**PROJECT 1**

**DoS**: Massimo Alfano

Title: Molecular fingerprint of human idiopathic male infertility

Curriculum: Experimental and Clinical Medicine

Link to OSR/UniSR personal page: https://research.hsr.it/en/institutes/urological-research-institute/extracellular-microenvironment.html

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

Azoospermia is the absence of spermatozoa in the seminal fluid and affects about 1% of all men. In 80% of cases, germ cells are completely absent and the cause is unknown, therefore the pathological state is defined as idiopathic Germ Cell Aplasia[1] (iGCA).

Epidemiological data show that azoospermic men have an increased risk of an early onset of age-related chronic diseases (e.g., cardiovascular disease, type II diabetes mellitus, autoimmune disease and cancers)[2,3] with a relative higher risk of death[2] compared to fertile men. Likewise, azoospermia emerges as one of the first manifestations of premature ageing, initially at the testicular level, and thereafter at a systemic level. Overall, these data support the concept that male infertility may be a proxy of men's health.

Single-cell RNAseq was used to unveil the occurrence of dysregulated transcriptional pathways in 7 testicular somatic cell populations in human idiopathic germ cell aplasia, and validated at tissue level by confocal microscopy. Our recent analyses (manuscript under revision) show that the somatic environment of azoospermic testes was characterized by chronic tissue inflammation, fibrosis and an overall senescent phenotype. Leydig cells seem to play a pivotal role. In fact, they show an immature phenotype (prepuberal/neonatal stage) at both transcriptional and protein level associated with deregulated expression of parentally imprinted genes, significant changes in the Lamin A/C transcript ratio and an active DNA Damage Response.

The PhD student will tackle iGCA starting from one of the following points:
- the deregulated expression of parentally imprinted genes located on different chromosomes indicate a genomewide defect recalling Multi-Locus Imprinting Disturbances; one of the topic that will be addressed is the identification of the molecular mechanism(s) leading to defects in imprinted gene expression
- identify the role of imprinted genes modulating the senescent cellular phenotype; knock-in and knock-out of the identified parentally imprinted genes will be performed in immortalized primary testes cells, and the read-out will be the expression of genes associated with the i) Senescent Activated Secretory Phenotype proteins, and ii) DNA damage response.
- our recent analysis clearly demonstrated a senescent phenotype of the somatic testes cells; to associate the infertile status with the early onset of age-related chronic diseases the senescent phenotype of the somatic cells will be searched in other human tissues and at the systemic level.
- the novel male infertility datasets that will be created will be compared with the existing datasets to search for signatures associated with age-related chronic diseases.

- use available preclinical models of DNA Damage and senescence in the testis to assess the connection with inflammation and germ cell aplasia
(https://www.frontiersin.org/articles/10.3389/fmolb.2020.00024/full)
(https://www.jax.org/strain/026629,)

The identification of the molecular pathways and signatures associated with idiopathic male infertility will create novel datasets to better understand the pathology of the primary disease as well as for the identification of patients at risk of early onset of age-related comorbidities. Likewise, the biomarkers might represent actionable targets to prevent the onset of the comorbidities.

Outcomes of this study will have impact on the early recognition of risk factors associated to the infertile status and onset of comorbid conditions, thus justifying earlier treatment of infertile men for reproductive purposes, while monitoring their general health status for preventive purposes as well, e.g. in the reproductive age (30-40 years) and before the onset of chronic and/or health threatening diseases.

**Skills to be acquired by the student:**

The student will learn to
- use, store and process human tissues and to manage clinical data.
-characterize cellular phenotypes in tissue sections by immunofluorescence and ImmunoHistochemistry, and analyse images obtained by confocal microscopy.
- apply basic molecular and cellular biology approaches as well as bulk and single-cell Next Generation Sequencing of DNA and RNA.
- perform experiments with murine preclinical models.
Being a multidisciplinary study, the student will receive support from scientists with experience in different scientific fields.

**References** (max. 3)

1 Lee, J. Y., Dada, R., Sabanegh, E., Carpi, A. & Agarwal, A. Role of genetics in azoospermia. *Urology* **77**, 598-601, doi:10.1016/j.urology.2010.10.001 (2011).
2 Glazer, C. H. *et al.* Male factor infertility and risk of death: a nationwide record-linkage study. *Hum Reprod* **34**, 2266-2273, doi:10.1093/humrep/dez189 (2019).
3 Choy, J. T. & Eisenberg, M. L. Male infertility as a window to health. *Fertil Steril* **110**, 810-814, doi:10.1016/j.fertnstert.2018.08.015 (2018).