucleus or cell degradation. Moreover, a comparison of global transcriptomes showed that the expression of genes related to immunity and energy production was increased in YY fish semen, while genes related to meiosis and spermiogenesis were highly expressed in XY fish semen. Then, we compared the semen of YY fish and red crucian carp (RCC), in which the sperm has normal structure but the different developmental potential for different ploidy. Epigenetic analysis revealed that di/tri-methylation on histone H3 lysine 9 (H3K9me2/3) exhibited higher levels in YY fish semen than in RCC semen. Consisting with the variation of H3K9me2/3, expression of histone methylase gene *setdb1b* increased, while demethylase gene *kdm4a* decreased in YY fish semen. Taken together, this is the first time reported the molecular dominance of diploid sperm of YY fish, which will be helpful for the following practice aquaculture breeding application.

1. Introduction

Super-male fish (YY fish), which can be used to obtain all male offspring, played a significant role in theoretic research related to sex control and producing economic efficiency in practice, especially in species that show obvious growth and size advantages in male than female individuals. Scientists have produced super-male fish successfully in some fish species, such as Nile tilapia (Varadaraj and Pandian, 1989), goldfish (Yamamoto, 1975), Atlantic salmon (Fjelldal et al., 2020), medaka (Hamilton et al., 1969), rainbow trout (Kotula-Balak et al., 2008), yellow catfish (Liu et al., 2013) and blotched snakehead (Zhao et al., 2021). Moreover, the sperm quality, testis development state and molecular characteristic have been compared between super-male fish and ordinary male fish in some species. The testis of YY fish exhibited earlier maturation and superior reproductive capacity than XY fish for its bigger testis, larger spermatogenic cyst, more spermatogenic cells, and more spermatids in rainbow trout, red tilapia and yellow catfish (Kotula-Balak et al., 2008; Salirrosas et al., 2017; Wu et al., 2015). While the sperm of XY and YY males displayed similar quality in Nile tilapia (Gennotte et al., 2012). Thus, there are still no consistent conclusion related to the gonad developmental advantages of YY fish.

In theory, androgenetic technology, in which the offspring only inherits the paternal genome and forms a paternal nuclear clone, is the best strategy to produce super-male fish (Komen and Thorgaard, 2007). However, most of the above YY fish was obtained by a combination of the endocrine sex reversed technique and sex breeding or gynogenesis, followed by lots of screening work with sex molecular markers. Why the best strategy, androgenetic technology, was given up to choose the latter

* Corresponding author at: State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University, Changsha 410081, China.
E-mail address: lsj@hunnu.edu.cn (S. Liu).

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one? Usually, the diploid fish produced haploid sperm, which could not directly develop into individuals during androgenetic process, but should double chromosomes by physical or chemical methods, thus resulting lower survival rate of offspring.

In the previous studies, androgenetic technology is reported with the diploid sperm of allotetraploid and autotetraploid crucian carp, which are obtained via distant hybridization (Sun et al., 2007; Zhou et al., 2015). The diploid sperm can develop into individuals directly without doubling chromosomes. Among these diploid male androgenetic offspring, we found partial fish (YY fish), derived from autotetraploid crucian carp, produced milky semen and form all male individuals when hybridized with other female fish (Zhou et al., 2015). Moreover, these diploid androgenetic YY fish interestingly produce diploid sperm. Therefore, this fish strain can be continued by androgenetic technology and possessed irreplaceable advantages in fish breeding.

Over a long period in the past, sperm was thought to be a vehicle of paternal DNA for its highly compacted nucleus and little cytoplasm. Recently, researchers found the critical role of sperm mRNA storage and the epigenetic state of sperm DNA in fertilization and following early embryonic development coupled with the development of high throughput sequencing technology (Herráez et al., 2017; Jenkins and Carrell, 2012; Ostermeier et al., 2002).

Based on the above characteristics of YY fish sperm, the related molecular advantages of YY fish sperm have not been reported. Here, to evaluate the advantages of the YY fish sperm, we firstly compared the structural features and RNA profiles between YY and XY fish semen. We found that YY fish sperm displayed more intact microstructure, and showed high expression of immunity-related genes, while XY fish sperm exhibited abnormal structure and obvious defects in meiosis and spermiogenesis-related gene expression. To explore the mechanism related to developmental advantage of diploid YY sperm, we then compared the epigenetic state of diploid and haploid sperm. We found that the level of H3K9me2/3 increased in diploid sperm, and the corresponding methylase gene *setdb1b* expressed higher and demethylase gene *kdm4a* expressed lower. This study is the first time report the morphological and molecular advantages of diploid YY sperm, which would provide us with new insights and deep application of super male fish, especially for yielding polyploidy fish.

2. Materials and methods

2.1. Ethics statement

All experiments were approved by the Animal Care Committee of Hunan Normal University. The Administration of Affairs Concerning Animal Experimentation guidelines received approval from the Science and Technology Bureau of China, and the methods were carried out under these approved guidelines.

2.2. Animals

The experimental fish (Red crucian carp (abbreviated as RCC), XY and YY fish) were cultured in the ponds at the Protection Station of Polyploidy Fish, Hunan Normal University, and fed with artificial feed once per day. Two-year-old fish, which is sexually matured, was used to manually extrude semen in April. The semen was frozen in liquid nitrogen, and then stored at −80 °C for the following total RNA isolation.

2.3. Scan electronic microscopy (SEM)

The manually isolated fresh semen was centrifuged at 3500 rpm for 5 min at 4 °C. After removing the supernatant, the pellet was fixed with three volumes of 2.5% glutaraldehyde solution and stored at 4 °C overnight. The morphological phenotype was observed by SEM according to Qin et al. described (Qin et al., 2014). Briefly, then the sample experienced dehydration, dropping, desiccation, and atomized gliding finally observed with SEM (X-650, Hitachi, Germany). For the limited number of normal sperm included in XY water-like semen, the sample volume of XY fish semen used for centrifugation is far more than YY fish semen.

2.4. Transmission electronic microscopy (TEM)

The first sample treatment is the same with the above description in SEM. Following procedure is according to the treatment reported by Xu et al. (2015). After dehydration, the sample was embedded in Epon812. Then, the ultrathin sections were cut and stained with uranyl acetate and lead citrate. The ultrastructure of samples was observed with TEM (JEOL-1230, Japan Electron Optics Laboratory Co. Ltd., Japan).

2.5. Total RNA extraction

The total RNA was extracted according to the instruction of TRIzol (Invitrogen). The frozen semen from three different individuals was homogenized with four volumes TRIzol to avoid degradation by glass homogenizer and centrifuged with 12,000 *g* for 10 min at 4 °C. Then add 1/5 volume chloroform to the supernatant to separate RNA form protein and DNA followed by centrifugation. RNA was precipitated by 1/2 volume isopropanol and centrifuged. The RNA pellet was washed with 70% alcohol and dissolved in RNase-free water. The quality of RNA was checked by spectrophotometer and agarose electrophoresis.

2.6. RNA-Seq

After a series of stringent detection to evaluate the purity, integrity, and quantity of RNA, we used 200 ng RNA to construct the cDNA library by the low initial method for the quantity limit of semen RNA. Firstly, magnetic beads with Oligo(dT) were used to enrich mRNA. And then, mRNA was fragmented and used to synthesize the first and second-strand cDNA with random primers. After purification with AMPure XP beads, tail repair, and linking the sequence adapter, the double-strand cDNA was used as the template for the following PCR amplification to obtain the final library. At last, the insert size and effective concentration of the library were detected by Agilent 2100 and Q-PCR. The qualified library was used for the following Illumina Hiseq sequencing. The raw sequencing data from this study have been deposited in the Genome Sequence Archive in BIG Data Center (http://bigd.big.ac.cn/), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under the accession number: CRA008618.

2.7. Real-Time PCR (RT-PCR)

Quantitative RT-PCR was performed as previously described with minor modulation (Zhou et al., 2013). At first, we used random primer for reverse transcription to synthesize the first strand of the cDNA template with PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa). The following RT-PCR was performed with PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific) using the ABI 7500 PCR System (Applied Biosystems). 18S rRNA was used as the internal control. The sequences of primer pairs are listed in Table S1. Every sample has been performed for triplicate repetitions.

2.8. Western blotting

Western blotting was performed as previously described (Zhou et al., 2013). 5 mg histone proteins were loaded on 15% SDS-PAGE for the following separation and then transferred to PVDF membranes for blotting. Histone H3 worked as the internal control. Anti-histone H3 (ab201456), anti-H3K4me3 (ab8580), anti-H3K9me2 (ab176882), anti-H3K9me3 (ab176916), anti-H3K27me3 (ab192985), anti-H3K36me2 (ab176921), anti-H3K36me3 (ab9050) were purchased from Abcam.

2.9. Statistical analysis

All the data, expressed as means ±SD, were analyzed by one-way ANOVA. Significant differences were determined with Duncan's multiple range tests. All tests were performed using SPSS 13.0 software. *P* values <0.05 were taken to indicate statistical significance.

3. Results

3.1. Morphological and structural comparison between sperm of XY and YY fish

The androgenetic offspring of male autotetraploid fish have been reported (Zhou et al., 2015). Although, in theory, there are three different genotypes (XX, XY, and YY), in practice, the morphological phenotype is similar and hard to distinguish. After sex maturation, we observed that the male androgenetic offspring could produce milky semen (YY fish) and water-like semen (XY fish). The homogametic characteristic of YY fish has been proved by the sex ratio of progeny in previous study (Zhou et al., 2015).

Here, we continued to compare the difference between XY and YY fish semen. The fertilization and hatching indexes are the evaluation standard for sperm quality. In practical breeding experiments, we observed high fertilization rate (~78.0%) and hatching rate (~70.0%) in the cross of RCC (♀) × YY fish (♂). While in the cross of RCC (♀) × XY fish (♂), the fertilization rate (~19.0%) and hatching rate (~4.0%) were very low. Results revealed by SEM showed that all observed sperms in XY and YY fish semen have the head, midpiece, and flagellum tail. The head of the two kinds of fish sperm is round with a diameter 2.17–2.27 μm (Fig. 1), which proved that both XY and YY fish sperm are diploid.

Moreover, results observed by TEM revealed that there is a compact nucleus with a litter gap in the head, many mitochondria, and few vesicles in the sleeve of the midpiece of YY fish sperm, the axoneme around with vesicles appeared in the central space of sleeve (Fig. 2A-C). Partial normal XY fish sperm displayed similar structures as YY fish sperm (Fig. 2D-F). We also observed some abnormal spermatids wrapped in the impurities of the XY fish semen (Fig. 2G-I). In Fig. 2G and H, some spermatids shew round and oval shape, whose nuclear-cytoplasmic ratio and nuclear density are significantly lower than the normal sperm. In Fig. 2I, the spermatid showed degradation in the nucleus with many vacuole bodies. Based on the above results, we supposed that not all sperm in XY semen completed the spermiogenesis process.

3.2. Library construction and sequence assembly

Based on the structural differences observed by SEM and TEM between the sperm of XY and YY fish, we used semen of the two fish for the following deep sequencing to compare the variation at the molecular level. For the low quantity of total RNA, the libraries were constructed with 200 ng initial and amplified for more cycles in the followed PCR. The final library was used for sequencing. After filtration, we have obtained 44,814,664 clean reads for YY fish semen and 45,872,146 clean reads for XY fish semen. For the hybridization genetic background of their paternal fish (the autotetraploid crucian carp), there was no useful reference genomes. Thus, the clean reads were mixed and assembled by Trinity with no reference (Grabherr et al., 2011), and then clustered by Corset (Davidson and Oshlack, 2014). Finally, 321,902 unigenes with the length expanding from 201 bp to 12,492 bp have been got according to the rule that selecting the longest transcripts of each gene to label as the unigene. 65,396 unigenes have been obtained only in XY fish semen, and 54,404 unigenes only in YY fish semen. 171,930 unigenes have been shared in both samples. The correlation between the two samples was only 0.156.

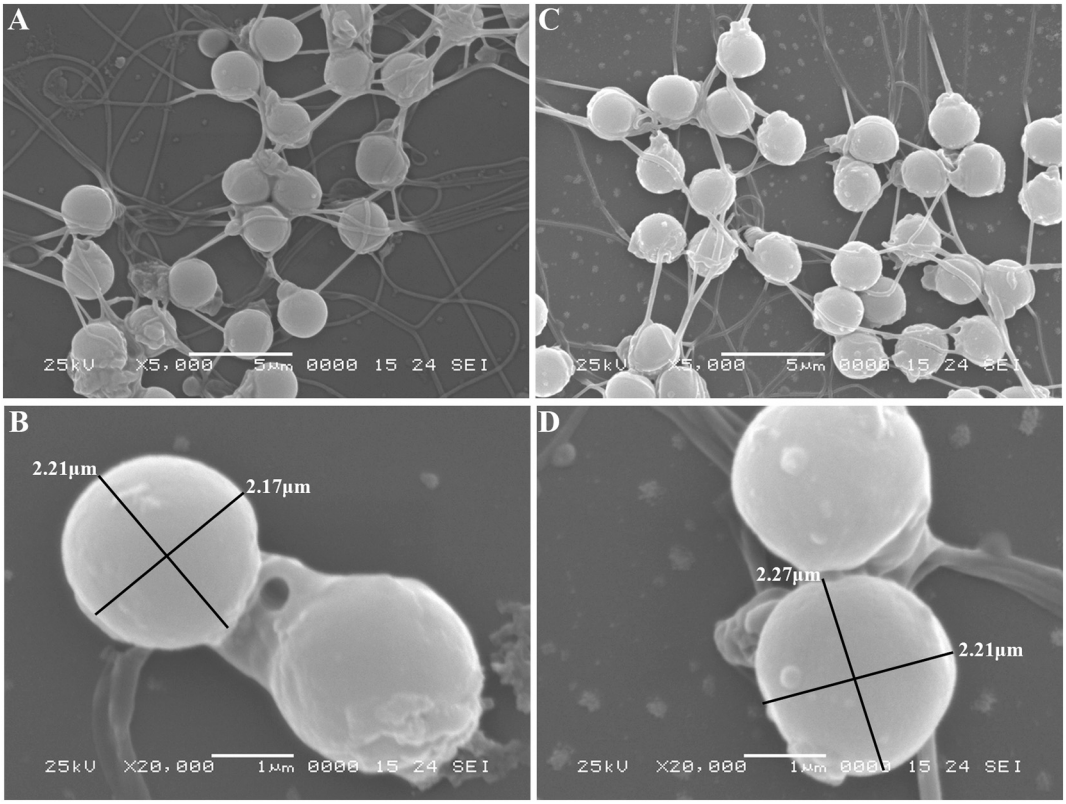

Fig. 1. Morphology of spermatozoa at different magnification scales revealed by SEM. (A and B) spermatozoa of YY fish; **(C and D)** spermatozoa of XY fish. The diameter of sperm is indicated in the pictures. The magnification and scale bar are labeled in the pictures.

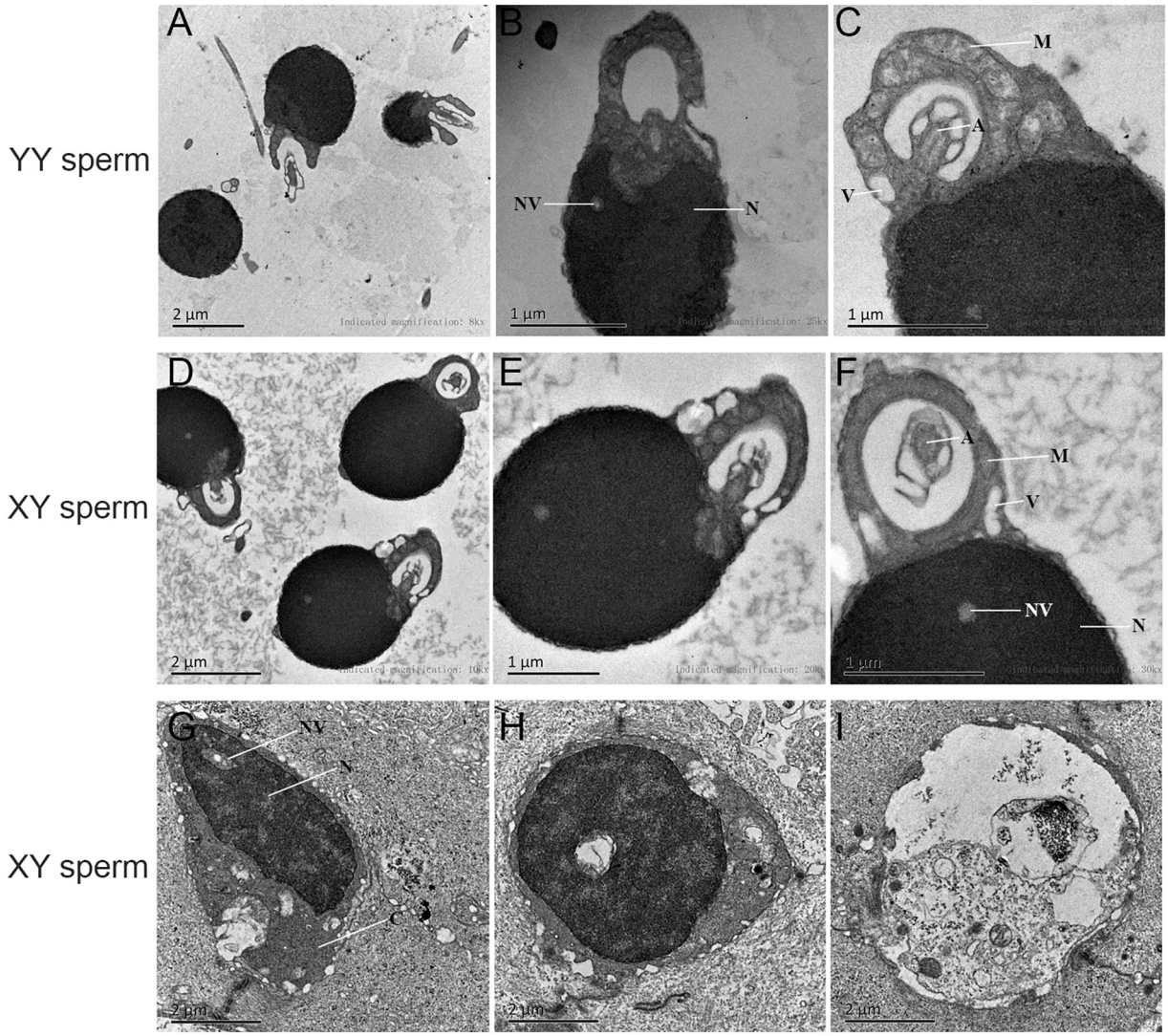

Fig. 2. Microstructure of spermatozoa revealed by TEM. (A-C) spermatozoa of YY fish with different amplification factors. **(D-F)** normal spermatozoa of XY fish at different magnification scales. **(G-I)** abnormal spermatozoa of XY fish. (G and H) sperm with an uncompacted nucleus; (I) apoptotic sperm. The magnification is labeled in each picture. N, nucleus. NV, nuclear vacuole. M, mitochondrion. V, vacuole. A, axoneme. C, cytoplasm.

3.3. Gene annotation and enrichment analysis

To well know the gene function comprehensively, seven databases, including Nr, Nt, Pfam, KOG/COG, Swiss-Prot, KEGG, and GO, have been used to annotate the function of unigenes. 98.37% of unigenes have been annotated in at least one database, while 14.1% of unigenes have been annotated in all databases. About 74% of unigenes can be blasted in zebrafish.

GO database, which covered the biological process, cellular component, and molecular function, is a classification system of describing the characteristics of genes and their products in biological life comprehensively. According to the annotation result, 45.59% of unigenes have been annotated in the GO database. Moreover, the annotated genes mainly focused on the cellular process, metabolic process and single-organism process of biological process part, in cell and cell part of cellular component part, in binding and catalytic activity of molecular function part.

Classification statistic of genes based on the annotation with KOG database revealed that 23.08% of genes have been annotated and the annotated genes mainly distributed in signal transduction mechanisms, general function prediction only, secondary metabolites biosynthesis, transport and catabolism, transcription and intracellular trafficking, secretion, and vesicular transport.

KEGG pathway database described the metabolic pathway network of biological molecules in the cell. The top three pathways, annotated by genes, included signal transduction, immune system, and endocrine system.

3.4. DEG gene analysis

To further compare the differential expressed genes between the two fish semen, we firstly statistic the number of read count of each gene and based on this to evaluate the value of FPKM. Combining the q value <0.005 and $\log_2$(fold change) > 1 as the filter conditions for differential expression genes, we have obtained 790 up-regulated genes and 2124 down-regulated genes in YY fish semen (Fig. 3) (Table S2). GO enrichment analysis revealed that the up-regulated genes mainly focused on biological process and molecular function, such as hydrolase activity (GO:0016787), ion transport (GO:0006811), substrate-specific transporter activity (GO:0022892), cation transport (GO:0006812), substrate-specific transmembrane transporter activity (GO:0022891) (Fig. 4A). While, the down-regulated genes enriched mostly in cellular component, such as cell (GO:0005623), cell part (GO:0044464), intracellular (GO:0005622), intracellular part (GO:0044424), organelle

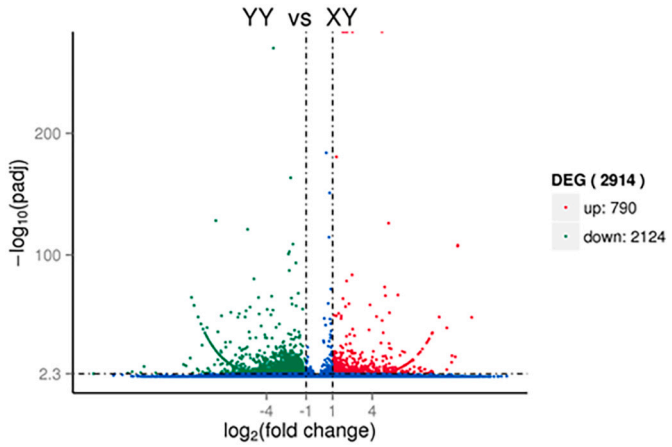

Fig. 3. The volcano plot of different expressional genes between YY and XY fish semen.

(GO:0043226) (Fig. 4B). Statistics of KEGG pathway enrichment displayed that the up-regulated genes mainly focused on enzyme related (phagosome, lysosome), apoptosis, immune-related (rheumatoid arthritis, influenza A, tuberculosis, antigen processing and presentation, vibrio cholerae infection), and energy-related (oxidative phosphorylation) pathways (Fig. 5A). However, the down-regulated genes in YY fish mostly dominated in meiosis-related (cell cycle, FoxO signaling pathway, progesterone-mediated oocyte maturation, oocyte meiosis) and Huntington's disease pathway (Fig. 5B).

3.5. RT-PCR validated the molecular advantages of YY sperm

To validate the expression of DEG genes, we chose genes related to meiosis (*sycp1*, *sycp2*, *msh5*, *mlh3*, *piwi1l*, *spag5*), spermiogenesis (*ift74*, *ift81*, *spef2*, *dnah1*), chromatin condensation (*tssk6*), protein synthesis (*rps6ka*) and immunity (*nf-kb*, *cd74*, *itgb2*, *ifi44l*), and employed RT-PCR to compare the gene expression among YY, XY and RCC semen (Fig. 6). Consistent with the sperm quality, genes related to meiosis and spermiogenesis (biogenesis of sperm head and flagellogenesis) expressed significantly lower in YY fish and RCC with milky semen than in XY fish with water-like semen. Genes related to chromatin condensation, protein synthesis and immunity expressed higher in semen of YY fish than XY fish. The results of RT-PCR exhibited similar variation tendency with DEG genes expression of RNA-seq. Compared to the RCC semen, genes related to protein synthesis and immunity also expressed higher, while genes related to chromatin condensation expressed lower in semen of YY fish. The results revealed that the two kinds of milky semen (RCC and YY fish) still have different molecular characteristics.

3.6. Differential histone modification state between YY and RCC sperm

Recent research revealed that the epigenetic state of sperm DNA also could contribute to early embryonic development (Wu et al., 2011). YY sperm and RCC sperm exhibit different developmental potential for their different ploidy. We firstly compared the histone methylation levels on K4/9/27/36 of histone H3 in YY sperm and RCC sperm via Western

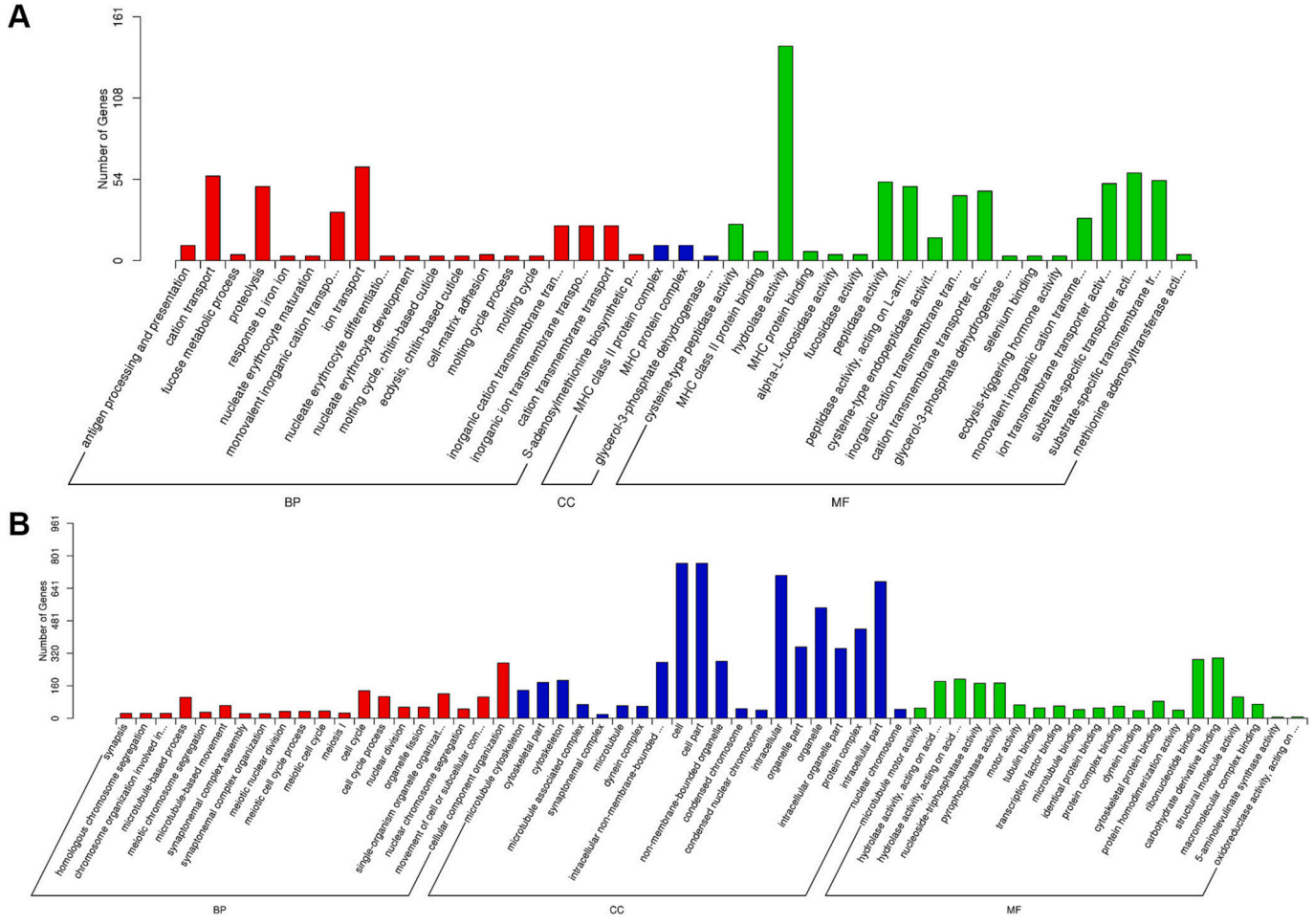

Fig. 4. GO enrichment analysis of up-regulated genes (A) and down-regulated genes (B) in YY fish semen.

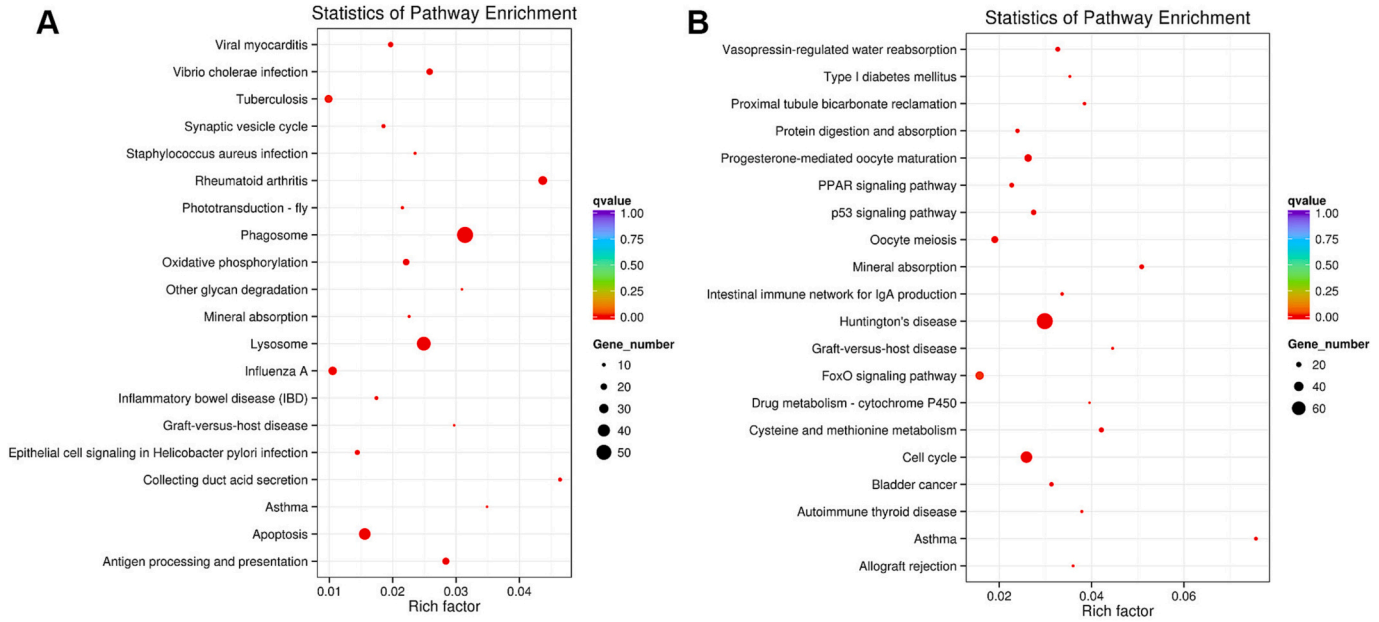

Fig. 5. KEGG pathway enrichment analysis of up-regulated genes (A) and down-regulated genes (B) in YY fish semen.

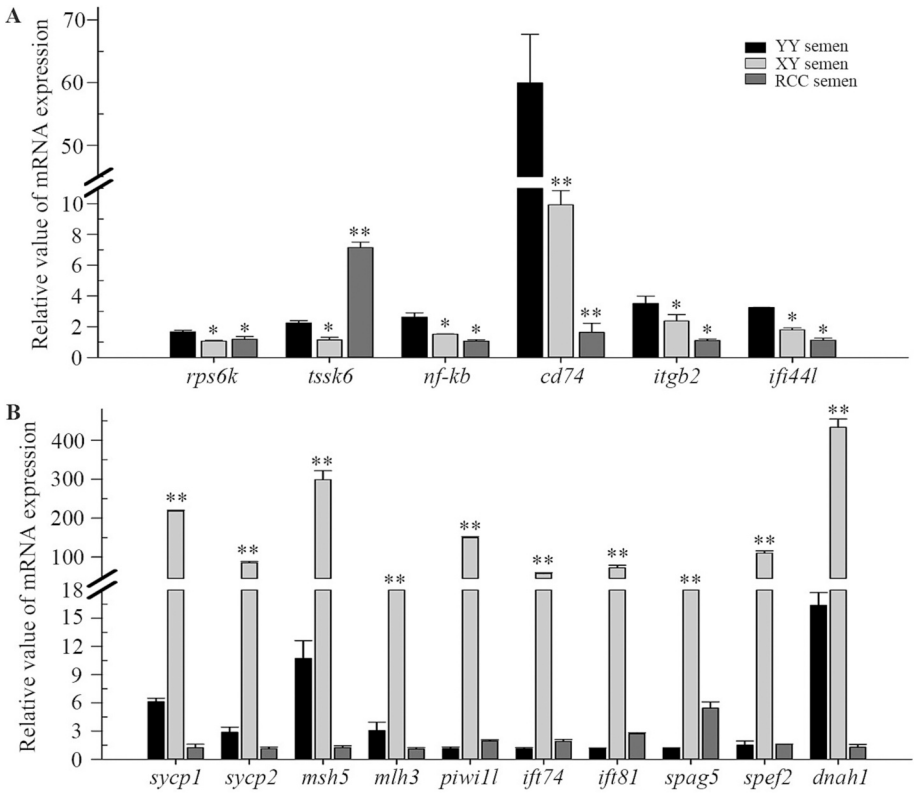

Fig. 6. Real-Time PCR revealed the relative expression of different expressional genes among YY, XY, and RCC semen. (A) Up-regulated genes in YY fish semen, **(B)** Down-regulated genes in YY fish semen.

blotting. Methylation levels of H3K9me2/3 were higher in YY semen (Fig. 7A and B). Then, we further analyzed the expression of corresponding enzyme genes and found that the related methylase gene *setdb1b* expressed higher, and demethylase gene *kdm4a* expressed lower in YY fish (Fig. 7C).

4. Discussion

The diploid male fish, obtained by androgenetic technology with the diploid sperm of tetraploid male fish derived from distant hybridization, was of great value in fish breeding practice for producing unreduced diploid sperm (Sun et al., 2007; Zhou et al., 2015). Duan et al. have reported improving the allotetraploid population by using these

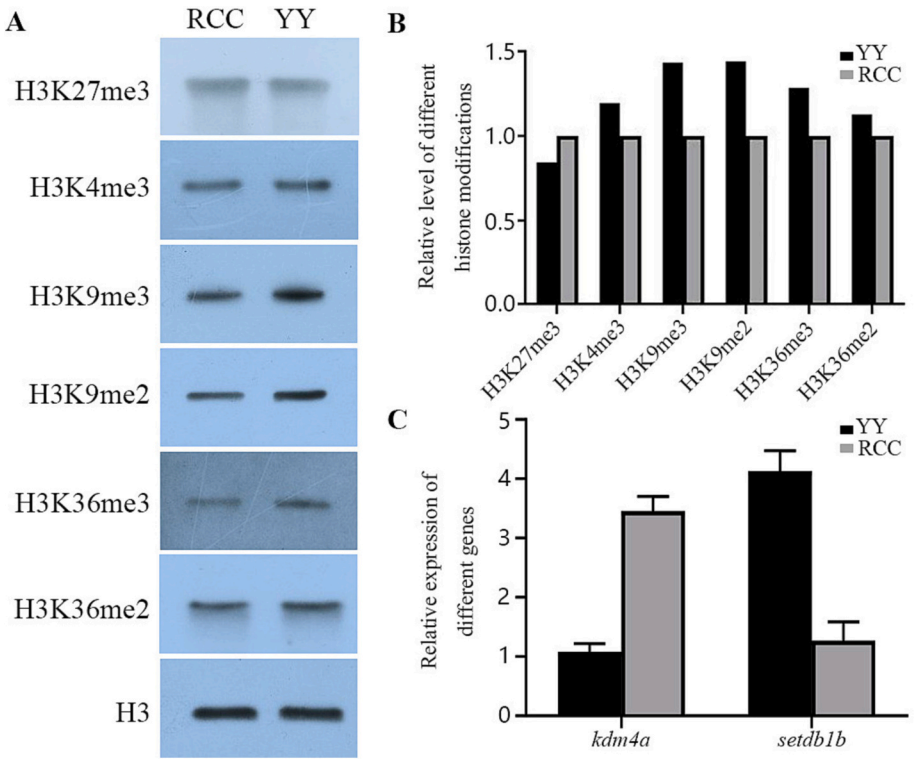

Fig. 7. Histone methylation and related enzyme gene expression in RCC and YY fish semen. (A) Changes of histone methylation level revealed by WB. **(B)** Quantified bar graph of (A). **(C)** Expression of methylase and demethylase genes on H3K9.

androgenetic diploid fish (Duan et al., 2007). The diploid YY fish have been used to produce all male triploid fish and tetraploid fish (Zhou et al., 2015).

Previous studies found that both the YY fish and XY fish can produce sperm, while the testis development and sperm quality of YY fish were better than XY fish in many species (Salirrosas et al., 2017; Wu et al., 2015). Here, we also found the reproductive advantages of YY fish with milky semen and normal structure of sperm revealed by SEM and TEM. However, the semen of XY fish is water-like, and partial sperm of XY fish displayed abnormal structure revealed by TEM, including problems in chromosome condensation. Moreover, the XY semen expressed abnormally high level of meiosis and spermiogenesis genes. The above phenomenon has not been reported in other species. This might be correlated to its paternal fish, autotetraploid fish. Although Qin et al. reported that this autotetraploid fish contained two sets of RCC chromosomes (Qin et al., 2014). The body color of autotetraploid fish is different from RCC. The FISH analysis also indicated that there might have mutations in one set of the RCC chromosomes of the autotetraploid fish derived from distant hybridization (Liu, 2022). Therefore, XY fish might have defects in meiosis and spermiogenesis for heterogametic genetic background. While the YY males can produce offspring successively for their homogametic characteristic.

A study in yellow catfish has compared the different development of testis between XY and YY fish and found that PI3K-AKT and G protein-coupled receptor signaling pathways might promote the earlier maturation of YY testis (Wu et al., 2015). Here, in our study, we chose semen as the analysis sample, which includes the seminal plasma and sperm, thus can directly reflect the characteristics of extruded sperm self and its micro-environment (Ciereszko et al., 2016). Consistent with the results in yellow catfish, we also found genes related to the above pathway, such as spleen tyrosine kinase (*syk*) gene and β2-Integrin (*itgb2*) gene, expressed higher in YY fish semen than in XY fish semen. For the in vitro fertilization modes, semen was released in water, the sperm should face the complex environment and fertilize with the egg, so advantages in immunity defense and energy production could improve sperm competition. Previous studies have reported that diploid sperm showed longer life spans and fast swimming time when compared to haploid sperm, and exhibited high expression of energy metabolism-related genes and proteins (Duan et al., 2016; Hu et al., 2017). In YY semen, we also found an obvious high expression of ATPase genes and genes related to oxidative phosphorylation. Moreover, the immunity-related genes expressed higher in YY semen than in both XY and RCC semen. All the above indicated that YY sperm is of good quality.

The sperm experienced chromosome condensation to form compacted nuclei during spermiogenesis. Tssk6 is required for post-meiotic chromatin remodeling mainly for DNA condensation (Jha et al., 2017). Partial XY sperm exhibited abnormal uncompacted nuclei, thus *tssk6* expressed lower in XY semen than in YY semen. However, expression of *tssk6* gene is higher in RCC semen even than in YY semen. This result indicated that the diploid YY sperm DNA condensation is looser than haploid RCC sperm, which might be related to sequential activation of paternal genes during the following early embryonic development of androgenesis.

The sperm DNA is fully compacted with histones in fish. The paternal epigenome changed by histone modifications played a relevant role during early embryonic development (Herráez et al., 2017; Wu et al., 2011). To explore mechanism under the developmental potential of diploid and haploid sperm, we compared the epigenetic state and found that H3K9me2/3 significant higher in YY diploid sperm than in RCC haploid sperm. Although H3K9me3 is usually associated with transcriptional repression on promoters to mark the heterochromatin (Andersen et al., 2012). H3K9me3 can also serve as active gene body marker to play roles in inhibiting spurious transcription from cryptic intragenic start sites (Andersen et al., 2013). ZGA (zygotic gene activation), which is important for early embryonic development in bisexual reproduction, is absent in androgenesis and gynogenesis for the damaged nuclei of the oocytes or sperm. How to modulate the epigenetic state of genome is the key point of successfully development. Ma et al. reported the difference of promoter's DNA methylation state of *ntl* gene is related to successive development in gynogenesis (Ma et al., 2011).

During the early embryonic development, H3K9me3 significantly increased after the onset of ZGA (Lindeman et al., 2011). Here, high levels of H3K9me2/3 in YY diploid sperm might be correlated to sequential paternal gene expression during embryonic development. Moreover, the expression patterns of *setdb1b* as the methylase gene of H3K9 in zebrafish are increased before ZGA and decreased after ZGA (Aanes et al., 2011). In our study, the storage of mature transcripts for H3K9 methylase (*setdb1b*) and demethylase genes (*kdm4a*) supported the level of this epigenetic modification. Therefore, the YY diploid sperm can directly develop into individuals.

5. Conclusions

In conclusion, our study is the first time reporting the molecular mechanism underlying advantages of diploid androgenetic YY fish by comparatively analyzing global transcriptome of semen and its epigenetic state. This study would provide new insight into how to obtain YY fish and how to apply the advantages of YY fish in aquaculture breeding practice.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739397.

CRediT authorship contribution statement

Rong Zhou: Conceptualization, Validation, Writing – original draft, Writing – review & editing, Funding acquisition. **Ying Chen:** Investigation, Validation. **Shudong Yang:** Investigation, Validation. **Kaikun Luo:** Resources. **Conghui Yang:** Formal analysis. **Rurong Zhao:** Resources. **Qinbo Qin:** Resources. **Shaojun Liu:** Conceptualization, Writing – original draft, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have shared the link to my data at the Attach file step

Acknowledgments

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References

Aanes, H., Winata, C.L., Lin, C.H., Chen, J.P., Srinivasan, K.G., Lee, S.G., Lim, A.Y., Hajan, H.S., Collas, P., Bourque, G., 2011. Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. Genome Res. 21 (8), 1328–1338. https://doi.org/10.1101/gr.116012.110.

Andersen, I.S., Reiner, A.H., Aanes, H., Alestrom, P., Collas, P., 2012. Developmental features of DNA methylation during activation of the embryonic zebrafish genome. Genome Biol. 13 (7), 1–13. https://doi.org/10.1186/gb-2012-13-7-r65.

Andersen, I.S., Lindeman, L.C., Reiner, A.H., Østrup, O., Aanes, H., Alestrom, P., Collas, P., 2013. Epigenetic marking of the zebrafish developmental program. Curr. Top. Dev. Biol. 104, 85–112. https://doi.org/10.1016/B978-0-12-416027-9.00003-6.

Ciereszko, A., Dietrich, M.A., Nynca, J., 2016. Fish semen proteomics — new opportunities in fish reproductive research. Aquaculture 472 (1), 81–92. https://doi.org/10.1016/j.aquaculture.2016.03.005.

Davidson, N.M., Oshlack, A., 2014. Corset: enabling differential gene expression analysis for *de novo* assembled transcriptomes. Genome Biol. 15 (7), 410. https://doi.org/10.1186/s13059-014-0410-6.

Duan, W., Qin, Q.B., Chen, S., Liu, S.J., Wang, J., Zhang, C., Sun, Y.D., Liu, Y., 2007. The formation of improved tetraploid population of red crucian carp x common carp hybrids by androgenesis. Sci China C Life Sci 50 (6), 753–761. https://doi.org/10.1007/s11427-007-0090-5.

Duan, W., Xu, K., Hu, F.Z., Zhang, Y., Wen, M., Wang, J., Tao, M., Luo, K.K., Zhao, R.R., Qin, Q.B., Zhang, C., Liu, J.H., Liu, Y., Liu, S.J., 2016. Comparative proteomic, physiological, morphological, and biochemical analyses reveal the characteristics of the diploid spermatozoa of allotetraploid hybrids of red crucian carp (*Carassius auratus*) and common carp (*Cyprinus carpio*). Biol. Reprod. 94 (2), 35. https://doi.org/10.1095/biolreprod.115.132787.

Fjelldal, P.G., Hansen, T.J., Wargelius, A., Ayllon, F., Glover, K.A., Schulz, R.W., Fraser, T., 2020. Development of supermale and all-male Atlantic salmon to research the *vgll3* allele - puberty link. BMC Genet. 21 (1), 1–13. https://doi.org/10.1186/s12863-020-00927-2.

Gennotte, V., Franĉois, E., Rougeot, C., Ponthier, J., Deleuze, S., Mélard, C., 2012. Sperm quality analysis in XX, XY and YY males of the Nile tilapia (*Oreochromis niloticus*). Theriogenology 78 (1), 210–217. https://doi.org/10.1016/j.theriogenology.2012.02.002.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29 (7), 644–652. https://doi.org/10.1038/nbt.1883.

Hamilton, J.B., Walter, R.O., Daniel, R.M., Mestler, G.E., 1969. Competition for mating between ordinary and supermale Japanese medaka fish. Anim. Behav. 17 (1), 168–176. https://doi.org/10.1016/0003-3472(69)90126-2.

Herráez, M., Ausió, J., Devaux, A., González-Rojo, S., Fernández-Díez, C., Bony, S., Saperas, N., Robles, V., 2017. Paternal contribution to development: sperm genetic damage and repair in fish. Aquaculture 472, 45–59. https://doi.org/10.1016/j.aquaculture.2016.03.007.

Hu, F.Z., Xu, K., Zhou, Y.F., Wu, C., Wang, S., Xiao, J., Wen, M., Zhao, R.R., Luo, K.K., Tao, M., Duan, W., Liu, S.J., 2017. Different expression patterns of sperm motility-related genes in testis of diploid and tetraploid cyprinid fish. Biol. Reprod. 96 (4), 907–920. https://doi.org/10.1093/biolre/iox010.

Jenkins, T.G., Carrell, D.T., 2012. The sperm epigenome and potential implications for the developing embryo. Reproduction 143 (6), 727–734. https://doi.org/10.1530/REP-11-0450.

Jha, K.N., Tripurani, S.K., Johnson, G.R., 2017. TSSK6 is required for γH2AX formation and the histone-to-protamine transition during spermiogenesis. J. Cell Sci. 130 (10), 1835–1844. https://doi.org/10.1242/jcs.202721.

Komen, H., Thorgaard, G.H., 2007. Androgenesis, gynogenesis and the production of clones in fishes: a review. Aquaculture 269 (1), 150–173. https://doi.org/10.1016/j.aquaculture.2007.05.009.

Kotula-Balak, M., Zielińska, R., Glogowski, J., Kowalski, R.K., Sarosiek, B., Bilińska, B., 2008. Aromatase expression in testes of XY, YY, and XX rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 149 (2), 188–196. https://doi.org/10.1016/j.cbpa.2007.11.009.

Lindeman, L.C., Andersen, I.S., Reiner, A.H., Li, N., Aanes, H., Østrup, O., Winata, C., Mathavan, S., Müller, F., Alestrom, P., Collas, P., 2011. Prepatterning of developmental gene expression by modified histones before zygotic genome activation. Dev. Cell 21 (6), 993–1004. https://doi.org/10.1016/j.devcel.2011.10.008.

Liu, S.J., 2022. Fish Distant Hybridization. Springer, Singapore.

Liu, H.Q., Guan, B., Xu, J., Hou, C.C., Tian, H., Chen, H.X., 2013. Genetic manipulation of sex ratio for the large-scale breeding of YY super-male and XY all-male yellow catfish (*Pelteobagrus fulvidraco* (Richardson)). Mar. Biotechnol. (NY) 15 (3), 321–328. https://doi.org/10.1007/s10126-012-9487-7.

Ma, S.S., Huang, W.X., Zhang, L., Zhao, S.F., Tong, Y., Liu, Z.H., Sun, L., Chen, H.Y., Luo, C., 2011. Germ cell-specific DNA methylation and genome diploidization in primitive vertebrates. Epigenetics 6 (12), 1471–1480. https://doi.org/10.4161/epi.6.12.18177.

Ostermeier, G.C., Dix, D.J., Miller, D., Khatri, P., Krawetz, S.A., 2002. Spermatozoal RNA profiles of normal fertile men. Lancet 360 (9335), 772–777. https://doi.org/10.1016/S0140-6736(02)09899-9.

Qin, Q.B., Wang, Y.D., Wang, J., Dai, J., Xiao, J., Hu, F.Z., Luo, K.K., Tao, M., Zhang, C., Liu, Y., Liu, S.J., 2014. The autotetraploid fish derived from hybridization of *Carassius auratus red var.* (Female) × *Megalobrama amblycephala* (Male). Biol. Reprod. 91 (4), 93. https://doi.org/10.1095/biolreprod.114.122283.

Salirrosas, D., Leon, J., Arqueros-Avalos, M., Sanchez-Tuesta, L., Rabanal, F., Prieto, Z., 2017. YY super males have better spermatic quality than XY males in red tilapia *Oreochromis niloticus*. Sci. Agropec. 8 (4), 349–355. https://doi.org/10.17268/sci.agropecu.2017.04.06.

Sun, Y.D., Zhang, C., Liu, S.J., Duan, W., Liu, Y., 2007. Induced interspecific androgenesis using diploid sperm from allotetraploid hybrids of common carp×red crucian carp. Aquaculture 264 (1), 47–53. https://doi.org/10.1016/j.aquaculture.2006.07.004.

Varadaraj, K., Pandian, T.J., 1989. First report on production of supermale tilapia by integrating endocrine sex reversal with gynogenetic technique. Curr. Sci. 58 (8), 434–441. https://www.jstor.org/stable/24098971.

Wu, S.F., Zhang, H.Y., Cairns, B.R., 2011. Genes for embryo development are packaged in blocks of multivalent chromatin in zebrafish sperm. Genome Res. 21 (4), 578–589. https://doi.org/10.1101/gr.113167.110.

Wu, J.J., Xiong, S.T., Jing, J., Chen, X., Wang, W.M., Gui, J.F., Mei, J., 2015. Comparative transcriptome analysis of differentially expressed genes and signaling pathways between XY and YY testis in yellow catfish. PLoS One 10 (8), e0134626. https://doi.org/10.1371/journal.pone.0134626.

Xu, K., Wen, M., Duan, W., Ren, L., Hu, F.Z., Xiao, J., Wang, J., Tao, M., Zhang, C., Wang, J., Zhou, Y., Zhang, Y., Liu, Y., Liu, S.J., 2015. Comparative analysis of testis transcriptomes from triploid and fertile diploid cyprinid fish. Biol. Reprod. 92 (4), 95. https://doi.org/10.1095/biolreprod.114.125609.

Yamamoto, T.O., 1975. A YY male goldfish from mating estrone-induced XY female and normal male. J. Hered. 66 (1), 2–4. https://doi.org/10.1093/oxfordjournals.jhered.a108564.

Zhao, J., Ou, M., Wang, Y.P., Liu, H.Y., Luo, Q., Zhu, X.P., Chen, B.X., Chen, K.C., 2021. Breeding of YY super-male of blotched snakehead (*Channa maculata*) and production of all-male hybrid (*Channa argus* ♀ × *C. maculata* ♂). Aquaculture 538, 736450. https://doi.org/10.1016/j.aquaculture.2021.736450.

Zhou, R., Fan, Y., Chen, D.F., Sun, Y.X., Yang, J.S., Yang, W.J., 2013. Acetylation of chromatin-associated histone H3 lysine 56 inhibits the development of encysted artemia embryos. PLoS One 8 (6), e68374. https://doi.org/10.1371/journal.pone.0068374.

Zhou, R., Xiao, J., Qin, Q.B., Zhu, B., Zhao, R.R., Zhang, C., Tao, M., Luo, K.K., Wang, J., Peng, L., Liu, S.J., 2015. YY super sperm lead to all male triploids and tetraploids. BMC Genet. 16 (1), 68. https://doi.org/10.1186/s12863-015-0230-z.