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Mercury Disturb Reproductive Functions of Primary Endometrial Stromal Cells (ESC).

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Abstract:

Introduction: Mercury (Hg) exposure has been related to reproductive alterations in both animal models and epidemiological studies (Rzymski et al., 2015, Ann Agric Environ Med). Increased levels of Hg in endometrium have been associated with pathological conditions (Guyot et al., 2015, Plos One). So far it has been described that exposure to Hg induces oxidative stress and perturbs the structure of the cytoskeleton in Ishikawa cells (Guyot et al., 2015, Plos One). However, the effect of Hg on primary endometrial stromal cells (ESC) has not been tested yet. This study aims to analyze the effects of in vitro exposure to Hg on ESC viability, ROS production and decidualization capacity.

Methods: Primary ESC were isolated by gravity sedimentation from endometrial biopsies collected from healthy oocyte donors, the day of ovarian puncture (n=12). Cell viability of ESC (n=4) exposed to Hg (0-500 nM) up to 72h was measured each 24h using colorimetric MTS assay (Promega). The acute production of cellular Reactive Oxygen Species (ROS) was detected by dichlorofluorescin diacetate (DCFDA) a fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species ROS activity within the cell (DCFDA Cellular ROS Detection Assay Kit, Abcam) and then measured with a fluorescence microscopy in ESC (n=4) exposed to Hg (0-500nM) for 24h. For the in vitro decidualization study, ESC (n=4) were pre-treated with Hg (0-500 nM) for 24 h. Then, decidualization was induced with P4+E2 for 8 days in the presence of their respective doses of Hg. Decidualization was checked by prolactin (PRL) secretion in culture media by ELISA (Abnova). Cell integrity was assessed by F-actin immunostaining with Rhodamine-Phalloidin (Abcam), and their proliferative status was checked using Ki67 immunostaining (Merk Millipore).

Results: In ESC exposed to Hg (500 nM), cell viability was significantly reduced ($p<0.05$) at 48 h and 72 h. ROS production was significantly increased ($p<0.01$) in ESCs exposed to Hg (500nM) for 24h. After 8 days of decidualization, PRL secretion was significantly decreased ($p<0.05$) at 250nM and 500nM. Accordingly, altered actin cytoskeleton was observed at 250nM and 500nM in a dose dependent manner. There was also a decrease in Ki67 positive cells, indicating a decrease in cell proliferation at 500nM ($p<0.01$) which reflect alterations in the correct differentiation of these cells.

Conclusion: High doses of Hg acutely affect the physiology of endometrial cells by decreasing cell viability, possibly related to an increase of ROS production. At low-medium doses, Hg can act as an endocrine disruptor, inhibiting ESC decidualization and disturbing actin cytoskeleton. Funded by APOTIP/2018/010, PFIS (PI/00009), Miguel Servet Contract (CPII18/00002) and ISCIII FIS project (PI17/00931).

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Category (Complete): 11.2 - Basic Reproductive Biology: Implantation, Endometrium
Presentation Preference (Complete): Either Oral or Poster
Awards (Complete):
SRI President's Plenary Awards (open to all In Training) : True
SRI Travel Awards (open to all In Training) : True
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Translational Value: Yes
Please describe the translational relevance below : This research is focused on the effect of mercury, an environmental pollutant, on human endometrium. The outcomes could aid to improve women reproductive health

Keyword (Complete): Mercury (Hg) ; Decidualization ; Endocrine disruptor
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