PhD proposal 2: Effects of particulate and endocrine-disrupting metals on fertility

1. Consortium

- University awarding the degree: University of Trieste
  Proposed supervisor: Dr. Lorella Pascolo (IRCSS Burlo Garofolo)
- Proposing CERIC partner facility: Elettra Sincrotrone Trieste
  Lead proponent: Dr A. Gianoncelli

- Contributing CERIC Partner facility: Ruđer Bošković Institute (RBI), Particle-Inducted X-ray Emission and Rutherford Backscattering, Zagreb, Croatia
  Lead collaborator: Dr. Iva Božičević Mihalić

- Contributing CERIC Partner facility: National Institute of Materials Physics (NIMP),HRTEM facilities, Bucharest-Magurele,Romania
  Lead collaborator: Dr. Corneliu Ghica

- Contributing CERIC Partner facility: Elettra Sincrotrone Trieste, SISSI- Chemical and LifeSciences branch, Trieste, Basovizza, Italy
  Lead collaborator: Dr. Lisa Vaccari

- Contributing CERIC Partner facility: Elettra Sincrotrone Trieste, SYRMEP beamline, Trieste, Basovizza, Italy
  Lead collaborator: Dr. Giuliana Tromba

- Contributing CERIC Partner facility: Elettra Sincrotrone Trieste, Nanoinnovation Laboratory, Trieste, Basovizza, Italy
  Lead collaborator: Dr. Loredana Casalis

2. Scientific background

Over the past decades, there has been an increasing concern about the effect of environmental pollutants exposure on human fertility. The environment is contaminated with numerous endocrine-disrupting compounds which for instance may disturb from gametogenesis to intrauterine development, resulting in irreversible effects. It is not clear yet how those compounds may affect human beings, although there is some evidence that they have the potential to induce deleterious changes in the human reproductive system. We propose then an *in vitro* and *in vivo* study (collecting samples from real patients) to increase the scientific knowledge on the topic. In particular the project will be focused on monitoring metalloestrogens (Aluminium, Antimony, Arsenic, Barium, Cadmium, Chromium, Cobalt, Copper, Lead, Mercury, Nickel, Selenium, Tin, Vanadium) and metal containing nanoparticles (Silver, Zinc, Titanium) [Toxics 2015, 3, 390-413; Oncology reports 36.2 (2016) 603-612] both in clinical samples (from surgeries and donors) and experimental cell models exposed to such chemicals. A widespread advanced analysis platform will be used taking advantage of the CERIC-ERIC consortium.

We expect that this study will increase the understanding of pathologies induced by endocrine disregulations (endometriosis, ovary tumor, testicular cancer) and infertility.

3. Outline of the experimental protocol

This project starts from an already ongoing collaboration with Burlo Hospital in the reproductive medicine field [Journal of Synchrotron Radiation, Vol. 26, pp. 1322-1329 (2019); NIMB, Vol. 459, pp. 120-124 (2019); X-Ray Spectrometry, Vol. 48 - 5, pp. 413-421 (2019); Reproductive BioMedicine Online, Vol. 37 - 2, pp. 153-162 (2018)].

The clinical specimens will be collected (after obtaining written consent) from patients of the Obstretics and Ginecology Unit of IRCCS Burlo Garofolo (Prof. G. Ricci MD) in collaboration with the clinical staff: sperms and seminal fluid; ovary tissue and endometrium samples from surgery of benign pathologies; placental tissue at delivery.

In parallel *in vitro* studies will be conducted on primary and immortalised cell lines (commercially available): human BeWo tumor derived placental cells, HTR-8/SVneo trophoblasts, Human Caov-3 ovary cells, human NTERA-2 cl.D1 testis cells etc. In particular the research involves the investigation of the accumulation mechanisms and the toxicological characterization following morphological and chemical changes.

Tissues and cells will be studied under the following setups:

- soft X-ray microscopy and X-ray Fluorescence for morphological and elemental assessment (TwinMic beamline at Elettra) monitoring morphology and light elements distribution (such as Al, Na, Mg);

- Complementary PIXE analysis, combined with RBS imaging to detect heavier chemical elements and molecular imaging of cells and tissues with MeV SIMS (RBI, Croatia);

- Biochemical characterizations by micro and nano-infrared spectro-microscopy (SISSI-bio beamline at Elettra);

- X-ray micro-tomography and complementary IR tomography on gamete cells and tissue samples (SYRMEP beamline at Elettra, SISSI-bio beamline at Elettra)

- Possible tissue stiffness comparisons by AFM (Nanoinnovation Lab).

- Combination with TEM morphological analysis (Bucarest, Romania)

4. The expected impact of the proposed research on the overall quality and capability of CERIC

The project requires the use and combination of a varitety of analytical techniques and imaging methods, which will complement standard laboratory protocols and tests. The proposed research will further boost the potential of CERIC consortium in Life Sciences, and in particular those with clinical applicability, allowing to optimise sample preparation protocols and data collection work flow, to highlight current CERIC capabilities and to further push state of the art instrumentation, together with an increase of the bio-user community.

5. **Estimated cost, if relevant** (tuition fee, salary/stipend, travel)

Year	Tuition fees	Salary	10% *	Stay abroad	Tot per year
1st	€ 496.00	€ 18,850.00			€ 19,346.00
2nd	€ 496.00	€ 18,850.00	€ 1,534.00	€ 2,356.00¥	€ 22,740.00
3rd	€ 496.00	€ 18,850.00	€ 1,534.00		€ 20,384.00
Total					€ 62,470.00
* Funds available for the research activity of the PhD, per regulation					
¥ Funds available for 3-months abroad, that is the minimum imposed per regulation					

Annex 1:

CURRICULUM VITAE: Lorella PASCOLO

email: lorella.pascolo@burlo.trieste.it

Address: Institute for Maternal and Child Health, IRCCS Burlo Garofolo,

Via dell'Istria, 65/1 – 34137 Trieste (Italy)

CURRENT POSITION

Health Researcher at the Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Department of Obstetrics and Gynaecology since January 2020

Main research activity: reproductive toxicology

PREVIOUS POSITIONS

2011-2015; 2017-2019 Researcher at the Institute for Maternal and Child Health, IRCCS Burlo Garofolo. Main research activity: Chemical imaging and Spectroscopic analyses to investigate quality of gametes in ART and mechanisms in infertility. Research activity on oncology mechanisms, intestinal diseases and toxicology. Asbestos and nano-toxicology research. Dismetabolism of iron and other metals. Tutoring activity for the Nanotechnology PhD school of the University of Trieste.

2015-2017: Research Associate – Histology. University of Trieste (and research activity at IRCCS Burlo Garofolo). Main research activity: Chemical imaging and Spectroscopic analyses to investigate quality of gametes in ART and mechanisms in infertility.

October 2010-January 2011. Fellowship in medical pathology. Research activity: Spectroscopy in the study of asbestos toxicity.

2008 to April 2010. Contract at Elettra, Sincrotrone Trieste. Research activity in the field of drug and nanotoxicology, using X-ray techniques.

2008-2009: Teacher at the University of Trieste, faculty of Pharmacy (Clinical Biochemistry).

2001-2004, 2005-2008: Assistant Professor – Clinical biochemistry (BBCM; University of Trieste)

1999-2001: Research Associate – Clinical biochemistry (BBCM; University of Trieste)

1995-1999: PhD student course and Fellowship – Biochemistry (BBCM; University of Trieste)

1993-1995: Research Activity; Fellowships supported by Ferderchimica (University of Trieste)

EDUCATION

2000- PhD degree, Biochemistry: Department of Biochemistry, Biophysics and

Chemistry of Macromolecules (BBCM), University of Trieste (1995-1999).

1992- Degree in Pharmacy: University of Trieste. Department of Food Chemistry

CURRENT RESEARCH INTERESTS

Reproductive Toxicology: Quality of gametes; endometriosis; infertility

Preservation of Fertility: Protocols for cryopreservation of ovary tissue and gametes

Nanotoxicology and Asbestos toxicity: Toxicity of occupational and environmental materials.

Advances imaging and spectroscopic techniques: XRF microscopy; Total Reflection XRF; x-ray micro and nano-tomography; FTIR; UV-Raman

PARTICIPATION IN ONGOING FINANCED RESEARCH PROJECTS

-Regional grant 2015-2019 (extended) financed from Commissione Regionale Amianto FVG: Mesotelioma (participant)

-2019, Programma "5 per mille anno 2015" Bando di ricerca sanitaria "Salute della bambina, dell'adolescente e della donna" for the project entitled "Studio del ruolo del ferro e di altri metalli ell'endometriosi mediante una piattaforma diffusa di analisi avanzate". 20.000 Euro (main proposer)

Full papers: 67 ; H index 22; citations : 1594

Advisor of several degree and PhD dissertations.