All'attenzione del Collegio dei Docenti
Dottorato di Ricerca in "Medicina Molecolare"
Direttore: Prof.ssa Antonella Naldini

XXXII Ciclo
A.A.2016-2017

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In the first year of my PhD in Molecular Medicine, I focused my attention on these topics:

1. *The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in women's fertility*

1.1 The role of MMPs and TIMPs in follicular microenvironment as a potential biomarkers of oocyte quality

MMPs and TIMPs are responsible for the degradation and remodeling of the extracellular matrix and they have been reported as key proteins in follicle bioactivities triggering proteolytic cascades throughout development, maturation, apex rupture, corpus luteum formation and regression, and follicle atresia.

For this reason, we have studied MMP2, MMP9 and MMP11 and their inhibitors TIMP1 and TIMP2 mRNA expression both in granulosa and cumulus cells collected from patients who underwent *in vitro* fertilization. mRNA was extracted from each sample and analyzed by qRT-PCR. Results show MMP2, MMP9 and MMP11 expression was significantly higher in granulosa than in cumulus cells. When the mRNA expression levels of TIMPs was analyzed in the same samples, we didn't find any difference for TIMP1 between GC and CC, whereas cumulus TIMP2 expression resulted significantly higher than in GC. These results are under revision in *Fertility and Sterility.*

1.2 The role of MMP2 and TIMP2 in endometrium of women affected by endometriosis

Endometriosis is a benign chronic gynecological disorder characterized by the presence of uterine endometrial tissue outside of the normal location.

A lot of genes have been identified to be abnormally expressed in endometrium of this women, among others, MMPs responsible for the degradation of extracellular matrix, which play a key role in the implant of endometrial tissue in ectopic sites as well as in the blastocyst invasion of

endometrium. The selective proteolytic activity of MMPs is finely regulated by TIMPs and the imbalance between MMPs and TIMPs expression has been involved in various pathological conditions.

In fact, it has been demonstrated that MMPs can activate several cytokines and inflammation mediators (which are involved in the pathogenesis of endometriosis) such as the precursor of tumor necrosis factor (TNFα).

Endometrial biopsies were collected from healthy women and women with endometriosis. mRNA was extracted from each sample and analyzed by qRT-PCR to evaluate MMP2/TIMP2 expression. Gene expression analysis was also carried out in cultured endometrial stromal cells from healthy and eutopic tissues treated with diffent concentration of TNFα.

Our preliminary results revealed that MMP2 expression in ovarian endometriotic lesions is increased when compared to deep infiltrating lesions. In primary endometrial stromal cells, obtained from healthy and endometriosis affected women, *in vitro* treated with increasing TNFα doses, we revealed that MMP2 and TIP2 expression are modulate by this inflammatory stimulus. These results are the subject of a poster presented at SEUD congress 2017.

2 *The expression of selected molecular markers for the neurogenesis in uterine tissues of women with uterine fibromatosis.*

Uterine leiomyoma, or fibroid, is the most common benign gynecological neoplasia in premenopausal women and it is characterized by increased myometrial cells proliferation and by an excessive deposition of extracellular matrix.

The clinical symptoms include pelvic pain, discomfort and menstrual disorders, as well as infertility. Because of one of the main symptoms is pain, I investigate on the direct association between pain and nerve fibers. Hence, in order to asses if a correlation between fibromatosis and neurogenesis could be identified, I planned to evaluate the expression of some neuronal markers such as Nerve Growth Factor (NGF, involved in the regulation of growth and differentiation of sympathetic and certain sensory neurons.), Synaptophisine (SYP, which encodes an integral membrane protein of small synaptic vesicles in brain and endocrine cells.) and Microtubule-associated protein 2 (MAP2, which encodes a protein that belongs to the microtubule-associated

protein family. The proteins of this family are thought to be involved in microtubule assembly, which is an essential step in neurogenesis.).

For this purpose, RNA from healthy and eutopic endometrium, myometrium and leiomyomas was extracted. Preliminary data demonstrated significant difference in NGF expression levels in healthy compared to eutopic endometrium and in myometrium compared to leiomyomas. A significant difference there is also in MAP2 levels in in myometrium compared to leiomyomas; by contrast, no significant difference was observed in endometrium.

About SYP, there are no significant difference between uterine tissues.

Poster:

- 3th congress of the Society of Endometriosis and Uterine Disorders (SEUD), 6 – 8 April 2017.
  MMPs and TIMPs gene expression in the pathogenesis of endometrial diseases.
  Pomboni P., Marrocco C., Luddi A., Semplici B., Centini G., Petraglia F.
  Best Poster Award

Partecipazione a congressi:

- 33rd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE), 2 - 5 July 2017.

Pubblicazioni:

- *Matrix Metalloproteinases and theirs inhibitors in human cumulus and granulosa cells as biomarkers of oocyte's reproductive potential*
  Luddi A, Gori M, Marrocco C, Capaldo A, Pavone V, Bianchi L, Focarelli R, Morgante G, De Leo V, Piomboni P.
  (Manuscript under review (*major revision after first submission*) in Fertility and Sterility)

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