

## **Proteomic Analysis of Oviduct Cilia: Identification of New Sperm-interacting Candidates**

Coline Mahé<sup>1</sup>; Aleksandra Maria Zlotkowska<sup>2</sup>; Karine Reynaud<sup>1</sup>; Régis Lavigne<sup>3,4</sup>; Emmanuelle Com<sup>3,4</sup>; Charles Pineau<sup>3,4</sup>; Pascal Mermillod<sup>1</sup>; Jennifer Schoen<sup>2</sup>; Marie Saint-Dizier<sup>1,5</sup>

1. CNRS, IFCE, INRAE, Université de Tours, PRC, 37380, Nouzilly, France
2. Department of Reproduction Biology, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany
3. Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR-S 1085, F-35000 Rennes, France
4. Protim, Univ Rennes, Biosit – UMS 3480 CNRS, US 018 Inserm, F-35000 Rennes, France
5. Tours University, Faculty of Sciences and Techniques, Tours, France

The cilia of mammalian oviduct epithelial cells play critical roles in gamete and embryo transport. Binding to cilia in the caudal part of the oviduct, named the isthmus, maintains sperm alive until ovulation in a reservoir. However, very few sperm receptors on cilia have been identified so far. Our aim was to compare the cilia proteome of bovine oviducts between anatomical regions (ampulla vs. isthmus) at pre- and post-ovulatory stages of cycle to identify new receptor candidates for sperm binding.

Oviducts from cyclic adult cows were collected at a local slaughterhouse. Only pre- and post-ovulatory oviducts ipsilateral to ovulation were used (4 cows/stage). After 6-h fixation and paraffin embedding, 6- $\mu$ m serial sections of the isthmus and ampulla were used for cilia laser microdissection. Ciliary proteins were digested and analyzed by nano liquid chromatography coupled with tandem mass spectrometry. Proteins were identified using the Uniprot *Bos taurus* database and considering minimum two unique peptides. Proteins were quantified by label-free extracted ion chromatogram. Differences in protein abundance were analyzed by t-tests ( $p \leq 0.05$ , fold-change  $\geq 1.5$ ). Prediction of subcellular localizations and molecular interactions were performed using TMHMM, MBPPred and STRING tools.

A total of 505 proteins were identified in oviduct cilia, of which 53 predicted to be transmembrane proteins. After statistical analysis, 29 proteins were found overabundant in the

isthmus compared to the ampulla, while 17 were overabundant at pre-ovulatory compared to post-ovulatory stages. Furthermore, 18 proteins were predicted to interact with membrane phospholipids, including ANXA1,2,4,5,8, LRBA, GSN and EZR, and five were predicted to interact with the sperm-coating proteins BSPs, including PEBP1 and PSMA6.

This is the first oviduct cilia proteome reported in mammals. This study highlights changes in ciliary proteins along the oviduct during the periconception period and reports new candidates involved in the oviduct sperm reservoir.