Obesity prone mice present impaired steroidogenesis associated with SOCS3- mediated Nodal downregulation in ovarian theca-stromal compartment

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Obesity is a major health issue and presents multiple comorbidities, amongst them infertility. Obesity leads to systemic hormonal imbalance and diabetes, as well as ovarian failure comprising lipid accumulation, cellular stress and inflammation which culminates with decreased ovulation and infertility. Furthermore, we have previously shown that diet- induced obesity (DIO) in mice leads to the establishment of ovarian leptin resistance. Presently, we hypothesise that ovarian performance during obesity is linked to leptin and Nodal signalling disturbances. Using mouse models with contrasting responses to DIO, we unravelled the cross talk between leptin signalling modulator SOCS3 and Nodal signalling underpinning impaired steroidogenesis in obese mothers.

We treated either obesity prone C56BL/6J (B6) mice or obesity resistant 129S1 (129) mice with chow diet (CD) and high fat diet (HFD) for 16 weeks (wk), and characterised the animal phenotype, profiling body weight (BW) and food intake (FI). Additionally, after culling the animals we collected blood, gonadal fat, ovaries, ovarian granulosa cells (GC) and theca/stroma cell fraction (TC) after puncturing the ovaries. Furthermore we used pharmacologically hyperleptinemic (LEPT) mouse model, in which animals were intraperitoneally injected with saline (C) or 100μg of leptin/ day (L), as well as model of genetic obesity with leptin deficiency (ob/ob) and collected ovaries from wild type (+/+) or mutants (-/-) mice. Despite presenting greater FI then the lean counterparts ($p<0.0001$), the 129 HFD mice did not gain BW, in contrast with B6 HFD mice, which doubled their BW after DIO (38g) regarding B6 CD (20g). Both strains had increased plasma levels of leptin, but to a greater extent in B6 HFD ($p<0.0001$). Moreover, increased BW gain in B6 HFD after DIO was associated with the upregulation in mRNA levels of white adipose tissue expansion markers *Polymerase I and transcript release factor* (*Cavin)*, *Secreted frizzled-related protein 5 (Sfrp5), Mesoderm specific transcript* (*Mest,* $p<0.01$) and the proinflammatory genes *NLR Family Pyrin Domain Containing 1* (*Nlrp1)* and *C-C motif chemokine ligand 5* (*Ccl5,* $p<0.01$). Furthermore, obesity prone B6 mice presented decreased expression of *Luteinizing hormone* (*Lh*) receptor ($p<0.05$) in ovarian extracts and *Prolactin receptor* (*Prlr)* mRNA in TC ($p<0.01$). Moreover mRNA expression of *Steroidogenic acute regulatory protein* (*Star*), *3 beta- hydroxysteroid dehydrogenase* (*3bhsd)* and *17bhsd* ($p<0.05$) was decreased in TC in B6 HFD mice compared to CD, as well as *progesterone receptor* (*PR,* $p<0.01$) and *estradiol receptor a* (*Era,* $p<0.05$) mRNA. No significant differences were noted in 129 mice. Finally, we confirmed the upregulation of *suppressor of cytokine signalling 3* (*Socs3)* from B6 HFD comparing to CD ($p<0.001$) and associated downregulation of *Nodal* ($p<0.01$) and its receptors *Activin receptor type-2B* (*Acvr2b)* and *Activin A receptor* (*Alk7*, $p<0.05$). Moreover, LEPT model demonstrated

increased *Socs3* expression in ovarian extracts but decreased *Nodal* and *Alk4* expression comparing to controls, while the extremely obese -/- *ob/ob* mice demonstrated no changes in *Nodal* or its receptors.

Overall, impaired steroidogenesis in obesity prone mice is associated with dysregulation of Nodal signalling in TC, which is mediated by the overexpression of SOCS3. Hence, the cross talk between leptin and Nodal signalling may suppress steroidogenesis and subsequently impact oocyte development and quality in obese mothers. Further studies are needed to explore the underlying interactions.

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