Chemical induction of haploid gynogenesis in sterlet Acipenser ruthenus

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Chromosomal manipulations in sturgeons, particularly gynogenesis, are interesting due to the potential to change female ratio in progeny that can be useful for caviar production. The optimization of UV treatment for induction of gynogenesis is complicated due to high and variable optical density of the milt due to differential spermatozoa concentration, and because of sensitivity of spermatozoa' s motility apparatus. Therefore in this study we compared chemical methods of sperm treatment as an alternative to short wave-length UV treatment; evaluation considers impact on spermatozoa motility, DNA integrity, and efficiency of DNA inactivation. Dimethyl sulfate (DMS) in concentrations of 2.5–30mM was applied to spermatozoa in order to inactivate DNA. Also ethidium bromide (EB), psoralen (PS), and 4' -aminomethyl-4,5' ,8-trimethylpsoralen (AMT) were used to increase sensitivity of spermatozoa' s DNA to long wavelength UV-A light (360 nm). CASA analyses of treated sperm showed strong negative effects on spermatozoa motility with the increasing concentration of active substances. Additionally in case of PS, EB, and DMS treatment comet assay did not reveal significant DNA damage of sperm at the range of concentrations relatively safe for spermatozoa motility. Flow cytometric analysis of relative DNA content in larvae resulting from activation of normal ova of sterlet with the treated sperm showed low efficiency of haploid gynogenesis induction. The putative gynogenetic larvae were found after treatment with PS in concentrations higher than 18μM and EB higher than 10μM followed by UV-A irradiation at the dose of 900 J/m^2 and DMS up to 5mM. Because of an overwhelming impact on the sperm motility and relatively low DNA damage, treatment of sperm with PS, EB or DMS did not prove efficient compared with a widely used UV-C irradiation treatment. In contrast, treatment with AMT followed by UV-A showed lower influence on spermatozoa motility and higher efficiency of DNA damaging resulting in the higher percentage of gynogenotes in the progeny, thus could be considered as a possible substitution for UV-C treatment.

Keywords:

sturgeons; chromosomal manipulation; dimethyl sulfate; psoralen; comet assay

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