PUBLISHABLE SUMMARY

SUMMARY OF THE CONTEXT AND OVERALL OBJECTIVES

The FREIA project aims to provide better test methods to identify human-made chemicals that disturb hormones and their actions on development and function of the reproductive system in women. Currently available test methods do not work well, which is partly the reason why the effects of such endocrine disrupting chemicals (EDCs) on female reproductive health are often overlooked in regulatory chemical safety assessments. This means that women's reproductive health is at risk globally. Our EU-funded project FREIA will increase our understanding of how EDCs can harm female reproductive health. We will use this information to provide better test methods that enable fit-for-purpose chemical regulation. We will also share our knowledge widely to improve the reproductive health of women globally.

WORK PERFORMED AND MAIN RESULTS

In the FREIA project, the development of better test methods consists of two phases. In the first, "Discovery phase" we will look for biological characteristics (biomarkers) for female reproductive toxicity and develop a test strategy using existing knowledge as well as new data collected from our laboratory studies. For this, we will use two well understood EDCs, diethylstilbestrol (DES, a potent estrogen receptor activator) and ketoconazole (KTZ, a blocker of steroid hormone production). In the second, "Testing phase" of the FREIA project, we will assess how well our testing strategy works. The first Periodic Report describes the work done in the first 18 months of the FREIA project (January 2019 – June 2020).

Cells from human fetal, child and adult ovarian tissues have been cultured in the lab to identify which cell types are present in the human ovary and delineate harmful effects of DES and KTZ in human ovaries at various life stages. Fragments of human fetal ovaries (6-12 weeks after conception) were cultivated with various concentrations of KTZ for one week. We observed that KTZ decreased the percentage and the total number of immature germ cells, irrespective of the age of the ovary. Similar initial experiments with DES showed no obvious toxic effect on ovarian cells. We will study the effects of KTZ and DES better by assessing specific ovarian cellular markers, screening the hormones produced by the ovarian cells and by analysing ovarian gene expression profiles. Methods were established to measure known and suspected EDCs, e.g. perfluorinated chemicals, phthalates, bisphenols and parabens, and to measure biomarkers in biological fluid that surrounds the developing oocyte in the adult ovary. These follicular fluid samples were retrieved from women undergoing fertility treatment in Sweden and Estonia. In the samples of the Swedish women, we already have discovered associations between chemical exposure and the number of oocytes retrieved from the patient in the fertility treatment, as well as the chance of the treatment resulting in a live birth. Together these data from human tissues will pinpoint changes in biological characteristics in human ovarian cells at various life stages upon exposure to EDCs.

A rat study was conducted to define biomarkers that predict the onset of female reproductive toxicity. Female rats were orally exposed to three dose levels of DES or KTZ from day 7 of pregnancy until 22 days after giving birth (postnatal day - PND 22). Both DES and KTZ caused endocrine disrupting effects in the pups. The onset of puberty was delayed in the female pups in all DES groups and the middle KTZ group, which was demonstrated by a delay in vaginal opening. No influence on ovary weights at PND 14, 22 or 90 was detected. The pulsatory release of Gonadotropin Realising Hormone (GnRH), a hallmark for puberty, was studied in the brain of the female pups at PND 22, 42 and 90. At PND 22, the two highest doses of DES and KTZ induced a delay in the time between GnRH pulses. At PND 42, but not PND 90, this was also observed for DES. Interestingly, our finding of delayed GnRH pulses confirms the delay in onset of puberty indicated by examination the age at vaginal opening. A much lower number of animals were needed to see

effect on GnRH release than on age at vaginal opening, which provides an important starting point for improving test methods for female reproductive toxicity assessment.

Several cell-based and computational methods are being (further) developed that may help the identification of EDCs that are toxic to female reproduction. A sensitive reporter gene assay for modulation of estrogen receptor (ER)-beta activity and a G-coupled protein estrogen receptor (GPER) activity assay are being developed that allow fast screening of potential EDCs on these receptors. An assay looking at multiple cellular markers in human ovarian cell lines showed distinct results on mitochondrial function and oxidative stress upon DES and KTZ exposure. Clear harmful effects were observed on the maturation of bovine oocytes exposed to DES in a culture dish, which lead to the decreased ability to develop into an embryo after fertilisation. A sensitive analytical method to measure steroid hormones is being optimised that will be applied to blood from the rat studies and cell culture media. Computational quantitative structure-activity relationship (QSAR) models to predict the effects of EDCs on aromatase inhibition and peroxisome proliferator-activated receptor (PPAR)-gamma activation are being improved using existing data sets. How these methods can be used in a test strategy to predict female reproductive toxicity will be explored further. Through thorough review of the available literature, we have now constructed ten possible pathways to explain how EDCs can cause female reproductive disorders. These so-called putative adverse outcome pathways (pAOPs) have been published (doi: 10.1007/s00204-020-02834-y). These pAOPs together with the results from the ovarian cell studies and rat studies will help us to pinpoint which test methods are needed to predict a chemical's effect on female reproductive development and health and improve test strategies for future use in regulatory chemical safety assessment.

FREIA is one of the eight projects on test method development for EDC identification within the EURION cluster (www.eurion-cluster.eu). FREIA coordinates the EURION working group on rat studies. This working groups helps to share tissues from rat studies that are not directly relevant to the FREIA project. On the FREIA website (www.freiaproject.eu), general background information on EDCs and female reproductive health can be found as well as project specific information, including a webinar recording, 7 peer-reviewed scientific publications and the FREIA factsheet (available in 5 languages) and infographic (14 languages).

PROGRESS BEYOND THE STATE OF THE ART AND POTENTIAL IMPACTS

FREIA provides the unique possibility to investigate hormonal processes in human ovaries from fetal to adult age to improve scientific knowledge on the causes of female reproductive toxicity. Our committed collaborators for policy, advocacy and communicating actions to promote women's health and a healthy society allow FREIA to have a huge societal impact. The FREIA approach will strongly support the work of European regulatory agencies, or even globally through the EURION cluster activities. The tools we are developing perfectly fit the needs of modern-day toxicity testing with a clear regulatory application in mind. Together, the FREIA outcomes will support testing, identification and assessment of EDCs that are toxic for female reproduction.

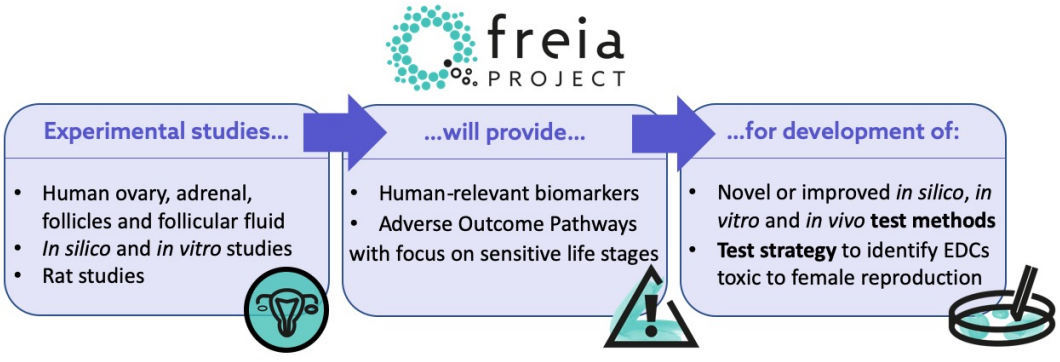

Figure from doi.10.3390/ijms21093215