SUMMARY

The role of leptin signalling pathway on NLRP3 inflammasome genes and proteins expression changes in the ovary of obese mice.

Obesity results from a disproportion between calorie intake and energy expenditure. The growth of white adipose tissue during obesity leads to an increase in leptin secretion, which regulates ovarian function controlling steroidogenesis, folliculogenesis or corpus luteum development. Obesity is associated with increased inflammation in the ovaries, anovulation, and decreased oocyte quality. Furthermore, maternal obesity is also associated with impaired embryo development or increased risk of fetal death. Thus, a better understanding of the pathogenesis of inflammation in the ovary may unravel the mechanisms underlying ovarian failure and infertility during the development of obesity. One of the factors involved in the maturation of pro-inflammatory cytokines such as interleukin-1 β (IL-1β) and IL-18 is NLRP3 (NOD-like receptor protein 3) inflammasome, whose role in the ovary is poorly understood. Moreover, recent studies showed the establishment of leptin resistance in the ovaries of obese mice. Thus, the following hypothesis was set: leptin signalling pathway mediates regulation of the NLRP3 inflammasome in the ovary of obese mice. The aim of the present study was to characterise the expression of the NLRP3 inflammasome components at the mRNA and protein level in the ovaries of obese mice, particularly instigating the crosstalk with leptin signalling.

The following models were used in the experiments: model DIO: C57BL/6J (B6) mice fed for 4 weeks with a standard diet (CD, 4wkCD) or a high fat diet (HFD, 4wkHFD) and 16wkCD or 16wkHFD; model of genetic obesity: leptin receptor knockout mice (*db/db*) and a group of control mice (*db/+*); leptin knockout mice (*ob/ob*) and a group of control mice (*ob/+*); model of pharmacological hiperleptinemia: B6 mice treated intraperitoneally with 100 μg of leptin for 16 days (16L) or saline (16K). In order to characterize the profile of NLRP3 inflammasome activation throughout cyclicity, the expression of inflammasome NLRP3 components was determined in the ovaries of mice in either the *oestrus* and *diestrus* stages. Increased protein expression of NLRP3 and IL-18 in the ovaries of mice in *diestrus* stage was observed. In addition, NLRP3 immunolocalization was confirmed in granulosa cells (GCs), theca cells (TCs) and oocytes. Next, the crosstalk between leptin signaling pathway and the activation

of NLRP3 inflammasome in the ovaries of obese mice was instigated using mouse models of DIO, genetic obesity and pharmacological hyperleptinemia. In the DIO mice, the protein expression of NLRP3 inflammasome components (NLRP3, IL-18 p24) was increased in the 4wkHFD group, but decreased in the 16wkHFD group. In *db/db* mice, no differences in the expression of NLRP3 inflammasome components were found, except for the decreased mRNA of *Il-1β*. Moreover, in *ob/ob* mice, the protein expression of NLRP3 and IL-1β concentration was significantly reduced. This observation contrasted with findings in the hyperleptinemic mouse, in which we found increased mRNA and protein expression of NLRP3 inflammasome components and IL-1β concentration in the ovaries of 16L mice. In the next experiment, transcriptomic analysis of GCs collected from ovaries from *db/db* mice and *ob/ob* mice was performed. Despite the similarities in body weight in the obese homozygous mice (average body weight 40 grams while control *db/+* and *ob/+* mice around 20 grams), global gene expression profile was distinct between *db/db* and *ob/ob*, as well as, concerning specific genes involved in follicular development and ovulation. In the GCs of *db/db* mice, there was reduced expression of genes regulating steroidogenesis and related to prostaglandin action, while in the *ob/ob* mice increased expression of genes regulating steroidogenesis and lipid metabolism. Moreover, in the model of early phase of obesity (4wkHFD) and 16L the effect of leptin on the expression of NLRP3 inflammasome genes in cumulus cells (CCs) was determined. Transcriptomic analysis of the CCs of 4wkHFD and 16L mice showed common genes modulating the activity of the NLRP3 inflammasome. In addition, leptin increases the expression of NLRP3 inflammasome genes in the CCs of 16L mice. In the last experiment, the effect of leptin on the expression of genes and proteins of the NLRP3 inflammasome in the liver of obese mice partially contrasted with that in the ovary. Increased expression of NLRP3 inflammasome components at the protein level in the liver only after 16wkHFD mice was observed, whereas remained decreased in *ob/ob* mice corroborating the association with leptin signalling.

In conclusion, increased NLRP3 inflammasome activity was demonstrated in the ovaries of mice during *diestrus* stage. Confirmed localization of NLRP3 in the GCs, TCs and oocytes, suggests a potential role of NLRP3 inflammasome in follicles development and corpus luteum function. In addition, active leptin signaling pathway was shown to regulate the activity of the NLRP3 inflammasome in the ovaries of mice in early obesity. Conversely, after leptin resistance establishment in the ovaries of mice in late

obesity, the activity of NLRP3 inflammasome was decreased. It shows that leptin regulates NLRP3 inflammasome in the ovaries of obese mice. Transcriptomic analysis of the GCs of the *db/db* and *ob/ob* mice showed that disruption of the leptin signaling pathway altered the expression of genes crucial for mouse follicle development. Both strains of *db/db* and *ob/ob* mice have different pattern of leptin action and different responses. For instance non-canonical leptin action in *db/db* mice, which lacks leptin receptor, results in impaired steroidogenesis and prostaglandins activity, what may also impact on ovulation process and possibly involvement of NLRP3 inflammasome, while total lack of leptin in *ob/ob* mice additionally affect lipid metabolism that is crucial for oocyte development. It shows that dynamic changes of leptin signaling throughout obesity can differently affect ovarian function. Transcriptomic analysis of the CCs of 4wkHFD and 16L mice confirmed the presence of common genes regulating the activity of the NLRP3 inflammasome. Moreover, NLRP3 inflammasome activation in the mice ovaries in early obesity contrasted with its activation exclusively during late obesity in the liver. The obtained results suggest new avenues for treatment of infertility in obese females, particularly concerning assisted reproduction techniques in humans and animals.