In eukaryotes, chemical modifications of chromatin proteins and DNA, referred to as epigenetic modifications, are one component controlling the correct genome expression within a given cell type. DNA methylation in the promoter region of some genes is associated to irrevocable transcriptional silencing of these genes. Improper methylation of specific DNA regions in mammalian spermatozoa is associated to infertility or altered embryo development. In zebrafish, hypomethylated DNA was found at the promoter region of most developmental transcription factors, likely to enable early transcription of the genes during embryo development. This suggests that as in mammals, sperm DNA methylation pattern in fish is important for embryo development. In this context, it is important to ensure that the use of reproductive biotechnologies will not alter this pattern. The aim of this study was to describe to which extent the use of a cryoprotectant bearing methyl groups in fish, MeOH, will alter the DNA methylation pattern of cryopreserved spermatozoa. This study was conducted on two fish species, the zebrafish (Danio rerio) and the goldfish (Carassius auratus), and global DNA methylation was measured by the restriction enzyme method followed by LUMA analysis. We found that in zebrafish, cryopreservation with MeOH increased the methylation level of sperm DNA. No such tendency could be observed in goldfish where cryopreserved sperm had methylation levels indiscriminately lower or higher than the fresh controls. Consequences on embryo development quality have to be assessed with regards to a possible species-specific sensitivity. This will be developed in the CRB-Anim program (ANR-11-INBS-0003).