Transmission of oocyte DNA damage to preimplantation embryos after in vivo mouse exposure to daunorubicin and cytarabine.

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O-295 Transmission of oocyte DNA damage to preimplantation embryos after in vivo mouse exposure to daunorubicin and cytarabine

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Study question: Does oocyte DNA damage induced by a previous in vivo mouse exposure to chemotherapy agents is transmissible to preimplantation embryos?

Summary answer: DNA damage was observed in preimplantation embryos issued from mice previously exposed to daunorubicin and cytarabine.

What is known already: In acute leukemia, the emergency to start a chemotherapy don't allow a fertility preservation at the time of diagnosis. Some authors have proposed to cryopreserve mature oocytes or embryos after a controlled ovarian stimulation applied shortly after the induction chemotherapy, which is mainly composed by daunorubicin and cytarabine, and reputated to be less gonadotoxic than alkylant agents. We previously observed DNA damage on mouse oocytes issued from antral follicles exposed in vivo to daunorubicin and cytarabine. Little is known about the risk of transmission of oocyte DNA damage to preimplantation embryos after fecundation of oocytes recently exposed to chemotherapy.

Study design, size, duration: By three time, two groups of mice (n = 11) were exposed for four days to cytarabine (10 mg/kg IP) or every two days to daunorubicin (1 mg/kg IV). Each group was compared with a negative control group (n = 11) and with a positive control group (n = 11) injected with cyclophosphamide (75 mg/kg IP). Females were mated one week after exposure and preimplantation embryos were collected by flushing the oviducts.

Participants/materials, setting, methods: 4 weeks female CD1 mice were mated one week after exposure for studying embryos conceived from oocytes exposed to chemotherapy at late pre-antral stage of follicular development. Cytotoxicity has been assessed by ovulation and fertilization rates and by embryo morphology. DNA embryonic damage was assessed by: (i) alkaline comet assay to quantify the tail DNA (ii) fluorescent immunohistochemical staining in blastomeres to quantify accumulating γH2AX foci.

Main results and the role of chance: In mouse, a recent exposure to daunorubicin and cytarabine did not alter the ovarian response to controlled ovarian stimulation with no adverse impact on the fertilization rate and the number of embryo conceived. Ovulation and fertilization rates in mice previously exposed to daunorubicin and cytarabine were similar to those in our negative control group. One week after exposure, we observed with the comet assay a significant increase of embryonic DNA damage after exposure to daunorubicin (16.57 ± 1.3, p = 0.0003) and cytarabine (16.46 ± 1.4, p = 0.0003) Vs 26.16 ± 2.5 after cyclophosphamide exposure (p < 0.0001) and 7,01 ± 1,1 in negative control group exposed to an injection of sterile saline solution. The analysis γ-H2AX on embryos showed a significant increase of foci corresponding to DNA double-strand breaks, after exposure to daunorubicin (7.97 ± 1.1; p = 0.001), cytarabine (6.47 ± 0.7, p = 0.0039), cyclophosphamide (5.92 ± 0.9; p = 0.0148) compared with negative control group (2,8 ± 0,7).

Limitations, reasons for caution: Mouse oocyte DNA is not exactly similar to human oocyte DNA, and would be more sensitive to genotoxic effects of chemotherapy agents. After chemotherapy, the kinetic of DNA repair before and after fertilization has to be studied by further assays in exposed oocyte and in embryos.

Wider implications of the findings: DNA damage in preimplantation embryos conceived from oocytes exposed to chemotherapy at late pre-antral stage of follicular development lead us to hypothese a transmission of oocyte DNA damage to preimplantation embryo. In acute leukemia, we strongly advise to not cryopreserve mature oocytes or embryo early after induction chemotherapy.

Trial registration number: Experimental protocols and animal handling procedures were reviewed by the French National Ethics Committee on Animal Experimentation (N° 2017033010523688).