# IDENTIFICATION OF LACTADHERIN IN THE PORCINE OVIDUCTAL SECRETION

<u>A. Guillén-Martínez</u><sup>1</sup>; O.S. Acuña <sup>1</sup>; C. Moros<sup>1</sup>; B. Algarra-Oñate, <sup>1</sup>; L. Jara<sup>1</sup>; C. Soriano-Úbeda<sup>2</sup>; O. López Albors <sup>3</sup>; M. J. Izquierdo-Rico <sup>1</sup>; M. Avilés<sup>1</sup>

<sup>1</sup>Department of Cell Biology and Histology, Faculty of Medicine and Nursing.

<sup>2</sup>Department of Physiology. Veterinary Faculty.

<sup>3</sup>Department of Anatomy and Comparative Pathology, Veterinary Faculty. University of Murcia,

<sup>1,2</sup> University of Murcia, Campus Mare Nostrum and IMIB, Murcia, Spain.

# INTRODUCTION

The oviductal secretions have an active role in the gamete maturation, sperm capacitation and fertilization. Previous studies have reported that genetically modified sperm in mice are sterile in *in vitro* assay; however, they are fertile *in vivo* or after incubation of the sperm with uterine secretions. These studies strongly suggest that the female tract secretions contribute to the sperm fertility. Lactadherin is a protein associated with the sperm plasma membrane secreted by the male genital tract. This protein was involved in the sperm binding to the zona pellucida. The aim of this study was to analyze the presence of lactadherin protein in the porcine oviduct.

### MATERIALS AND METHODS

<u>Sample collection</u>: Porcine oviducts at the preovulatory phase of the estrous cycle were collected at the abattoir. The cyclic stage of animals was assessed on the basis of ovarian morphology. <u>Immunohistochemistry</u>: The ampullary and the isthmic region of the Fallopian tube were fixed in 10% formalin in PBS and embedded in paraplast. The sections were incubated with a rabbit anti-lactadherin polyclonal antibody and incubated with a secondary antibody conjugated with peroxidase. <u>Western blot analysis</u>: Oviductal fluids were separated by SDS-PAGE electrophoresis under reducing condition. The proteins were electrotransferred to Immobilon-P membrane and they were incubated with specific antibodies. <u>Proteomic analysis</u>: The oviductal fluids were separated by SDS-PAGE electrophoresis blue. The band with a molecular weight of approximatively 50 kDa was cut and processed for MS/MS proteomic analysis.

# RESULTS

A moderate immunoreactivity was detected in the non-ciliated cells of both ampulla and isthmus. A specific band of 50 kDa has identified by Western-blot analysis. The presence of lactadherin was confirmed by the identification of different peptides by mass spectrometry analysis.

# CONCLUSION

In this study, we provide evidences of the presence of the lactadherin in the oviductal fluid and its secretion by the non-ciliated cells of the oviductal epithelium. This protein could modify the sperm membrane during the sperm transit through the female genital tract with a potential effect on the fertilization.

This work was supported from the MINECO (AGL2012-40180-C03-01-02-03), FEDER/ERDF and Fundación Séneca (04542/GERM/06).