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在线全文

# 母体年龄相关的卵母细胞老化对生育力的影响机制：斑马鱼模型的转录组学测序分析*

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**【摘要】 目的** 以斑马鱼为模型，研究母体年龄相关的卵母细胞老化对生育力的影响，寻找随年龄增长的生育力下降的潜在分子机制。**方法** 随机选取6月龄、12月龄、18月龄雌性斑马鱼各8条，均与6月龄雄性斑马鱼交配产卵。对各月龄组的结局指标进行比较。主要结局指标为胚胎受精率，次要结局指标为雌性斑马鱼单次产卵数量、胚胎死亡率和胚胎畸形率。收集不同月龄雌性斑马鱼的卵母细胞以及后代囊胚期胚胎进行转录组学测序分析。**结果** 18月龄组受精率（86.8±5.5）%低于6月龄组（94.9±3.6）%及12月龄组（92.3±4.2）%，差异有统计学意义（$P$均<0.05）。与6月龄组相比，12月龄与18月龄组雌性斑马鱼胚胎死亡率均升高（$P$均<0.05）。各组单次产卵数量和胚胎畸形率之间的差异无统计学意义。母体年龄相关的囊胚期胚胎差异性表达基因主要富集于MAPK信号通路以及脂肪酸降解等通路途径。母体年龄相关的卵母细胞差异性表达基因主要富集于细胞黏附分子，蛋白质消化吸收等通路途径。**结论** 母体年龄可能是卵母细胞受精能力降低及早期胚胎死亡率升高的影响因素，母体年龄相关的卵母细胞老化影响生育力及后代胚胎的发育。

**【关键词】** 斑马鱼 母亲年龄 受精 生育力

**Mechanisms of the Effect of Maternal Age-Related Oocyte Aging on Fertility: Transcriptomic Sequencing Analysis of a Zebrafish Model**

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**【Abstract】 Objective** Female fertility gradually decreases with the increase in women's age. The underlying reasons include the decline in the quantity and quality of oocytes. Oocyte aging is an important manifestation of the decline in oocyte quality, including *in vivo* oocyte aging before ovulation and *in vitro* oocyte aging after ovulation. Currently, few studies have been done to examine oocyte aging, and the relevant molecular mechanisms are not fully understood. Therefore, we used zebrafish as a model to investigate oocyte aging. Three different age ranges of female zebrafish were selected to mate with male zebrafish of the best breeding age. In this way, we studied the effects of maternal age-related oocyte aging on fertility and investigated the potential molecular mechanisms behind maternal age-related fertility decline. **Methods** Eight female zebrafish aged between 158 and 195 d were randomly selected for the 6-month age group (180±12) d, 8 female zebrafish aged between 330 and 395 d were randomly selected for the 12-month age group (360±22) d, and 8 female zebrafish aged between 502 and 583 d were randomly selected for the 18-month age group (540±26) d. Male zebrafish of (180±29) d were randomly selected from zebrafish aged between 158 and 195 d and mated with female zebrafish in each group. Each mating experiment included 1 female zebrafish and 1 male zebrafish. Zebrafish embryos produced by the mating experiments were collected and counted. The embryos at 4 hours post-fertilization were observed under the microscope, the total number of embryos and the number of unfertilized embryos were counted, and the fertilization rate was calculated accordingly. The numbers of malformed embryos and dead embryos were counted 24 hours after fertilization, and the rates of embryo malformation and mortality were calculated accordingly. The primary

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outcome measure was the embryo fertilization rate, and the secondary outcome measures were the number of embryos per spawn (the total number of embryos laid within 1.5 hours after the beginning of mating and reproduction of the zebrafish), embryo mortality, and embryo malformation rate. The outcome measures of each group were compared. The blastocyst embryos of female zebrafish from each group born after mating with male zebrafish in their best breeding period were collected for transcriptomics analysis. Fresh oocytes of female zebrafish in each group were collected for transcriptomics analysis to explore the potential molecular mechanisms of maternal age-related fertility decline. **Results** Compared with that of the 6-month group (94.9%±3.6%), the embryo fertilization rate of the 12-month group (92.3%±4.2%) showed no significant difference, but that of the 18-month group (86.8%±5.5%) decreased significantly ($P$<0.01). In addition, the fertilization rate in the 18-month group was significantly lower than that in the 12-month group ($P$<0.05). Compared with that of the 6-month group, the embryo mortality of the female zebrafish in the 12-month group and that in the 18-month group were significantly higher than that in the 6-month group ($P$<0.000 1, $P$<0.001). There was no significant difference in the number of embryos per spawn or in the embryo malformation rate among the three groups. The results of the transcriptomics analysis of blastocyst embryos showed that some genes, including *dusp5*, *bdnf*, *ppip5k2*, *dgkg*, *aldh3a2*a, *acsl1a*, *hal*, *mao*, etc, were differentially expressed in the 12-month group or the 18-month group compared with their expression levels in the 6-month group. According to the KEGG enrichment analysis, these differentially expressed genes (DEGs) were significantly enriched in the MAPK signaling pathway, the phosphatidylinositol signaling system, and the fatty acid degradation and histidine metabolism pathway ($P$<0.05). The analysis of the expression trends of the genes expressed differentially among the three groups (the 6-month group, the 12-month group, and the 18-month group in turn) showed that the gene expression trends of *fancc*, *fancg*, *fancb*, and *telo2*, which were involved in Fanconi anemia pathway, were statistically significant ($P$<0.05). In the results of oocyte transcriptomics analysis, the genes that were differentially expressed in the 12-month group or the 18-month group compared with the 6-month group were mainly enriched in cell adhesion molecules and the protein digestion and absorption pathway ($P$<0.05). The results of the trends of gene expression in the zebrafish oocytes of the three groups (the 6-month group, the 12-month group, and the 18-month group in turn) showed that three kinds of gene expression trends of declining fertility with growing maternal age had significant differences ($P$<0.05). Further analysis of the three significantly differential expression trends showed 51 DEGs related to mitochondria and 5 DEGs related to telomere maintenance and DNA repair, including *tomm40*, *mpc2*, *nbn*, *tti1*, etc. **Conclusion** With the increase in the maternal age of the zebrafish, the embryo fertilization rate decreased significantly and the embryo mortality increased significantly. In addition, with the increase in the maternal age of the zebrafish, the expression of mitochondria and telomere-related genes, such as *tomm40*, *mpc2*, *nbn*, and *tti1*, in female zebrafish oocytes decreased gradually. Maternal age may be a factor contributing to the decrease in oocyte fertilization ability and the increase in early embryo mortality. Maternal age-related oocyte aging affects the fertility and embryo development of the offspring.

**【Key words】** Zebrafish　Maternal age　Fertilization　Fertility

随着年龄的增长，女性生育力逐渐下降[1]，不孕不育症风险升高。女性生育力随年龄下降的潜在原因包括卵母细胞数量与质量下降、子宫维持妊娠的能力下降等[2]。有研究表明卵母细胞质量随年龄增长而下降，可能是由于慢性应激诱导端粒缩短，继发卵母细胞线粒体功能障碍，从而引起卵母细胞老化导致的[3]。但卵母细胞老化的研究较少，其涉及的分子途径不甚清楚。目前，年龄相关的生育力下降因辅助生殖技术的广泛应用得到一定程度的缓解，但并不能完全解决年龄相关的生育力下降带来的一系列问题，随年龄增长的生育力下降的潜在分子机制有待进一步研究。

斑马鱼是一种小型热带鱼，与人类基因组具有高度同源性，其体型小易操作，繁殖能力强，胚胎发育快，是良好的动物模型[4]。本文以斑马鱼为动物模型，研究年龄对女性生育力的影响及潜在分子机制，以期为进一步解决女性生育力低下提供参考。

## 1　材料与方法

### 1.1　实验动物

本研究实验动物为四川大学华西第二医院西部妇幼医学研究院“四川大学-香港中文大学生殖医学联合实验室”饲养的AB品系野生型斑马鱼。斑马鱼饲养条件为28.5 ℃，光照周期14 h/10 h。实验室饲养条件下，斑马鱼通常在发育第3～4个月达到性成熟并开始产卵[5]，早期产卵质量和数量较低。斑马鱼在发育第6～12个月是自然交配的最佳繁殖期[6]，而在发育约14个月繁殖能力开始下

降。因此本研究选取发育时间处于最佳繁殖初期、最佳繁殖末期以及繁殖能力下降的3个年龄段雌性斑马鱼(约为6月龄、12月龄、18月龄)用以研究母体年龄对生殖能力的影响。本研究获得四川大学华西第二医院实验动物管理与伦理委员会的批准,伦理批准号为2023105。

### 1.2 实验方法

#### 1.2.1 斑马鱼分组及交配产卵

在交配繁殖前,斑马鱼在各自饲养缸中以相同实验室条件饲养。从年龄范围为158 ~ 195 d的斑马鱼中随机选取8条雌性斑马鱼为6 月龄组〔(180±12) d〕;从年龄范围为330 ~ 395 d的斑马鱼中随机选取8条雌性斑马鱼为12月龄组〔(360±22) d〕;从年龄范围为502 ~ 583 d的斑马鱼中随机选取8条雌性斑马鱼为18 月龄组〔(540±26) d〕。样本数量根据自由度(E)和既往斑马鱼生殖能力相关研究[7]综合决定(E为总样本量与分组数的差值,当10≤E≤20表示样本量合适)。从年龄范围为158 ~ 195 d的斑马鱼中随机选取雄性斑马鱼〔(180±29) d〕与各组雌性斑马鱼配对繁殖。一条雌鱼与一条雄鱼随机配对,于前一天傍晚放置在斑马鱼繁殖缸内以隔板将雌鱼与雄鱼分开,经历过一个暗周期后,在第二天早晨开始接受光照时撤去繁殖缸内隔板,使其相互追逐繁殖产卵,撤去隔板1.5 h后收集沉至繁殖缸底的斑马鱼胚胎。显微镜下观察产卵后4 h胚胎,计数胚胎总个数及未受精胚胎个数,计算受精率。受精后24 h计数畸形胚胎及死亡胚胎个数,计算胚胎畸形率与死亡率。

#### 1.2.2 结局指标

主要结局指标:胚胎受精率。次要结局指标:单次产卵数量(斑马鱼开始交配繁殖后1.5 h内产卵总数)、胚胎死亡率、胚胎畸形率。

$$\text{受精率} = (\text{胚胎总个数} - \text{未受精胚胎个数})/\text{胚胎总个数} \times 100\% \quad (1)$$

$$\text{死亡率} = \text{死亡胚胎个数}/(\text{胚胎总个数} - \text{未受精胚胎个数}) \times 100\% \quad (2)$$

$$\text{畸形率} = \text{畸形胚胎个数}/(\text{胚胎总个数} - \text{未受精胚胎个数} - \text{死亡胚胎个数}) \times 100\% \quad (3)$$

#### 1.2.3 转录组学测序分析

收集6、12、18 月龄组斑马鱼的卵母细胞及后代囊胚期胚胎进行转录组学测序,样本处理及cDNA文库建立由广州基迪奥生物科技有限公司完成。提取样本RNA制备PCR文库并过滤低质量数据获得高质量数据后,利用HISAT2软件将测序数据与参考基因组Ensemble_108进行比对分析统计样本基因数目;根据HISAT2的比对结果,利用Stringtie重构转录本,并利用RSEM计算每个样本中所有基因的表达量。样本间差异分析时首先对样本基因表达量read count数据进行标准化,计算假设检验概率($P$)以及错误发现率(false discovery rate, FDR);再以FDR< 0.05, $|\log_2 FC| > \log_2 2$为阈值筛选样本间显著差异基因;最后对样本间显著差异基因进行KEGG功能富集性分析并以$Q$ value≤0.05为阈值筛选差异基因显著富集的KEGG通路。样本趋势分析时以6、12、18 月龄组的顺序首先对差异基因表达模式进行聚类,并以$P$≤0.05为阈值筛选出基因显著差异表达趋势;再对各趋势中的基因进行KEGG功能富集分析,以$Q$ value≤0.05为阈值筛选趋势中基因显著富集KEGG通路。

#### 1.2.4 统计学方法

采用GraphPad Prism 9软件进行统计分析,实验数据以$\bar{x} \pm s$表示,采用单因素方差分析(ANOVA)进行多组间比较,然后用Tukey's法进行两两比较。$P$< 0.05为差异有统计学意义。

## 2 结果

### 2.1 母体年龄影响胚胎受精率及死亡率

斑马鱼受精胚胎自受精后,1-细胞依次卵裂为2-细胞、4-细胞、8-细胞等,于受精后4 h形成囊胚期胚胎;而斑马鱼未受精胚胎细胞不会出现卵裂,始终停留在1-细胞期直到细胞死亡(图1A)。对于产卵量,6 、12 、18月龄组间雌性斑马鱼单次产卵量差异无统计学意义(图1B)。但是在斑马鱼后代胚胎受精率方面,与6月龄组(94.9±3.6)%相比,12月龄组(92.3±4.2)%差异无统计学意义,而18月龄组下降〔(86.8±5.5)%, $P$< 0.01〕,且18月龄组与12月龄组比较差异有统计学意义($P$< 0.05, 图1C)。在斑马鱼后代胚胎24 h死亡率方面,与6月龄组相比,12月龄组与18月龄组均升高($P$< 0.000 1或$P$< 0.001, 图1D和图1E),但18月龄组与12月龄组比较差异无统计学意义。观察斑马鱼胚胎受精后24 h胚胎表型,3组斑马鱼后代畸形胚胎量少,3组斑马鱼后代组间畸形率差异无统计学意义(图1F)。

### 2.2 母体年龄对胚胎发育的影响

对不同年龄斑马鱼母体的后代囊胚期胚胎进行转录组分析,数据显示不同年龄斑马鱼组的后代囊胚期胚胎大量基因差异性表达。与6 月龄组的胚胎相比,12 月龄组胚胎共有5 847个差异性表达基因,其中1814个基因差异性表达上调,4 033个基因差异性表达下调;18 月龄组胚胎与6 月龄组相比,共有3 160个差异性表达基因,其中1 058个基因差异性表达上调,2 102个基因差异性表达下

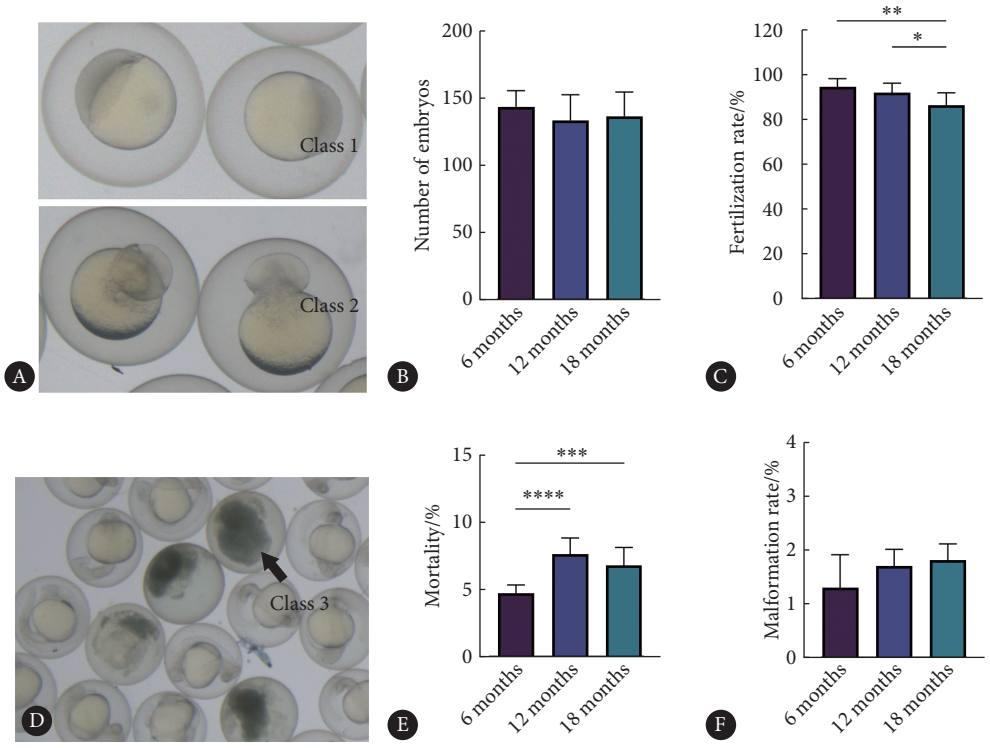


**图 1　雌性斑马鱼年龄影响胚胎受精率及死亡率**

**Fig 1　The age of female zebrafish affects embryo fertilization rate and mortality**

A, Class 1: fertilized eggs, Class 2: unfertilized eggs; B, the number of embryos of single mating; C, fertilization rate; D, Class 3: dead embryos; E and F, mortality and malformation rate of embryos of 24 h. $n$=8 in each group. $^{****}P$<0.000 1, $^{***}P$<0.001, $^{**}P$<0.01, $^{*}P$<0.05.

调(图2A)。对差异性表达基因进行信号通路的KEGG功能富集分析,在12 月龄组胚胎中,差异性表达基因富集的前20条KEGG通路中仅前两条MAPK信号通路和磷脂酰肌醇信号系统通路富集有统计学意义($Q$ value≤0.05),涉及包括*dusp5*、*bdnf*、*ppip5k2*、*dgkg*等基因(图2B,附表1)。同样与6 月龄组相比,18 月龄组差异性表达基因在前两条KEGG通路——脂肪酸降解通路及组氨酸代谢通路中富集有统计学意义($Q$ value≤0.05),涉及包括*aldh3a2a*、*acsl1a*、*hal*、*mao*等一系列基因(图2C,附表2)。分析3组胚胎基因表达趋势,8种基因表达趋势中有2种趋势具有统计学意义($P$< 0.05),分别包含2 272和1 721个基因(图2D),对此两种差异表达趋势涉及基因进行通路显著性KEGG功能富集分析,趋势1中差异表达基因富集的前20条通路中的第一条范科尼贫血通路富集具有统计学意义($Q$ value≤0.05),涉及*fancc*、*fancg*、*fancb*、*telo2*等基因差异性表达(图2E,附表3);趋势2中差异性表达基因富集的前20条通路中的前5条通路富集具有统计学意义($Q$ value≤0.05),其中包括磷脂酰肌醇信号系统通路以及胰岛素信号通路等,涉及*mtmr3*、*mtmr7a*、*calm14b*、*insra*、*insrb*等基因差异性表达(图2F,附表4)。所有附表请见网络资源附件。

### 2.3　母体年龄对卵母细胞质量的影响

与6 月龄组斑马鱼卵母细胞相比,12 月龄组卵母细胞有2 638个差异性表达基因,18 月龄组有2 921个差异性表达基因(图3A)。对差异性表达基因进行KEGG功能富集分析,12 月龄组卵母细胞差异性表达基因在前两条亚油酸代谢和细胞黏附分子通路富集具有统计学意义($Q$ value≤0.05,图3B),涉及*pla2g1b*、*cyp3c3*、*cldn23a*、*esamb*等(附表5);18 月龄组卵母细胞差异性表达基因如*esamb*、*cldn23a*、*slc1a1*、*col5a3b*等在前两条KEGG途径——细胞黏附分子和蛋白质消化吸收通路富集有统计学意义($Q$ value≤0.05)(图3C,附表6)。3组不同年龄斑马鱼的卵母细胞基因表达趋势分析结果显示,3种随母体年龄增长而表达量下降的基因差异表达趋势有统计学意义($P$< 0.05)(图3D),包含*ttn.1*、*hnf1a*、*mhc1zea*、*atp1a1a.4*、*me3*、*pde10a*等基因,*ttn.1*参与扩张型心肌病及肥厚性梗阻型心肌病,*mhc1zea*涉及1型糖尿病,*me3*参与代谢通路及PPAR信号通路途径(附表7 ~ 附表9)。进一步分析此3种显著性差异表达趋势中与线粒体和端粒相关的差异表达基因,共筛选出51个与线粒体相关的差异性表达基因,其中10个基因在3组斑马鱼卵母细胞中表现为趋势0,16个基因表现为趋势1,25个基因表现为趋势3(附表10);

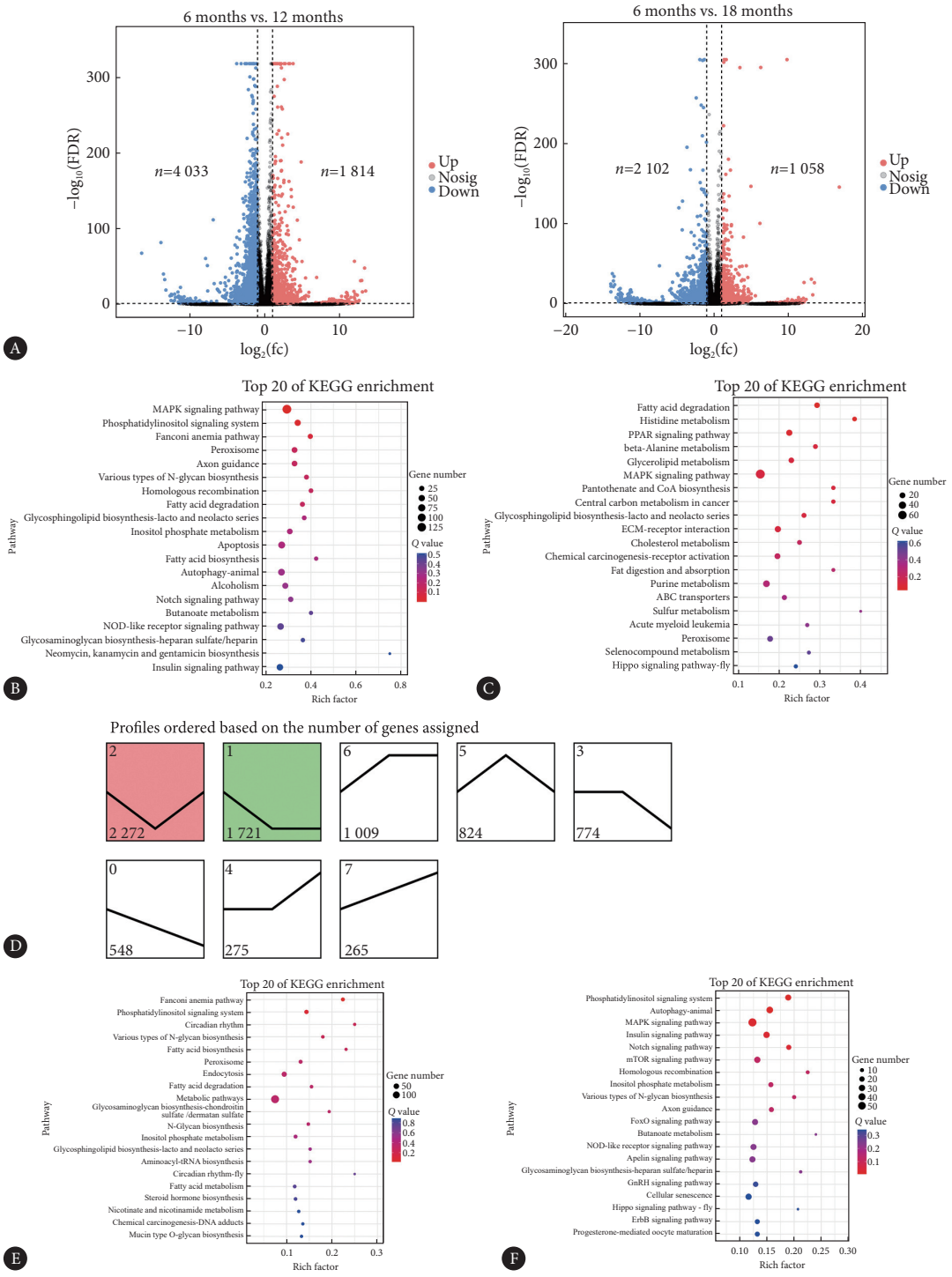


**图 2 不同年龄雌性斑马鱼囊胚期胚胎的转录组学测序分析**

**Fig 2 Transcriptomics analysis of blastocyst embryos of female zebrafish of different age**

A, The volcano plot of differentially expressed genes (DEGs) in the 12-month and 18-month groups compared with the 6-month group. The top 20 KEGG-enriched DEGs in the 12-month group (B) and 18-month group (C) compared with the 6-month group; D, 8 profiles of DEGs expression trends from 6 months vs. 12 months vs. 18 months. Each graph showed the expression patterns of DEGs in three consecutive groups (in a specific maternal age sequence, 6 months-12 months-18 months) with certain biological characteristics (such as a continuous increase in gene expression). Red and green indicated that the gene expression trend was significant, while white indicated that the gene expression trend was not significant. The starting point, the midpoint, and the end point of the line from left to right represented the 6-month, the 12-month and 18-month groups, respectively. The positive or negative slope of the line indicated that the expression trend of DEGs between groups was increasing or decreasing, and the magnitude of the slope indicated the degree of change in the expression of DEGs between groups. The number above the line was the number assigned for the 8 profiles of DEGs expression, and the number below the line was the number of DEGs matching the trend of DEGs expression in the specific graph. The trends of profile 1 and profile 2 have significant difference ($P$<0.05). The top 20 KEGG-enriched DEGs in profile 1 (E) and profile 2 (F). Nosig: no significance.

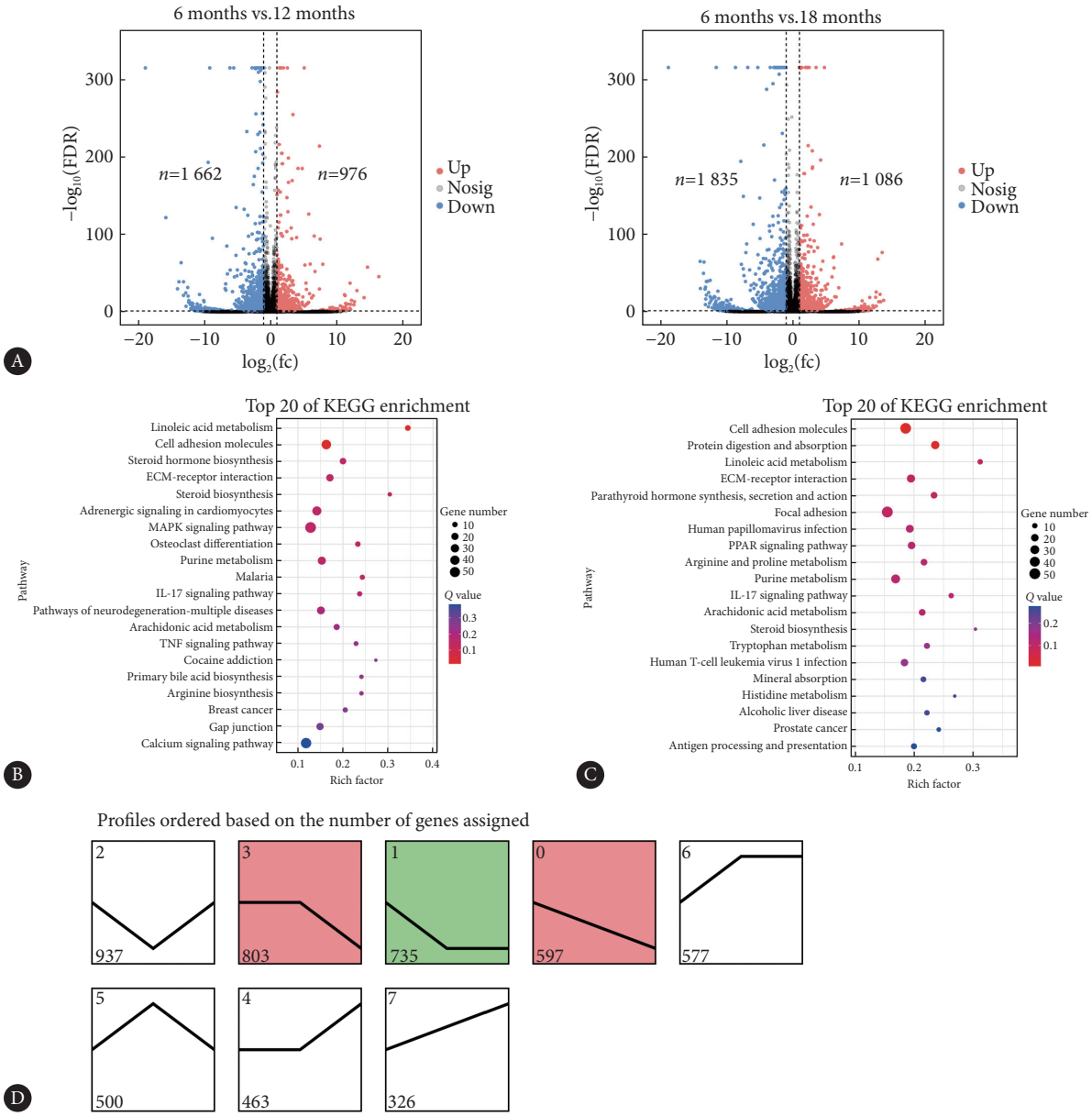


**图 3 不同年龄雌性斑马鱼卵母细胞的转录组学测序分析**

**Fig 3 Transcriptomics analysis of oocytes of female zebrafish of different age**

A, The volcano plot of DEGs in the 12-month and 18-month groups compared with the 6-month group; The top 20 KEGG-enriched DEGs in the 12-month group (B) and the 18-month group (C) compared with the 6-month group; D, 8 profiles of inter-group DEGs expression trends in the 6-month, 12-month, and 18-month groups. Each graph showed the expression patterns of DEGs in three consecutive groups (in a specific maternal age sequence, 6 months-12 months-18 months) with certain biological characteristics (such as a continuous increase in gene expression). Red and green indicated that the gene expression trend was significant, while white indicated that the gene expression trend was not significant. The starting point, midpoint, and ending point of the line from left to right represented the 6-month, 12-month, and 18-month groups, respectively. The positive or negative slope of the line indicated that the expression trend of DEGs between groups was increasing or decreasing, and the magnitude of the slope indicated the degree of change in the expression of DEGs between groups. The number above the line was the number assigned for the 8 profiles of DEGs expression, and the number below the line was the number of DEGs matching the trend of DEGs expression in the specific graph. The trends of profile 0, profile 1, and profile 3 showed significant difference ($P<0.05$). Nosig: no significance.

包含*tomm40*, *mpc2*等基因涉及神经退行性疾病及糖尿病性心肌病等途径; 此外, 3种随年龄增长而基因表达下调的趋势中包含5个与端粒维持及DNA修复相关的基因, 如*nbn*、*tti1*等(附表11)。

## 3　讨论

女性生殖老化在很大程度上是基于与年龄相关的卵巢功能变化而非子宫的生殖能力, 卵巢老化表现为卵母

细胞数量与质量的下降[8]。目前通过超声和内分泌标记物可以准确反映卵泡数量的变化,但以卵泡数量变化判断女性生育力的能力有限,卵母细胞质量是影响年龄相关的女性生育力的重要因素。研究表明卵母细胞质量下降会导致受精率、胚胎质量和生殖结果变差[9]。卵母细胞老化是质量下降的重要表现,包括排卵前体内卵母细胞老化及排卵后体外卵母细胞老化。有关卵母细胞排卵后老化的小鼠研究表明,在卵母细胞排卵后老化8 h内,卵母细胞发育为2-细胞胚及胚泡期胚胎的能力没有明显影响[10]。而在与卵母细胞排卵后老化相关的胚胎质量差及子代异常结局中,氧化应激诱导的线粒体功能受损扮演重要角色,保护线粒体功能有利于减轻卵母细胞老化的影响[11-12],但其具体分子机制尚不清晰。本文选用不同年龄的斑马鱼研究卵母细胞排卵前老化对生育力的影响,为分析女性年龄相关的生育力下降提供理论依据。本研究选取年龄依次递增的6月龄、12月龄、18月龄正常健康雌性斑马鱼为研究对象研究年龄因素对生殖能力的影响。根据斑马鱼单次产卵量及后代胚胎畸形率实验结果,表明在雌性斑马鱼发育6 ~ 18个月大时,年龄因素不会影响雌性斑马鱼单次产卵量及后代胚胎正常表型。在受精率方面,18月龄组后代胚胎受精率与6月龄组相比下降($P$< 0.05),18月龄组与12月龄组相比受精率依然下降($P$< 0.05),表明卵母细胞受精率随母体年龄增长而下降,母体年龄是卵母细胞受精率的影响因素。而在死亡率方面,12月龄组和18月龄组与6月龄组相比死亡率均上升,但18月龄组与12月龄组相比死亡率差异无统计学意义,结合受精率结果表明,12 ~ 18月龄段的年龄增长主要通过在早期影响卵母细胞受精能力而不是受精后胚胎死亡率使生育力下降。本研究结果证实了在6 ~ 18个月年龄段内斑马鱼母体年龄在对后代胚胎死亡率产生一定程度影响的基础上,随年龄增长,卵母细胞受精率降低。后代胚胎死亡率在母体年龄12 ~ 18个月内不随年龄增长而上升的原因可能是由于年龄增长导致的卵母细胞质量下降,在第一步受精阶段就受阻而无法发育形成受精胚胎。研究结果提示随年龄增长而下降的卵母细胞质量可能主要通过影响卵母细胞受精能力影响女性自然生育力。

随着女性年龄增长,患不孕不育、流产风险升高。即使怀孕,较高年龄的孕产妇也面临更多的妊娠并发症,且其子代患心血管疾病、糖尿病、唐氏综合征等风险增加[13-16]。大量研究表明21-三体综合征及18-三体综合征等先天性染色体障碍疾病可能与母体衰老导致的卵母细胞染色体非整倍体改变有关,而纺锤体、微管蛋白、着丝粒均是母体衰老导致的染色体非整倍性的影响因素,具体分子机制尚不十分清楚[17-19]。卵母细胞中线粒体DNA约占三分之一,而精子线粒体DNA在胚胎发育中不发挥作用,因此,女性卵母细胞线粒体DNA异常经过母系遗传,可能是导致卵母细胞受精异常以及早期胚胎发育障碍的潜在分子机制[20-21]。端粒缩短诱发染色体分离异常同样是与年龄相关的卵母细胞质量下降及胚胎发育异常的潜在影响因素[22-23]。本研究发现在斑马鱼卵母细胞基因表达趋势分析中,涉及扩张型心肌病,1型糖尿病通路途径的基因如*ttn.1*、*mhc1zea*随母体年龄的增长而表达下降,提示与年龄相关的卵母细胞质量下降可能是后代患心血管疾病风险升高的影响因素。此外与线粒体、端粒相关的如*tomm40*、*mpc2*、*nbn*、*tti1*等基因随母体年龄增长在卵母细胞内表达下降,为进一步研究年龄相关的生育力下降的分子机制提供潜在靶点。

综上所述,本研究结果揭示了年龄相关的卵母细胞质量下降对卵母细胞受精率及胚胎发育的影响,提升了对卵巢衰老机制的认识,为找到年龄相关的卵母细胞质量下降预测指标提供理论依据。研究发现扩张型心肌病,1型糖尿病通路途径涉及的如*ttn.1*、*mhc1zea*等基因随母体年龄的增长而表达下降,这为进一步研究高龄产妇后代患心血管疾病,1型糖尿病等疾病的分子机制提供潜在靶点,具有一定的临床意义。

* * *

**作者贡献声明** 竹琳负责论文构思、调查研究和初稿写作,林子媛负责正式分析、研究方法和验证,刘燕燕负责经费获取、研究项目管理和监督指导,孙华钦负责经费获取、提供资源和初稿写作,孙春堂负责经费获取、提供资源和监督指导,陈凤负责论文构思、调查研究、初稿写作和审读与编辑写作。所有作者已经同意将文章提交给本刊,且对将要发表的版本进行最终定稿,并同意对工作的所有方面负责。

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