BACKGROUND. The sex cells (oocytes and sperm) have a low mutation rate in comparison with somatic cells. In mice, the number of *de novo* mutations per generation was estimated to be approximately 5×10^{-9} in the germline, while in somatic cells - more than an order of magnitude higher.

The reasons for the low mutation rates, especially in the female germline, are unclear and there might be several explanations: (i) the absence of replication in the female germline, (ii) higher abundance of repair machinery, (iii) strong elimination of cells with DNA damage during early phases, or (iv) other factors such as the accessibility of chromatin. While we are mostly lacking direct data on how *de novo* mutations arise. Mouse models indicate that oxidative damage of DNA can contribute substantially. Indeed, oxidative damage of DNA is a common phenomenon arising from both environmental factors and cell´s own metabolism. The repair of modified bases by base excision repair (BER) is intimately linked to single-strand DNA break (SSB) repair, with which it shares many components.

GOAL. The overall goal of the proposal is to improve our understanding of the mechanisms behind a lower mutation rate of the germline in comparison with somatic cells. The specific goals include:

(1) to measure the number of endogenous single-strand DNA breaks in oocytes and in somatic cells, and to measure the amount and localisation of critical SSB repair factors in oocytes and somatic cells,

(2) to determine the capacity of oocytes and zygotes to repair SSBs and compare this capacity with somatic cells derived from different tissues,

(3) to verify if the abundance of repair factors constitutes the main factor, which renders oocytes more resistant to damage.

HYPOTHESIS. The planned work is based on a hypothesis, which assumes that oocytes have a high content of DNA repair factors (significantly higher than somatic cells), therefore they can repair single-strand breaks very efficiently.

RESEARCH PLAN. To analyse the impact of the abundance of the repair machinery in oocytes and in other cell types, we will directly correlate it with the capacity of oocytes and somatic cells to sense and repair SSBs.

PRELIMINARY DATA. In pilot experiments we have demonstrated an ability to (i) detect individual single-strand breaks *in situ,* in somatic cells, by sSTRIDE technique, (ii) detect and distinguish single-strand breaks being actively repaired, and the ones that are not repaired when the number of lesions exceeds repair capacity of a cell, (iii) demonstrate how a shortage of a critical repair factor can influence a choice between repair pathways, and reduce cellular capacity to process a number of lesions simultaneously.

METHODS. We propose to use two newly developed methods – induction of known numbers of single-strand DNA breaks by laser microirradiation, and direct *in situ* detection of DNA ends by sSTRIDE technique. Finally, using somatic cell nuclear transfer, we will ask whether the high amount of repair components is the only factor responsible for high genome stability in oocytes.

SIGNIFICANCE. For the first time, we will functionally test the capacity of oocytes and descendant cells to repair SSBs in a direct comparison with somatic cells. These results are crucial for our understanding of physiological properties of oocytes and early embryos, mechanisms of induction of genetic disorders, and sensitivity to DNA damage in various somatic cell types.