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Altered ovarian transcriptome is linked to early mortality and abnormalities in zebrafish embryos after maternal exposure to gamma irradiation

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1 **Abstract**

2 Recent laboratory studies focusing on multigenerational approach demonstrated
3 drastic phenotypic effects after chronic fish irradiation exposure. No irradiation effect
4 at phenotypic scale was observed for F0 (reproductive performances) while early
5 mortality and malformations were observed in F1 offspring whether they were
6 irradiated or not. The objective was to study molecular mechanisms likely to be
7 involved in these phenotypic effects induced by parental irradiation. Thus, F0 adult
8 zebrafish were irradiated for ten days until reproduction and maternal involvement in
9 offspring development was assessed. Levels of maternal provided cortisol and
10 vitellogenin, needed for embryo development, were not impacted by irradiation.
11 However, maternal transcriptome highlighted irradiation effect on processes involved
12 in oocyte development, as well as on essential maternal factors needed for offspring
13 development. Therefore, this study highlighted the importance of parental exposure on
14 offspring fate and of the importance of multigenerational exposure in risk assessment.

15 **Keywords:** multigenerational, maternal transcripts, cortisol, vitellogenin, zebrafish,
16 irradiation

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26 **Altered ovarian transcriptome is linked to early mortality**
27 **and abnormalities in zebrafish embryos after maternal**
28 **exposure to gamma irradiation**

29

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40 **1. Introduction**

41 As aquatic ecosystems are particularly impacted by chemical pollutants, many data
42 are available on their phenotypic effects and toxic mechanisms of action. Recently,
43 multigenerational consequences of chemical exposure is a growing area of interest for
44 environmental risk assessment (ERA). Although nuclear accidents such as Chernobyl
45 and Fukushima represent an ecological threat for wildlife populations, much less is
46 known regarding the effects of ionizing radiations (IR) over generations (Beresford et
47 al., 2020; Car et al., 2022; Cunningham et al., 2021; Geras'kin et al., 2021).

48 We previously showed that laboratory exposure of adult zebrafish to high dose rate
49 (50 mGy.h⁻¹, 12 Gy) for 10 days did not alter their reproductive performances nor
50 survival (Guirandy et al. 2019). But it is likely that effects would be more tenuous since

51 DNA damages and oxidative stress were already detected following IRs exposure
52 (Simon et al., 2011). Moreover, these subtle effects could be maternally transmitted,
53 with potential consequences on offspring fitness. We indeed reported that the
54 irradiated or non-irradiated (recovery condition) offspring from irradiated genitors at a
55 high dose rate showed a high mortality from 24 hours post fertilization (hpf) (Guirandy
56 et al., 2019). Similar results were observed by Hurem et al. 2017 after an exposition to
57 53 mGy.h⁻¹, which induced non-viable offspring. Another study at a lower dose rate (5
58 mGy.h⁻¹, 30d, 3.6 Gy) showed an impact of IR on the irradiated or non-irradiated
59 offspring leading to high mortality rates at early stages of development (8 days post
60 fertilization (dpf)) as well as male-biased sex ratio at adulthood (Guirandy et al., 2022).
61 All these studies highlighted a high mortality of the offspring from irradiated parents.
62 The mechanisms of parental effect on offspring still remain unclear.

63 Strong maternal effects are likely to occur following exposure to IRs since mothers not
64 only provide genomic material but also many essential cytoplasmic components and
65 mRNA needed to achieve the proper embryonic development of the offspring. First,
66 females contain the future eggs, on which IR may induce oxidative stress and DNA
67 damages (Guirandy et al., 2019; Hurem et al., 2018, 2017). Damaged eggs would then
68 suffer higher mortality, as observed by Chen et al. 2015 and Vuori et al. 2008.
69 Secondly, some essential macro-molecules are provided by female and are needed
70 for embryo development ("Maternal Effect Genes in Development, Volume 140 - 1st
71 Edition," n.d.). Two macro-molecules are of particular interest, namely vitellogenin
72 (VTG) which needs to be cleaved by cathepsin D in order to form the yolk egg (Finn,
73 2007; Yilmaz et al., 2018); and cortisol (the main stress hormone in fish (Sadoul and
74 Geffroy, 2019)). VTG is the yolk egg precursor and the developing offspring of
75 oviparous animals are entirely dependent on this energy store. A defect in VTG

76 quantity or in its metabolism is known to be a cause of early mortality (Soares et al.,
77 2009; Yilmaz et al., 2018). Altered cortisol concentration could also play a key role in
78 the described effects since a proper maternally-provided quantity of cortisol is needed
79 for early embryo development (Faught and Vijayan, 2018; McCormick, 1999; Nesan
80 and Vijayan, 2016, 2012). Finally, maternal mRNAs support embryonic development
81 until activation of the zygote genome (Abrams and Mullins, 2009; “Maternal Effect
82 Genes in Development, Volume 140 - 1st Edition,” n.d.). Indeed, good quality eggs
83 production begins with oocyte development in females. For instance, many studies
84 highlighted the primordial role of maternal mRNAs and more generally of the Balbiani
85 body (Bb) in embryo axis establishment and progeny survival (Abrams and Mullins,
86 2009; Flores et al., 2008; Langdon and Mullins, 2011). Particularly, formation of the Bb
87 in developing oocytes allows establishment of the first polarity (animal/vegetal) of
88 embryo (Jamieson-Lucy and Mullins, 2019; Marlow, 2010). Two maternal genes are
89 particularly involved in Bb formation; (i) *buc*, which allows Bb assembly and (ii) *macf1*,
90 which allows Bb translocation and dispersal in oocyte. Maternal mRNA needed later
91 for embryo development are accumulated in the Bb before to be dispersed to their
92 functional localization.

93 Here, we assessed maternally transmitted effects following parental exposure to
94 gamma irradiation with no offspring irradiation (recovery) as well as irradiation of both
95 genitors and offspring (irradiated). Maternal transcriptome was then analysed to get
96 mechanistic insights into the impact of irradiation. This study represents a first step to
97 define parental effects after irradiation, by assessing mother involvement. Hence, after
98 a gamma irradiation performed at 50 mGy.h⁻¹ for 10 days, we sampled ovaries and
99 eggs to study irradiation-induced impacts (i) on maternally deposited material through
100 cortisol and VTG measurements, (ii) on oocyte development and (iii) on specific

101 maternal transcripts needed for offspring development by analysing ovary
102 transcriptome (RNA-Seq) in order to get insights into the mechanisms that could
103 explain the transmitted effects from female to offspring. 50 mGy.h⁻¹ dose rate was
104 used to underpin effects observed in our previous study and to assess a mechanistic
105 study at high dose rate not observed in the environment.

106 **2. Materials and methods**

107 ***2.1. Fish husbandry***

108 The project (APAFIS#15821) was authorized by the Institut de Radioprotection et de
109 Surêté Nucléaire ethics committee no. 81 (EU 0520) in an application under the
110 directive 2010/63/UE relating to animal care. The study was conducted on AB strain
111 zebrafish that were kept, reproduced, and irradiated in a zebrafish housing system
112 (Zebtec Tecniplast Stand Alone) with recirculating oxygenated freshwater. Adult fish
113 were acclimatized over 3 weeks to tap water + 20% demineralized water renewed daily
114 (Aquadem; pH 7.4 ± 0.2, conductivity = 398 ± 2 µS cm⁻¹, temperature = 28.4 ± 0.3 °C),
115 with a 12:12-h light:dark cycle photoperiod. The fish were fed ad libitum three times a
116 day with GEMMA Wean (Skretting®).

117 ***2.2. Adult and embryo exposure***

118 Nominal dose rate was 50 mGy.h⁻¹ for the irradiated condition. Gamma-rays were
119 emitted from a ¹³⁷Cs source (444 GBq, 662 keV; Institut de Radioprotection et de
120 Surêté Nucléaire MICADO-Lab platform). Dose rate was simulated using MCNP5
121 software and measured using thermoluminescent dosimeters (Chiyoda
122 Technologies), and the values represented between 80 and 120% of the nominal
123 values. Control condition was kept in a separate room (60–80 nGy.h⁻¹). The population
124 density of adult fish was 0.7 L.g⁻¹.

125 Twenty-four adult fish per condition (12 females : 12 males) were irradiated over 10 d.
126 Daily controls and feeding were conducted as described in Guirandy et al., 2019.
127 F1 offspring were obtained from 12 F0 spawning couples (24 fish, 1 female:1 male)
128 (i.e., replicates) per condition. Mating and eggs viability were determined as described
129 in Guirandy et al., 2019. The 3hpf-embryos from 6 spawns per condition were
130 separated into 2 groups of 30 eggs per clutch. The first one F1 embryos were then
131 positioned in an incubator and irradiated at 50 mGy.h⁻¹ (irradiated) (Figure 1). The
132 second group was placed in non-irradiated incubator (recovery). F1 embryos were
133 maintained in both conditions over 6days. The same culture medium was used for
134 embryos and adults and 10% was replenished daily.
135 F0 adults were then placed in control condition and reproduction was done as
136 explained before, at 6 days (resilience_6d) and 36 days (resilience_36d). Each F1
137 obtained offspring were kept in control conditions for 4 days.

138 ***2.3. Ecological endpoints and morphological analysis***

139 Reproductive performances (reproductive success, fecundity: number of eggs laid per
140 female) of adults were measured after 10 days of exposure.

141 The progeny survival rate was assessed from 24 to 96 hours post fertilization (hpf) in
142 the control, irradiated, and non-irradiated (recovery) conditions (30 eggs from 6
143 spawns per condition).

144 Photographs of F1 larvae were recorded at 72hpf (6 to 30 larvae per condition) under
145 a stereomicroscope (Nikon SMZ800) connected to a high-resolution camera
146 (acA1300-60gm, Basler, Germany). Recording was performed using Media Recorder
147 Software (v.4.0.542.1, Noldus Information Technology, Netherlands) and the analysis
148 of photos was performed using the DanioScope Software (v.1.1.110, Noldus

149 Information Technology, Netherlands) in order to assess morphology measurements
150 (surface of yolk reserve) and malformations (cardiac edema). Analyses were made for
151 F1 irradiated, F1 recovery, F1 resilience 6d and F1 resilience 36d.

152 ***2.4. Sequencing library and transcriptomic analysis of ovaries***

153 Ovaries were dissected after reproduction and gonads were kept in RNALater®
154 (Sigma-aldrich) at -80°C until analysis. RNA extractions were made from female
155 gonads (8 per condition). Each sample was homogenized in 600 µL of ice-cold RTL
156 buffer (Qiagen) with 6 µL of β-mercaptoethanol using a tissue homogenizer for 3 times
157 15s Following centrifugation, DNA and RNA were extracted from the homogenate
158 using the AllPrep DNA/RNA kit (Qiagen) according to manufactures' guidelines, with
159 DNaseI's treatment (Qiagen) according to the manufacturer's recommendations.

160 Libraries were generated from 1 µg of total RNA following the TruSeq mRNA stranded
161 protocol (Illumina). RNA integrity (RIN) assessed using RNA Nano Chips (Bioanalyzer
162 2100, Agilent) did not show any sign of degradation (RIN > 7). Librarie quality and
163 concentration were measured on DNA1000 Chips (Bioanalyzer 2100, Agilent) and
164 sequenced on a NovaSeq 6000 platform to produce 50 bases long paired-end reads
165 (Clinical Research Sequencing Platform, Broad Institute, MIT, Cambridge, USA).
166 Good-quality reads (Q > 30) were produced for each sample (Table S1). Quality check
167 of paired end reads and quantification of the RNAseq data were performed as
168 described before (Murat El Houdigui et al., 2019). Briefly, read quality was assessed
169 with FastQC (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), adapter
170 sequences were removed with TrimGalore! v0.6.4
171 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and mapped against
172 the GRCz11 zebrafish reference genome using RNA-STAR v020201 and the known
173 exon–exon junctions from Ensembl release 103. Normalisation and differential

174 expression analysis were performed with DESeq2 v1.26.0. Genes with adjusted p-
175 value < 0.05 (false discovery rate) were considered as differentially expressed in all
176 analysis (DEG). Genes with $|\log_2\text{FoldChange}| \geq 1$ and adjusted p-value < 0.05 (false
177 discovery rate) were considered as up or down regulated.

178 **2.5. Enrichment analysis**

179 ClusterProfiler, a R package, was used to perform pathway enrichment using Gene
180 ontology terms (GO terms), Kyoto Encyclopedia of Genes and Genomes pathway
181 (KEGG pathway) on the DEG obtained from DESeq2 (Table S2). Enrichments with p-
182 value from Fisher's exact test ≤ 0.05 were considered significant. Dot plots were
183 produced using the R package ggplot2.

184 **2.6. RT-qPCR analysis**

185 RNA extraction for qPCR was performed as for the RNA-seq. A total of 1 μg of RNA
186 was used to synthesize first-strand cDNA using the GoScript Reverse Transcription
187 System (Promega), according to the manufacturer's guidelines. cDNA was then diluted
188 40-fold. Real-time PCR reactions were then performed in a thermocycler (LC480,
189 Roche; 95 °C for 2 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 1 min).
190 Each 15 μL reaction contained 7.5 μL of GoTaq qPCR master mix (Promega), 5 μL
191 template and the specific primer pairs at a final concentration of 0.33 μM each.
192 Amplification efficiencies for all primer sets were calculated to allow direct comparison
193 of amplification plots according to the $\Delta\Delta\text{Ct}$ method (Livak and Schmittgen, 2001).
194 Relative quantification of gene expression was achieved by amplification of
195 endogenous control genes chosen from our RNAseq dataset, i.e. genes for which their
196 transcription level was not impacted by irradiation (*aspa*, *pdia6*). During our
197 experiment, total RNAs were quantified and the same quantity was used to be reverse-

198 transcribed. During the subsequent qPCR amplifications, the output cycles of the
199 endogenous controls were examined, no significant difference was observed on the
200 output cycle of *aspa* and *pdia6* among conditions. The mean Ct value of the two latter
201 genes was used as reference. Primers used are available in Table S3 and are involved
202 in oogenesis and sex determination. Log2FC from RNAseq and qPCR analysis were
203 compared in order to validate RNAseq data (Figure S1); they varied similarly between
204 the two analyses.

205 ***2.7. Steroid extraction and cortisol measurement***

206 A half female's gonad per replicate was flash-frozen in liquid nitrogen and kept at -
207 80°C until extraction. Gonads were then thawed and transferred in sodocalcic glass
208 tube. 1ml of ethyl acetate/cyclohexane (50:50) was added per sample before grinding
209 for 2 min in a potter (Eurostardigital, IKA Labortechnik,) at 2000 Tr/min. Lysate was
210 then transferred to new sodocalcic glass tube. This step was carried out twice in order
211 to recover a maximum of material as recommended in Sadoul and Geffroy, 2019.
212 Tubes were vortexed for 10 sec then centrifuged for 5 min at 5000G. Supernatant was
213 collected in a sodocalcic glass tube. This step was performed twice. Each sample was
214 evaporated to dryness under nitrogen at 40 °C and resuspended in buffer from the
215 "Cortisol Express ELISA Kit" from Cayman Chemical. Cortisol assay were then
216 performed according to manufacturer's guidelines.

217 ***2.8. Protein extraction and VTG western blot analysis of 24hpf eggs***

218 Twenty 24hpf-eggs per replicate were quick frozen in liquid nitrogen and stored at -
219 80°C until extraction. For protein extraction, samples were placed in a modified RIPA
220 lysis buffer (Sigma Aldrich; with anti-protease mixture), with 0.5-mm diameter
221 zirconium beads and homogenized by three 5000-rpm cycles at 4°C in a Precellys

222 grinder system (Ozyme). After 30mn incubation on ice, crude extracts were centrifuged
223 for 10 minutes at 4°C at 5000g. Protein concentration was determined using the BCA
224 kit (Thermo Scientific) with BSA as a standard, according to the manufacturer's
225 instructions and then adjusted to a same concentration to ensure the loading of the
226 same amount of proteins for western blot analysis. After denaturation (15mns, 95°C
227 with Laemli buffer (Tris (0,31M), DTT (0,25M), BBP (0,02%), SDS (10%) and glycerol)
228 9µg of proteins from each sample were loaded in precast gels (4-20%? TGX Biorad)
229 and electrophoresis was run in TGS buffrer during 50min (80V for 15 min and 150V
230 35min). 5µL of load marker (RNP800E, ECL Plex Fluo rainbow) were added in one
231 well. After migration, gel was transferred on a polyvinylidene fluoride (PDVF, GE
232 Healthcare) membrane (1h, 4°C, transfer buffer (TGS 1X, ethanol (20%)), for 1 hours
233 at 100V. Membrane was then blocked with BSA 1% (TBST-BSA buffer (TBS1X,
234 pH=7.5 – Tween 0.1% - 1% filtered BSA) for 1hand incubated overnight at 4°C in
235 primary mouse antibody JE-10D4 (Abcam ab36804, dilution 1:1000 in TBST-BSA)
236 followed by incubation in secondary anti-mouse antibody Cy5® (Goat Anti-Mouse IgG
237 H&L preadsorbed (ab6563)dilution 1:6000 in TBST-BSA) for 2h at 4°C. Membrane was
238 finally rinsed and results were visualized with Typhoon FLA 9500 (GE Healthcare) at
239 670nm, PMT ().Integrations of bands (volume without background) were done using
240 ImageQuantTL software (1D gel analysis module). Recent bibliography shows that the
241 use of housekeeping protein to normalization is difficult (Eaton et al., 2013; Moritz,
242 2017). These findings were proved in our case with traditional proteins (β -actin and α -
243 tubulin) which were highly impacted by gamma irradiation (Figure S2). Reproducibility
244 of lane was proved by multiple gel migrations, as described by (Eaton et al., 2013;
245 Moritz, 2017; Richon et al., 2000), using total protein gel stained in parallel (Table S4)
246 since the use of housekeeping protein to normalization is difficult after exposure to a

247 stressor (Eaton et al., 2013; Moritz, 2017). Homogeneity of variances was tested using
248 bartlett's test for each membrane ($p\text{-val}>0.05$) and variances of two gels were tested
249 using Anova ($p\text{-value}>0.05$).

250 **2.9. Statistical analysis**

251 For F1 survival rate, F1 morphology, F1 maturation products of vitellogenin intensity
252 and F0 cortisol concentrations, data were presented as mean values \pm SE, with
253 significance taken as $p < 0.05$. For F1 vitellogenin ratio, results were presented by the
254 ratio 30kDa band/ 26kDa band. Concerning data relating to the F1 generation (survival
255 rate and vitellogenin ratio), conditions were compared using a GLM (Generalized
256 Linear Model, glm function in R) with binomial distribution. Concerning data related to
257 morphology, vitellogenin density results and cortisol, conditions were compared using
258 Anova with BoxCox transformation when normality and homogeneity were not verified.
259 A TukeyHSD, post-hoc test, was then used. Concerning qPCR data, data were
260 presented as log2FoldChange where FoldChange (FC) = $(\text{mean } 2^{-\Delta\text{Ct}_{\text{irradiated}}} - \text{mean } 2^{-\Delta\text{Ct}_{\text{control}}}) / \text{mean } 2^{-\Delta\text{Ct}_{\text{control}}}$. Conditions were compared using Anova with BoxCox
261 transformation when normality and homogeneity were not verified. A Tukey (HSD),
262 post-hoc test, was then used. When normality was not verified even after data
263 transformation, a non-parametric test was used (kruskal wallis). A Dunnet test with
264 Bonferroni correction was then used as post hoc test.

266 The analyses were performed using R software (R Core Team, 2021) with the following
267 packages : "tidyverse", "here", "knitr", "lme4", "MASS", "car". Figures were produced
268 using the package "ggplot2".

269 **3. Results**

270 **3.1. F0 reproductive performances and F1 survival**

271 F0 adult fish (n=24 per condition) and F1 embryos (n=30 eggs X 6 spawns per
272 condition) were irradiated to a cumulative dose of 12Gy and 4.8Gy, respectively. The
273 direct exposure to IRs did not induce F0 adult mortality over the experiment. The
274 reproductive success (RS) of F0 irradiated adults was lower (n=12 mating pairs, 75%),
275 but not significantly different to control fish (n=12, 92%). No effect on fecundity was
276 observed after exposure to gamma irradiation (n=9, 367±170), compared to the control
277 group (n=11, 344±153).

278 At 24 hpf, the percentage of F1 mortality for control, irradiated and recovery groups
279 reached 54.9%±5.6, 71.7%±10.7 and 77.0%±7.0, respectively (Figure 2). Despite
280 enhanced mortality for both irradiated and recovery conditions, no significant difference
281 was observed compared to the control group. However, an important variability was
282 observed for all groups. The same trend was observed at 48 hpf (control: 56.1%±5.9;
283 irradiated: 77.8%±8.2; recovery: 83.0%±5.7), but a significant difference ($p < 0.05$) was
284 observed only for the recovery condition. A significant difference in mortality ($p < 0.05$)
285 was observed from 72 hpf for both irradiated (89.9%±4.9) and recovery (98.4%±0.7)
286 groups compared to controls (56.1% ± 5.9). No statistical difference was observed
287 between the irradiated and recovery conditions. Control's mortality remained similar
288 until the last day (6 dpf) of the experiment (57.2%±5.7), whereas it reached 100% for
289 the irradiated group and 99.5%±0.5 for the recovery group.

290 291 **3.2. F1 abnormalities after parental exposure**

292 Malformations (pericardium edema and yolk reserve edema) were observed in F1 72
293 hpf larvae after parental exposure to IRs (Figure 3A). A significant increase ($p < 0.05$)
294 of yolk reserve area was observed in both conditions, irradiated (n=9, 0.319mm² ±

295 0.007) and recovery (n=12, 0.349mm² ± 0.019), compared to the control group (n=6,
296 0.244mm² ± 0.014), with no difference between them (Figure 3B). After setting F0
297 irradiated adult fish in control condition for 6 days, their offspring (Resilience_6d) still
298 showed a significant increase (p < 0.05) in yolk reserve area (n=11, 0.400mm² ± 0.020)
299 compared to the control group (n=11, 0.241mm² ± 0.009). On the other hand, after 36
300 days of setting the adults in control condition, no morphological defects were observed
301 in their offspring (Resilience_36d). For pericardial area, statistical difference (p < 0.05)
302 was observed only for resilience_6d compared to control group (Figure 3C).

303 **3.3. Transmitted macro-molecules from F0 to F1**

304 *3.3.1. Glucocorticoid regulation in the gonad*

305 Cortisol concentration in F0 female gonads after reproduction was not impacted by IR
306 (Figure 4A). Indeed, its concentration reached 0.036ng.mg⁻¹ ± 0.005 for irradiated
307 group (n=6) and 0.041ng.mg⁻¹ ± 0.006 for control group (n=6). However, at the
308 transcriptional level (RNAseq), a down-regulation of *nr3c1*, the glucocorticoid receptor,
309 was observed in F0 female gonads (n=8) after irradiation (Figure 4B). Moreover, many
310 genes involved in cortisol biosynthesis were also down-regulated (*cyp11a1*, *cyp11a2*,
311 *apoa1a*, *apoeb*, *npc2*, *fdx1*, *fdx1b*).

312 *3.3.2. VTG analysis in eggs*

313 Western-blot analysis, using an anti-VTG antibody on 24 hpf F1 embryos (n=7 per
314 condition), revealed two bands at around 26 and 30 kDa (Figure S3), but not the band
315 corresponding to the whole VTG (around 150 kDa). Whatever the band considered,
316 i.e. total one, 30kDa band intensity or 26 kDa band intensity, or the ratio between
317 intensities, no significant difference was observed between control and irradiated
318 groups (Figure S4). However, the transcription level of the gene encoding for the

319 enzyme responsible for VTG cleavage, cathepsin D (*ctsd*), was down-regulated
320 (Log2FoldChange= -0.5, FDR <0.01).

321 3.4. **Ovarian transcriptome**

322 Differential gene expression was assessed in pairwise comparison between irradiated
323 and control ovaries (n=8 per condition). The total number of Differentially Expressed
324 Genes (DEG) (FDR < 0.05), up and down regulated genes with a fold change superior
325 to 2 ($|\log_2\text{fold change}| \geq 1$ and FDR < 0.05), up regulated genes ($\log_2\text{fold change} \geq 1$
326 and FDR < 0.05) and down regulated genes ($\log_2\text{fold change} \leq -1$ and FDR < 0.05)
327 reached 9182, 5348, 3234 and 2114, respectively.

328 Pathways enrichment analyses were then performed using GO repositories (with
329 zebrafish orthologues) and the KEGG database (with human orthologous genes).

330 Considering all dysregulated genes (FDR <0.05) or only up regulated or down
331 regulated genes ($|\log_2\text{fold change}| \geq 1$ and FDR < 0.05), 304, 394 and 83 GO terms
332 were enriched, respectively (Table S2). The most dysregulated pathways from
333 dysregulated genes were related to ribosomes (GO:0022613, ribonucleoprotein
334 complex biogenesis, p.adjust=1.74E-22) and DNA damage (GO:0006281, DNA repair,
335 p.adjust= 7.25E-11) (Figure 5A). GO terms linked to ribosomes synthesis were
336 principally represented by down regulated genes (GO:0042254, ribosome biogenesis,
337 p.adjust= 5.28E-09). We also found other GO terms associated with DNA damage and
338 genotoxicity that were highly dysregulated (GO:0051403, stress-activated MAPK
339 cascade, p.adjust= 0.02; GO:0097190, apoptotic signalling pathway, p.adjust= 0.02;
340 GO:0006302, double-strand break repair, p.adjust= 2.72E-04). For DNA damage
341 group, transcripts of the p53 pathway were all up-regulated (Figure 5B, *tp53bp1*,
342 *tp53bp2a*, *tp53bp2b*, *deltaNp63*) while other genes involved in DNA repairs were

343 down-regulated (*gadd45ab*, *rad51*, *xrcc6*, *xrcc5*, *ddb2*, *creb1a*, *creb1b*, *polb*).
344 Transcripts involved in the fight against oxidative stress were also down-regulated
345 (*gpx1a*, *gpx1b*, *gsta.1*, *sod1*, *sod2*).

346 GO terms linked to reproduction (GO:0022414, reproductive process, p.adjust= 6.21E-
347 04; GO:0048477, oogenesis, p.adjust= 3.12E-04) and germ line production
348 (GO:0007281, germ cell development, p.adjust=0.03) were also impacted by
349 irradiation.

350 Dysregulated genes involved in oogenesis and ribosomes are presented in Figure 5B.
351 They were mostly down-regulated (*buc*, *rpl*, *rps*) while *macf1a* was up-regulated.

352 Among the upregulated genes, many were involved in morphogenesis (GO:0060560,
353 developmental growth involved in morphogenesis, p.adjust= 1.13E-04) and embryo
354 axis establishment (GO:0009952, anterior/posterior pattern specification,
355 p.adjust=0.01; GO:0009953, dorsal ventral pattern formation, p.adjust= 2.73E-03,
356 GO:0016055, Wnt signalling pathway, p.adjust= 9.96E-03). Transcripts related to
357 embryonic development, and in particular in axis development, were mainly up-
358 regulated (Figure 5B, *ctnnb2*, *runx2b*, *smad* genes), but some important factors for axis
359 establishment were down-regulated (*grip2a*, *sybu*, *pou5f3*, *ndr1*, *gdf3*). Transcripts
360 related to the maternal to zygotic transition (MZT) stage were also up-regulated
361 (*drosha*, *dicer1*, *ago2*, *ago3b*, *ago4*, *ythdf2*).

362 Considering dysregulated genes (FDR <0.05), or only up regulated genes or down
363 regulated genes 20, 15 and 5 KEGG pathways were enriched, respectively and are
364 presented in Figure 6. KEGG enrichment analysis with only upregulated genes were
365 consistent to GO terms enrichment since pathway linked to embryo development were
366 impacted (dre04068, FoxO signalling pathway, p.adjust= 0.04; dre04330, Notch

367 signalling pathway, p.adjust= 4.88E-03; dre04340, Hedgehog signalling pathway,
368 p.adjust= 3.53E-03; dre04310, Wnt signalling pathway, p.adjust= 3.90E-04; dre04012,
369 ErbB signalling pathway, p.adjust= 1.56E-05). As previously described, many
370 dysregulated genes or down regulated genes were involved in oogenesis (dre04914,
371 Progesterone mediated oocyte maturation, p.adjust= 4.23E-03; dre04114, Oocyte
372 meiosis, p.adjust= 4.23E-03) and ribosomes (dre03008, Ribosome biogenesis in
373 eukaryotes, p.adjust= 3.61E-06).

374 **4. Discussion**

375 In order to investigate the molecular cascade that induce drastic effects on offspring,
376 a high dose rate of 50 mGy.h⁻¹ was purposely chosen. Here we detected significant
377 differences on offspring survival rates at 72 hpf (Figure 2). Moreover, malformations,
378 mainly yolk reserve edema, were observed for irradiated, recovery and resilience_6d
379 conditions after parental irradiation (Figure 3). Offspring effects were therefore
380 observed even when only genitors were irradiated. Moreover, Resilience_6d condition
381 show altered offspring, while genitors were in control condition. This supports that
382 deleterious effects in genitors induce non-viable offspring. Several maternal factors
383 being transmitted to embryo could explain these phenotypes. This is why this study
384 initially focused on maternal involvement. Paternal involvement cannot be totally
385 excluded but seems less obvious to us in the present case where effects are very early,
386 probably before zygote genome activation.

387 ***4.1. IR altered maternal provided molecules***

388 *4.1.1. IR led to oxidative stress and DNA damages in ovary*

389 The RNAseq analysis on female gonads showed an evidence of DNA damage,
390 confirming previous results using COMET assay (Guirandy et al., 2019; Hurem et al.,

391 2018). Enrichment analysis on GO terms (Figure 5A) revealed typical biological
392 processes related to DNA damage and oxidative stress (« stress-activated MAPK
393 cascade »). Enrichment analysis on KEGG pathways (Figure 6) showed IR-induced
394 effects on « FoxO signalling pathway » and « ErbB signalling pathway », which are
395 involved in oxidative stress and cancer development, respectively, and already known
396 to be impacted by radiations (Dent et al., 2003; Luo et al., 2007).

397 Several genes involved in apoptosis via the P53 pathway (*tp53bp1*, *tp53bp2*,
398 *deltaNp63*) (Elersek et al., 2022)) were up-regulated (Figure 5B). p53 genes, required
399 for radiation-induced apoptosis in mouse (Lowe et al., 1993), are involved in cell cycle
400 arrest but also in damage repair in zebrafish (Canedo and Rocha, 2021; Sandrini et
401 al., 2009). However, several genes involved in DNA repair mechanisms, mainly in
402 double strand break reparation (Canedo and Rocha, 2021; Reinardy et al., 2013;
403 Sandrini et al., 2009) were also down regulated (Figure 5B, *gadd45ab*, *rad51*, *xrcc6*,
404 *xrcc5*, *ddb2*, *creb1a*, *creb1b*, *polb*). In addition, genes involved in the fight against
405 oxidative stress (Figure 5B, *gpx1a*, *gpx1b*, *gsta.1*, *sod1*, *sod2*) were down regulated,
406 suggesting an alteration of the antioxidant response and ROS imbalance. The altered
407 oxidative balance in genitors did not seem to disturb the reproductive performances of
408 adults but could contribute to adverse effects on oocyte DNA, as explain before, or on
409 offspring development, as proposed by W. Huang et al. 2020. Thus, a direct effect of
410 ROS or an induced effect on the integrity of the maternal DNA in oocytes could occur,
411 thereby producing a future non-viable offspring. However, fertilization and first stages
412 of embryo development (gastrulation) seemed to occur normally.

413 4.1.2. IR did not impact cortisol and VTG

414 Maternal cortisol is necessary for proper embryo development (Colson et al., 2015;
415 Faught and Vijayan, 2018). Altered maternal concentration of cortisol may yield cardiac

416 oedema as described by Nesan and Vijayan, 2012. In our study, even if cardiac
417 oedema were observed in offspring at 72 hpf for resilience-6d (Figure 3E), no effect
418 was observed in ovarian cortisol level (Figure 4A). However, maternal glucocorticoid
419 receptor transcripts (*nr3c1* or *gr*), that participate in the maternal programming of
420 embryo development (Pikulkaew et al. 2011), were down regulated in female gonads
421 (Figure 4B). Furthermore, other transcripts involved in cortisol biosynthetic process
422 were also differentially regulated (Figure 4B, *cyp11a1*, *cyp11a2*, *apoa1a*, *apoeb*, *npc2*,
423 *fdx1*, *fdx1b*) (Facchinello et al., 2017; Nesan and Vijayan, 2012). Thus, even if these
424 molecular effects were not associated with changes at the metabolite level, an effect
425 of cortisol could not be totally discarded.

426 Moreover, yolk reserve oedemas (Figure 3) were observed in offspring at 72 hpf for
427 irradiated, recovery and resilience-6d, suggesting a nutrient reserve disruption
428 (observed for many pollutants in fish). The gene encoding for the enzyme responsible
429 for VTG cleavage, cathepsin D (*ctsd*), was down regulated, suggesting less VTG
430 conversion into storage forms that are required for early development (Yilmaz et al.,
431 2018). However, the total quantity of vitellogenin products that can bind to the antibody
432 was not impacted by irradiation, and yolk reserve areas were higher after irradiation
433 suggesting rather a non-consumption of reserves in larvae from irradiated parents.
434 Since maternally-provided *ctsd* transcripts allows proper treatment and consumption
435 of yolk proteins by embryos (Follo et al., 2011), down-regulation of this gene could
436 explain the non-consumption. Cortisol and VTG were non-impacted by IRs and cannot
437 explain mortality and malformation. Molecular alteration on cortisol biosynthesis and
438 VTG degradation encourage us to further investigations.

439 Another hypothesis to identify mechanisms of transmitted effects was that altered
440 maternal transcriptome, after irradiation exposure, could have early and delayed
441 consequences on embryo development.

442 **4.2. IR altered oocyte development**

443 Enrichment analysis showed GO terms and KEGG pathways linked to reproduction
444 and oogenesis, including « oocyte meiosis » and « oocyte development » (Figure 5A,
445 Figure 6). Embryo development success is dependent on mRNA, molecules,
446 germplasm and organelles (golgi and mitochondria) aggregated in the Balbiani body
447 (Bb) during maternal oogenesis (Jamieson-Lucy and Mullins, 2019; Marlow, 2010).
448 Three maternal genes are involved in Bb development (Marlow, 2010). Among them,
449 *bucky ball* (*buc*), that is necessary to establish the polarity of the egg (animal-vegetal
450 axis), was down regulated after irradiation (Figure 5B) (Marlow, 2010). Maternal *buc*
451 deficiency is known to induce polyspermy with normal cell division after fertilization and
452 ultimately embryo death (Jamieson-Lucy and Mullins, 2019). This could explain death
453 at around 72 hpf here and at around 24 hpf in the study of Guirandy et al., 2019.
454 Microtubule actin crosslinking factor 1 (*macf1*) is critical for Bb disassembly ensuring
455 the dispersion of mRNA along the animal-vegetal axis (Jamieson-Lucy and Mullins,
456 2019). In our study, *macf1a* was up-regulated (Figure 5B), potentially leading to
457 disturbed mRNA distribution and finally to embryo death.

458 Oogenesis also allows the accumulation of maternal ribosomal proteins (RP) in order
459 to support early embryo development until zygotic genome activation (ZGA) (Mercer
460 et al., 2021). Precise control of ribosome biogenesis is vital for cell survival (Danilova
461 et al., 2008) and therefore for preventing abnormalities during zebrafish embryonic
462 development. For example, a deficiency in *rpl11* induces an increase in yolk reserve
463 size in mutants, reflecting a slow metabolism, and a decrease in larvae size (Palasin

464 et al., 2019). These two phenotypes were also observed in this study and by Guirandy
465 et al. 2019. Enrichment analysis (Figure 5A, Figure 6) highlighted different processes
466 linked to ribosome synthesis (« ribonucleoprotein complex biogenesis », « ribosome
467 biogenesis », « Ribosome biogenesis in eukaryotes »). Several RP transcripts that
468 were down-regulated (Figure 5B, *rpl10a*, *rpl11*, *rpl23*) are known to activate the p53
469 pathway (as observed in 4.1.1) (Chakraborty et al., 2009; Palasin et al., 2019). Down-
470 regulation of *rps19*, *rps8a*, *rps8b*, *rps11* and *rps18* were associated with an up-
471 regulation of deltaNp63, as previously observed (Danilova et al., 2008). RP deficiency
472 via its impact on cell division and on the p53 pathway, could represent a further
473 explanation for the observed early effects on offspring after maternal gamma
474 irradiation.

475 It is clear that irradiation altered proper oocyte development. Thus, it could produce
476 defective offspring. Next, we wondered if altered maternal mRNAs could induce later,
477 i.e; after spawning and fertilization, effects in addition to direct impacts on oocytes.

478 ***4.3. IR altered maternal mRNAs needed for offspring development***

479 *4.3.1. Embryo axis establishment*

480 Other maternal mRNAs needed for embryo development were also impacted by
481 irradiation. Among them, many are accumulated in Bb and are involved in embryo axis
482 establishment (Marlow, 2010). Enrichment analysis revealed GO terms and KEGG
483 pathways involved in embryo development (Figure 5A, Figure 6), in particular with
484 genes that were up-regulated. Several biological processes concerning the
485 morphogenesis were impacted (“cell projection morphogenesis”, “embryonic organ
486 morphogenesis”, “dorsal/ventral pattern formation”, “Wnt signalling pathway”). Since

487 oocyte polarity and maternal mRNAs were impacted (4.2), it appears not aberrant that
488 maternal mRNAs governing embryo axis establishment were also disturbed.

489 Maternal β -catenin and *grip2a* are the first indication of dorso-ventral polarity (Langdon
490 and Mullins, 2011). β -catenin genes (*ctnnb1* and *ctnnb2*) were down-regulated and up-
491 regulated, respectively (Figure 5B). *ctnnb1* is known to act upstream at the molecular
492 level and *ctnnb2* then acts on the physical establishment of the axis. Opposite
493 regulations for these two key stages could alter proper embryo development (Zhang et
494 al., 2012). For example, up-regulation of β -catenin leads to hyper-dorsalization (Kelly
495 et al., 1995; Nojima et al., 2004; Sumoy et al., 1999). *grip2a* that acts upstream or in
496 parallel to maternal β -catenin was down-regulated (Figure 5B). In the same way,
497 *syntabulin* (*sybu*) was down regulated (Figure 5B) and loss of maternal *sybu* was found
498 to induce ventralization and failure to activate the Wnt signalling pathway, which is
499 required to initiate dorsal cell fate specification (Langdon and Mullins, 2011).

500 In contrast, the Bone Morphogenetic Protein signalling pathway, involved in ventral
501 axis specification, was not impacted. However, an initiator gene of this pathway, *pou5f3*
502 (Abrams and Mullins, 2009; Flores et al., 2008; "Maternal Effect Genes in
503 Development, Volume 140 - 1st Edition," n.d.) was down-regulated (Figure 5B).
504 Moreover, several ventral genes were also dysregulated (Figure 5B, *runx2b*, *smad1*,
505 *smad2*, *smad4a*, *smad5*, *smad6a*) (Pelegri, 2003). As for dorsal axis specification,
506 genes involved in ventral axis specification seemed to be highly dysregulated that
507 could lead to the observed malformations and non-viable embryos.

508 Finally, the left/right axis, needed to organ asymmetry such as brain and heart, is
509 mainly governed by the nodal signalling pathway (Bisgrove et al., 2017; Miccoli et al.,
510 2017), which was not impacted. However, a key gene of this pathway, *ndr1* was down-

511 regulated (Figure 5B). This was not surprising since its positive co-factor, *gdf3*
512 (Bisgrove et al., 2017), was also down-regulated. *Gdf3* mutation leads to embryo
513 death. Gamma irradiation thus impacted maternal transcripts necessary to asymmetry
514 development and could explain observed mortality.

515 4.3.2. Maternal mRNA degradation

516 Failure of maternal mRNA degradation concomitantly to zygotic genome activation
517 (ZGA) can lead to miscoordinated gene expression (Chang et al., 2018; Walser and
518 Lipshitz, 2011). The best-known degradation mechanism is deadenylation. The
519 majority of maternal transcripts are therefore deadenylated and degraded between 4
520 and 6 hpf (Chang et al., 2018; Walser and Lipshitz, 2011) at the same time as ZGA.
521 Interestingly, enrichment analysis showed an impact on the biological process "positive
522 regulation of nuclear-transcribed mRNA catabolic process, deadenylation-dependent
523 decay" (Figure 5), which could reflect a deregulation of maternal mRNA catabolic
524 process after irradiation. We also detected the dysregulation of many factors involved
525 in the maturation of miRNA (*droscha*, *dicer1*, *ago2*, *ago3b*, *ago4*) as mentioned after
526 parental irradiation in Martín et al., 2021. As MiR-430 facilitates the deadenylation of
527 maternal mRNAs (Giraldez et al., 2006; Laue et al., 2019; Liu et al., 2020; Yao et al.,
528 2014), it is possible that deregulation of miRNA production contribute to the increased
529 in embryonic mortality . Another degradation way during maternal to zygotic transition
530 stage is uridylation, done by *tut7* which was down regulated (Figure 5B) (Chang et al.,
531 2018). Furthermore, *igf2bp3*, a gene encoding for a protein involved in maternal mRNA
532 stability (Ren et al., 2020), was up-regulated (Figure 5B). whereas *ythdf2*, involved in
533 maternal transcript degradation, was down-regulated (Figure 5B) (Ren et al., 2020).
534 These results suggest a disturbed mRNA degradation process in female gonads.
535 Maternal mRNA could thus be rapidly degraded and could not support early

536 development or could alter the temporal pattern of gene expression during early
537 development.

538 **5. Conclusions**

539 This paper highlighted transmitted effect from genitors to offspring after 10 days of
540 gamma irradiation at 50 mGy.h⁻¹. No phenotypic effect was observed in genitors,
541 notably on reproductive performances (reproductive success and fecundity). Early
542 mortality in offspring in link to parental transmitted effects was observed as no
543 difference was observed between irradiated and recovery conditions. Moreover, early
544 malformations were observed in offspring after parental irradiation. The main objective
545 was to get mechanistic insights into the impacts of irradiation at the cellular and
546 molecular levels in order to identify mechanisms behind phenotypic effects on
547 offspring.

548 Cortisol level in F0 female gonads after reproduction was not impacted by irradiation.
549 However, it should be necessary to measure cortisol in embryos to refute its
550 involvement in F1 mortality. Despite a down-regulation of cathepsin D in maternal
551 gonads, VTG analysis in embryos did not highlight any different amount nor distribution
552 of maturation products which suggests no nutrition defect for embryos. This non-effect
553 should be however confirmed by measuring the whole VTG protein. Oogenesis and
554 maternal factors (mRNA) required for production of viable offspring and their
555 development were significantly altered, supporting maternal transmission of IR-
556 induced effects.

557 This first multigenerational approach to link phenotype to molecular events highlighted
558 an important effect of female irradiation on offspring viability with (i) an impact of
559 irradiation on oocyte development and (ii) an impact on maternal mRNAs needed later

560 for embryonic development. However, paternal contribution should not be excluded
561 and should be investigated in future experiments, since they were also exposed.
562 Further experiments would be to perform a cross-exposure (only female exposed) to
563 assess maternal role. Finally, offspring transcriptomic analysis before and after the
564 maternal-to-zygotic transition should be interesting to define more precisely the role of
565 maternal mRNAs. Our data could be used to improve the Adverse Outcome Pathways
566 and challenge the environmental risk assessment method currently deployed.

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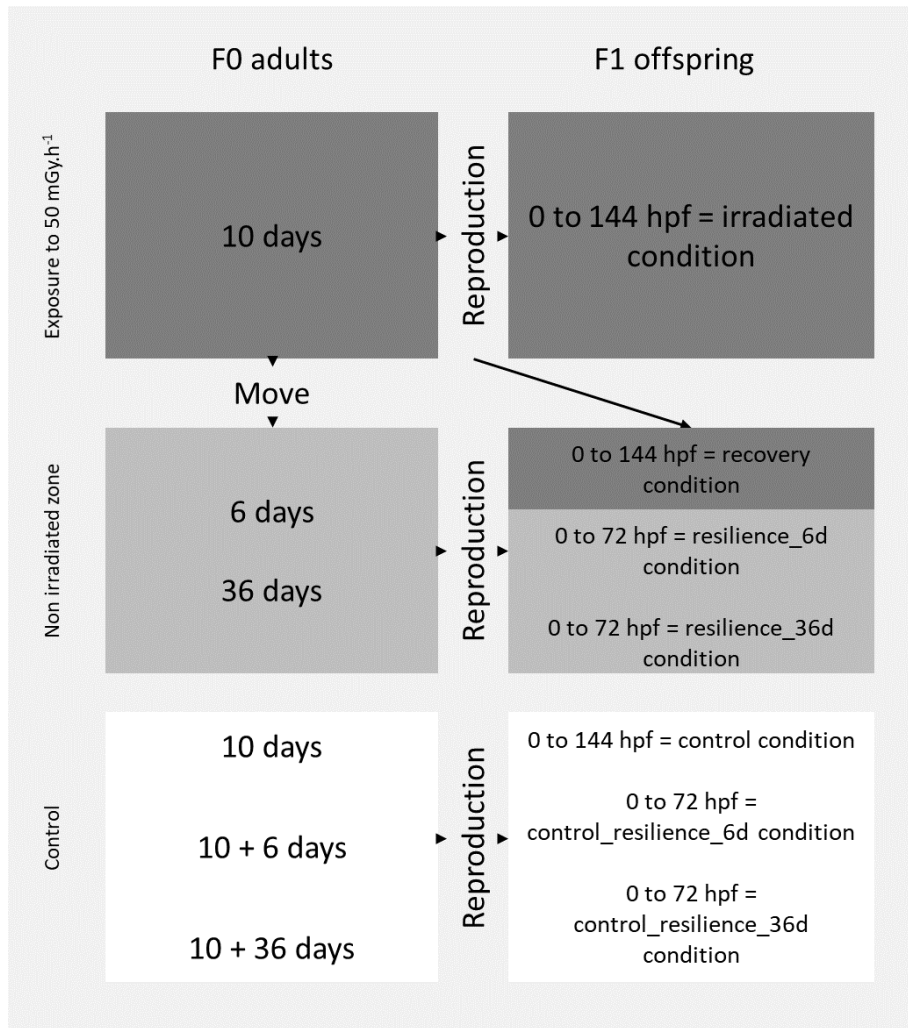
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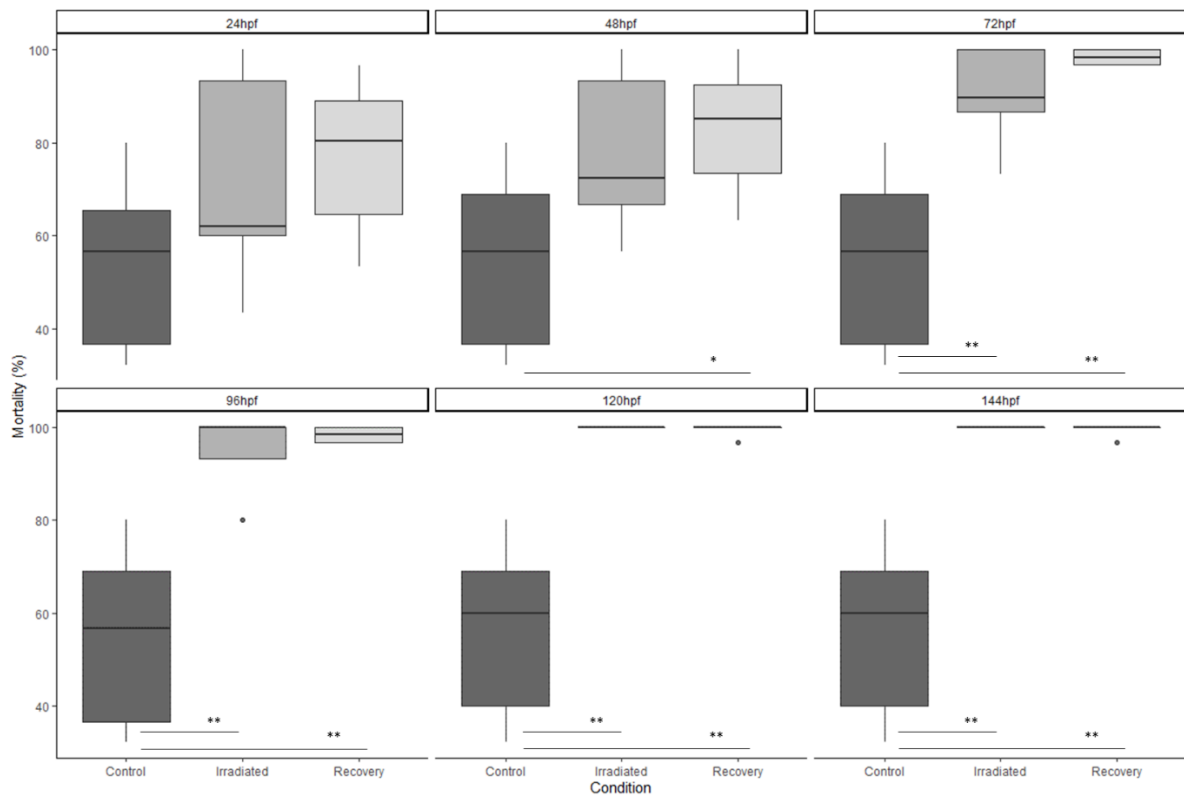
784 **Figures**

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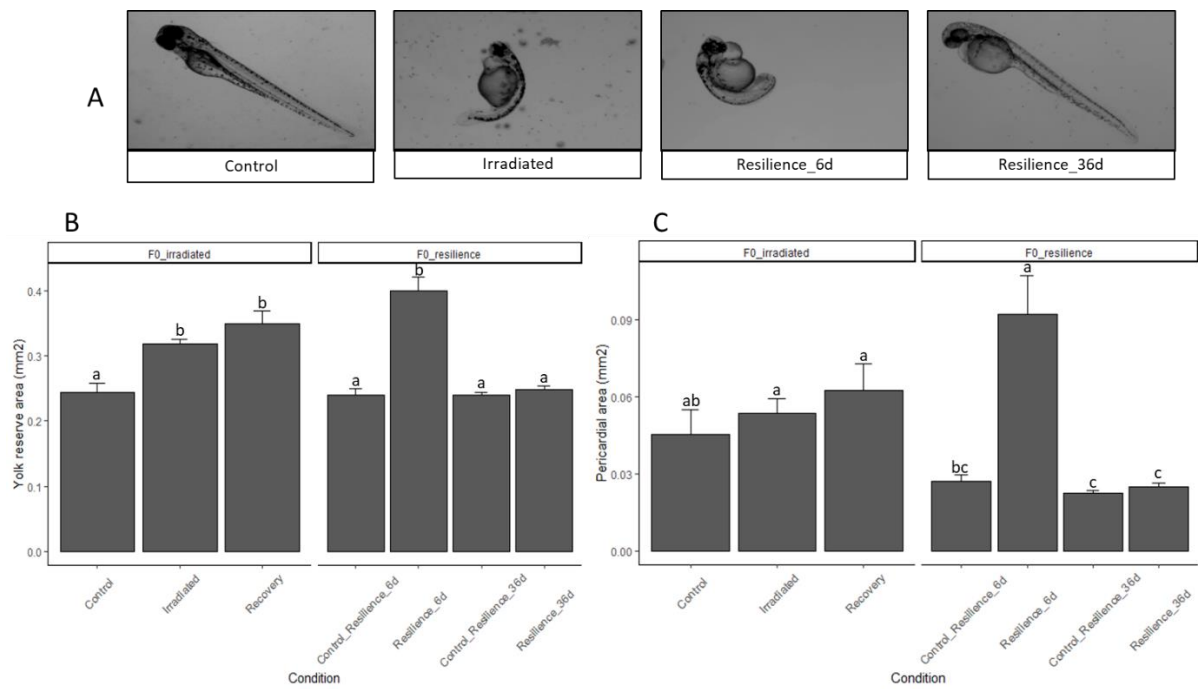
787 *Figure 1: Experimental design and exposure conditions (duration and dose rate (mGy.h⁻¹)). F0 adults (24 per*
 788 *conditions) were exposed over 10 days until reproduction. F1 progenies (30 eggs X 6 spawns) were then placed*
 789 *in irradiated (irradiated condition) and non-irradiated (recovery condition) exposure conditions over 6 days. F0 adults*
 790 *were then moved to non-irradiated condition for 6 and 36 days before reproduction. F1 progenies from 6 days F0*
 791 *(resilience_6d condition) and from 36 days F0 (resilience_36d condition) were then placed in control condition.*



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793 *Figure 2: Box-plot of cumulative mortality rates of F1 (%) at 24, 48, 72, 96, 120, 144 hpf after control, dose rate*
 794 *exposure to 50 mGy.h⁻¹ (irradiated) and recovery conditions. The boxplot represents the 25th and the 75th*
 795 *percentile with the median indicated by the horizontal black line. For control, irradiated and recovery, n=9, n=5, n=6,*
 796 *respectively. * (p < 0.05), ** (p < 0.01), *** (p < 0.001).*

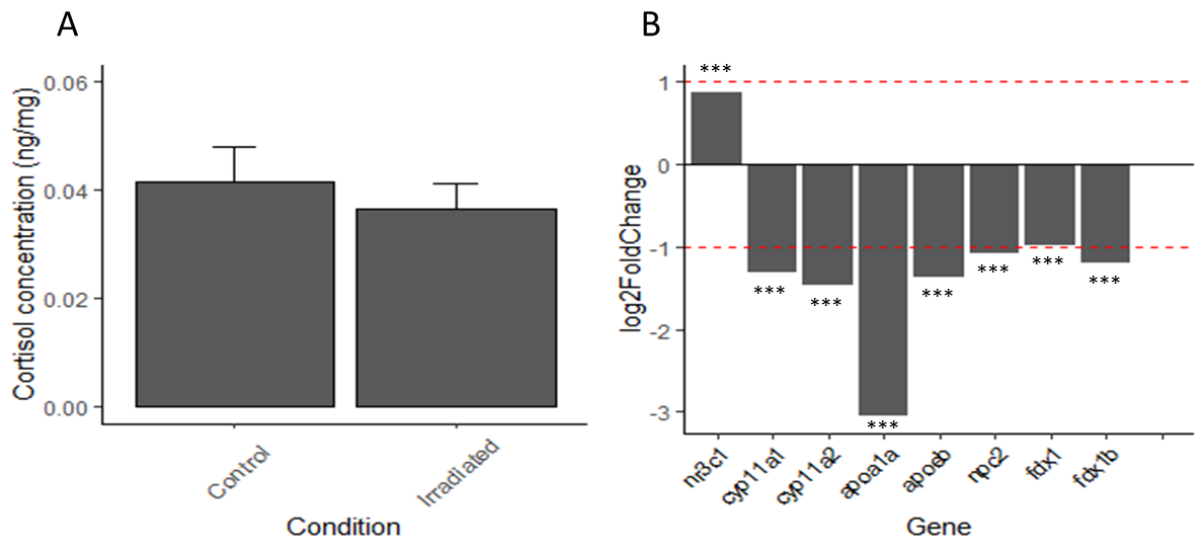
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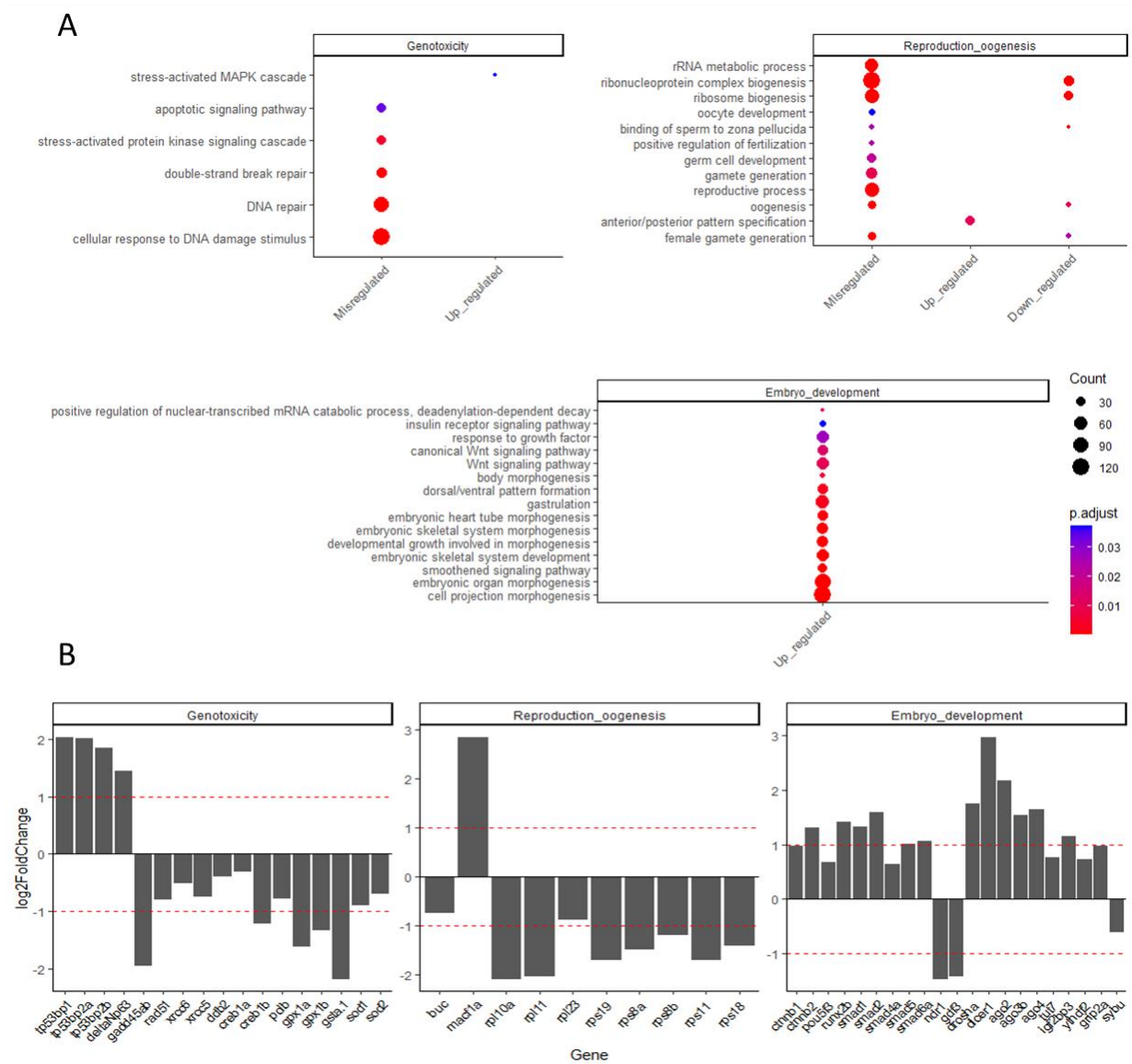
800 Figure 3: (A) Photographs of F1 larvae at 72 hpf after Control, F0 exposure to 50 mGy.h⁻¹ (10 days) with F1 exposure
 801 to 50 mGy.h⁻¹ (Irradiated), F0 exposure to 50 mGy.h⁻¹ (10 days) follow by non-irradiation 6 days (Resilience_6d) or
 802 to 36 days (Resilience_36d). (B and C) Bar plot of yolk reserve area (mm²) and pericardial area (mm²) for control
 803 (n=6), control_resilience_6d (n=11), control_resilience_36d (n=45), irradiated (F0 + F1 exposed to 50 mGy.h⁻¹,
 804 n=9), recovery (F0 exposed to 50 mGy.h⁻¹, F1 non-irradiated, n=12), resilience_6d (F0 exposed to 50 mGy.h⁻¹ follow
 805 by non-irradiation exposure 6 days, F1 non-irradiated, n=11), resilience_36d (F0 exposed to 50 mGy.h⁻¹ follow by
 806 non-exposure 36 days, F1 non-exposed, n=32). Letters indicate statistical differences (p < 0.05) among conditions
 807 (Anova test and Tukey post-hoc).

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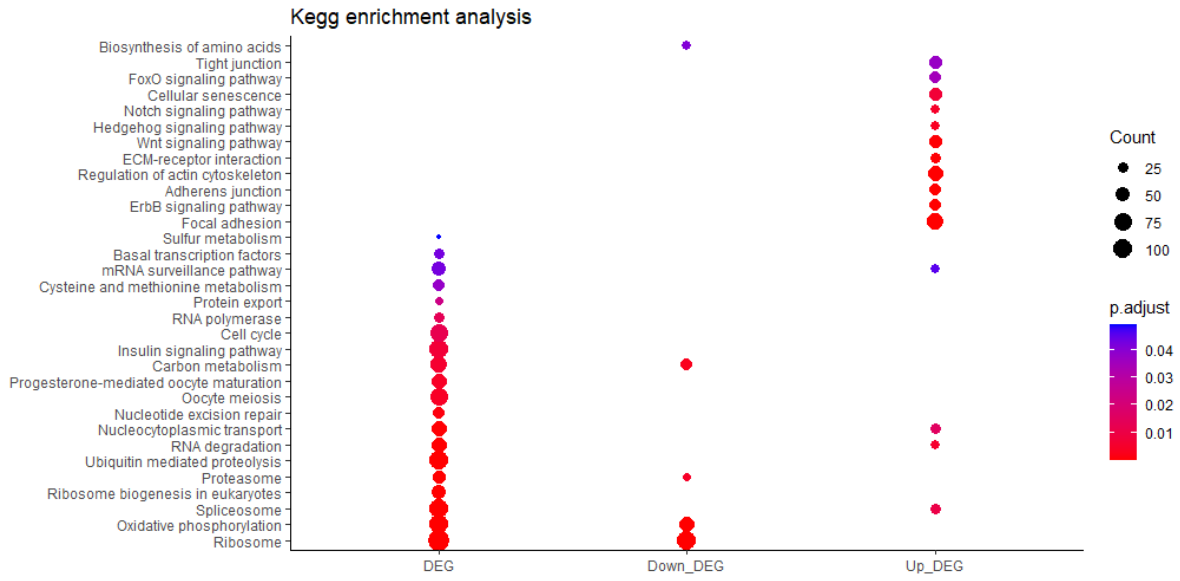
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811 *Figure 4: (A) Bar plot of F0 ovaries cortisol concentration after control and exposure to 50 mGy.h⁻¹ (irradiated), n=6*
 812 *per condition. (B) Barplot of log₂FoldChange (as compared to controls) for genes involved in cortisol metabolism;*
 813 *n=8 per condition. Data came from transcriptomic data on F0 ovaries. Red line represents cut off value chosen for*
 814 *interpretation of gene regulation. * (p < 0.05), ** (p < 0.01), *** (p < 0.001).*



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816 *Figure 5: (A) Dot plot of selected GO (Gene Ontology) term enrichment from dysregulated, up and down genes in*
 817 *exposed F0 ovaries. Colours indicate the p-values from Fisher's exact test, and dots size is the number of genes*
 818 *constituting the given pathway. n=8 per condition. (B) Change in gene transcription levels (FC as compared to*
 819 *controls) in female gonad F0 from transcriptomic data. For all genes, FDR<0.01. Red line represents cut off value*
 820 *chosen for interpretation of gene regulation. n=8 per condition.*



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822 *Figure 6: Dot plot of selected KEGG enrichment using human orthologues from misregulated, up and down genes*
 823 *in irradiated ovaries. Colours indicate the enrichment p-values from exact Fisher's test, and dots size is the number*
 824 *of genes constituting the given pathway. n=8 per condition.*

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