2. ABSTRACT

Endocrine disrupting compounds that adversely affect biological semen parameters are one of the cause of declining male fertility. Their main mechanism is based on steroid-signaling pathway alterations. This group of compounds includes BPA, which, as has been proven, migrates from the plastic to the human body. Due to the data of BPA toxicity, its alternatives - BPF and BPS - were introduced to the industry. The aim of the study was to determine the mechanisms of BPA, BPF and BPS action, both individually and in a mixture on the *in vitro* model of male reproductive cells. The GC-2spd(ts) cell line of murine spermatocytes was used for the study. Cell viability was assessed by MTT test, changes in mitochondrial membrane potential and cell cycle were examined by the cytometric method. Cell damage after exposure was assessed using LDH lactate dehydrogenase activity in the cell media. The gene expression profile was examined by the RT-PCR. Exposure to the bisphenols was also conducted after the pretreatment with selective agonists, inhibitors and antagonists.

All the tested compounds affect the cell viability, causing not only cytotoxic but also some stimulating effect, depending on the concentrations used and the duration of the exposure. The LDH activity test after exposure of cells to BPA, BPF and BPS confirmed their cytotoxic effect, and the effects were similar for all substances tested. Analysis of changes in mitochondrial membrane potential showed that all tested compounds influenced membrane depolarization by inducing apoptosis. No changes in the cell cycle after exposure to BPA, BPF and BPS have been observed. Evaluation of cell viability after estrogen receptor agonists treatment confirmed their opposite action in the process of apoptosis. None of the inhibitors and antagonists used (for ER, GPR30, EGFR, MEK1/2) abolished BPA cytotoxic effect, which indicates the participation of other pathways in its mechanism of action. Exposure to the BPF and BPS showed an additive effect between estrogen receptors ESR and GPR30.

BPA, BPF and BPS also influenced GC-2 cell steroid receptor and steroidogenesis related genes expressions. Exposure to the combination of BPA, BPF and BPS confirmed a dose addition effect between them. The changes caused by the mixture were similar to those caused by a single compound.

These data show that exposure to BPA and its main substitutes- BPF and BPS dysregulated multitude of pathways. The similarity of these compounds clearly indicates that both BPF and BPS are not a safe alternative to BPA in terms of male reproductive health and their use should be limited.