ABSTRACTS

SESSION 2: SEX CONTROL

GENETIC ARCHITECTURE OF SEX DETERMINATION IN TURBOT (*SCOPHTHALMUS MAXIMUS*)

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Abstract

Turbot (*Scophthalmus maximus*) is a flatfish with increasing aquaculture value in Europe and China. This species shows extreme sexual growth dimorphism, females growing faster and becoming sexually mature later than males, hence the interest of industry in producing all-female populations. Sex determination (SD) of turbot shows a major genetic component, the main quantitative trait loci (QTL) being located at linkage group (LG) 5, but minor QTLs and temperature influence have also been reported. Available data suggest a ZZ/ZW system of recent evolutionary origin, since recombination is not restricted and no genetic divergence at this region is observed males and females. In this study, we carried out a GWAS analysis of SD in this species using 18,165 SNPs in a large set of 36 families. Although, previous findings were confirmed (major SD region at LG5), ~30% of families showed a different pattern suggesting association with other LGs or environmental influence. Standard SD LG5 families were used to narrow down to 531 kb the region where the master gene is putatively located. This region was deeply analyzed by re-sequencing ZZ and WW individuals to look for a diagnostic difference between sexes related to SD. Furthermore, candidate genes were scrutinized for structural differences and their expression profiles studied along the critical SD period. All data support that SD in this species meets to a complex trait with the major locus being recently recruited.

Keywords: Turbot, sex determination, genetic architecture, environmental factors, sex control

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THE FIRST NON-MAMMALIAN Y CHROMOSOME SEQUENCE REVEALS BCAR1 AS THE CANDIDATE SEX DETERMINATION GENE IN CHANNEL CATFISH

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Abstract

Channel catfish (*Ictalurus punctatus*) has a XY male heterogametic sex determination system, but the molecular basis of its sex determination is unknown. Here we took a genomic approach to determine the sex determination gene(s). First, the sex determination locus was mapped through genetic linkage mapping to LG4. Genome-wide association studies (GWAS) were used to pin down the size of the sex determination locus using genetically unrelated wild catfish. Then we attempted to determine the sex determination gene through sequence analysis. In order to generate X chromosome sequence, a gynogen catfish was used as sequencing template. In order to obtain the Y chromosome sequence, YY male catfish was first produced through sex reversal of a XY male catfish to a phenotypic female; its mating with a normal male catfish allowed generation of the YY male catfish. This YY male catfish was used as the sequencing template. Using the third generation sequencing technologies, we generated, assembled and annotated the YY genome sequence of channel catfish. This represents the very first Y chromosome sequence among teleost fish, and one of the few Y chromosome sequences among vertebrate species. The genome sequence assembly had a contig N50 size of 2.7 Mb and a scaffold N50 size of 26.7 Mb. Comparative analysis of the channel catfish X and Y chromosome sequences was conducted. However, no sex-specific genes were found. Comparative RNA-Seq analysis between females and males revealed exclusive sex-specific expression of an isoform of BCAR1 gene in the male during early sex differentiation. Coupling of positional and expression candidates suggest the candidacy of BCAR1 as the sex determination gene, and experimental knockout of BCAR1 gene converted genetic males (XY) to phenotypic females. Thus, the present research supports BCAR1 as a candidate locus for sex determination in channel catfish.

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EVIDENCE FOR MULTIPLE MECHANISMS CAPABLE OF CONFERRING MALENESS IN ATLANTIC SALMON

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Abstract

The master regulator of sex determination in salmonids has been identified as the *sdY* gene, which forms the basis of DNA diagnostics aiming to diagnose the sex of immature Atlantic salmon. Routine analysis of *sdY* has been conducted in fish from the SALTAS selective breeding program that have established phenotypic assignment of sex. This identified a number of phenotypic males that test negative for the presence of *sdY*, and other animals which appear to carry only partial sequence of the gene. This prompted us to explore if our existing *sdY* diagnostics are correctly reporting the presence / absence of the gene, using whole genome sequencing and development of independent PCR assays. Next, we sought to determine if 10 *sdY* test negative males were capable of generating viable progeny of both sexes. A test cross was performed and progeny raised as a single management group to 14 months of age, before sex was assigned by dissection. Our findings confirmed the appearance of test progeny that are phenotypically male, and DNA testing of these fish confirmed they lack *sdY* and indeed appear not to carry the entire male specific region of the genome. Our results strongly suggest the presence of a mechanism in Atlantic salmon sex determination that acts to confer maleness in the absence of a functional *sdY* gene. This has consequences for the operation of the selective breeding program and raises a biological question concerning the extent of plasticity in sex determination in this economically important aquaculture species.

GENETIC ARCHITECTURE OF SEX REVERSAL IN HALF-SMOOTH TONGUE SOLE (Cynoglossus semilaevis)

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Abstract

Sex reversal in insects, amphibians, reptiles, and fishes is a complicated and interesting biological phenomenon. Sex reversal changes the sex ratio of populations and may complicate breeding schemes. In the Chinese tongue sole (*Cynoglossus semilaevis*), genetic females may change into pseudomales, thereby increasing aquaculture costs because of the lower growth rate of the males than that of the females. Here, we identify two single nucleotide polymorphisms (SNP) (named Cyn_Z_6676874 and Cyn_Z_8564889) on Z chromosome associated with sex reversal; which are located in the third intron of the F-box and leucine rich repeat protein 17 (*FBXL17*) gene and in the third intron of the doublesex and mab-3 related transcription factor 1 (*Dmrt1*) gene, respectively. SNP Cyn_Z_6676874 has two alleles, A and T. Genetic females with Z^AW genotypes will never reverse into phenotypic males, but those with Z^TW genotypes can sometimes undergo sex reversal. SNP Cyn_Z_8564889 has two alleles, A and G, but heterogeneous genotype AG were found in some ZW fish, indicating the duplication of *Dmrt1* gene. Genetic females with $Z^{GG}W$ genotypes will never reverse into phenotypic males, but those with $Z^{AG}W$ or $Z^{AA}W$ genotypes can sometimes undergo sex reversal. Considering both SNP Cyn_Z_6676874 and Cyn_Z_8564889, we found they interactively regulated sex reversal. The genetic females simultaneously carrying the T allele of Cyn_Z_6676874 and the A allele of Cyn_Z_8564889 changing into pseudomales. We also confirmed pseudomale cannot produce W sperm. The interaction and linkage between Cyn_Z_6676874 and Cyn_Z_8564889 and the absence of W sperm from pseudomales unravel the genetic architecture of sex reversal in *C. semilaevis*, and these findings are beneficial to improve female percentage in tongue sole aquaculture.

Keywords: genetic architecture, genetic interaction, tongue sole, genome-wide association study

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MOLECULAR ANALYSIS OF HEAT-INDUCED MASCULINIZATION IN TELEOSTS

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Abstract

For aquaculture species that have desirable sexual dimorphic traits, control of the sexual development is crucial. For example, a male tilapia grows almost twice as fast as a female, which translates into shorter culturing time and more uniform harvest size. The commonly used method for getting an all-male tilapia population is by oral administration of the synthetic male sex hormone (17α-methyltestosterone). However, there are concerns about environmental and human health impacts from the overuse of sex hormones. Heat-induced masculinization, which is a common phenomenon in many teleost species, is a hormone-free alternative but its underlying mechanism is unclear. Using the model organism zebrafish, we established the conditions for heat-induced masculinization and performed in-depth gonadal transcriptomic analysis. It was observed that the degree of masculinization varied among different zebrafish families, indicating interactions between the genotype and the environment (GxE). Major gonadal transcriptomic reprogramming was observed in juvenile zebrafish after exposure to elevated temperature and in some fish this effect persisted for a prolonged period of time even after temperature returned to normal. These findings in zebrafish can serve as reference for the implementation of heat-induced masculinization for aquaculture species.

Keywords: masculinization, sex control, zebrafish, fish reproduction, temperature

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MASS PRODUCTION OF ALL FEMALE YELLOW DRUM (*NIBEA ALBIFLORA*) USING SEX CONTROL

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Abstract

Yellow drum (*Nibea albiflora*) is one of the most important species for commercial fisheries and a promising candidate for aquaculture in China. This fish exhibits significant sexually dimorphic growth and the efficiency and profitability of its aquaculture could be maximized by all-female production. Consequently, we developed an effective protocol to induce meiotic gynogenesis and further produced sex-reversed fish (neo-males) using gynogenetic fish. Spermatozoa from rock bream (*Oplegnathus fasciatus*) were used and the optimal UV-irradiation dose for genetically inactivating the spermatozoa was observed at a UV dose of 45 mJ/cm^2. The critical parameters for preventing extrusion of the second polar body were optimized, and the best yield of gynogens was obtained with a cold-shock at 4ºC for a period of 8 min, initiated 2 min after fertilization. Flow cytometry and microsatellite analyses demonstrated that our protocol successfully obtained 100% gynogenetic individuals. Then, immersion treatments with 17a-methyltestosterone (MT) were applied to gynogenetic yellow drum to induce phenotypic sex reversal fish. Gynogenetic yellow drum at an undifferentiated sex stage (total length, 1.63 ± 0.16 cm) were immersed in 17α-methyltestosterone (17α-MT) for 2 h/day from 30–90 dph (60 days), producing 100% neo-males. Furthermore, crossing of gynogenetic sex-reversed males with normal females produced 100% females, demonstrating the potential efficiency of this protocol for reversing the sex of yellow drum and establishing large-scale production of all-female populations in yellow drum.

Key words: *Nibea albiflora*, gynogenesis, neo-males, sex control

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THE NATIONAL BREEDING PROGRAM OF SERIOLA LALANDI FOR DIVERSIFYING THE CHILEAN AQUACULTURE AND THE DEVELOPMENT OF GENOMIC RESOURCES.

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Abstract

Developing efficient breeding programs, invoke interesting questions regarding the biology of wild species, that are used for the diversification of aquaculture. A private-public consortium was developed in order to develop *Seriola lalandi* aquaculture in Chile. However, this species lacks sufficient genomic resources required for assessing broodstock relationships, genetic contributions, population assessment and genomic selection. This is a very important issue in marine species, where, it is not possible to keep family records during the first stages of development due to communal spawning. Thus, inbreeding and relatedness can increase dramatically if algorithms for maintaining the rate of inbreeding when selecting broodstock are not used. We are aiming at developing the required genomic tools using whole genome and transcriptome sequencing for transcript discovery and annotation of the genome. A draft genome sequence for each sex was developed. The assembly of about 70x genome coverage was produced, with a genome length equal to 700Mbp for the female sequence, which is similar to the predicted genome size for the species. Using sequence data from about 50 full sequenced genomes of both sexes, we determined with high confidence the sex determination region using GWAS and expected haplotype diversity differing between sexes. We obtained estimates of genetic diversity using individuals obtained recently from fisheries that are going to be used as founders of the Chilean breeding program of this species, using a SNP array of more than 90K SNPs. The individual inbreeding was found to be relatively high in some individuals, but the haplotype structure suggests a relatively high effective population size. Nevertheless, the LD is efficiently capture with the number of SNPs used in the array, given the genome size. The genome was annotated using transcriptomic data, in particular genes expressed of skeletal deformities revealing a different pattern of variability. Overall the national breeding program will incorporate all these advancements in order to diversify the Chilean aquaculture, in particular deterministic simulations, suggest than genomic selection is the most promising method, when considering gain and rates of inbreeding, for increasing the profitability of the Seriola production.

Keywords: Genome, sex determination, haplotype diversity.

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SEX CONTROL IN LOBSTERS AND PRAWNS – LESSONS LEARNT AND THE ROAD AHEAD

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Abstract

Unlike fish where sex can be manipulated by shifting the steroid balance, crustaceans have a different mode of sexual differentiation. In the past decade a lot of research attention was given to the androgenic gland (AG). Exclusive to male crustaceans, it elicits masculinity by producing and secreting an insulin-like hormone. In the giant freshwater prawn *Macrobrachium rosenbergii*, identification of genetic sex markers, together with silencing of the gene encoding the hormone, enabled a commercially viable production of all-male populations (via neo-females). The same marker was also instrumental in developing all-female populations of the same species by transplanting AG cells of males into females to produce neo-males. Outside this species, no additional species was reported to have been successfully manipulated with these approaches despite the intensive research effort. These attempts include the case of the rock lobsters. Genetic sex markers prove to be the first cardinal step in establishing sex control of any given species and in the Eastern rock lobster *Sagmariasus verreauxi* these markers were identifiable in two ways: one, through random screening of the genome (RAD-Seq). using this approach, which could be applied to any given species, enabled the identification of 30 male-specific markers (five of which were validated via PCR), with none in females, strongly indicative of XX/XY mode of inheritance. In another approach, screening of transcriptomic data identified the first case of a transcription factor encompassing the sex determination conserved DM domain on the Y chromosome. It was thus termed iDMY. This is the fourth case in nature where a Dmrt type gene is found to be the master sex determining factor. Research is underway to develop techniques to establish all-female populations of *S. verreauxi*, specifically targeting this novel gene. Given the high variation in sex determination mechanisms, it appears that the best strategy is to establish the mode of inheritance using RAD-Seq or similar approaches and in parallel identify species-specific tools to manipulate sex. The lobster iDMY provides one such example, placing the Dmrts at the forefront for exploring sex determining genes of interest.

Keywords: Genetic sex markers, RAD-Seq, Sex determining gene, iDMY

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A TRANS-SPECIES SNP IN A STEROIDOGENIC GENE IS ASSOCIATED WITH SEX DETERMINATION IN *Seriola* SPECIES.

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It is obvious that steroids play critical roles in sex differentiation in vertebrates. However, the involvement of steroidogenesis in an earlier process, sex-determination, has been a controversial topic in reproductive biology more than half a century. Since the 1950', when Tokio Yamamoto postulated that sex steroids are direct determinants of sex (i.e., substances that induce gonadal sex) in medaka fish, a large number of experiments have investigated the roles of steroids in sex determination in many fish species. However, the steroids hypothesis has been hard to rigorously test due to difficulties in discriminating the role of steroids in sex determination and sex differentiation. Indeed, manipulation of sex steroids in either process often results in the same phenotype: gonadal sex-reversal. *Seriola* fishes are the most important aquaculture species in Japan, accounting for 50–60% of the annual aquaculture production. We investigated the sex-determining locus in two *Seriola* species in which sex is determined by a ZZ/ZW system. We identified a SNP in the gene encoding 17β-hydroxysteroid dehydrogenases 1 (*Hsd17b1*) as the sole polymorphism that was almost perfectly correlated with phenotypic sex in a high-resolution association mapping. Based on this and subsequent analyses using biochemical, computational and histological approaches, we concluded that steroids themselves can serve as direct determinants of the sex in a natural system. Our results also suggested that the SNP in *Hsd17b1* will be useful for precocious sex identification, facilitating the breeding programs of *Seriola* fishes.

Key words: Steroids, sex-determining gene, sex determination, sex differentiation

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BIOTECHNOLOGY FOR ALL-FEMALE PRAWNS – FIRST TIME IN CRUSTACEAN AQUACULTURE

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Abstract

Monosex culture is common in animal husbandry and highly advantageous in crustacean aquaculture as many cultured species exhibit distinct size variation between males and females. In aquaculture of the giant freshwater prawn (*Macrobrachium rosenbergii*), mixed cultures are difficult to intensify due to complex social structure being dominated by large, aggressive, territorial males which inhibit the growth performance of the rest of the animals in the pond. On the other hand, the yield from all-female cultures is higher since females are less aggressive, less territorial and exhibit a homogenous body size in the end of the grow-out period. Therefore, selective harvest during or in the end of the grow-out period is not required. In the present study the first ever large-scale field study comparing mixed and all-female prawn cultures was performed thanks to the use of our novel biotechnology that included the following three steps: (1) a single injection of androgenic gland cell suspension caused fully functional sex-reversal of females into 'Neo-males' bearing the feminine WZ genotype; (2) crossing Neo-males with normal females (WZ), yielded progenies containing ~25% WW females as validated by sex-specific DNA markers and (3) WW females were crossed with normal males (ZZ), which gave rise to all-female progenies. All-female cultures showed better performance in all parameters including higher survival rate, higher yield per hectare and uniform body size leading to more marketable animals from a given pond. Also, the fact that no males or berried females were found in the all-female ponds reflects the reliability of this novel technology to achieve sustainable monosex female prawn aquaculture. Further applications regarding this biotechnology will be discussed.

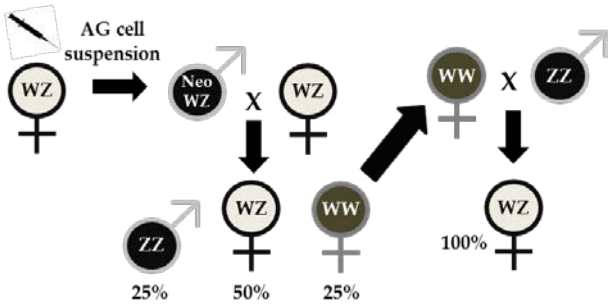

Figure 1: Reproduction scheme of Neo-male (WZ) with normal female (WZ) and of normal male (ZZ) with "super female" (WW)

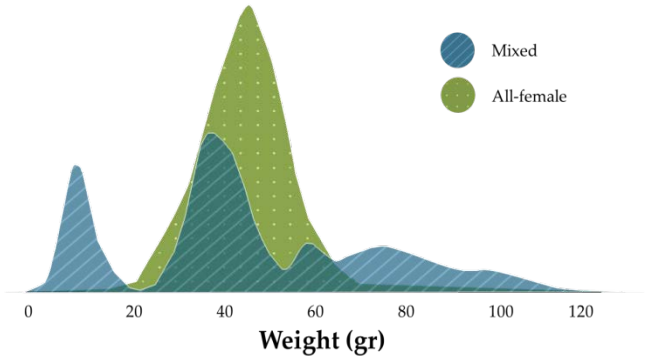

Figure 2: Size distribution of all-female culture compared to mixed culture

Keywords: All-female population; Androgenic gland; Intensification; Macrobrachium rosenbergii.

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QTL RESULTS REVEAL OVERLAPPED GENOMIC REGIONS FOR SEX DETERMINATION IN SILVER CARP AND BIGHEAD CARP

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Abstract

Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmicthys nobilis*) are genetically close aquaculture fish in the same genus of Cyprinidae, and they have been confirmed to hold female homogametry or male heterogametry sex determination (XX/XY) by both gynogenesis and sex-specific markers. However, genomic regions for sex in these two fish were not localized in previous QTL studies for growth due to difficult sexing for young fish. In this study, by using sex-specific markers recently identified, we performed sexing for previous families and QTL mapping for sex in silver carp and bighead carp, respectively. In silver carp, we mapped a genomic region on LG 21 (73.746-87.906 cM) significantly associated with sex, and 9 SNPs were in strong linkage disequilibrium with the sex with phenotypic variance explained (PVE) of 81.7-96.1%. In bighead carp, a genomic region (69.787-73.650 cM) was mapped on LG 19, and 6 SNPs were in strong linkage disequilibrium with the sex with PVE of 95.7-100%. Comparative genomic analyses indicated that LG 21 of silver carp is homologous to LG 19 of bighead carp and Chr 25 of zebrafish. This study showed that highly similar or overlapped genomic regions for sex determination may exist in both silver carp and bighead carp, which would provide clues to identify potential sex determining genes in the two carp species and elucidate genomic architecture and evolutionary mechanism of sex determination in cyprinid fishes.

Key Words: Silver carp ; bighead carp; sex determination region; QTL; Comparative mapping

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OPTIMIZATION OF TRIPLOIDY INDUCTION IN BENNI (*MESOPOTAMICHTHYS SHARPEYI*), USING HEAT AND COLD SHOCKS.

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Abstract

Chromosome manipulation produce triploidy, which is expected to improve fish yield in aquaculture. A study was done to identification of optimum temperature for triploidy induction in *Mesopothamichthys sharpeyi* by altering the duration and timing of application of heat and cold shocks. Heat shocks (34, 36 and 38 °C) and cold shocks (2 & 4 °C) were used for 3 and 5 minutes on recently fertilized eggs in two intervals (2 & 5 minutes after fertilization) which formed a total of 24 treatments in three replicates in addition to the control group, which developed without any shocks. Eggs were incubated under hatchery conditions at 23°C. The highest triploid yield was obtained in 34°C, 2 & 5 minutes after fertilization which lasted for 5 minutes. In triploid individuals, the indices of the nucleus increased significantly, confirming that the use of the RBC method could be used as a diagnostic method for the polyploidy state. The results of this study showed that induction of triploidy by thermal shocks especially heat, can lead to the production of triploid individuals in Benni. Regarding this result, in order to investigate the effect of triploidy on growth status and its comparison with diploid individuals it is recommended to continue the culture process until the maturity stage for future studies.

Keywords: Barbus, Benni, Heat shocks, polyploidy

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ANDROGENETIC DEVELOPMENT IN FISH - RADIATION-INDUCED ALTERATIONS IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) EGGS

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Abstract

Ionizing radiation (IR) is applied to inactivate nuclear genome in the rainbow trout eggs during androgenesis. However, it has been considered that doses of IR used to damage maternal chromosomes may also affect maternal RNA deposited in the cytoplasm during oogenesis and lead to the post ovulatory oocyte aging. To verify this assumption, quality and quantity of RNA extracted from the irradiated rainbow trout eggs were studied using standard molecular techniques and RNA-Seq approach. Moreover, distribution of lipid droplets in the irradiated and non-irradiated rainbow trout eggs and morphology of larvae that hatched from androgenetic and control groups were compared. Eggs were irradiated 350 Gy of X-rays, inseminated and exposed for the high hydrostatic pressure (HHP) shock to develop as androgenetic doubled haploids (DHs). RNAs from irradiated and non-irradiated eggs were extracted and their integrity evaluated and compared. A total of 800 ng of purified RNA was used as input for TruSeq RNA Sample Prep v2 kit (Illumina). Validated and normalized libraries were sequenced using TruSeq SBSv3 Sequencing kit (Illumina). No significant differences in RNA quantity were observed between control and irradiated eggs. Nevertheless, minor transcriptome alterations in the irradiated and non-irradiated eggs were shown. Irradiation of rainbow trout eggs with 350Gy altered transcripts of 754 genes analyzed on a pointwise level ($p<0.05$). On a genome-wide level, statistically significant ($p<0.01$) differences between irradiated and non-irradiated rainbow trout eggs concerned only few transcripts. In irradiated eggs, transcript of *nmrk2* (nicotinamide riboside kinase 2) gene showed reduced expression level while transcripts of genes known as "immediate early genes"; *ier2* (immediate early response 2) and *egr1* (early growth response 1) were found to be upregulated. Most of the non-irradiated eggs exhibited rather equal distribution of the oil droplets whereas eggs studied after irradiation had coalesced lipid droplets, a pattern found in the eggs with reduced quality. Incidences of abnormally developed larvae were more frequently observed among fish that hatched from the irradiated eggs. Provided results exhibited irradiation of rainbow trout eggs in order to inactivate maternal chromosomes may also affect maternal RNA and lead to rapid post ovulatory oocyte aging.

Key words: androgenesis, lipid droplets, maternal RNA, larvae, transcriptome

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