PROJECT 1

DoS: Silvia Gregori

Title: Define the immune drivers of chronic inflammation and early onset of comorbidities in idiopathic infertile men

Curriculum: BAIO

Link to OSR/UniSR personal page: https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/mechanisms-of-peripheral-tolerance/silvia-gregori.html

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):

Male infertility accounts for the 40% of total cases of inability of a couple to achieve pregnancy. Despite several clinical and genetic parameters are currently used to diagnose male infertility, in the 20% of the cases the etiology is still unknown, and these subjects are classified as idiopathic infertile men (1). Males with idiopathic infertility have been shown to have higher risk to develop comorbidities (e.g., autoimmunity, malignancies, and metabolic diseases) compared to fertile men (2-5) and to display markers of chronic inflammations in testis and peripheral blood (6). Both the comorbidities and the pro-inflammatory status observed in these subjects, could be linked to disfunctions of the immune system such as alterations of the regulatory and/or exhaustion of the immunogenic compartment (7-8). We have a long-lasting experience in the identification and characterization of specific subsets of tolerogenic and immunogenic immune cells in peripheral blood and target organs, and we reported that altered number or impaired function of tolerogenic cells are associated with disease onset or progression (9-11). Preliminary data in the lab using the above approach indicate the presence of immune dysregulation in idiopathic infertile men.

Based on these premises, the goal of the proposed project is to better define the systemic (peripheral blood) and tissue specific (seminal fluid) immunological profile of idiopathic infertile men in comparison with age-matched fertile men, and to identify potential predictive immune-related markers of the development of comorbidities in infertile men. An extensive assessment of the frequency and function of regulatory and effector/exhausted immune cells in peripheral blood and seminal fluid of idiopathic infertile men and controls will be performed. Single cell RNAseq analysis of specific subsets of immune cells as well as of hematopoietic cells isolated from seminal fluids will define the overall immune cell composition. Functional characterization of adaptive and innate immune cells will be performed in *in vitro* assays. These data will be correlated with clinical variables, hormonal and semen parameters, and genetic signatures of the enrolled patients.

The overall study will define, for the first time, the systemic and local immunological asset of idiopathic infertile men and will possibly lead to the identification of specific biomarkers related to male infertility and/or predictive of developing comorbidities.

Skills to be acquired by the student (*Number of characters*, *including spaces*: max 600):

The candidate will:

1) become able to manipulate different types of human samples;

2) learn how to isolate, keep in culture, and characterize immune cell populations;
3) deal with techniques starting from highly standardized multicolor flow cytometry and ELISA protocols to the more advanced multiplex bead arrays and RNA single-cell Next Generation Sequencing techniques;
4) interact with internal and international collaborators leaders in the field;
5) be encouraged to acquire a critical attitude and to refine the abilities required for the proper design and interpretation of generated data.

References (max. 15)

1) Jungwirth et al., https://d56bochluxqnz.cloudfront.net/media/EAU-Pocket-Guidelines-Male-Infertility-2019.pdf

2) Salonia et al., Eur Urol 2009, PMID: 19297076;

3) Ventimiglia et al., Fertil Ster 2015, PMID: 26006735;

4) Eisenberg et al., Fertil Ster 2015, PMID: 25497466;

5) Glazer et al., Semin Reprod Med 2017, PMID: 28658712;

6) Alfano et al., Nat Commun 2021, PMID: 34471128;

7)Dominguez-Villar, Nat Immunol 2018, PMID: 29925983;

8) Crespo et al., Curr Opin Immunol 2013, PMID: 23298609;

9) Roncarolo et al., Immunity 2018, PMID: 30566879;

10) Amodio et al., Front Immunol 2021, PMID: 34659254;

11) Amodio and Mugione et al., Hum Immunol 2013, PMID: 23238214.