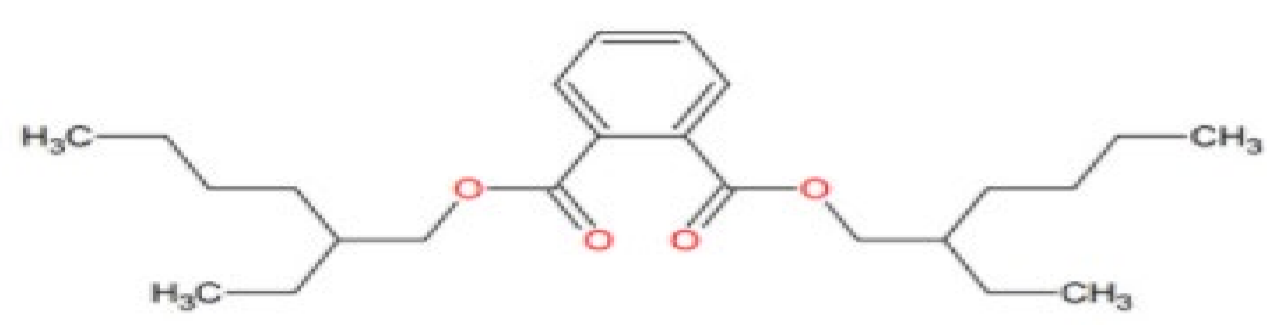


## INTRODUCTION AND OBJECTIVES

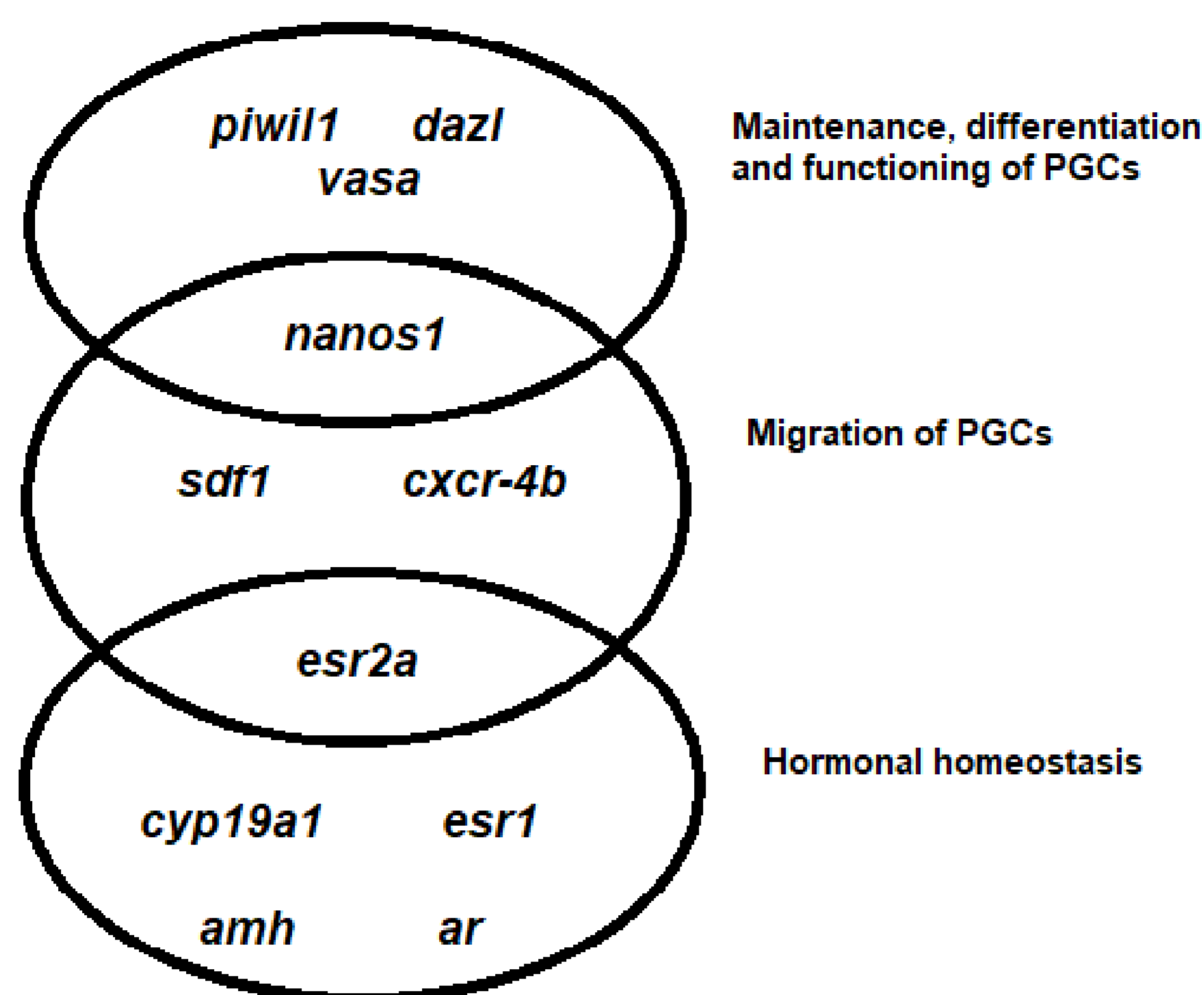
**Di(2-Ethylhexyl)Phthalate (DEHP)** belongs to the group of synthetic chemicals with ubiquitous exposures because of its use in plastics and cosmetics. It has been shown that reproduction and development of reproductive system is highly sensitive to DEHP exposure.



**Figure 1. Structural formula of DEHP** (<https://oxoplast.com/en/plasticizers-what-is-the-difference-between-deht-and-dehp/>)

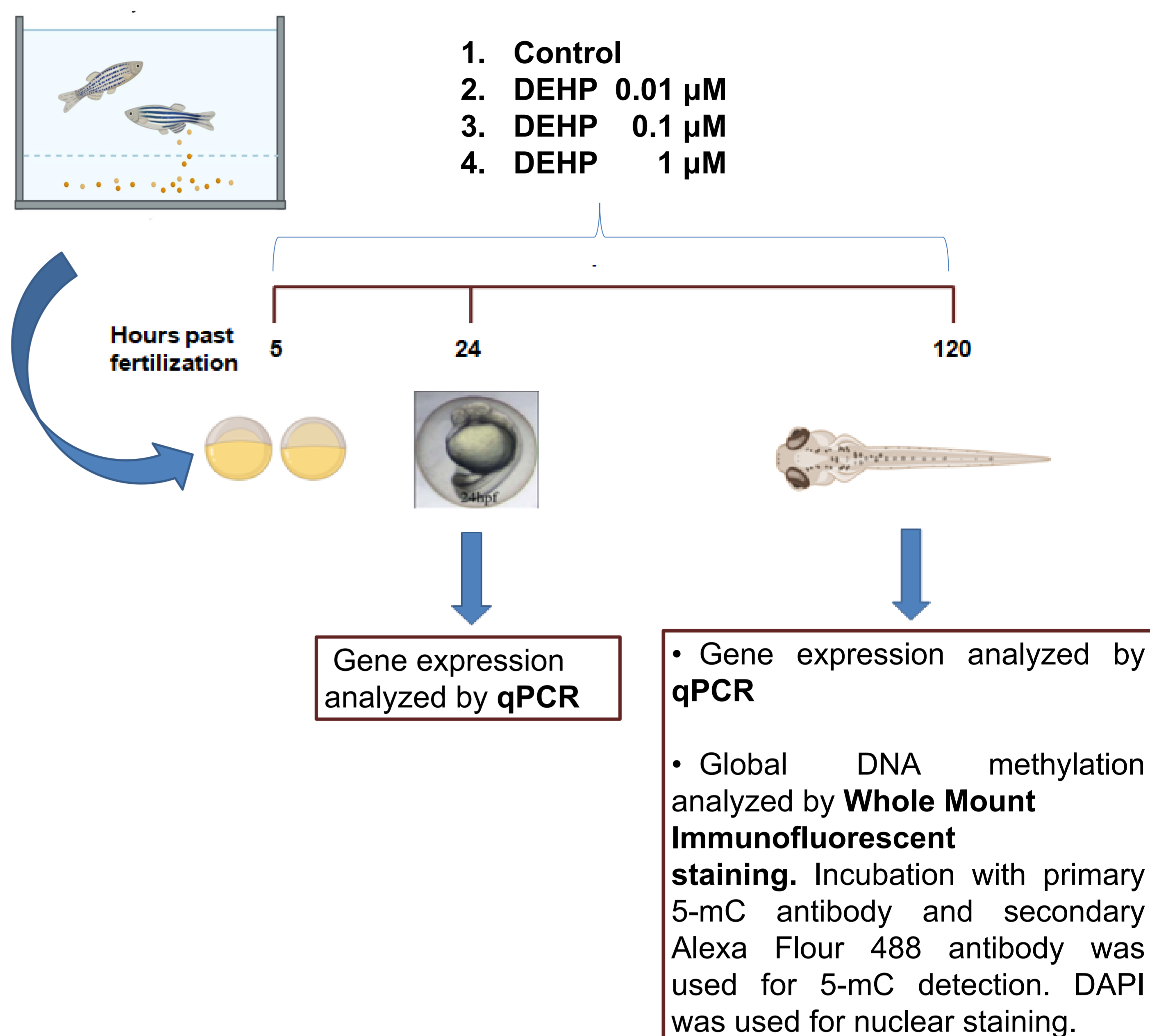
Development of zebrafish reproductive system begins with migration of embryonic primordial germ cells (PGCs) to genital ridge during 24 hours post fertilization (hpf), where PGCs govern the process of gonad formation. During embryogenesis, migration, number and proper function of PGCs are crucial events in the development of reproductive system. Zebrafish sex determination is also dependent on environmental influence.

**OBJECTIVES:** To investigate effects of DEHP on zebrafish PGC by evaluating the expression of genes crucial for migration at 24 hpf, maintenance, differentiation and functioning of PGC as well as hormonal homeostasis at 120 hpf.



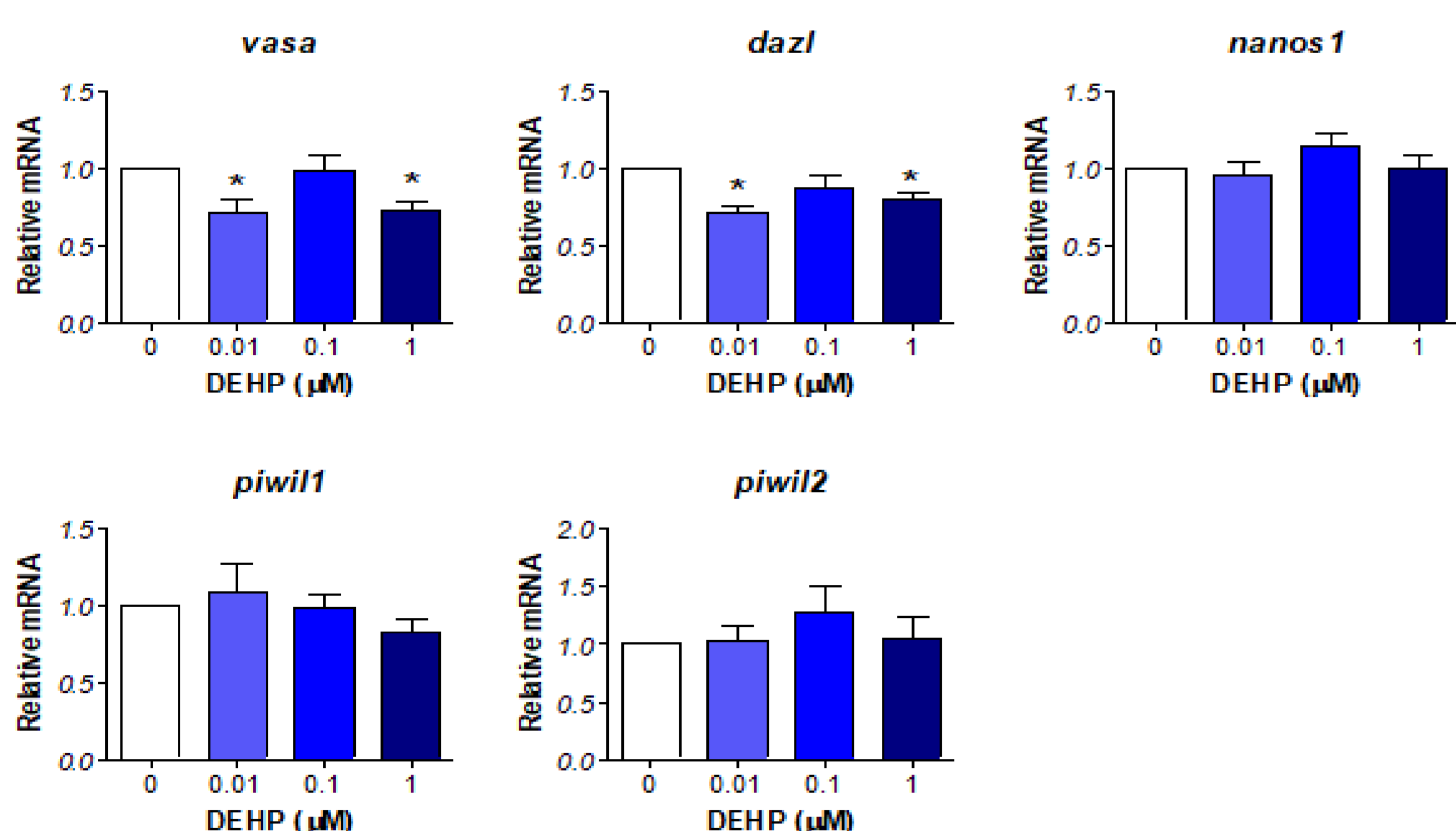
Global DNA methylation as a screening tool of the DEHP induced global effects on zebrafish genome was also analyzed at 120 hpf.

## MATERIAL & METHODS



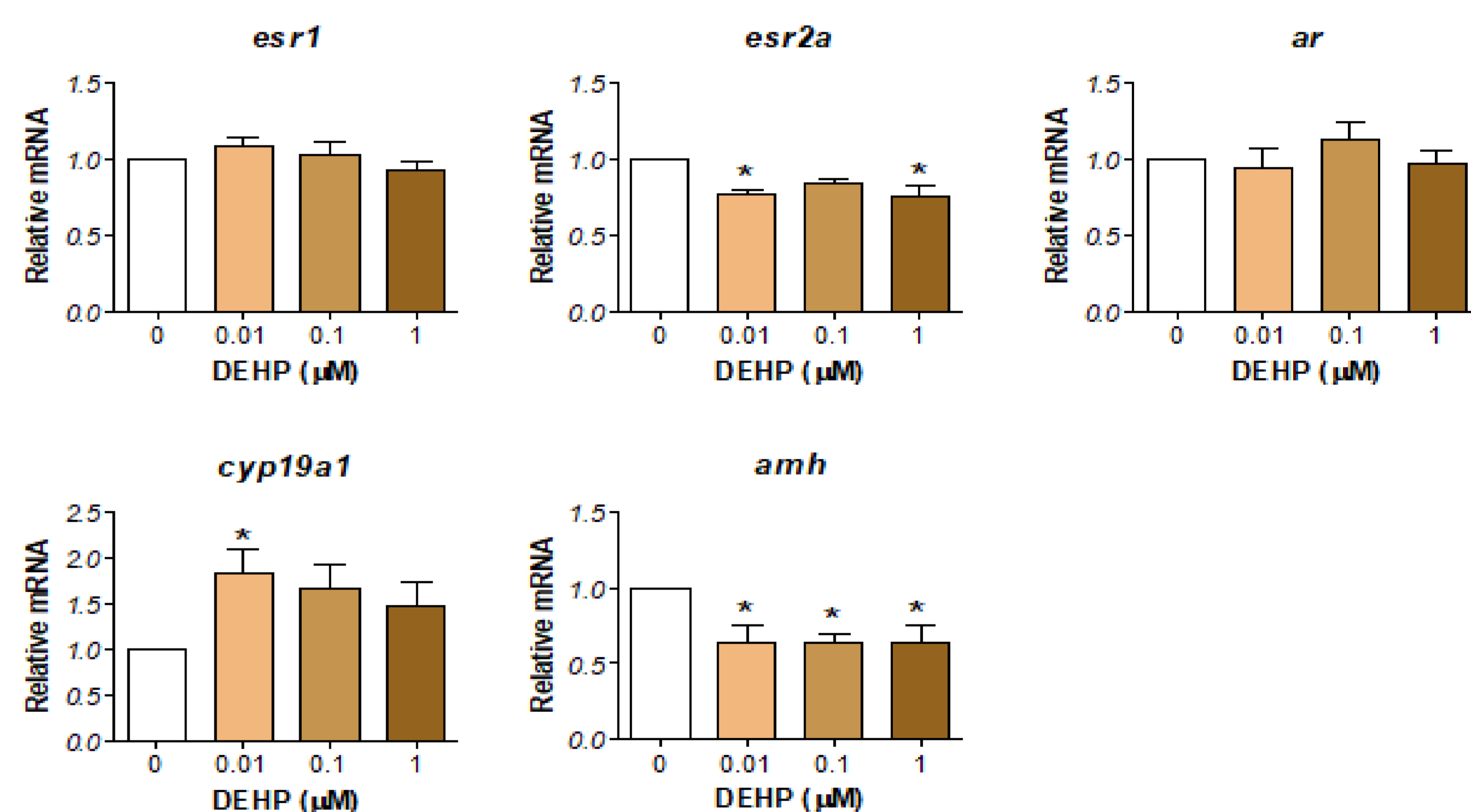
## RESULTS & DISCUSSION

### Effects of DEHP on expression of genes crucial for maintenance, differentiation and functioning of PGC



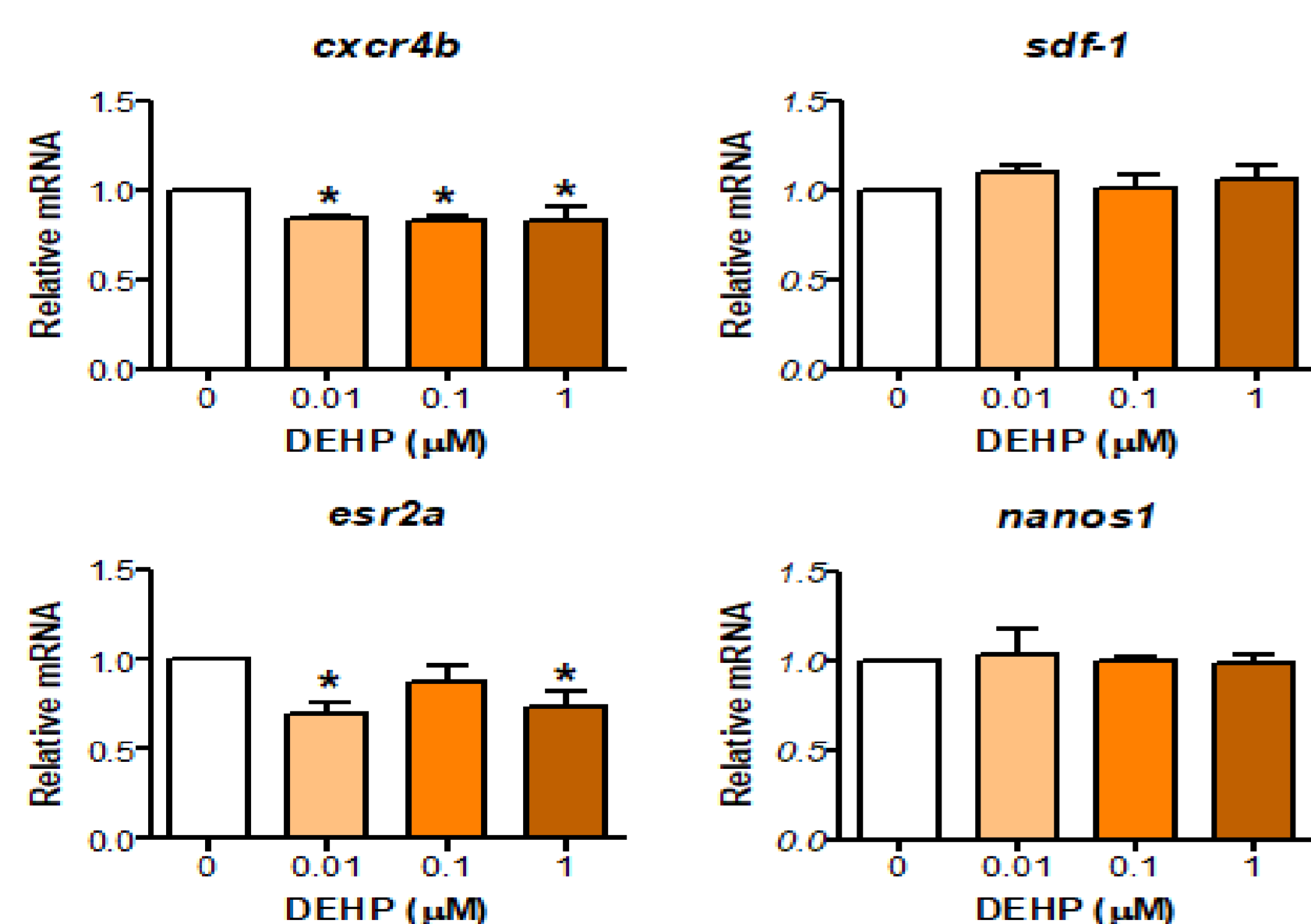
**Figure 2. Effects of DEHP on mRNA expression of *vasa*, *dazl*, *nanos1*, *piwil1* and *piwil2* genes** when exposed to treatment during 120 hpf. DEHP inhibited the gene expression of *vasa* and *dazl* genes, which are important for maintenance, proliferation and differentiation of PGC.

### Effects of DEHP on expression of genes crucial for hormonal homeostasis



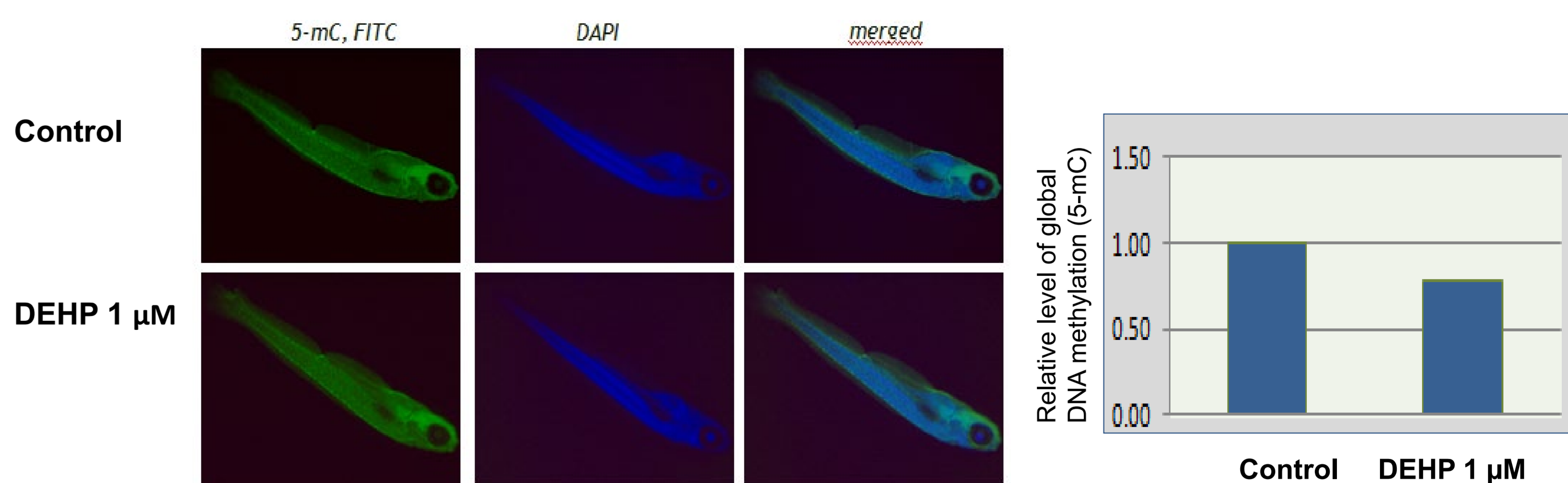
**Figure 3. Effects of DEHP on mRNA expression of *esr1*, *esr2a*, *ar*, *cyp19a1* and *amh* genes** when exposed to treatment during 120 hpf. DEHP inhibited the gene expression of *cyp19a1* and *amh*, involved in sex determination in zebrafish. DEHP also inhibited the expression of *esr2a* gene, which is involved in cellular responses to estrogens.

### Effects of DEHP on expression of genes crucial for migration PGC



**Figure 4. Effects of DEHP on mRNA expression of *cxcr4b*, *sdf1*, *esr2a* and *nanos1* genes** when exposed to treatment during 24 hpf. DEHP inhibited the expression of *cxcr4b* gene, which encodes receptor on PGC cell surface crucial for their migration to genital ridge and *esr2a* gene, which mediates estradiol coordinated distribution of PGC.

### Effects of DEHP on global DNA methylation



**Figure 5. Effects of DEHP on global DNA methylation** when exposed to treatment during 120 hpf. Analysis was performed by Whole Mount Immunofluorescent staining. ImageJ software was used for fluorescence quantification. FITC signals were normalized to corresponding DAPI signals. No significant change in global DNA methylation was detected.

## CONCLUSIONS

✓ DEHP can disrupt mRNA expression of *cxcr4b* and *esr2a* genes involved in regulation of migration, *vasa* and *dazl* gene involved in maintenance and functioning of PGCs as well as *esr2a*, *cyp19a1*, *amh* genes involved in hormonal homeostasis during the early embryonic development of zebrafish.

✓ This may have lasting effects on their expression and can be reflected in the further development and functioning of the reproductive system.



## Acknowledgments