ESRF	**Deciphering the dynamics of exchange between Zn(II) pools in normal and pathological human seminal plasma (DyZem)**	**Experiment number**: **30-02-1126**
Beamline: BM30B	**Date of experiment**: from: July 2017, 13th to: July 2017, 18th	**Date of report**: *Received at ESRF:*
Shifts: 15	**Local contact(s)**: Olivier Proux (olivier.proux@esrf.fr)	

Names and affiliations of applicants (* indicates experimentalists):

Dr Safouane Hamdi (ITAV, CNRS/UPS, Toulouse, France)
Dr Christelle Hureau (LCC, CNRS/UPS, Toulouse, France)
Pr Emmanuel Guillon (ICMR, CNRS UMR6229, Reims, France)
Dr Stéphanie Sayen (ICMR, CNRS UMR6229, Reims, France)
* Dr Elena Atrian-Blasco (LCC, CNRS/UPS, Toulouse, France)
* Dr Laurent Sabater (LCC, CNRS/UPS, Toulouse, France)
* Amandine Conte-Daban (LCC, CNRS/UPS, Toulouse, France)
* Valentina Borghesani (LCC, CNRS/UPS, Toulouse, France)

Report: Zinc ion, one of the most common metal ion, is found in human body and has been involved in male reproductive physiology. In seminal fluid, concentration in Zinc ion is very high in comparison to concentration in human blood. It appears that Zinc ion is of considerable importance in key functions of spermatozoa fertilization from motility to activation. Seminal fluid is a highly dynamic system and Zinc is involved during most steps of the complicated transformations of semen such as ejaculation, coagulation and liquefaction. In seminal plasma and during fertilization process, Zinc is supposed to be finally bound by Low Molecular Weight Pepetides (LMWP) after being bound to higher molecular weight proteins (mainly Semenogelin) ; this modulates the zinc availability in the different steps of reproduction and impacts modification of semen. The LMWP are issued from cleavage of parent Semenogelin protein. Such biochemical mechanisms are under studies to better understand this very complex process, with the objective to fight against infertility.

The main goal of this study was to characterize Zinc environment in differents biological pools and to establish simple models of Zinc coordination to better disentangle transformations that occurs during fertilization. Zn^{2+} cation was explored in different samples from biological pools to simple mix of peptides (LMWP). The semenogelin protein, one of the most abondant and important protein in interaction with zinc ion, is degraded during the liquefaction of the semen. This protein is cleaved into peptides and some of them can interact with Zn^{2+}. Three key peptides were identified from the degradation of semenogelin: a 40 amino-acid sequence (TL-40) which can be split in a 18 amino-acid sequence (TY-18) and a 22 amino acid sequence (DL-22). Zinc coordination with these peptides was explored during this experiment. As for amyloid-β, the TL40 contains a part able to bind Zn ion (TY-18) and a part able to agregate (DL-22). As we previously did in case of Azheimer's disease with amyloid-β peptide, we aim at better understanding the processes that occur during fertilization from metal coordination to agregation/disagregation steps.

Main results : Zn signature is recorded in three biological matrices: two pools of normal seminal plasma gathering pools from several dozen of anonymous donors (Pool 2015 and Pool 2016) and a special pool (Azoo) made from donors which present an obstruction of seminal pathways leading to the absence of Semenogelin (and thus the LMWP issued for its cleavage). Thus Azoo seminal fluid contains only prostatic fluid and no spermatozoid nor semenogelin. This biological matrix will be used to reproduce the environment of LMWP in the normal pools; and synthetic LMWP will be added to this biological matrix to identify the Zn^{2+} binding peptides/sequence by comparison of the spectroscopic signatures with the one of the normal pool.

Biological pools 2015 and 2016 (containing about 2mM of Zn), different mix of LMWP (TL-40; TY-18 and/or DL-22) with Zn added and finally the Azoo pool with added LMWP were recorded at the Zn k-edge in XANES and EXAFS.

First, as shown in Figure 1, XANES spectra at Zn k-edge show significant differences between Zn bound to LMWP (in buffer only) and biological pools. In biological pool (Fig 1a), a well defined peak around 9660 eV, indicating a coordination number N = 5/6 is observed. Between 9670 and 9750 eV, a specific shape can be observed, which is not observed on the LMWP (Fig. 1b).

Second, three different signatures are observed with the three peptides (Fig 1b). For TY-18 and TL-40 with 1eq. Zn at 2mM, a wider and smaller peak is observed caracteristic of a coordination number N = 4, whereas for DL-22 and 1eq. of Zn at 2mM a narrower and higher peak is detected, corresponding to N = 5/6.

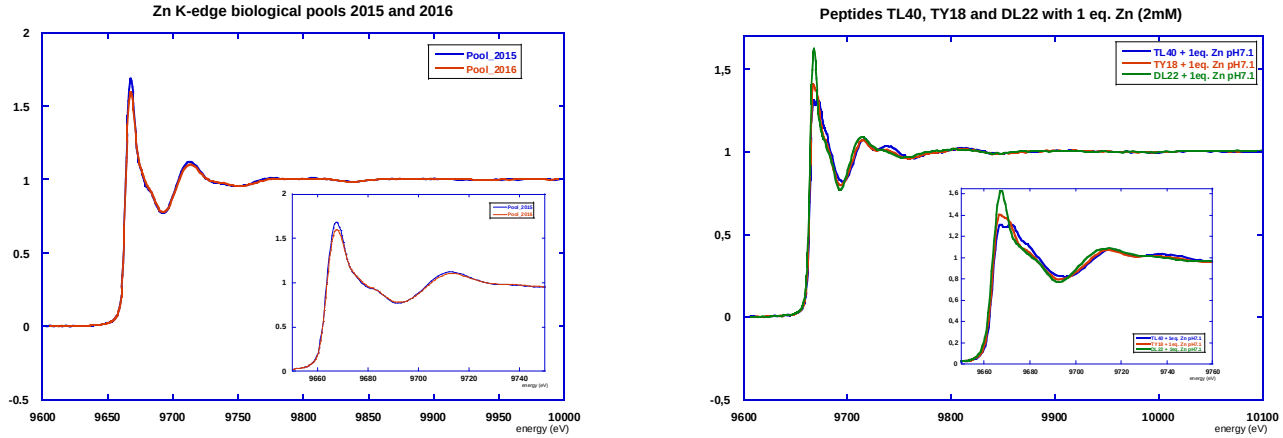

Figure 1: Zn K-edge XANES normalized spectra of : a) pools 2015 and 2016 (Zn 2mM), b) peptides TY-18, DL-22 and TL-40 with 1eq. Zn^{2+} (2mM)

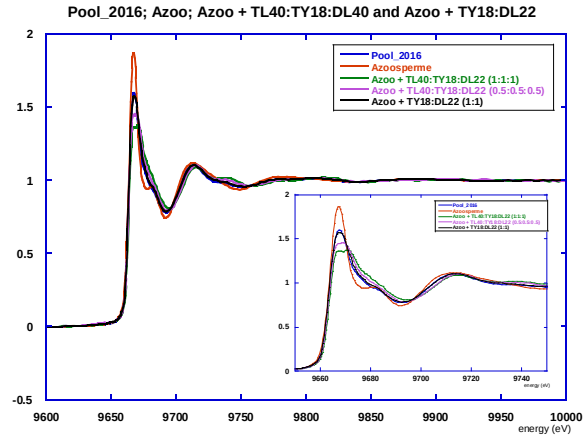

Figure 2 : comparision of Pool_2016, Azoo, Azoo with addition of TL40/TY18/DL22 and Zn (0.5 eq. or 1 eq. of each peptide against Zn) and Azoo with the addition of TY18 and DL22 (1eq. of each peptide vs. Zn).

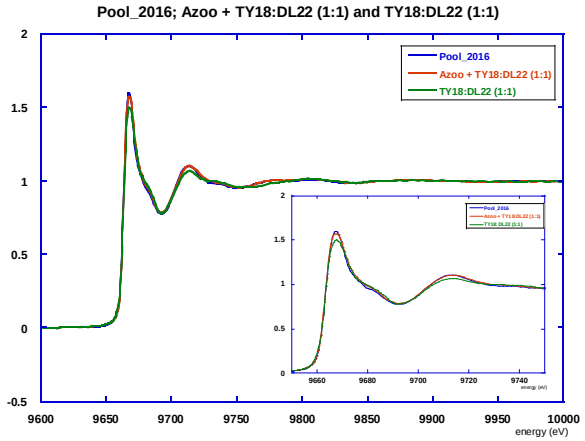

Figure 3 : comparision of Pool_2016, Azoo with addition of TY18:DL22 (1:1) and TY18:DL22 (1:1) + 1eq. of Zn / peptide.

Lastly, Zinc in azoosperme pool have a clearly different environment than in biological pools 2015 and 2016 (See Figure 2). It exhibits a higher and narrower Zn k-edge signal (corresponding to N=6). In addition, the shape after the edge is very different showing hamp and valley at different energies compared to pool 2015/2016. Addition of several mixtures of LMWP serioulsy impact the signature, and a 1:1 mixture of TY18 and DL22 (added as equimolar ratio with Zn ions) lead to a quasi-perfect reproduction of the signature of the normal pool 2015/2016. Because the signature of Zn bound to TY18 and DL22 doesn't reproduce the signature of the normal pools when mixed in buffer but does when mixed in the Azoo pool, we conclude that another component (apart from the peptides) of the biological fluid is required for Zn binding.

Experimental details: Zn k-edge XANES and EXAFS spectra were recorded on the FAME beamline (BM30B) during a 15-shifts session in July 2017. The measurements were performed on mM (in Zinc ions) solutions at low temperature (He-cryostat) in the fluorescence mode using a 30-element high-purity Ge detector. The energy was calibrated by the measurement of Zn foil spectra in transmission. For each sample, at least 3 XANES/EXAFS spectra were recorded and averaged.

Conclusion and perspectives: During this beamtime, biological pools 2015 and 2016 and abnormal Azoosperme pool have been recorded at Zn k-edge. The main difference between Azoosperme and Pools 2015/16 is that Azoosperme is free of semenogelin. Signatures of peptides sequences (TL40, TY18 and DL22) from semenogelin decomposition were recorded and compared to biological pool to determine parts able to coordinate Zinc during fertilization phases coincident to semenogelin degradation. A signature for the Zinc environment very close to biological pools was obtained using Azoosperme with addition of the two smalls peptides TY-18 and DL-22. These sequences have been identified and reported in the literature during semenogelin decomposition studies. We thus expect to publish a paper on the identification of the Zn coordinating sequence. In future experiment, we plan to clearly identify the Zn coordination site using mutants peptides similarly to what were done for Amyloid-β during experiment 20130207.