**Transgenerational effects of endocrine disruptors combined to an *in utero* exposure to high fat diet**

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Endocrine disrupting chemicals (EDCs) are ubiquitous environmental pollutants that can alter puberty, fertility or energy balance. Our laboratory recently documented the effects of transgenerational exposure to EDCs on the hypothalamic control of puberty and reproduction1. Given the increase in prevalence of obesity worldwide and the impact of gestational high fat diet on metabolic risk in the descendance2,3 , our current project aims at characterizing the effect of transgenerational exposure to a mixture of EDCs combined with gestational high fat diet (HFD). F0 dams were orally exposed to a mixture of 13 EDCs at environmentally relevant doses or to oil (controls), 2 weeks before mating, during gestation and lactation. F2 dams were than exposed to HFD (45% fat) or a normal diet during gestation and the first week of lactation. We exposed 7 F2 dams in each groups. Data were analyzed by two-way ANOVA.

Gestational exposure to HFD was associated with a significant lower weight in F3 pups between PND7 and PND40 (p < 0,0001). This phenotype was not significantly worsened by ancestral exposure to EDCs. In addition, we observed an increased neonatal mortality rate in rats exposed to HFD compared to normal diet (p < 0,05).

F3 females exposed to HFD *in utero* showed a significantly greater anogenital distance at P10 (*F=*47,83; p < 0,0001) and a shorter time between vaginal opening and first estrus (*F=*5,501; p=0,02) compared to controls. Gestational exposure to HFD also altered the estrous cyclicity in adult F3 (n=7/groups). Females cycled less regularly when exposed to HFD *in utero* with a significant decrease in time spent in proestrus (*F=*9,516; p=0,0036). This phenotype was not worsened by ancestral exposure to EDCs.

F3 males gestationally exposed to HFD showed significant pubertal delay, characterized by the age of balanopreputial separation (*F=*43,60; p < 0,0001). The same group presented a significant decrease in testicular weight at P25 (*F=*13,69; p = 0,0012) and at 7 months (n= 7/groups, *F=*33,99; p < 0,0001), but those effects were not worsened by ancestral exposure to EDC. The combination of transgenerational EDC exposure and in utero HFD induced a testicular descent et earlier age descent (n=7/group). No difference in gonadotropin or testosterone levels or sperm count were measured between the control, HFD or EDC groups at 7 months (n=7/groups).

In conclusion, we have shown that gestational exposure to HFD affected postnatal growth as well as pubertal development in male and female rats.

1. López-Rodríguez, D. *et al.* Multi- and transgenerational outcomes of an exposure to a mixture of endocrine-disrupting chemicals (EDCs) on puberty and maternal behavior in the female rat. *Environ. Health Perspect.* **129**, (2021).

2. do Carmo Rodrigues Virote, B. *et al.* Obesity induction in adult zebrafish leads to negative reproduction and offspring effects. *Reprod. Camb. Engl.* **160**, 833–842 (2020).

3. Rodríguez-González, G. L. *et al.* Maternal obesity and overnutrition increase oxidative stress in male rat offspring reproductive system and decrease fertility. *Int. J. Obes. 2005* **39**, 549–556 (2015).