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RESEARCH

Comparative Transcriptome Analysis Reveals the Role of Ribosome Reduction in Impeding Oogenesis in Female Triploid *Crassostrea Gigas*

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Abstract

The fecundity of triploid female *Crassostrea gigas* exhibited significant variation and was lower compared to diploid individuals. Previous studies categorized mature stage triploid female *C. gigas* into two groups: female α, characterized by a high number of oocytes, and female β, displaying few or no oocytes. To investigate the molecular mechanisms underlying irregular oogenesis and fecundity differences in triploid *C. gigas*, we performed a comparative analysis of gonad transcriptomes at different stages of gonadal development, including female α, female β, and diploids. During early oogenesis, functional enrichment analysis between female diploids and putative female β triploids revealed differently expressed genes (DEGs) in the ribosome and ribosome biogenesis pathways. Expression levels of DEGs in these pathways were significantly decreased in the putative female β triploid, suggesting a potential role of reduced ribosome levels in obstructing triploid oogenesis. Moreover, to identify regulatory pathways in gonad development, female oysters at the early and mature stages were compared. The DNA repair and recombination proteins pathways were enriched in female diploids and female α triploids but absent in female β triploids. Overall, we propose that decreased ribosome biogenesis in female triploids hinders the differentiation of germ stem cells, leading to the formation of a large number of abnormal germ cells and ultimately resulting in reduced fecundity. The variation in fertility among triploids appeared to be related to the degree of DNA damage repair during female gonad development. This study offers valuable insights into the oogenesis process in female triploid *C. gigas*.

Keywords Triploid · *Crassostrea gigas* · Ribosome · Oogenesis

Introduction

Triploid technology has gained widespread use in aquaculture, encompassing various species such as triploid Atlantic salmon (*Salmo salar*) (Murray et al. 2018), triploid Chinese catfish (*Clarias fuscus*) (Qin et al. 1998), triploid rainbow trout (*Oncorhynchus mykiss*) (Weber et al. 2014), triploid shrimp (Manan and Ikhwanuddin 2021) and triploid oysters (Nell 2002). Triploid animals are characterized by gonadal sterility, which allows them to allocate more energy towards growth and maintain superior meat quality (Piferrer et al. 2009), resulting in significant economic and ecological benefits in aquaculture.

The Pacific oyster (*Crassostrea gigas*) is one of the most extensively farmed shellfish worldwide. The utilization of triploid Pacific oysters offers numerous advantages, including enhanced growth rates in the reproductive seasons and increased marketability, resulting in a substantial share of the oyster culture industry (Nell 2002; Normand et al. 2008; Dégremont et al. 2016). Research on triploid *C. gigas* has been a prominent focus in aquaculture studies since its initial introduction. Numerous investigations have been conducted on the growth, development, and stress resistance of triploid *C. gigas* (Li et al. 2022; Nell 2002; Normand et al. 2008; Yang et al. 2018).

The fecundity of triploid *C. gigas* has been consistently reported to be significantly lower compared to diploid

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individuals (Beaumont and Fairbrother 1991; Guo and Allen 1994; Jouaux et al. 2010). Guo and Allen (1994) evaluated triploid fertility and found that the relative fecundity of triploid females was only around 2% compared to female diploid controls. Jouaux et al. (2010) measured reproductive effort using the gonadal tubule index and found over 81.6% for diploid oysters, while the maximum value for female triploids was 53.9%. The gonadal development of female triploid *C. gigas* exhibits complexity and high variation (Allen and Downing 1990; Jouaux et al. 2010; Normand et al. 2008; Yang et al. 2022a). Allen and Downing (1990) observed high variation in the number of oocytes, while Normand et al. (2008) found considerable variation in the extent of gonad development between diploid and triploid *C. gigas* during the reproductive season. Jouaux et al. (2010); Yang et al. (2022a) categorized females into two groups: female α, which produced a large number of oocytes and exhibited fertility, and female β, which was completely sterile and produced few or no oocytes. Extensive histological analysis revealed approximately 10% of triploids were classified as females α, while over 40% were classified as female β (Yang et al. 2022a).

Notably, previous studies identified abnormal oogenesis in triploids by conducting histological analyses comparing diploid and triploid oysters (Jouaux et al. 2010; Matt and Allen 2021; Yang et al. 2022a). These studies reported the presence of abnormal germ cells in triploids, characterized by condensed and rod-shaped chromosomes located along the inner side of the gonadal tubules. Jouaux et al. (2010) suggested that these cells represented a blockage of gonial mitosis events in triploids. Matt and Allen (2021) noted the consistent presence of these germ cells in female triploids and hypothesized that they represented irregular female germ cells. Yang et al. (2022b) analyzed the cell cycle of gonadal cells in triploids and found that their cell cycle might be arrested before the G2 phase. Based on these findings, the reduced fertility in female triploids is suggested to be associated with the presence of these abnormal germ cells, and the early gametogenesis stage may be the key to understanding the molecular mechanism of oogenesis abnormalities in triploids.

To unravel the molecular mechanism behind the abnormal oogenesis in female triploids of *C. gigas*, we conducted a comparative analysis of the transcriptome between diploids and triploids, with a particular focus on the early gametogenesis stage. Our results provide fresh perspectives and novel insights into the sterility of triploid oysters.

Materials and Methods

Sample Collection, Ploidy Verification

The triploid and diploid oysters were collected from March to June 2021, from a commercial hatchery, in Qingdao, Shandong, China (36.2°N, 120.6°N). Before sample collection, the ploidy of triploids was verified. Approximately 1 mm^3 of gill tissue was cut up as possible in PBS (Phosphate buffer saline) for cell suspension and DAPI solution (final concentration: 0.6 μg/mL) was used to stain cells. The ploidy of triploids was verified via flow cytometry (CytoFlex Beckman Coulter, US). The gonad tissue from each oyster was sampled and fixed in Bouin's solution for histological analysis, and the rest was frozen in liquid nitrogen and stored at -80 °C for RNA extraction.

Histological Analysis

The gonads of oysters were fixed in Bouin's solution for 24 h, then dehydrated in successive dilutions of ethanol and embedded in paraffin wax at last. Sections of five micrometers thickness were cut and stained with Hematoxylin-Eosin. The slides were observed through Olympus BX53 microscope (Olympus, Japan). The sex and gonad development stages of the diploid and triploid Pacific oysters were determined according to the new classification criteria (Yang et al. 2022a). To compare the oogenesis of diploids and triploids, the early stage of oogenesis (S1) and the mature stage (S3) of female diploids and female triploids were focused. In addition, each stage of female triploids was further divided into two types. The female triploids at the mature stage were subdivided into 3nα with plenty of oocytes and 3nβ without or with few oocytes. For female triploids in the S1 stage, those with oocytes were identified as putative 3nα, while females with follicles containing β gonia were classified as putative 3nβ.

RNA Extraction, Library Construction, and Sequencing

Total RNAs from gonad tissues were extracted with Trizol reagent (Invitrogen). RNA concentration and integrity were detected using an RNA Nano 6000 Assay Kit and a kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA), respectively. Three duplicate individual gonad samples of six groups were prepared and named Ds1F_1, Ds1F_2, Ds1F_3, P-Ts1Fα_1, P-Ts1Fα_2, P-Ts1Fα_3, P-Ts1Fβ_1, P-Ts1Fβ_2, P-Ts1Fβ_3, Ds3F_1, Ds3F_2, Ds3F_3, Ts3Fα_1, Ts3Fα_2, Ts3Fα_3, Ts3Fβ_1, Ts3Fβ_2, Ts3Fβ_3. The libraries were sequenced using 150 bp paired-end reads on an Illumina NovaSeq 6000 platform.

The cDNA libraries of female diploid and triploids at the mature stage were obtained from our previous study (Sun et al. 2022) with accession numbers SRR13374889, SRR13374888, SRR13374879,SRR13374878,SRR13374877,SRR13374876, SRR13374875, SRR13374874, SRR13374873. The raw read data of female diploids and triploids at the early gametogenesis have been submitted to the NCBI SRA database with the project accession number PRJNA994674.

Differential Gene Expression Analysis

The quality of the raw reads from the 18 libraries was assessed using fastp v0.20.1 package (Chen et al. 2018). After removing adapter sequences, poly-N sequences, and low-quality reads, the clean reads and reports of the Q20, Q30, and GC content of the clean data were produced. All the downstream analyses were based on these high-quality clean data. Then, all the RNA-seq reads were mapped to the oyster genome (GenBank accession No. GCA_902806645.1) with the HISAT2 v2.2.1 (Kim et al. 2015). The produced SAM files were converted to BAM files and sorted by SAMtools v1.10 (Li et al. 2009). Based on the *C. gigas* genome sequence, transcript assembly was performed by StringTie v2.1.2 (Pertea et al. 2015), with which StringTie estimated expression abundance for input to IsoformSwitchAnalyzeR v1.13.05 (Vitting-Seerup and Sandelin, 2019). Then, raw read counts of all genes were extracted with the IsoformSwitchAnalyzeR, and the fragments per kilobase per million mapped reads (FPKM) of each gene were also calculated. The abundances of each gene in read count values were normalized using the transcripts per million (TPM) method. Principal component analysis (PCA) was used to examine variations in the gene expression patterns of samples among the different time points. Correlations among all samples were analyzed using RStudio v1.4.1717 software. The DESeq2 R package was used to analyze differential expression patterns (Love et al. 2014). Differentially expressed genes (DEGs) were identified using the following criteria: adjusted $P < 0.05$ and $|\log2(\text{fold change})| > 1$.

Differentially Expressed Genes Enrichment Analysis

GO and KEGG enrichment analysis of differentially expressed genes was implemented by the clusterProfiler R package (3.8.1). To establish the latest local protein databases of Gene ontology (GO) (Khatri and Draghici 2005) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2007), all the metazoan protein sequences with GO numbers were downloaded from NCBI protein database, and those with K numbers were obtained through KEGG API (https://www.kegg.jp/kegg/rest/keggapi.html). Through BLASTP (cut-off E-value of 1e-5) against the resulting databases, deduced amino acid sequences of all the genes (FPKM > 1 in at least one group) were assigned with GO and KEGG numbers. GO and Kegg enrichment was done using clusterProfiler (Yu et al. 2012) with default parameters and then visualized.

Validation of the RNA-seq Analysis by qRT-PCR

Total RNAs from gonad tissues of diploid and triploid Pacific oysters were extracted with Trizol reagent (Invitrogen). The cDNA was synthesized using the PrimeScriptTM reverse transcription kit (Takara, Japan). Primer sequences designed for RT-qPCR were provided in Table S1. All RT-qPCR was carried out in a LightCycler® 480 real-time PCR system (Roche, Switzerland) using 2 × SYBR Premix ExTaq (Qiagen, Germany). Five commonly used housekeeping genes, EF-1a, EF2, RS18, RO21, and RL7, were evaluated for their expression stabilities with GeNorm v3.5 software (Vandesompele et al. 2002). The RS18 was finally the reference gene. The PCR reactions were initiated as follows: 95 °C for 5 min, 40 amplification cycles at 95 °C for 15 s, and 60 °C for 30 s. Melting curves were constructed to ensure accurate amplification. The relative expression levels (RNA abundance) of the target gene were calculated with $2^{-\Delta\Delta CT}$, and data were expressed as the mean ± SD. The significant differences ($P < 0.05$) were analyzed using one-way ANOVA, followed by a posthoc comparison with the Tukey HSD method.

Results and Discussion

Histological Analysis of Gonad Development in Diploid and Triploid Pacific Oysters

To study triploid oogenesis, the gonads of diploid and triploid oysters were analyzed during the early stage (Fig. 1a-c) and the mature stage (Fig. 1d-f) of gametogenesis. The developmental stages of all samples were determined according to our previous morphological classification criteria (Yang et al. 2022a). During the early stage, two types of triploids were identified, putative Ts1Fα and putative Ts1Fβ. The putative Ts1Fα triploids exhibited distinct previtellogenic oocytes, oocytes, and β gonia (Fig. 1b), while the putative Ts1Fβ triploids only showed β gonia and were considered sterile (Fig. 1c). At the mature stage, two types of female triploids were also observed: Ts3Fα, characterized by numerous mature oocytes (Fig. 1e), and Ts3Fβ, which produced few or no oocytes (Fig. 1f).

Transcriptome Assemblies

To identify differentially expressed genes in the gonads of triploid and diploid oysters, eighteen cDNA libraries were constructed at both the early gametogenesis and the mature stages. Through quality control, a total of 1146.7 million (Q30:

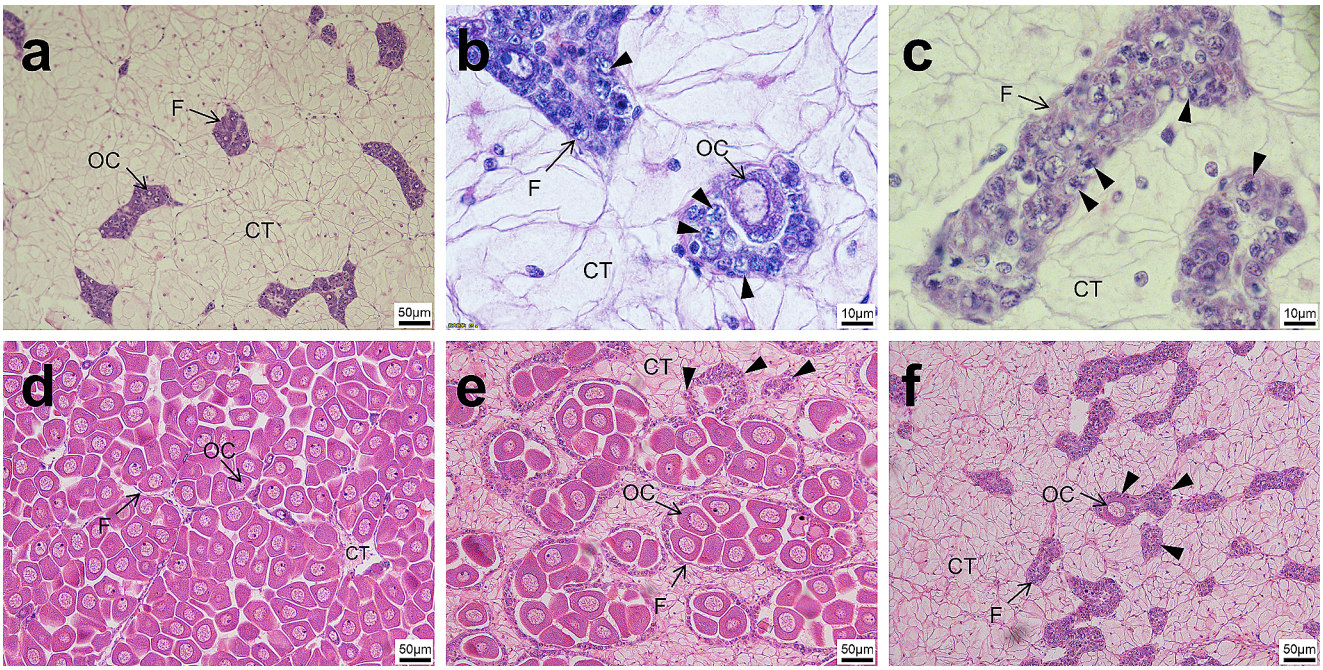

Fig. 1 Female diploids and triploids at the early gametogenesis (**a**-**c**) and the mature stage (**d**-**f**). (**a**) female diploids - Ds1F; (**b**) female triploids - P-Ts1Fα; (**c**) female β triploids - P-Ts1Fβ; (**d**) female diploids - Ds3F; (**e**) female α triploids containing numerous oocytes - Ts3Fα; (**f**) female β triploids containing few or no oocytes - Ts3Fβ. Oc: Oocyte; F: Gonadal follicle; CT: Conjunctive tissue; β gonia (arrowhead)

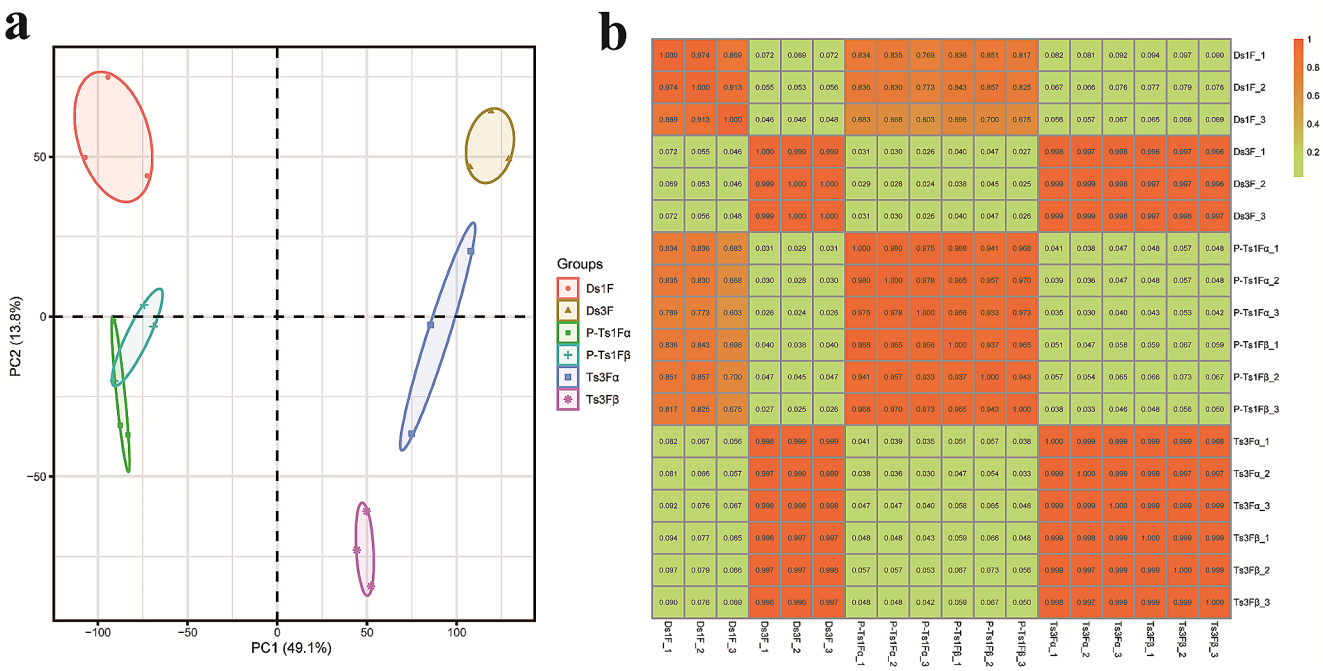

Fig. 2 Correlations among all samples. (**a**) Principal component analysis (PCA) of all normalized gene expression levels (TPM) clustered by different ploidy and development stages; (**b**) Correlations among all samples shown in a heatmap

91.61–96.32%) clean reads were obtained from 1154.4 million pair-end 150 bp raw reads across eighteen libraries (Table S2). After alignment, the principal component analysis (PCA) was employed to visualize the relative relationships among all samples (Fig. 2a). PC1, which accounted for 49.1% of the observed variation, effectively clustered the biological replicates together. The P-Ts1Fα group showed overlap with the P-Ts1Fβ group. In addition, a strong correlation was observed among samples at the same stage, whereas weaker correlations were evident between samples at different stages (Fig. 2b).

Identification of DEGs and Validation by qRT-PCR

The expression distribution of all transcripts is shown in Fig. 3. A total of 1740 DEGs in the comparison between Ds1F and P-Ts1Fβ was detected, including 950 up-regulated genes and 790 down-regulated genes (Fig. 3a). For the comparison between P-Ts1Fα and P-Ts1Fβ, 488 DEGs were detected, with 410 up-regulated genes and 78 down-regulated genes (Fig. 3b). In the Ds3F vs. Ts3Fβ comparison, 8256 DEGs were found, including 3985 upregulated genes and 4271 down-regulated genes (Fig. 3c). In the Ts3Fα vs. Ts3Fβ comparison, 2986 DEGs were identified, including 974 upregulated genes and 2012 down-regulated genes (Fig. 3d). The number of DEGs was higher in the mature stage of gonadal development compared to the early stage (Fig. 3e). A Venn diagram revealed the shared and unique DEGs among the Ds1F vs. P-Ts1Fβ, P-Ts1Fα vs. P-Ts1Fβ, Ds3F vs. Ts3Fβ, and Ts3Fα vs. Ts3Fβ comparisons (Fig. 3f).

To evaluate the accuracy of the RNA-seq results, we performed qRT-PCR on 20 DEGs involved in the gonadal development of oysters. The fold change values obtained from RNA-seq expression analysis were compared with those from qRT-PCR (Table S1). The fold-change values of these selected DEGs were similar, indicating that the RNA-seq expression analysis yielded accurate results.

GO Analysis

To further annotate the differentially expressed genes and assess their functions, GO enrichment analysis of DEGs was retrieved (Fig. 4). GO analysis revealed that a total of 54, 55, 297, and 204 GO terms were enriched among the Ds1F vs. P-Ts1Fβ, P-Ts1Fα vs. P-Ts1Fβ, Ds3F vs. Ts3Fβ, and Ts3Fα vs. Ts3Fβ groups, respectively (Fig. 4a-d). The number of enriched GO terms between diploids and triploids increased from the early oogenesis to the mature stage.

KEGG Pathway Enrichment Analysis

Comparative Analysis in the same Developmental Stage

During the progression of oogenesis, primordial germ cells (PGCs) differentiate into germline stem cells (GSCs), which ultimately give rise to oocytes (Cavelier et al. 2017). In the early stage, female triploid *C. gigas* exhibits abnormal gonad development compared to diploids, giving rise to abnormal germ cells referred to as β gonia (Jouaux et al. 2010; Yang et al.

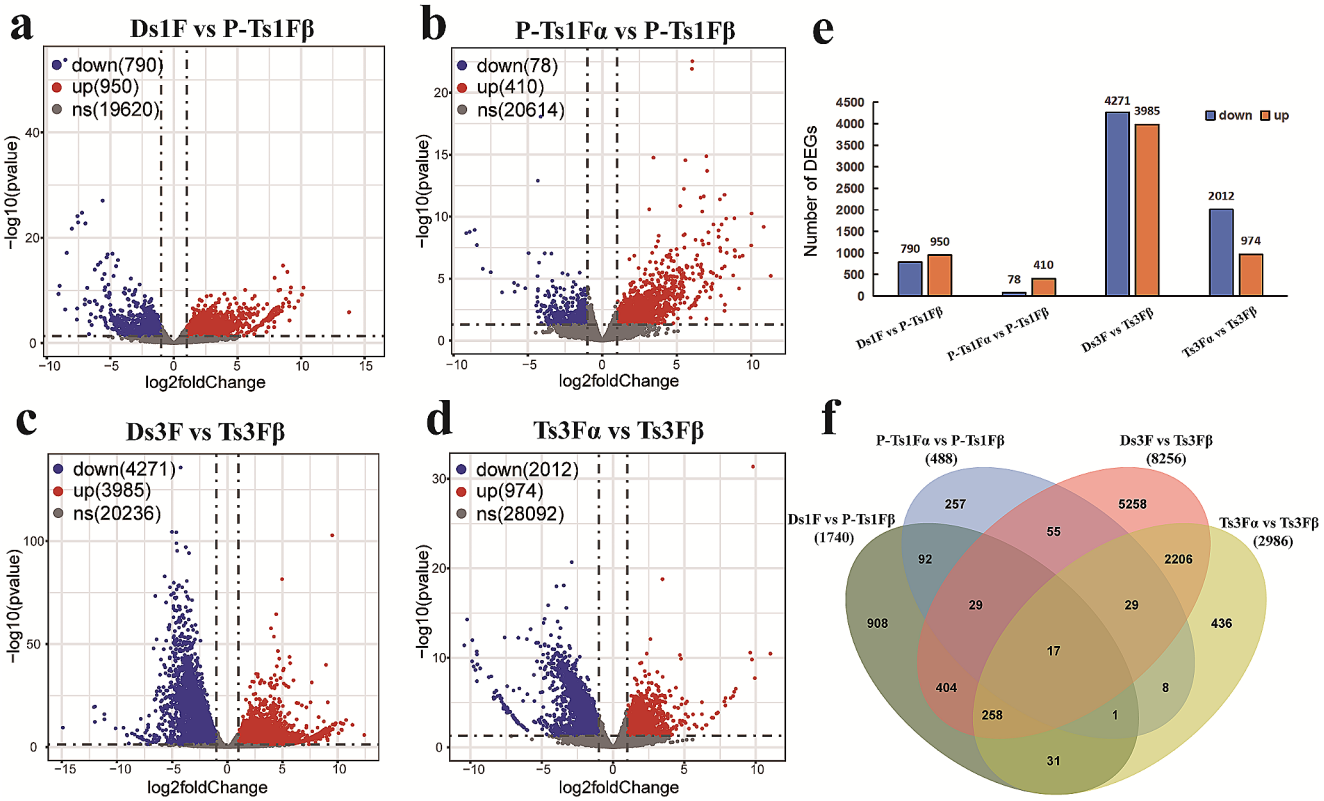

Fig. 3 Comparison of differentially expressed genes (DEGs). (**a-d**) Volcano plot of DEGs in the Ds1F vs. P-Ts1Fβ, P-Ts1Fα vs. P-Ts1Fβ, Ds3F vs. Ts3Fβ, and Ts3Fα vs. Ts3Fβ groups; (**e**) Numbers of up-regulated and down-regulated DEGs in each group; (**f**) Venn diagram of overlapping DEGs among these four groups

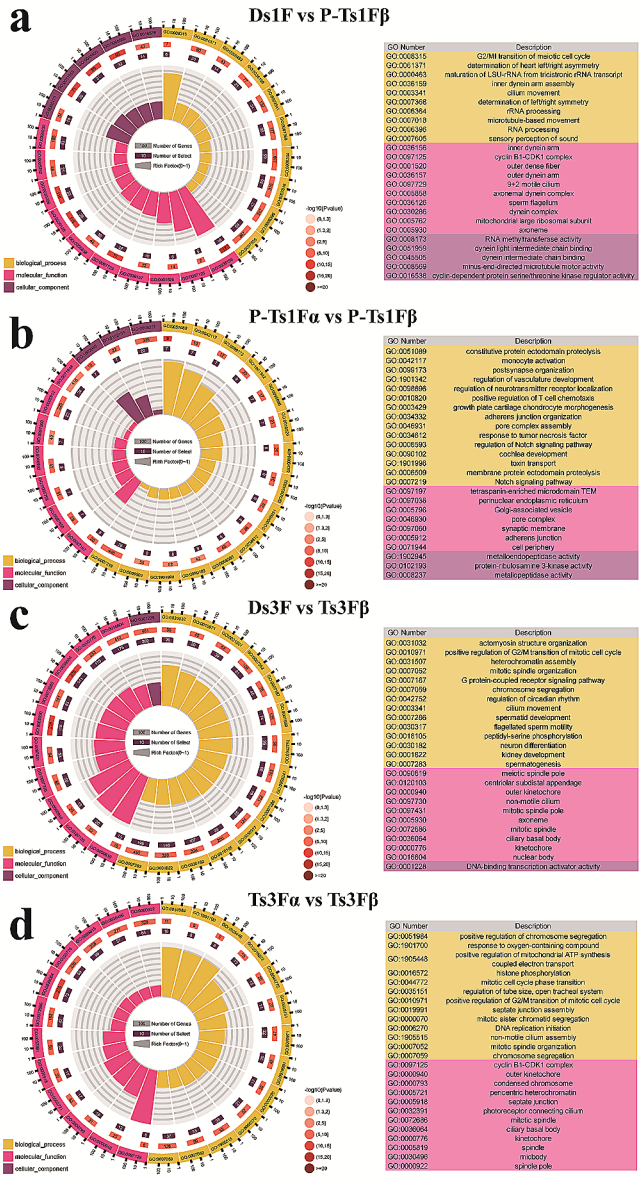

Fig. 4 The top 25 significantly enriched GO terms in the (**a**) Ds1F vs. P-Ts1Fβ, (**b**) P-Ts1Fα vs. P-Ts1Fβ, (**c**) Ds3F vs. Ts3Fβ, and (**d**) Ts3Fα vs. Ts3Fβ groups. The outermost circle represnts the top 25 GO terms enriched and assigned to three functional classes, biological processes (yellow), molecular functions (aubergine), and cellular components (purple). The second circle indicates the numbers of the genes in the background and *p* values for each term. The innermost circle indicates the number of DEGs with each GO term

2022a). To investigate the molecular mechanisms underlying this atypical gonad development in triploid *C. gigas*, a comparative transcriptomic analysis of triploid and diploid oysters during early oogenesis was performed.

The DEGs identified in the Ds1F vs. P-Ts1Fβ, P-Ts1Fα vs. P-Ts1Fβ, Ds3F vs. Ts3Fβ, and Ts3Fα vs. Ts3Fβ groups were found to be significantly enriched in 13, 5, 27, and 14 KEGG pathways, respectively (Fig. 5a-e). Notably, the significantly enriched signaling pathways in the DS1F vs. P-TS1β group were primarily associated with the ribosome (Ribosome biogenesis and Ribosome), which was consistent with the result obtained from the DS1F vs. P-TS1α group (Fig. S1). The majority of DEGs associated with these pathways were found to be down-regulated in P-Ts1Fβ compared to Ds1F (Fig. 6).

Ribosomes play a crucial role in the translation of mRNAs into proteins, essential for maintaining basic physiological activities in most organisms. Reduced ribosome levels have been shown to cause differentiation defects in various stem cell systems (Corsini et al. 2018; Khajuria et al. 2018; Zhang et al. 2014). In humans, decreased ribosome levels result in diseases due to improper differentiation of tissue-specific progenitor cells (Armistead and Triggs-Raine 2014; Brooks et al. 2014; Higa-Nakamine et al. 2012; Mills and Green 2017). An increasing number of studies have reported that the transition from self-renewal to differentiation of GSCS relies on enhanced ribosome biogenesis (Sanchez et al. 2016; Jang et al. 2021; Martin et al. 2022; Breznak et al. 2023). In *Drosophila*, knockdown of several ribosome assembly components (snoRNP, pre-90 S, pre-60 S, and pre-40 S components) in S2 cells led to the accumulation of undifferentiated cells and ovarioles containing fewer germ cells (Sanchez et al. 2016). Jang et al. (2021) reported that ribosomal protein paralogs, RpS5b, regulate the homeostasis of functional ribosomes to promote proper oocyte chamber development. Martin et al. (2022) discovered that the knockdown of Aramis, Athos, and Porthos (three DExD/H-box proteins) in GSCS led to a germline ribosome biogenesis defect, cell cycle arrest and resulted in the loss of differentiation and the formation of stem cysts during the oogenesis of *Drosophila*. The H/ACA small nuclear ribonucleoprotein (snRNP) complex, responsible for pseudouridylation of ribosomal RNA (rRNA), promotes this process. Depletion of the H/ACA snRNP complex components resulted in a specific loss of GSCs (Breznak et al. 2023).

Our previous studies have found cell cycle arrest in germ cells of triploids and speculated that this may be due to the presence of abnormal germ cells, β gonia (Yang et al. 2022b). These findings suggest that a low level of ribosomes may contribute to the blockage of oogenesis in female triploids. The ribosome serves as the primary site for protein synthesis, and the level of ribosomes directly impacts the rate of protein synthesis (Forchhammer and Lindahl 1971). In our study, we also detected differences in protein synthesis-related pathways, including Translation factors, Transcription machinery, and Transfer RNA biogenesis, between diploids and triploids at the early stage. Importantly, the expression of most genes within these pathways was significantly down-regulated in triploids compared to diploids.

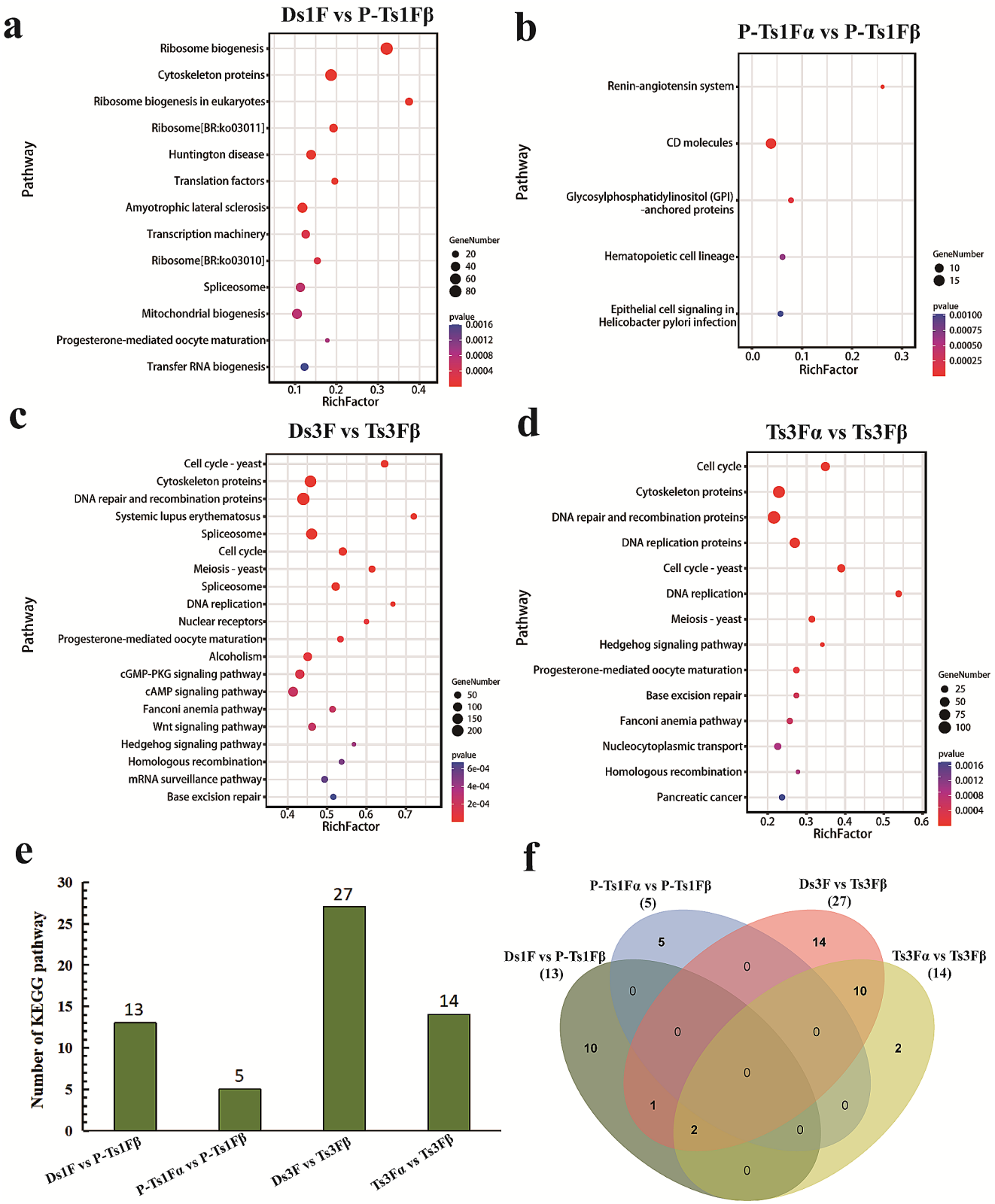

Fig. 5 KEGG enrichment analysis of DEGs in the (**a**) Ds1F vs. P-Ts1Fβ, (**b**) P-Ts1Fα vs. P-Ts1Fβ, (**c**) Ds3F vs. Ts3Fβ, and (**d**) Ts3Fα vs. Ts3Fβ groups. The Ds3F vs. Ts3Fβ (**c**) group only showed the top 20 pathways of KEGG enrichment analysis. (**e**) Some pathways of KEGG enrichment analysis in each group; (**f**) Venn diagram of overlapping pathways among these four groups

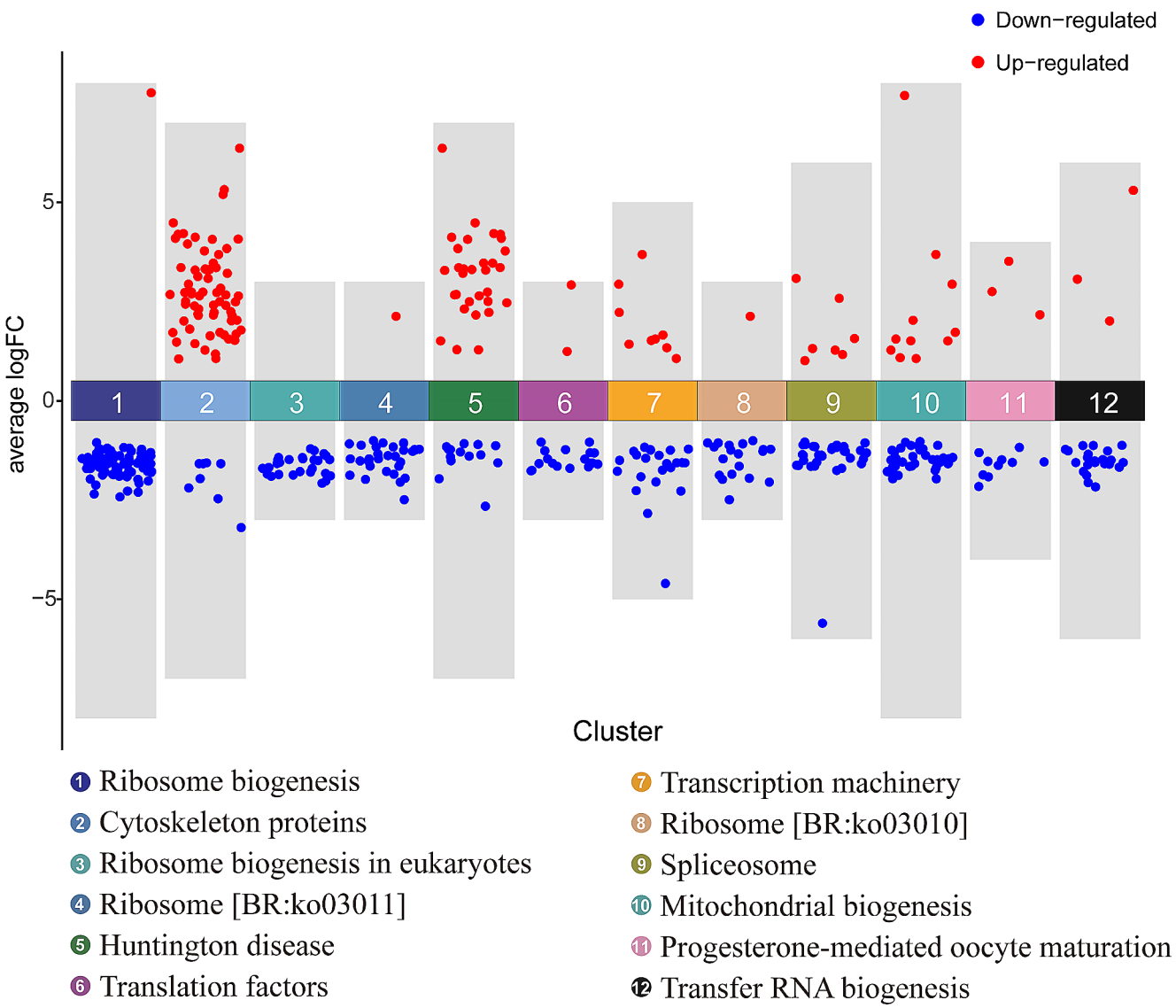

Fig. 6 Differential gene expression analysis showing up- and down-regulated genes of twelve KEGG enriched pathways in the Ds1F vs. P-Ts1Fβ group

Differentiation of germline stem cells relies on enhanced ribosome biogenesis and increased protein synthesis (Sanchez et al. 2016).

Based on these findings, we propose a hypothesis for the early gametogenesis of female triploids. Given the ribosome's pivotal role in protein synthesis, a reduction in ribosome level is anticipated to down-regulat genes associated with protein synthesis-related pathways, including Translation factors, Transcription machinery, and Transfer RNA biogenesis. Consequently, this impediment is likely to hinder the differentiation of germ stem cells, leading to the accumulation of a substantial number of abnormal germ cells identified as β gonia.

Comparative Analysis Between Different Developmental Stages

A notable feature of female triploid *C. gigas* is the variation in fecundity between individuals. Two types of triploids, female α and female β, have been identified, with female α being fertile and producing a significant number of mature eggs, while female β is sterile, producing few or no eggs (Yang et al. 2022a). To investigate the molecular mechanisms underlying this fecundity difference in triploid *C. gigas*, we performed KEGG enrichment analysis on the DS1F vs. DS3F, P-TS1Fα vs. TS3Fα, and P-TS1Fβ vs. TS3Fβ groups. The results showed that 66, 40, and 34 pathways were enriched in these groups (Fig. 7). Eleven pathways were found to be enriched only in DS1F vs. DS3F and P-TS1Fα vs. TS3Fα, but absent

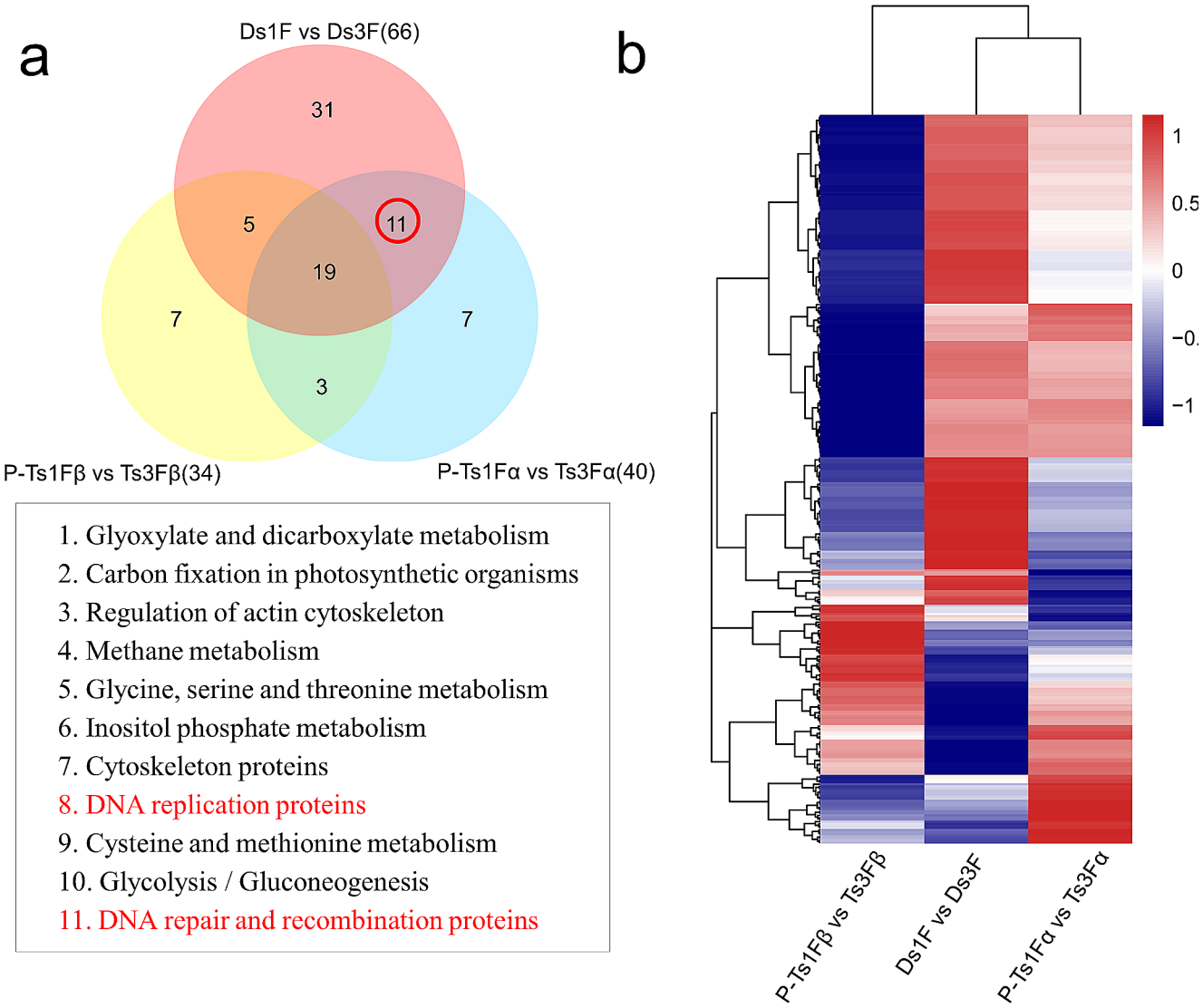

Fig. 7 (**a**) Venn diagram of overlapping pathways of KEGG enrichment analysis of DEGs in the Ds1F vs. Ds3F, P-Ts1Fα vs. Ts3Fα and P-Ts1Fβ vs. Ts3Fβ groups. (**b**) The expression patterns of all DEGs from pathways (DNA repair and recombination proteins and DNA replication) in the three groups

in P-TS1Fβ vs. TS3Fβ (Fig. 7). Notably, two of these pathways were related to DNA repair and recombination proteins, and DNA replication. The expression patterns of genes associated with these pathways were similar in diploids and female α triploids, while the expression levels in female β triploids showed opposite trends (Fig. 7b). All genes related to DNA repair and recombination proteins, as well as DNA replication, are detailed in Table S3. These results suggest that during the gonad development, DNA repair and replication play an important role in the regulation of oogenesis and gonadal development in diploids and female α triploids. However, in female β triploids, these two pathways are not enriched and most genes were down-regulated, indicating that they are not initiated and do not play a regulatory role, ultimately leading to a decrease in oogenesis.

Triploids possess an odd set of chromosomes compared to normal diploids, which leads to chromosomal instability during cell division (Zhang et al. 2010). Guo and Allen (1997) observed aneuploidy in triploid gametes by analyzing the number of chromosomes, and Zhang et al. (2010) detected heteroploid mosaics in triploid gill cells, suggesting clumping and abnormal segregation of chromosomes during mitotic divisions in triploids. Thus, the chromosomal stability of triploid germ cells during genesis is more compromised than that of diploid, potentially leading to increased accumulation of DNA damage. DNA repair mechanisms play important roles in the development and maturation of oocytes to ensure the integrity of the offspring's genetic material (Stringer et al. 2018; Aitken 2022). Dheilly et al. (2014) identified differentially expressed genes between triploid and diploid *C. gigas* using a microarray

and found upregulation of genes involved in DNA replication and recombination in fertile triploids and diploids compared to sterile triploids (Dheilly et al. 2014). In triploid *Nodipecten subnodosus* scallops, Galindo-Torres et al. (2022) performed a comparative transcriptome analysis between sterile triploids and diploids in different stages and found upregulation of genes related to the DNA damage response and double-strand break repair. They speculated that the sterility of triploid scallops may be associated with DNA damage and failure to repair during DNA replication in the initial gametogenesis of triploids. Therefore, the variation in fertility between triploid individuals may be related to DNA damage repair during the development of female gonads. The upregulation of DNA replication in fertile triploids may contribute to their ability to produce a larger number of gametes.

An increasing number of studies have revealed the crosstalk between the DNA damage response and ribosome biogenesis, indicating a dual role for conventional DNA repair proteins in ribosome biogenesis and vice versa (Ogawaa and Baserga, 2017). For example, FANCI, binding with FANCD2, activated for DNA repair by phosphorylation and monoubiquitination (Garner and Smogorzewska 2011; Joo et al. 2011; Kottemann and Smogorzewska 2013). FANCI is also localized to the nucleolus and involved in ribosome biogenesis (Sondalle et al. 2019). In our study, the decreased ribosome biogenesis in female triploid *C. gigas* may lead to the down-regulation of DNA repair due to the dual role of key ribosomal genes, and results in gametogenesis defect in female triploid *C. gigas* during the gonadal development.

In summary, this study performed a comparative analysis of gonad transcriptomes at different stages of gonadal development between female triploids and female diploids. Our results imply that the decreased ribosome biogenesis in female triploids *C. gigas* hinders the differentiation of germ stem cells, ultimately resulting in reduced fecundity. With the female gonadal development, variations in fertility among female triploid individuals emerge, possibly related to the level of DNA damage repair during oogenesis. This study offers valuable insights into the oogenesis process in female triploid *C. gigas*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10126-024-10283-2.

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Author Contributions Qiong Yang: Completion of the experiment, data analysis, and manuscript drafting. Hong Yu: Experimental design and coordination and manuscript revision. Qi Li: Experimental design and coordination and manuscript revision.

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Data Availability The data analyzed in this study are presented in this article and the supplementary information file.

Declarations

Competing Interests The authors declare no competing interests.

References

Aitken RJ (2022) Role of sperm DNA damage in creating de-novo mutations in human offspring: the 'post-meiotic oocyte collusion' hypothesis. Reprod Biomed Online 45:109–124

Allen SK, Downing SL (1990) Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. Can J Fish Aquat Sci 47:1213–1222

Armistead J, Triggs-Raine B (2014) Diverse diseases from a ubiquitous process: the ribosomopathy paradox. FEBS Lett 588:1491–1500

Beaumont AR, Fairbrother JE (1991) Ploidy manipulation in molluscan shellfish: a review. J Shellfish Res 10:1–18

Breznak SM, Peng Y, Deng L, Kotb NM, Flamholz Z, Rapisarda IT, Martin ET, LaBarge KA, Fabris D, Gavis ER, Rangan P (2023) H/ACA snRNP-dependent ribosome biogenesis regulates translation of polyglutamine proteins. Sci Adv 9:eade5492

Brooks SS, Wall AL, Golzio C, Reid DW, Kondyles A, Willer JR, Botti C, Nicchitta CV, Katsanis N, Davis EE (2014) A novel ribosomopathy caused by dysfunction of RPL10 disrupts neurodevelopment and causes X-linked microcephaly in humans. Genetics 198:723–733

Cavelier P, Cau J, Morin N, Delsert C (2017) Early gametogenesis in the Pacific oyster: new insights using stem cell and mitotic markers. J Exp Biol 220:3988–3996

Chen S, Zhou Y, Chen Y, Gu J (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890

Corsini NS, Peer AM, Moeseneder P, Roiuk M, Burkard TR, Theussl HC, Moll I, Knoblich JA (2018) Coordinated control of mRNA and rRNA processing controls embryonic stem cell pluripotency and differentiation. Cell Stem Cell 22:543–558

Dégremont L, Ledu C, Maurouard E, Nourry M, Benabdelmouna A (2016) Effect of ploidy on the mortality of *Crassostrea gigas* spat caused by OsHV-1 in France using unselected and selected OsHV-1 resistant oysters. Aquac Res 47:777–786

Dheilly NM, Jouaux A, Boudry P, Favrel P, Lelong C (2014) Transcriptomic profiling of gametogenesis in triploid pacific oysters *Crassostrea gigas*: towards an understanding of partial sterility associated with triploidy. PLoS ONE 9

Forchhammer J, Lindahl L (1971) Growth rate of polypeptide chains as a function of the cell growth rate in a mutant of Escherichia coli 15. J Mol Biol 55:563–568

Galindo-Torres P, Abreu-Goodger C, Llera-Herrera R, Escobedo-Fregoso C, García-Gasca A, Ibarra AM (2022) Triploid-induced complete sterility in the scallop *Nodipecten subnodosus* might be triggered by an early and sustained DNA damage response. Aquaculture 559:738422

Garner E, Smogorzewska A (2011) Ubiquitylation and the fanconi anemia pathway. FEBS Lett 585:2853–2860

Guo X, Allen SK (1994) Reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Biol Bull 187:309–318

Guo X, Allen SK (1997) Sex and meiosis in autotetraploid pacific oyster, *Crassostrea gigas* (Thunberg). Genome 40:397–405

Higa-Nakamine S, Suzuki T, Uechi T, Chakraborty A, Nakajima Y, Nakamura M, Hirano N, Suzuki T, Kenmochi N (2012) Loss of ribosomal RNA modification causes developmental defects in zebrafish. Nucleic Acids Res 40:391–398

Jang S, Lee J, Mathews J, Ruess H, Williford AO, Rangan P, Betrán E, Buszczak M (2021) The Drosophila ribosome protein S5 paralog RpS5b promotes germ cell and follicle cell differentiation during oogenesis. Dev 148

Joo W, Xu G, Persky NS, Smogorzewska A, Rudge DG, Buzovetsky O, Elledge SJ, Pavletich NP (2011) Structure of the FANCI-FANCD2 complex: insights into the Fanconi anemia DNA repair pathway. Science. 2011;333(6040):312–316

Jouaux A, Heude-Berthelin C, Sourdaine P, Mathieu M, Kellner K (2010) Gametogenic stages in triploid oysters Crassostrea gigas: irregular locking of gonial proliferation and subsequent reproductive effort. J Exp Mar Bio Ecol 395:162–170

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T, Yamanishi Y (2007) KEGG for linking genomes to life and the environment. Nucleic Acids Res 36:D480–D484

Khajuria RK, Munschauer M, Ulirsch JC, Fiorini C, Ludwig LS, McFarland SK, Abdulhay NJ, Specht H, Keshishian H, Mani DRR et al (2018) Ribosome levels selectively regulate translation and lineage commitment in human hematopoiesis. Cell 173:90–103

Khatri P, Draghici S (2005) Ontological analysis of gene expression data: current tools, limitations, and open problems. Bioinformatics 21:3587–3595

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357–360

Kottemann MC, Smogorzewska A (2013) Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. Nature 493:356–363

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079

Li Y, Xu C, Li Q (2022) Physiological and gene expression responses of diploid and triploid Pacific oyster (*Crassostrea gigas*) to heat acclimation. Aquac Res 53:6641–6650

Love MI, Huber W, Anders S (2014) Moderated estimation of Fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:1–21

Manan H, Ikhwanuddin M (2021) Triploid induction in penaeid shrimps aquaculture: a review. Rev Aquacult 13:619–631

Martin ET, Blatt P, Nguyen E, Lahr R, Selvam S, Yoon HAM, Pocchiari T, Emtenani S, Siekhaus DE, Berman A, Fuchs G, Rangan P (2022) A translation control module coordinates germline stem cell differentiation with ribosome biogenesis during *Drosophila* oogenesis. Dev Cell 57:883–900

Matt JL, Allen SK (2021) A classification system for gonad development in triploid *Crassostrea virginica*. Aquaculture 532:735994

Mills EW, Green R (2017) Ribosomopathies: there's strength in numbers. Science 358:6363

Murray DS, Kainz MJ, Hebberecht L, Sales KR, Hindar K, Gage MJG (2018) Comparisons of reproductive function and fatty acid fillet quality between triploid and diploid farm Atlantic salmon (Salmo salar). R Soc Open Sci 5

Nell JA (2002) Farming triploid oysters. Aquaculture 210:69–88

Normand J, Le Pennec M, Boudry P (2008) Comparative histological study of gametogenesis in diploid and triploid Pacific oysters (*Crassostrea gigas*) reared in an estuarine farming site in France during the 2003 heatwave. Aquaculture 282:124–129

Ogawa LM, Baserga SJ (2017) Crosstalk between the nucleolus and the DNA damage response. Mol Biosyst 13(3):443–455. 10.1039

Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nat Biotechnol 33:290–295

Piferrer F, Beaumont A, Falguière JC, Flajšhans M, Haffray P, Colombo L (2009) Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293:125–156

Qin JG, Fast AW, Ako H (1998) Growout performance of diploid and triploid Chinese catfish *Clarias fuscus*. Aquaculture 166:247–258

Sanchez CG, Teixeira FK, Czech B, Preall JB, Zamparini AL, Seifert JRK, Malone CD, Hannon GJ, Lehmann R (2016) Regulation of Ribosome Biogenesis and protein synthesis controls germline stem cell differentiation. Cell Stem Cell 18:276–290

Sondalle SB, Longerich S, Ogawa LM, Sung P, Baserga SJ (2019) Fanconi Anemia protein FANCI functions in Ribosome Biogenesis. Proc Natl Acad Sci USA 116:2561–2570

Stringer JM, Winship A, Liew SH, Hutt K (2018) The capacity of oocytes for DNA repair. Cell Mol Life Sci 75:2777–2792

Sun DF, Li Q, Yu H (2022) DNA methylation differences between male and female gonads of the oyster reveal the role of epigenetics in sex determination. Gene 820:146260

Vandesompele J, Preter KD, Pattyn F, Poppe B, Roy NV, Paepe AD, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:1–12

Vitting-Seerup K, Sandelin A, Berger B (2019) IsoformSwitchAnalyzeR: analysis of changes in genome-wide patterns of alternative splicing and its functional consequences. Bioinformatics 35:4469–4471

Weber GM, Hostuttler MA, Cleveland BM, Leeds TD (2014) Growth performance comparison of intercross-triploid, induced triploid, and diploid rainbow trout. Aquaculture 433:85–93

Yang H, Simon N, Sturmer LN (2018) Production and performance of triploid oysters for aquaculture. EDIS 2018:1–9

Yang Q, Yu H, Li Q (2022a) Refinement of a classification system for gonad development in the triploid oyster *Crassostrea gigas*. Aquaculture 549

Yang Q, Yu H, Li Q (2022b) Disruption of cell division prevents gametogenesis in triploid Pacific oysters (*Crassostrea gigas*). Aquaculture 560

Yu G, Wang LG, Han Y, He QY (2012) ClusterProfiler: an R package for comparing biological themes among gene clusters. Omi A J Integr Biol 16:284–287

Zhang Q, Yu H, Howe A, Chandler W, Allen SK (2010) Cytogenetic mechanism for reversion of triploids to heteroploid mosaics in *Crassostrea gigas* (Thunberg) and *Crassostrea ariakensis*. Aquac Res 41:1658–1667

Zhang Q, Shalaby NA, Buszczak M (2014) Changes in rRNA transcription influence proliferation and cell fate within a stem cell lineage. Science 343:298–301