



**HAL**  
open science

## Analysis of proteins in the secretome of the mouse developing testis

François Chevalier, Marie-Justine Guerquin, Jordane Depagne, Pascale  
Bertrand, René Habert, Gabriel Livera

► **To cite this version:**

François Chevalier, Marie-Justine Guerquin, Jordane Depagne, Pascale Bertrand, René Habert, et al..  
Analysis of proteins in the secretome of the mouse developing testis. SMAP 2009 - Congrès Français  
de Spectrométrie de Masse et d'Analyse Protéomique, Sep 2009, Dijon, France. hal-04244517

**HAL Id: hal-04244517**

**<https://hal.science/hal-04244517>**

Submitted on 16 Oct 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

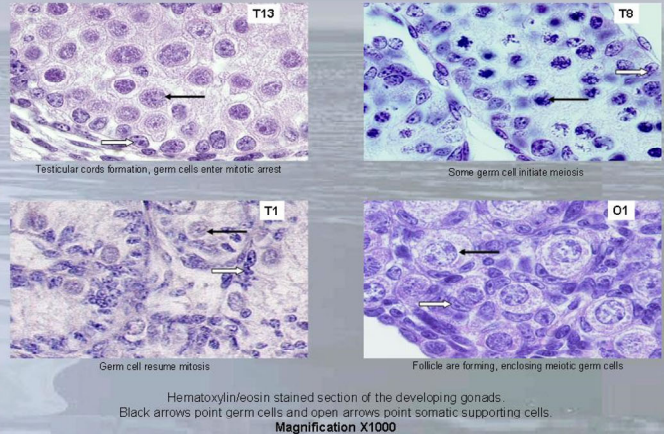
François Chevallier<sup>1</sup>, Marie-Justine Guerin<sup>2</sup>, Jordane Dépagne<sup>1</sup>, Pascale Bertrand<sup>1</sup>, René Habert<sup>2</sup> and Gabriel Livera<sup>2</sup>

1 Plateforme de Protéomique 2 Laboratoire du Développement des Gonades  
 Institut de Radiobiologie Cellulaire et Moléculaire - CEA/FAR - DSV/IRCM - 18, route du Panorama - BP N°6 - 92265 Fontenay aux Roses Cedex  
 Tel: 01-46-54-83-26 – Email: francois.chevallier@cea.fr

The development of the gonads is a complex process that relies on a dialogue between germ and somatic supporting cells. It has long been known that the somatic cells secretions are crucial to direct the fate and orchestrate the differentiation of the germ cells. However no study has ever investigated the composition of the secretome of the developing gonads.

In this study we set up a system to analyse the proteins secreted by the developing testis at different stages and by the neonatal ovary.  
 Three testicular stages were chosen:  
 - 13.5 days post-conception (T13) when the somatic cells formed cords containing germ cells that are entering mitotic arrest;  
 - 1 day (T1) post-partum (dpp) when germ cells resume mitosis  
 - 8 dpp (T8) in when the first meiotic male germ cells can be observed.

On the other hand secreted ovarian proteins were analysed using the 1 dpp ovary (O1) in which all germ cells had initiated meiosis and when somatic supportic cells start to differentiate.



## Analysis strategy



Samples: Testes - 13.5 days post conception (T13)  
 - 1 day post-partum (T1)  
 - 8 days post-partum (T8)  
 Ovaries - 1 days post-partum (O1)

**Organ culture and ex vivo incubations:** Mouse's gonads were removed aseptically under a binocular microscope and transferred to the in vitro culture system\*. Gonads or pieces of gonads were cultured on agar disks (1.5%) for 4 days in DMEM/HamF12 without serum. At the end of the culture, conditioned media were collected and centrifuged to get rid of possible floating cells.

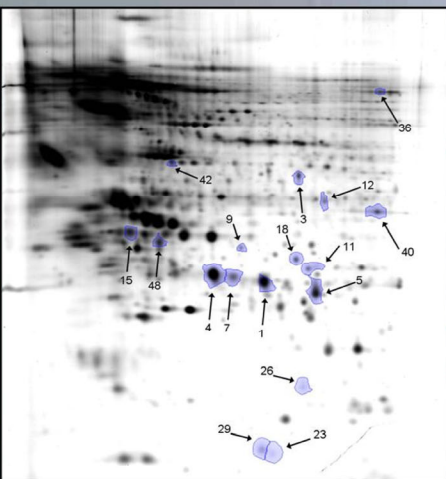
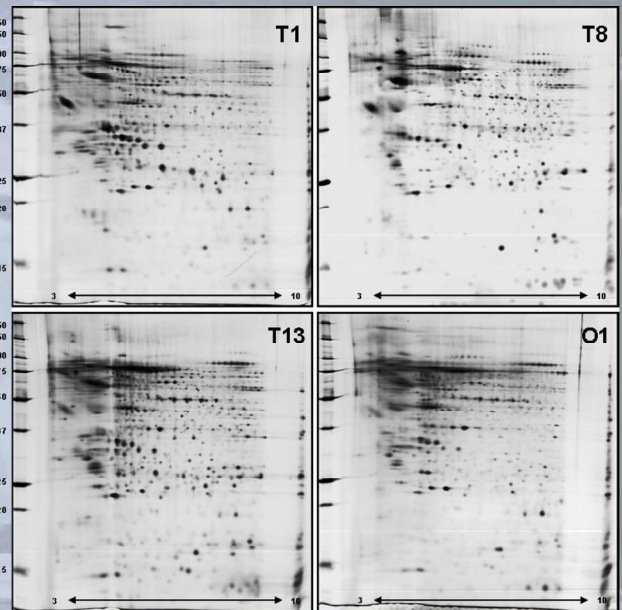
**Secretary proteins extraction:** TCA-NLS / THF protocol\*\*

1<sup>st</sup> D: 60 µg proteins; strips pl 3-10 NL; 18 cm; IEF Cell  
 2<sup>nd</sup> D: 12% acrylamide SDS-PAGE Dodeca Cell (silver stain)

**Gel analysis:** mean values of triplicate gels / Progenesis SameSpots software



**Mass spectrometry:** MALDI-TOF/TOF and nano-LC-MS/MS  
 Laboratoire de Protéomique Fonctionnelle / INRA Montpellier

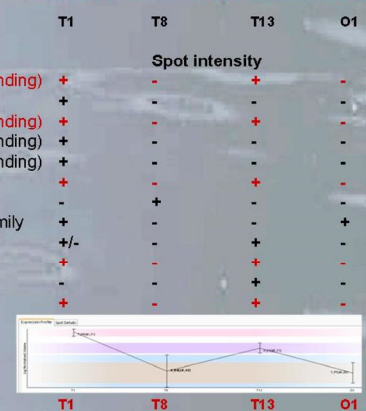


## Mass spectrometry results

Spot	Name	Accession	Location	Family
spot 1	Prostaglandin-D2 synthase	O09114	Secreted	Lipocalin (retinol binding)
spot 3	Anti-Mullerian hormone	Q5EC55	Secreted	TGF-beta family
spot 4	Prostaglandin-D2 synthase	O09114	Secreted	Lipocalin (retinol binding)
spot 5	Prostaglandin-D2 synthase	O09114	Secreted	Lipocalin (retinol binding)
spot 7	Prostaglandin-D2 synthase	O09114	Secreted	Lipocalin (retinol binding)
spot 11	Phosphoglycerate mutase 1	Q9DBJ1	cytoplasm	
spot 12	Aldose reductase	P45376	cytoplasm	
spot 18	Collagen alpha-2(IV) chain	P08122	Secreted	type IV collagen family
spot 29	Fatty acid binding protein	Q05816	cytoplasm	calycin
spot 34	Galectin-1	P16045	extracellular	Lectin
spot 40	L-lactate dehydrogenase A	P06151	cytoplasm	LDH family
spot 48	Anti-Mullerian hormone	Q5EC55	Secreted	TGF-beta family



**Proteins**  
 Matching profile between meiosis and spot intensity



high level at T1 and T13  
 low level at T8 and O1

## Findings / Perspectives

Several proteins known to be expressed by the developing testis were retrieved (Anti-mullerian hormone, Prostaglandin D2 synthase) and allowed the validation of the analysis strategy (developing period, ex-vivo culture conditions and proteins extraction/separation). This preliminary study has revealed several new proteins, unknown for being secreted by the developing testis (Galectin-1, Fatty acid binding protein, L-lactate dehydrogenase A). Their potential roles during male germ cell proliferation and differentiation will be further investigated as well as the identification of other interesting spots.