All'attenzione del Collegio dei Docenti del Dottorato in Medicina Molecolare

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In the first year of my PhD in Molecular Medicine, I have been involved in research projects regarding the study of male fertility and the oxidative stress (OS).

OS is produced by the imbalance between reactive oxygen species (ROS) and antioxidant defence and is involved in the pathophysiology of male infertility. Sperm membrane is rich in PUFAs, target of ROS, that cause lipid peroxidation (LPO).

At first, I studied 3 peptides, ghrelin, obestatin and resistin, in human semen and their relationship with OS. Ghrelin and obestatin are derived from the same precursor and are involved in many biological functions including reproduction.

Resistin is a pro-inflammatory adipokine, mainly secreted by adipose tissue and macrophages in humans, able to induce the expression of other proinflammatory cytokines.

We selected infertile patients with varicocele or leukocytospermia and fertile controls. Semen analysis was performed following WHO guidelines (2010) and sperm apoptosis and necrosis evaluation with AnnexinV/Propidium iodide assay. Seminal plasma samples were used to determine ghrelin/obestatin/resistin levels by immunological method, malondialdehyde levels (MDA) by HPLC analysis with UV detection, the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) by enzymatic method and catalase activity (CAT) by spectrophotometric method.

The results showed that in both infertile groups, semen parameters were significantly reduced, and sperm apoptosis and necrosis percentages were increased; concomitantly, ghrelin and obestatin were lower and resistin higher than those detected in semen of controls, the MDA concentration, marker of LPO, and CAT activity were raised, whereas GSH/GSSG ratio was diminished.

These results showed an interplay among ghrelin/obestatin and resistin with MDA, GSH/GSSG ratio and CAT activity in human semen. In particular, both ghrelin and obestatin play a protective role and this effect is probably due to their antioxidant activity known in different organs than male reproductive system.

In addition, we may hypothesize that the presence of leukocytes in semen and varicocele could cause increase of resistin levels concomitant with redox imbalance able to influence semen quality.

I also studied the effects of dietary plans enriched of n-3 PUFA sources, on New Zealand White rabbit bucks semen sample.

The experimental dietary groups included FLAX group fed with 10% of extruded flaxseed, FISH group fed with 3.5% of fish oil and control group.

Semen samples were collected and analysed weekly and fatty acids profile, isoprostanes and neuroprostanes concentrations were evaluated. Immunofluorescent analysis in sperm samples was performed using

antibodies anti docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These antibodies were chosen because the diets were enriched with DHA and EPA or their precursors.

Results showed that dietary plans improved motility characteristics and enriched in EPA and DHA sperm membranes, demonstrated also by immunofluorescence. F_2-isoprostanes and F_4-neuroprostanes levels were decreased and increased, respectively, vs control.

Study in progress are testing the effect of these diets on rabbit's testes.

Currently, I am working on *in vitro* tests to evaluate the possible antioxidant activity of some natural compounds used against OS induced in ejaculated human sperm.

Congress:

Poster: Noto D, Signorini C, Castellini C, Moretti E, Brecchia G, Cotozzolo E, Mattioli S, Belmonte G, Collodel G. "Effect of dietary n-3 source on reproductive traits of rabbit bucks". **Congress of Molecular Andrology, 24-26 September 2019**

Publications:

- "Defective spermatogenesis and testosterone levels in kinase suppressor of Ras1 (KSR1)-deficient mice." Moretti E, Collodel G, Belmonte G, Noto D, Giurisato E. Reprod Fertil Dev. 2019 Apr 15. doi: 10.1071/RD18386.
- "Resistin in human seminal plasma: relationship with lipid peroxidation, CAT activity, GSH/GSSG ratio and semen parameters." Moretti E, Micheli L, Noto D, Fiaschi AI, Menchiari A, Cerretani D. Oxidative Medicine and Cellular Longevity (accepted Oxidative Medicine and Cellular Longevity)
- "Relationships between ghrelin and obestatin with MDA, proinflammatory cytokines, GSH/GSSG ratio, catalase activity and semen parameters in infertile patients with leukocytospermia and varicocele." Lucia Micheli, Giulia Collodel, Daniela Cerretani, Andrea Menchiari, Daria Noto, Cinzia Signorini, Elena Moretti. Lucia Micheli, Giulia Collodel, Daniela Cerretani, Andrea Menchiari, Daria Noto, Cinzia Signorini, Elena Moretti (accepted to Oxidative Medicine and Cellular Longevity)