Oviductal secretions have a positive effect on fertilization in equine species, which involve Deleted in Malignant Brain Tumor 1 (DMBT1).

Douet C.,1, Ambruosi B.,1, Pascal G.,1, Monget P.,1, Moros C.,2, Holmskov U.,3, Goudet G.,1

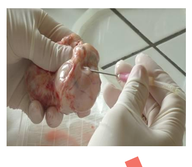
1: INRA, UMR85 Physiology of Reproduction and Behaviors, Nouzilly, France, 2 : Cell Biology and Histology, Faculty of Medicine, University of Murcia, Murcia, Spain, 3 : Institute for Molecular Medicine, University of Southern Denmark, Odense, Denmark

Introduction

Oviductal environment contains important molecules that affect sperm-oocyte interaction and the subsequent developmental competence of zygotes.
We showed previously the presence in oviductal fluid of Deleted in Malignant Brain Tumors 1 (DMBT1), a protein with functions in innate immunity and epithelial differentiation. No role in reproduction has been shown at the moment.
Our aim is to evaluate the effect of oviductal fluid and the possible involvement of DMBT1 on *in vitro* fertilization (IVF) in equine species.

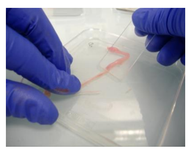
Materials & Methods

Equine ovaries collected from slaughtered mares



in vitro *maturation of oocytes*

Oviductal fluid (OF) collected from slaughtered sows 6 hours after ovulation



Exp 1) Does DMBT1 interact with oocytes ?

30 min co-incubation with OF

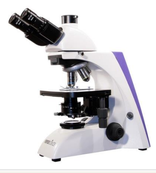
Detection of DMBT1 by immunocytochemistry using anti DMBT1 antibody

Labelled oocytes are observed with a confocal microscope

Exp 2) Does DMBT1 have an effect on IVF rate ?

30 min co-incubation with
- control or
- OF with or without anti DMBT1 antibody

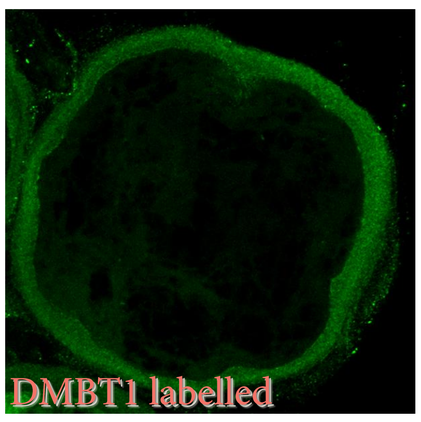
24h IVF with fresh equine semen treated with procaïne



Oocytes are stained with Hoechst and anti-lamin antibody

Results

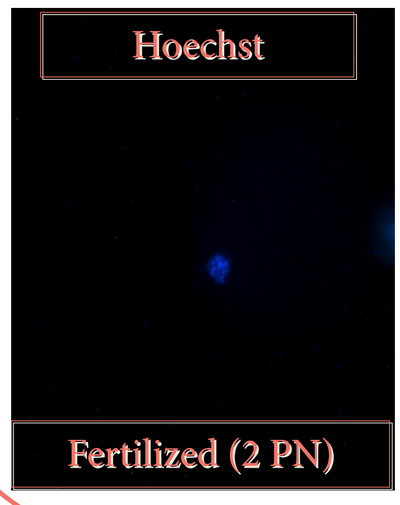
Exp 1) Does DMBT1 interact with oocytes ?

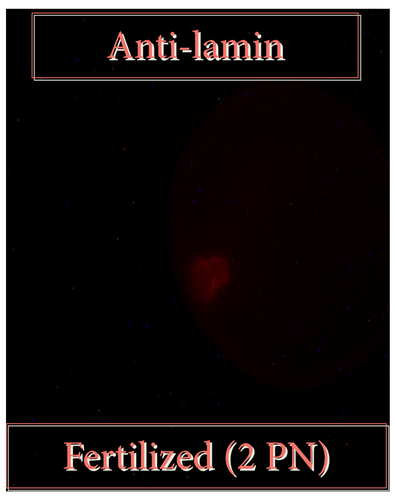


After co-incubation of *in vitro* matured oocytes in OF, DMBT1 is localized on zona pellucida of oocytes.

Exp 1)
DMBT1 interacts with oocytes.

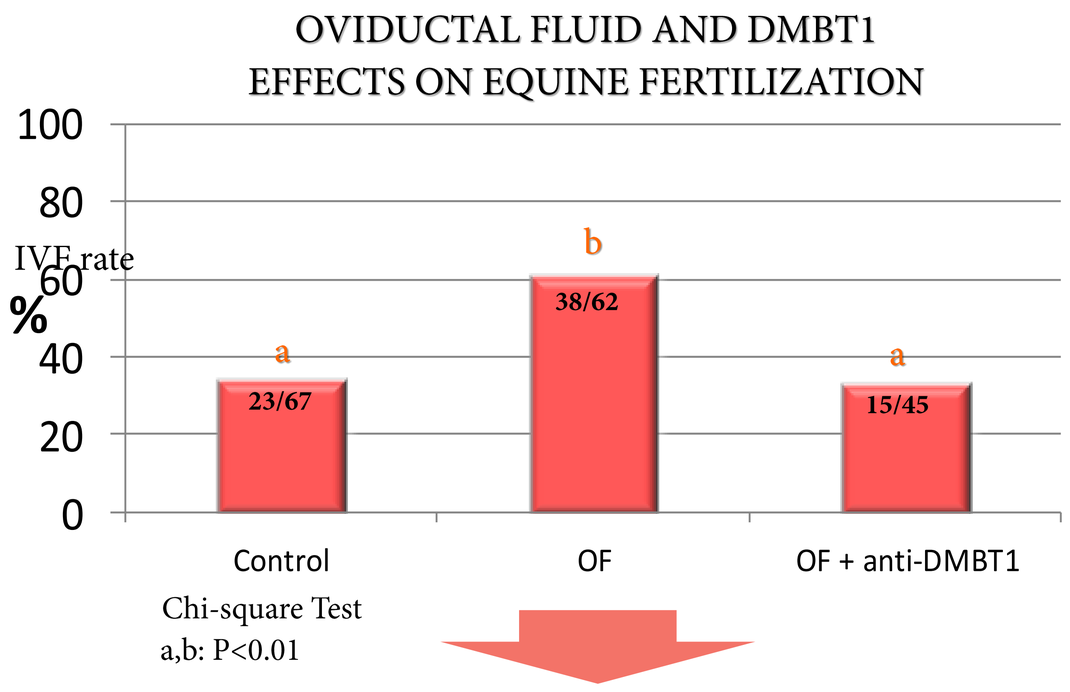
Exp 2) Does DMBT1 have an effect on IVF rate ?





Fertilized oocytes showed 2 pronuclei with DNA stained by Hoechst and membranes stained by anti-lamin antibody.

Exp 2) Does DMBT1 have an effect on IVF rate ?



Oocytes co-incubation with OF significantly increased IVF rates compared to control group.
The addition of anti DMBT1 antibody showed a significant decrease of IVF rates, cancelling the positive effect of OF.

Exp 2)
Oviductal fluid and its DMBT1 has a positive effect on IVF.
IVF achieved 60% of success with a very high repeatability.

Conclusions

Our study shows a beneficial effect of oviductal fluid on IVF in equine species and an involvement of DMBT1 in fertilization.
IVF technique, that has long been a major limitation in equine *in vitro* embryo production, has been validated in our lab with a high repeatability.

Centre de recherche Val de Loire,
UMR Physiologie de la Reproduction et des Comportements
37380 Nouzilly, France
www.val-de-loire.inra.fr
http://www7.tours.inra.fr/physiologie_reproduction_comportements