Executive Summary

Context and overall objectives of the project

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 are not fit for purpose, 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.

Main results of the work performed between January 2019 and June 2023

In the FREIA project, we first looked for biological characteristics (biomarkers) for female reproductive toxicity using two well-understood EDCs, diethylstilbestrol (DES, a potent estrogen receptor activator) and ketoconazole (KTZ, a blocker of steroid hormone production). Studies with cell cultures of fetal and adult human ovaries, bovine oocytes and immortalized ovarian cells show changes in several genes and proteins that may be good indicators of exposure to these EDCs. Some promising candidates are involved in cholesterol biosynthesis, which is needed to produce steroid hormones. We are currently testing with another potential EDC, propylparaben, in our cell models and animal models to verify the predictive value of our candidate biomarkers.

We previously showed that endpoints that are currently being assessed in rat studies in regulatory testing are not sufficiently sensitive to detect an endocrine disrupting effect. Our studies did reveal some findings that may improve existing test guidelines:

- Our data show that mammary glands shortly after birth (postnatal day 6) and in young adult (postnatal day 22) rats exposed in the womb to DES and KTZ were different compared to mammary glands of unexposed pups. Together with the platform for international standard-setting Organisation for Economic Co-operation and Development (OECD), we are assessing if inclusion of mammary gland analysis in existing test guidelines is valid and feasible.
- In test guidelines, effects on rat ovaries are evaluated by traditional histological assessment. We explored the possibility to use a relatively quick screening process called surface photo counting (SPC). Our data show that the SPC method has good predictive value in the assessment of ovulations and is simpler, faster, and more cost-effective than traditional histological assessment. SPC might open new possibilities for a fast and operator-friendly assessment of effects on ovaries that can help to prioritize exposure groups for more thorough histological evaluation (Li et al., 2023).
- We evaluated whether circulating steroid hormones may be an indicator of EDC effects. Clear age-dependent changes in hormone levels were observed in plasma of rats. However, exposure to KTZ and DES in the womb did not result in changes in circulating steroid hormone levels in female rats after birth. These data do not support inclusion of circulating sex steroid hormones in test guidelines. This does not mean that effects of chemicals on steroid hormone formation are irrelevant. In contrast, many of the effects of EDCs observed in our cell models revealed changes in steroid hormone formation, indicating an effect on reproductive cells directly. Our data do not indicate that an ovarian-specific cell model would improve EDC identification, but the existing steroidogenesis assay H295R may be improved by measuring more steroid hormones. Under supervision of the OECD, we are performing a study with other labs to investigate this.

Ultimately, we aim to integrate our newly identified biomarkers and sensitive endpoints with existing test systems from OECD to improve on future test methods and a strategy to determine the effect of an EDC on female reproductive development and health.

To provide further evidence on the effects of EDCs on female fertility, we explored this association in women attending fertility clinics in Sweden and Estonia. Levels of 59 known and suspected EDCs were analysed in follicular fluid, the biological fluid surrounding oocytes, of 185 Swedish women and 148 Estonian women undergoing fertility treatment. Multiple chemicals were detected in all follicular fluids. In >90% of the follicular fluids, 3 metabolites of the phthalate DEHP, methylparaben, and 6 PFAS (PFOS, PFOA, PFHxS, PFUnDA, PFNA and PFDA) were detected and used to link with female fertility parameters. The ovaries of women with higher levels of DEHP, methylparaben and possibly PFUnDA and PFOA responded less to fertility treatment, established by calculating the ovarian sensitivity index (OSI). There were indications that some PFAS lowered the success of fertility treatment, determined by chance of establishing a pregnancy or live birth. Overall, this study provides additional evidence that DEHP can negatively influence female fertility. In addition, several other chemicals, i.e. methylparaben and some PFAS, were identified that may harm ovarian function and contribute to female infertility (Bellavia et al, 2022). We also studied how these women were exposed to these chemicals. The Swedish women from this study answered a questionnaire that contained information on home-environment, occupation, lifestyle and diet. We found that frequent use of perfumes was associated with higher phthalate (MEP) levels. Henn's egg consumption led to higher PFAS exposure. PFAS levels were also associated with certain fish consumption. We did not observe any correlation between the semi-persistent chemicals and use of plastics in microwave heating of food or flooring material (Hallberg et al., 2023). We are currently collecting available scientific data on how humans, and women in particular, can be exposed to EDCs and what actions effectively can be taken to avoid exposure.

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 webinar recordings, peer-reviewed scientific publications and databases, the FREIA factsheet and infographics. FREIA is one of the eight projects on test method development for EDC identification within the EURION cluster (www.eurion-cluster.eu).

Progress beyond the state of the art and potential impacts

FREIA uniquely provides the opportunity to investigate hormonal processes in human ovaries from fetal to adult age in order 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.