Advances in the mechanism of sperm-oocyte interactions and cross-talk with the oviduct in the equine

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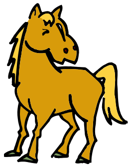
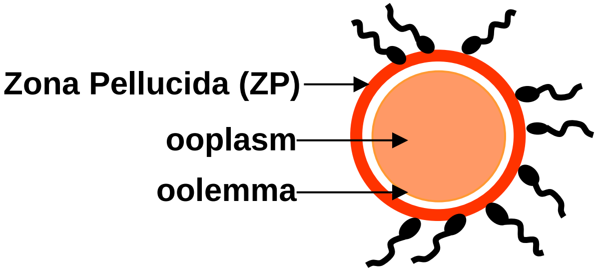
Clarify the **mechanism of fertilization** in the **equine**

⇨ interactions between spermatozoa and oocytes

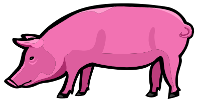
⇨ role of the oviduct during fertilization

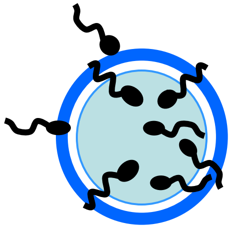
Develop a **comparative strategy** between 2 divergent models (equine and porcine) to identify **conserved** and/or **species-specific** molecular interactions that could highlight **key components** involved in the mechanism of fertilization





IVF rates are low (<60%)
Polyspermy is scarce





IVF rates are high (>80%)
Polyspermy rates are high (>50%)

1. comparison of the composition and structure of the equine and porcine ZP

A. Identification of ZP glycoproteins

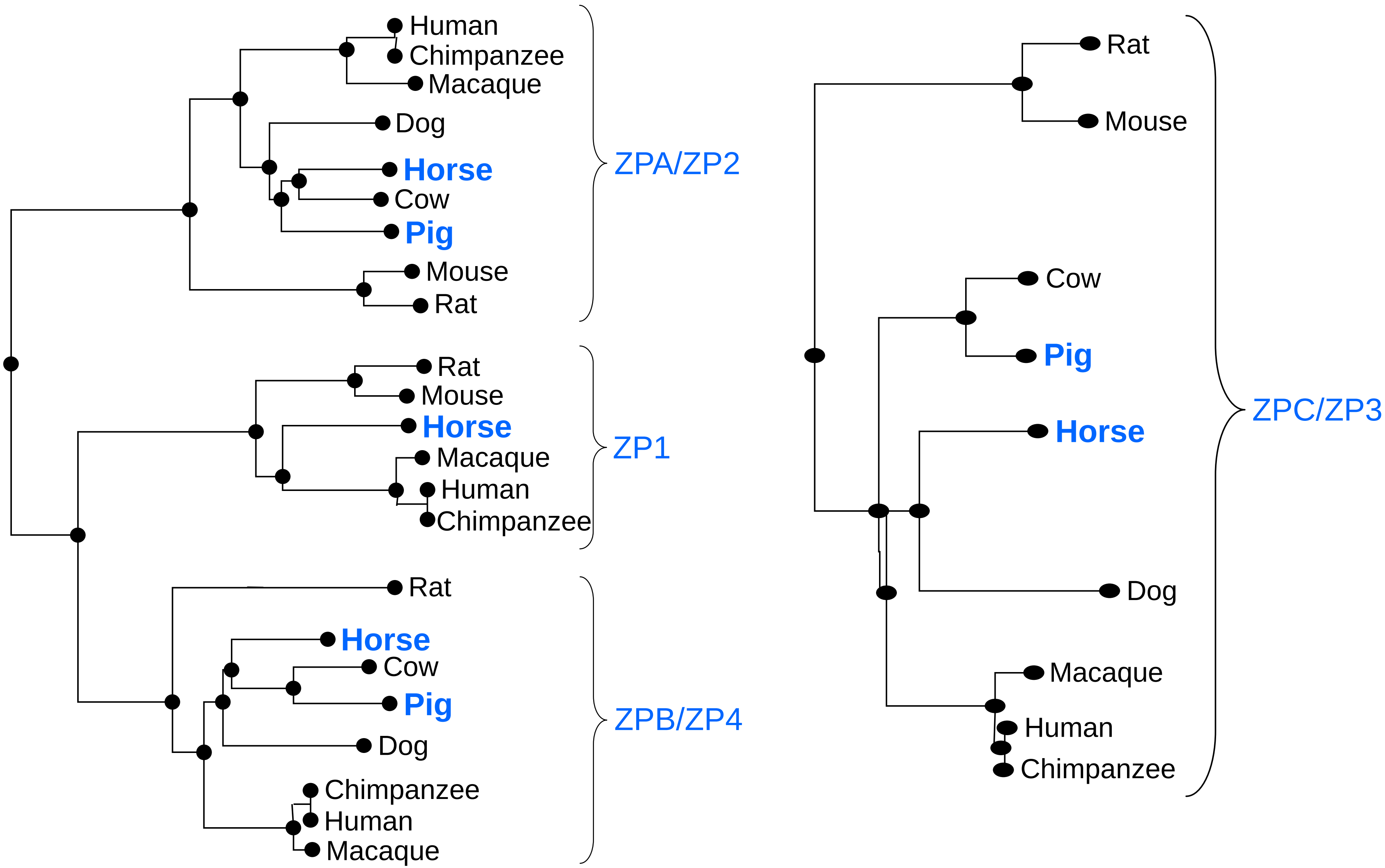
Bioinformatic analysis of ZP glycoproteins:

- phylogenetic trees using Figenix software
- updated list of the genes of the ZP family
- when one of the ZP proteins was not found, identification of pseudogenes:

BLAST against the genome to reveal the presence of stop codon or insertion/deletion

1. comparison of the composition and structure of the equine and porcine ZP

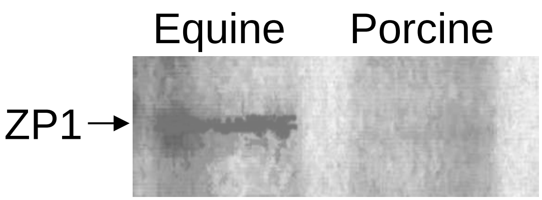
A. Identification of ZP glycoproteins

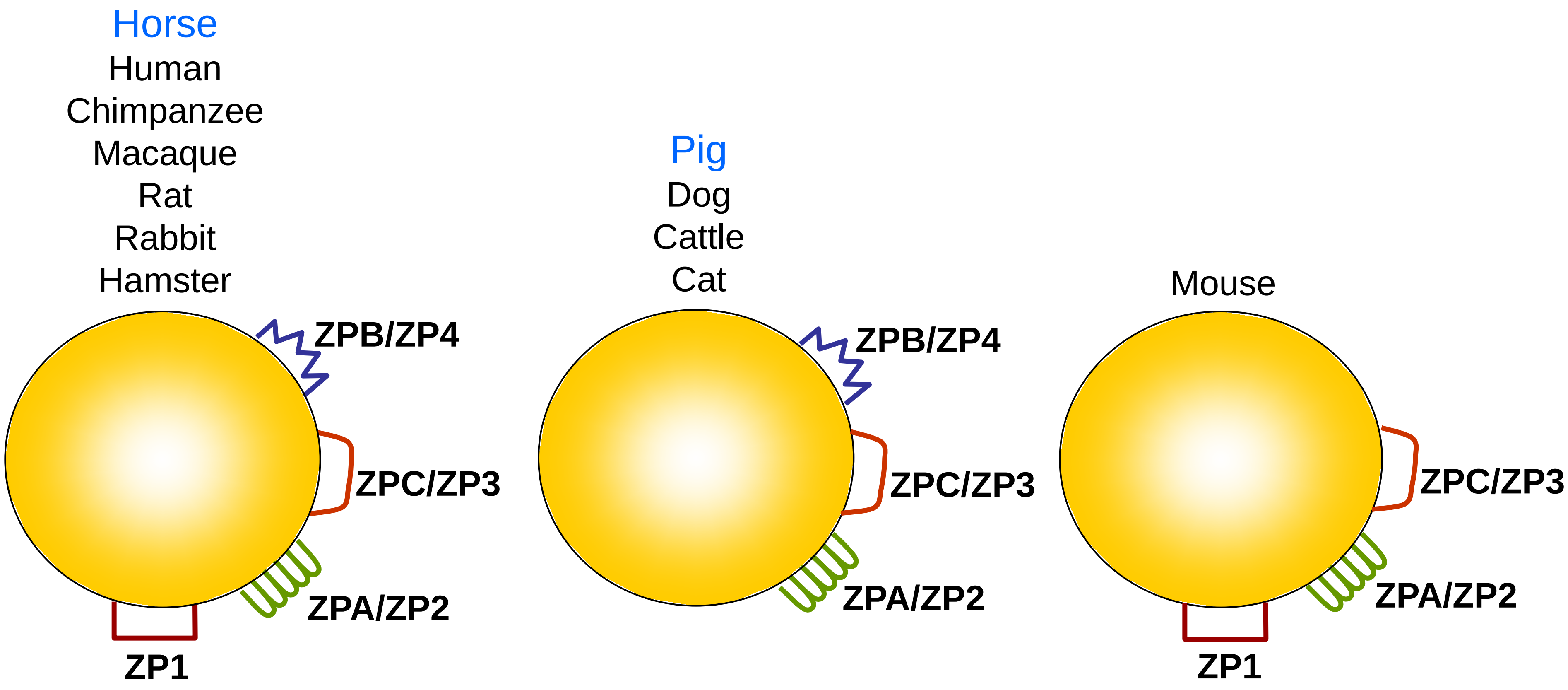


1. comparison of the composition and structure of the equine and porcine ZP

A. Identification of ZP glycoproteins

Western Blot with antibodies against human ZP1:

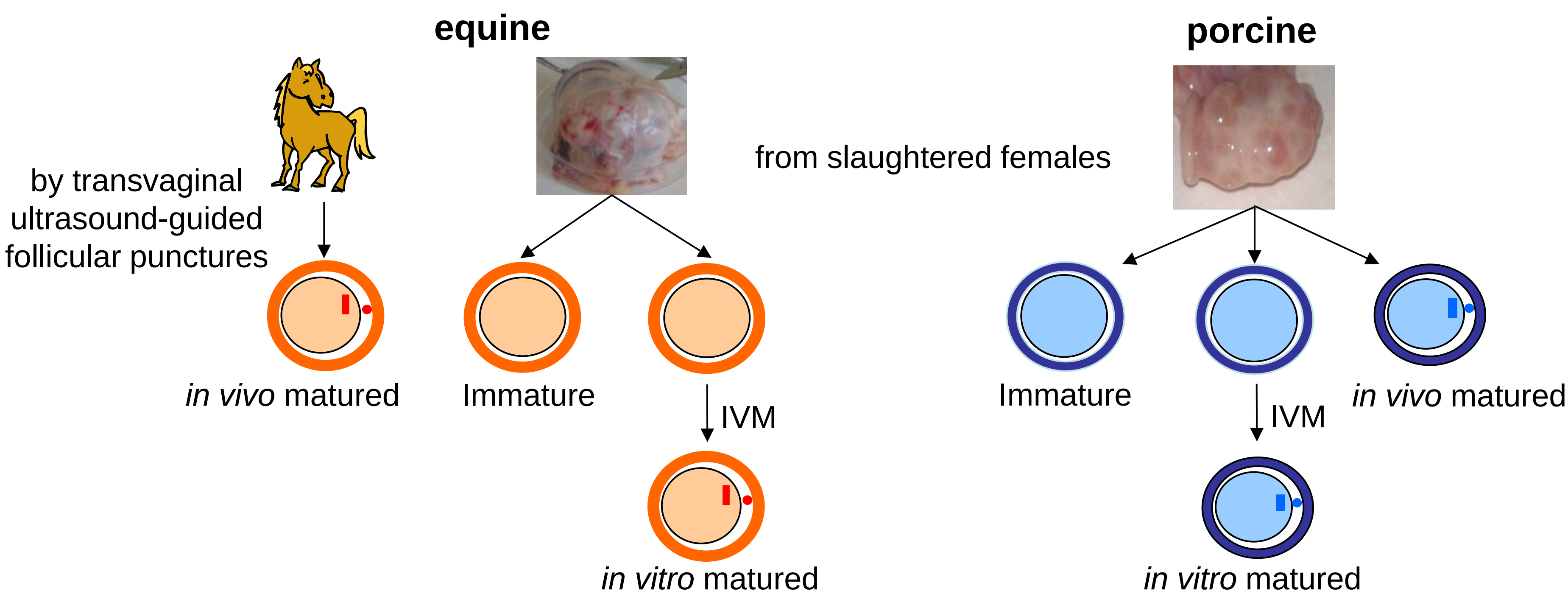




1. comparison of the composition and structure of the equine and porcine ZP

B. Localization of ZP glycoproteins

1) Collection of COCs



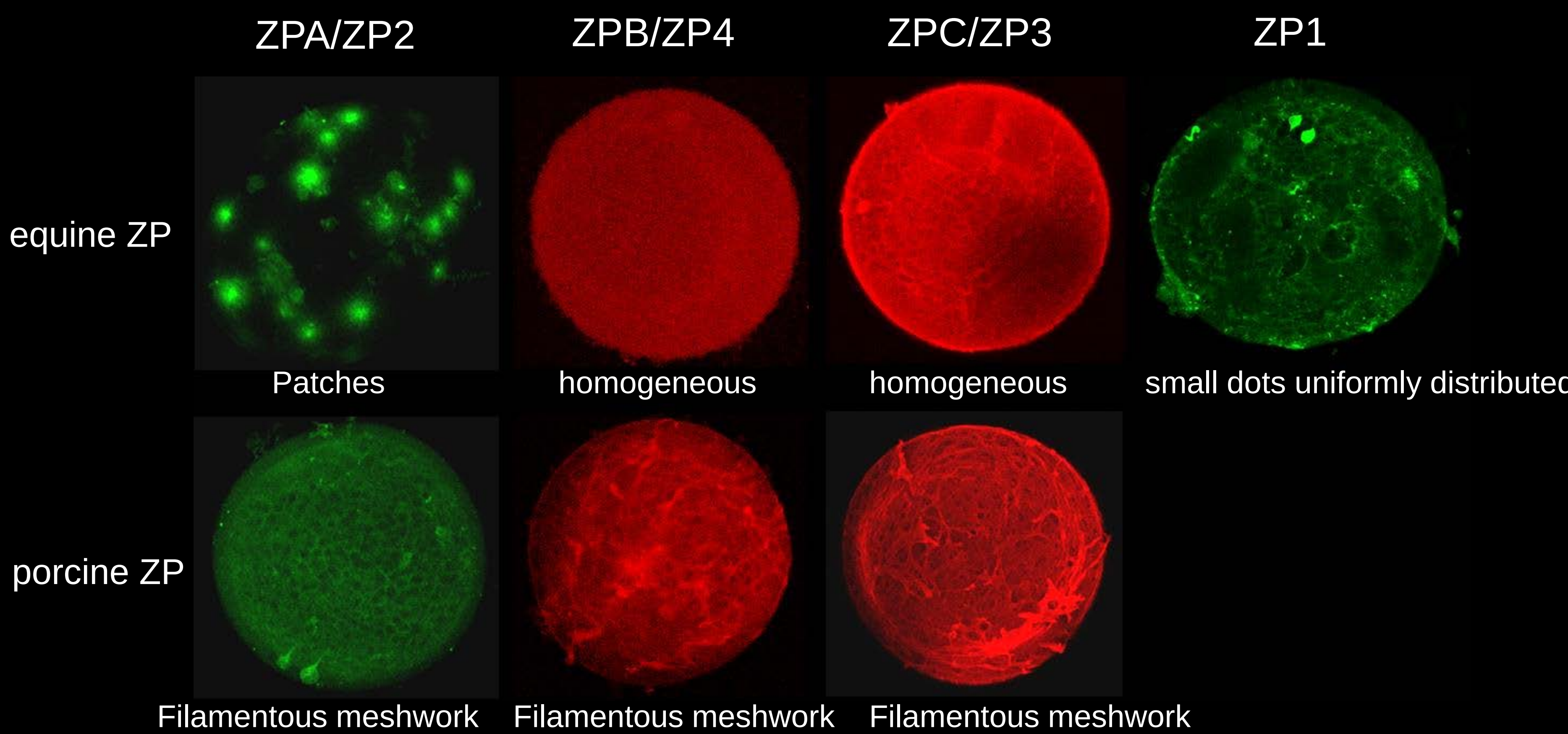
2) Removal of cumulus cells and fixation of oocytes

3) Incubation with anti ZPA/ZP2 or anti ZPB/ZP4 or anti ZPC/ZP3 or anti ZP1 antibodies and fluoprobes-conjugated secondary antibodies

4) Observation with a confocal microscope

Localization of ZPA, ZPB, ZPC and ZP1 on the equine and porcine ZP

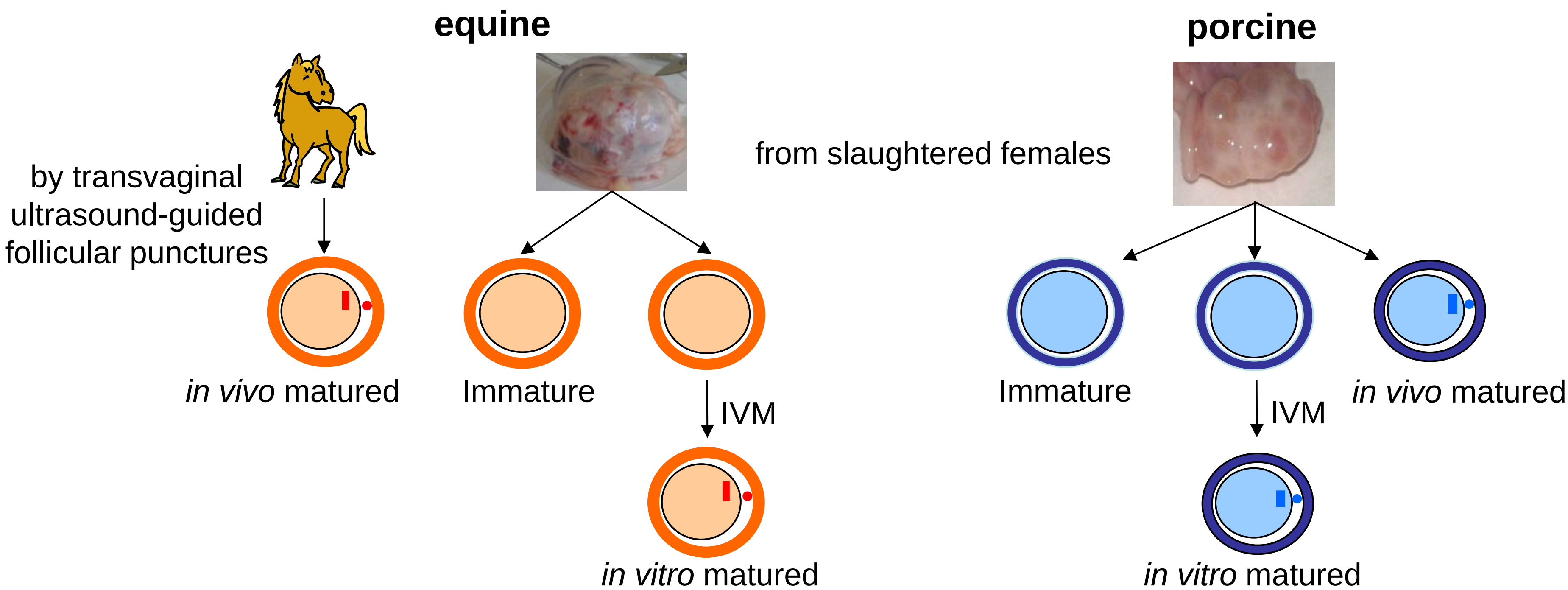
Similar patterns for immature, *in vitro* matured and *in vivo* matured oocytes



1. comparison of the composition and structure of the equine and porcine ZP

C. Structure of the ZP

1) Collection of COCs

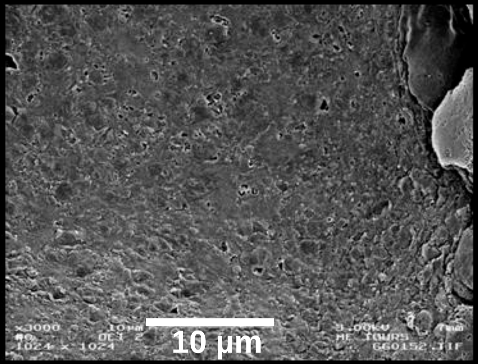
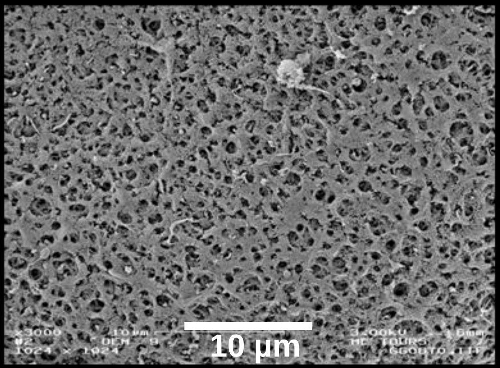
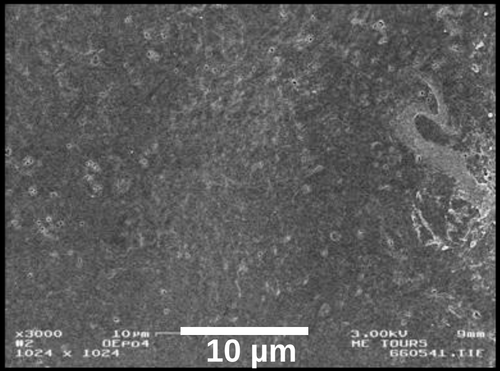
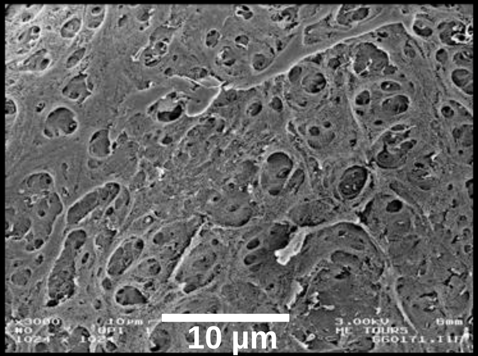
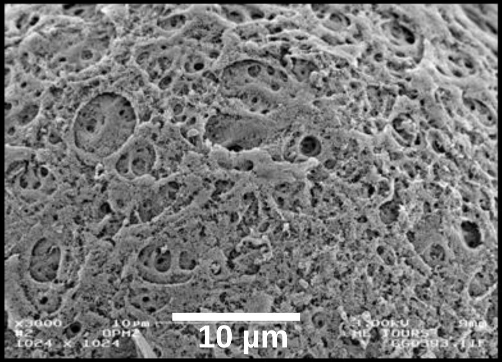
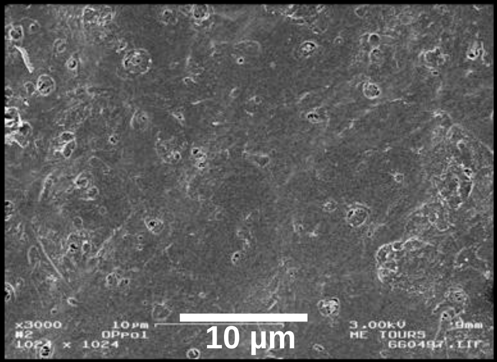


2) Removal of cumulus cells and fixation of oocytes

3) Preparation for scanning electron microscopy

4) Observation with a scanning electron microscope

Observation by scanning electron microscopy

	immature	*in vitro* matured	*in vivo* matured
equine ZP			
porcine ZP			

Equine ZP, immature and *in vitro* matured: Compact mesh-like structure with small pores

Equine ZP, *in vivo* matured: rough surface, mesh-like structure, small pores

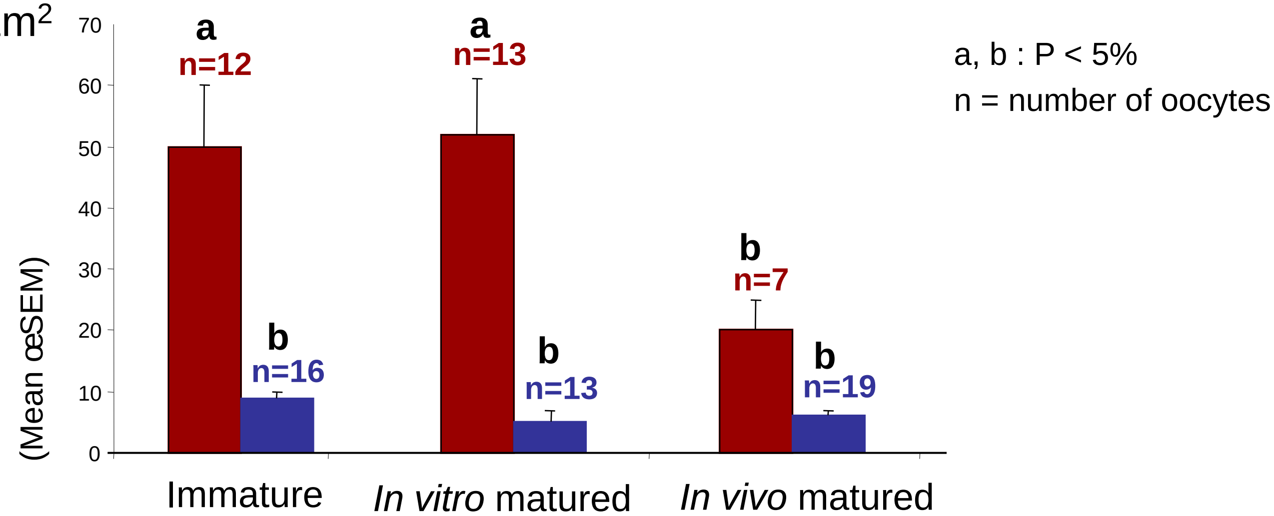
Porcine ZP, immature and *in vitro* matured: Distinct mesh-like structure with large pores

Porcine ZP, *in vivo* matured: rough surface, mesh-like structure, small pores

1. comparison of the composition and structure of the equine and porcine ZP

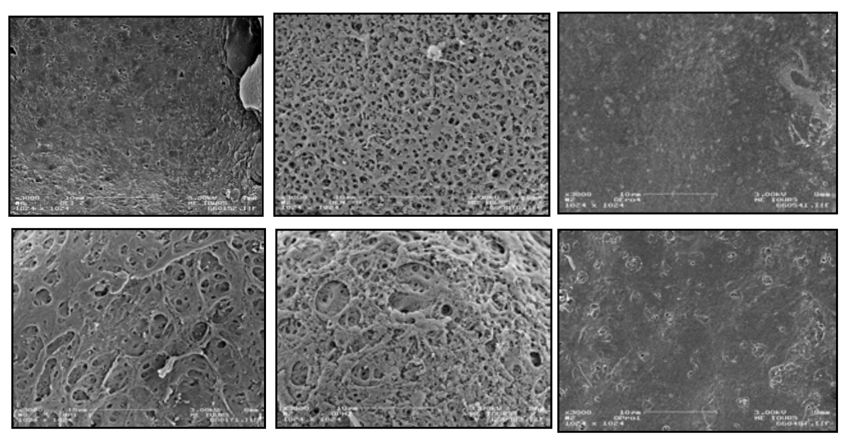
C. Structure of the ZP

Number of pores per 100µm^2



equine ZP

porcine ZP



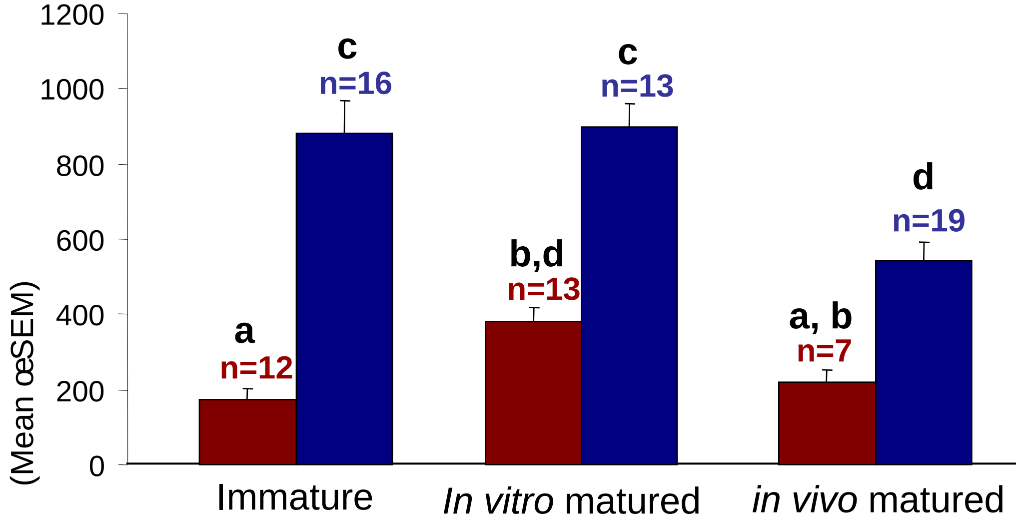
The number of pores was lower in the porcine ZP than in the equine ZP for immature and *in vitro* matured oocytes

In equine ZP, the number of pores was modified during *in vivo* but not *in vitro* maturation

1. comparison of the composition and structure of the equine and porcine ZP

C. Structure of the ZP

Diameter of pores

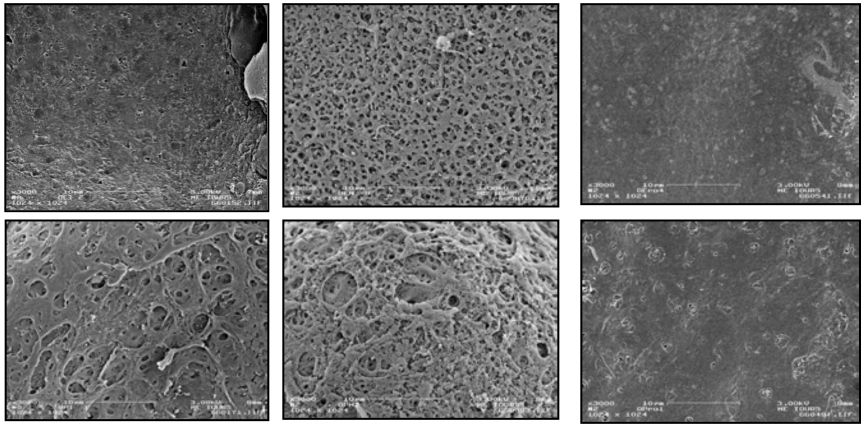


a, b, c, d : P<5%

n = nombre d’ovocytes

equine ZP

porcine ZP



The diameter of pores was larger in porcine ZP than in equine ZP

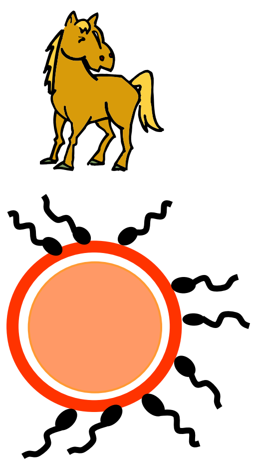
In equine ZP, the diameter of pores was modified during *in vitro* but not *in vivo* maturation

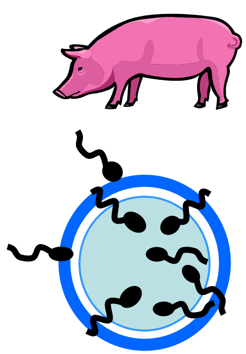
1. comparison of the composition and structure of the equine and porcine ZP

Conclusion

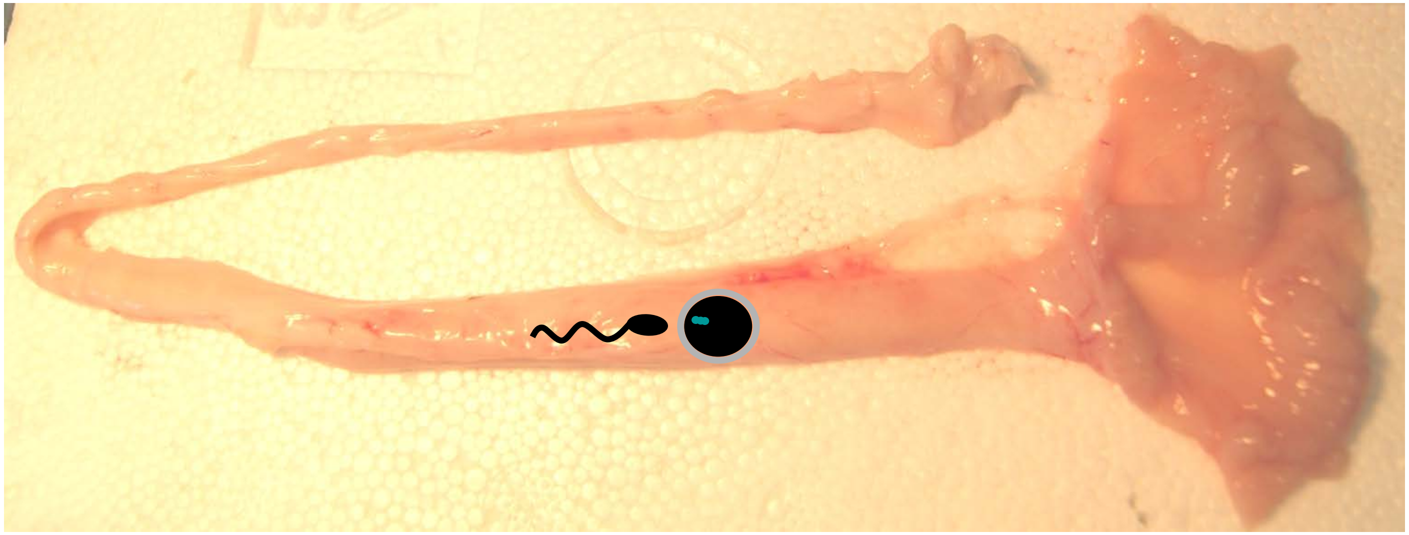
We observed differences in the number and localization of the ZP glycoproteins and in the mesh-like stucture of the ZP between equine and porcine species.

These differences might correlate with the differences in spermatozoa attachment and penetration rates between equine and porcine species.





2. influence of the oviductal secretions and identification of the molecules involved



In several mammals, oocytes and/or spermatozoa co-incubation with oviductal fluid increases monospermic IVF rates.

⇨ Are oviductal secretions involved in the mechanism of fertilization in the equine ?

⇨ Which are the molecules involved ?

One potential candidate molecule:

DMBT1 = Deleted in Malignant Brain Tumors 1
involved in innate immunity and epithelial differentiation.

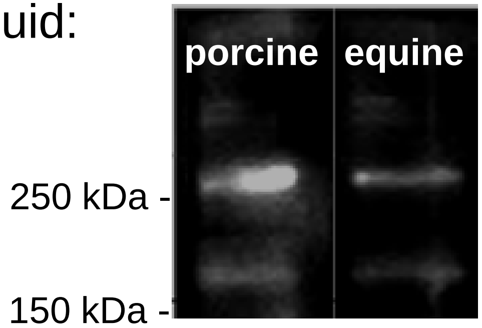
⇨ CUB Domain: domain found in sperm cell spermadhesin, which are implicated in mammalian sperm binding to the ZP

⇨ ZP Domain: domain which is present in oocyte ZP glycoproteins

⇨ Interaction with integrins, which are implicated in fertilization

⇨ Presence of DMBT1 in equine and porcine oviductal fluid:

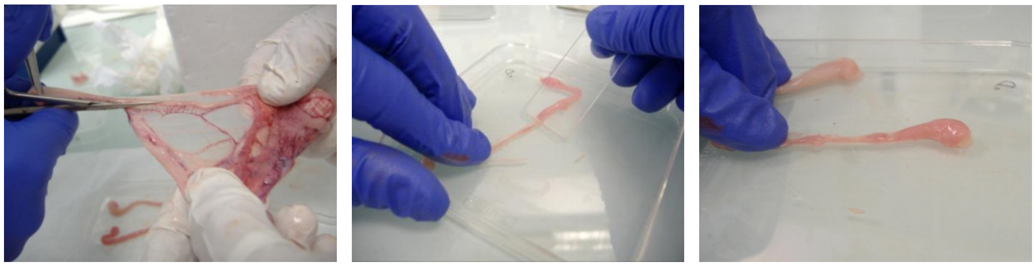
Gel electrophoresis and immunoblotting



Hyp: DMBT1 may be involved in the mechanism of fertilization

2. influence of the oviductal secretions and identification of the molecules involved

Oviductal fluid (OF) collection on sows slaughtered 6 hours after ovulation



Oviduct dissection, fluid expelled by squeezing with a slide

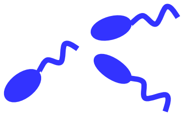
Oocyte collection and *in vitro* maturation: 44h for porcine COCs, 28h for equine COCs

2. influence of the oviductal secretions and identification of the molecules involved

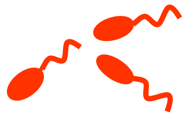
Oocytes pre-incubation for 30 min

+ control medium	+ control medium
+ oviductal fluid	+ oviductal fluid
+ oviductal fluid + 1 mg/ml anti-DMBT1	
+ oviductal fluid + 2 mg/ml anti-DMBT1	+ oviductal fluid + 2 mg/ml anti-DMBT1

Oocytes – spermatozoa co-incubation for 24 h

+ 

Frozen sperm
Percoll gradient
Caffeine

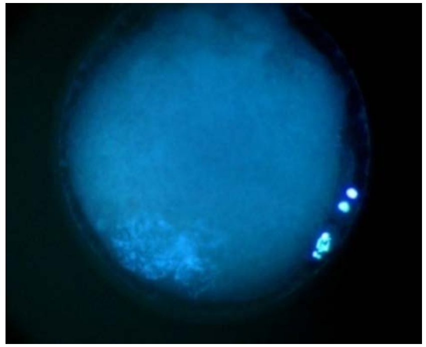
+ 

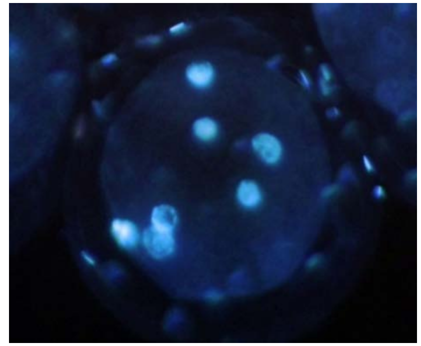
Fresh sperm
Centrifugations
Procaïne

Fixation in paraformaldehyde, staining with Hoechst

Observation under an epifluorescence microscope

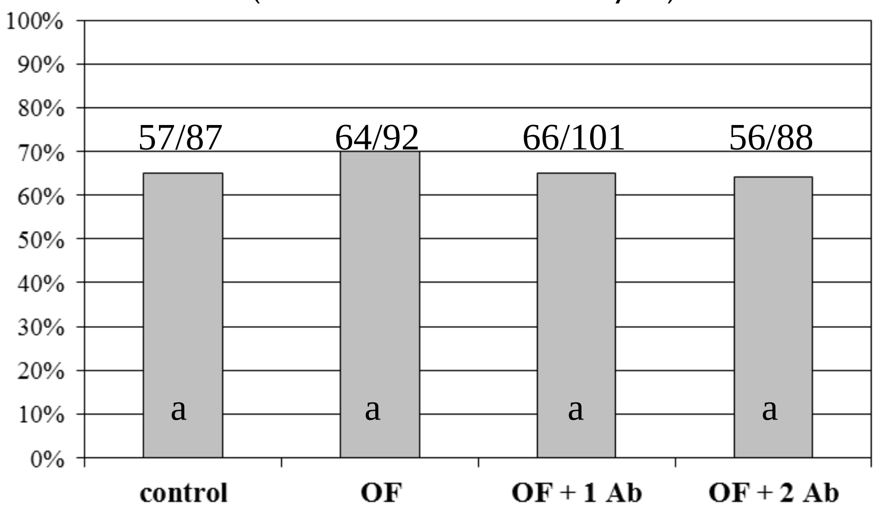
2. influence of the oviductal secretions and identification of the molecules involved





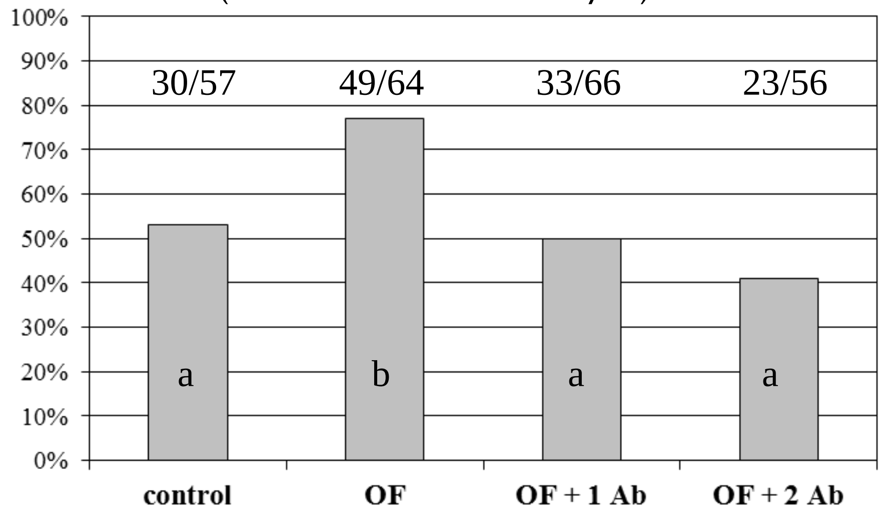
Fertilization rate

(Calculated on matured oocytes)



Monospermic fertilization rate

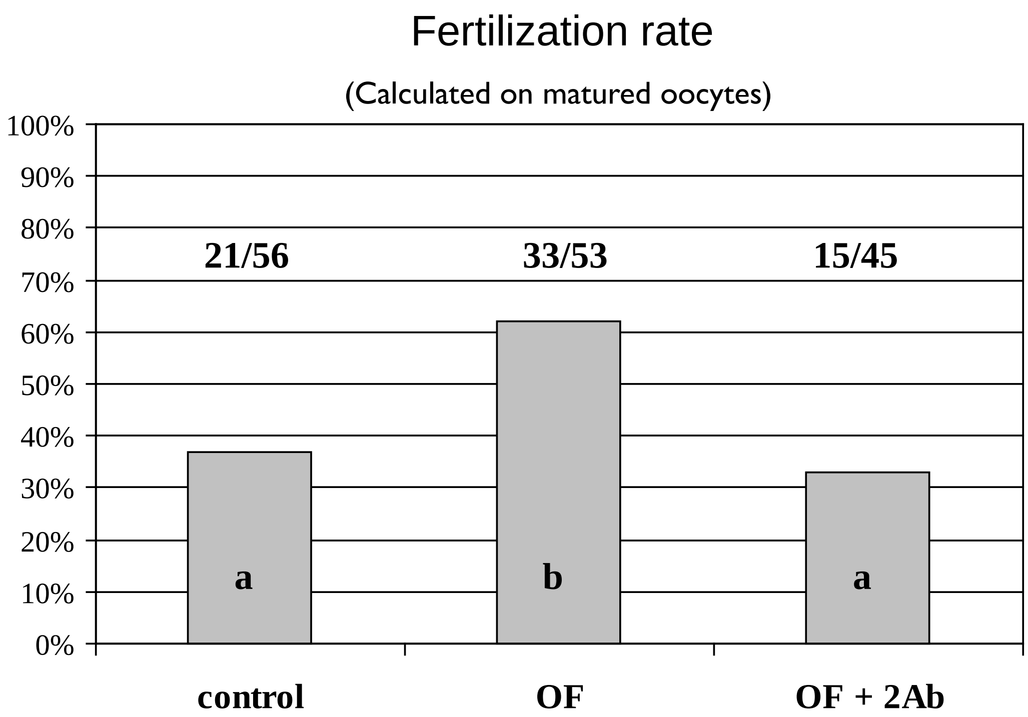
(Calculated on fertilized oocytes)



Chi-square test: a,b: $P<0,05$

Oocytes pre-incubation with oviductal fluid increased monospermic IVF rates. Addition of anti-DMBT1 Ab decreased monospermy rates compared to OF group, cancelling the positive effect of oviductal fluid.

2. influence of the oviductal secretions and identification of the molecules involved



Chi-square test: a,b: P<0,001

Oocytes pre-incubation with oviductal fluid increased monospermic IVF rates. The addition of anti-DMBT1 Ab decreased IVF rates compared to OF group, cancelling the positive effect of oviductal fluid.

2. influence of the oviductal secretions and identification of the molecules involved

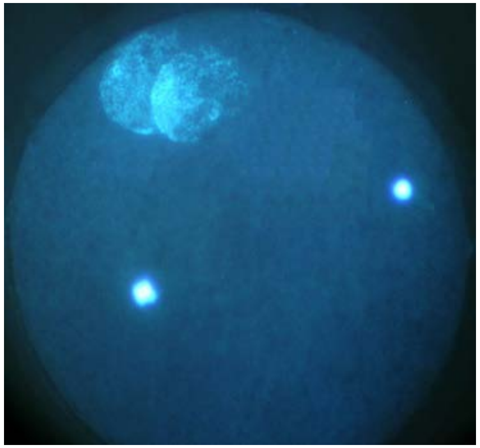
Conclusion

• Oocytes pre-incubation with oviductal fluid recovered from sows at post ovulatory stage increases monospermic IVF rates in equine and porcine species.

• DMBT1 is present in the oviduct and involved in the mechanism of fertilization in the equine and porcine species.

• With this equine IVF technique, the fertilization rate is higher than 60%.

• An efficient and repeatable IVF technique is now available for the production of equine embryos.



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