Oligosaccharides of human zona pellucida glycoproteins

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OBJECTIVES OF THIS STUDY :

1/ Analysis of human zona pellucida biotinylated-glycoproteins

- : METHODS

1D-SDS-PAGE, 7% and 12% gels,

Western blot with anti-ZP polyclonal antibody.

2/ Analysis of human zona pellucida oligosaccharides

: METHODS

Cytochemistry and blotting with lectins,

Western blot with anti-fucosylated oligosaccharides mAb.

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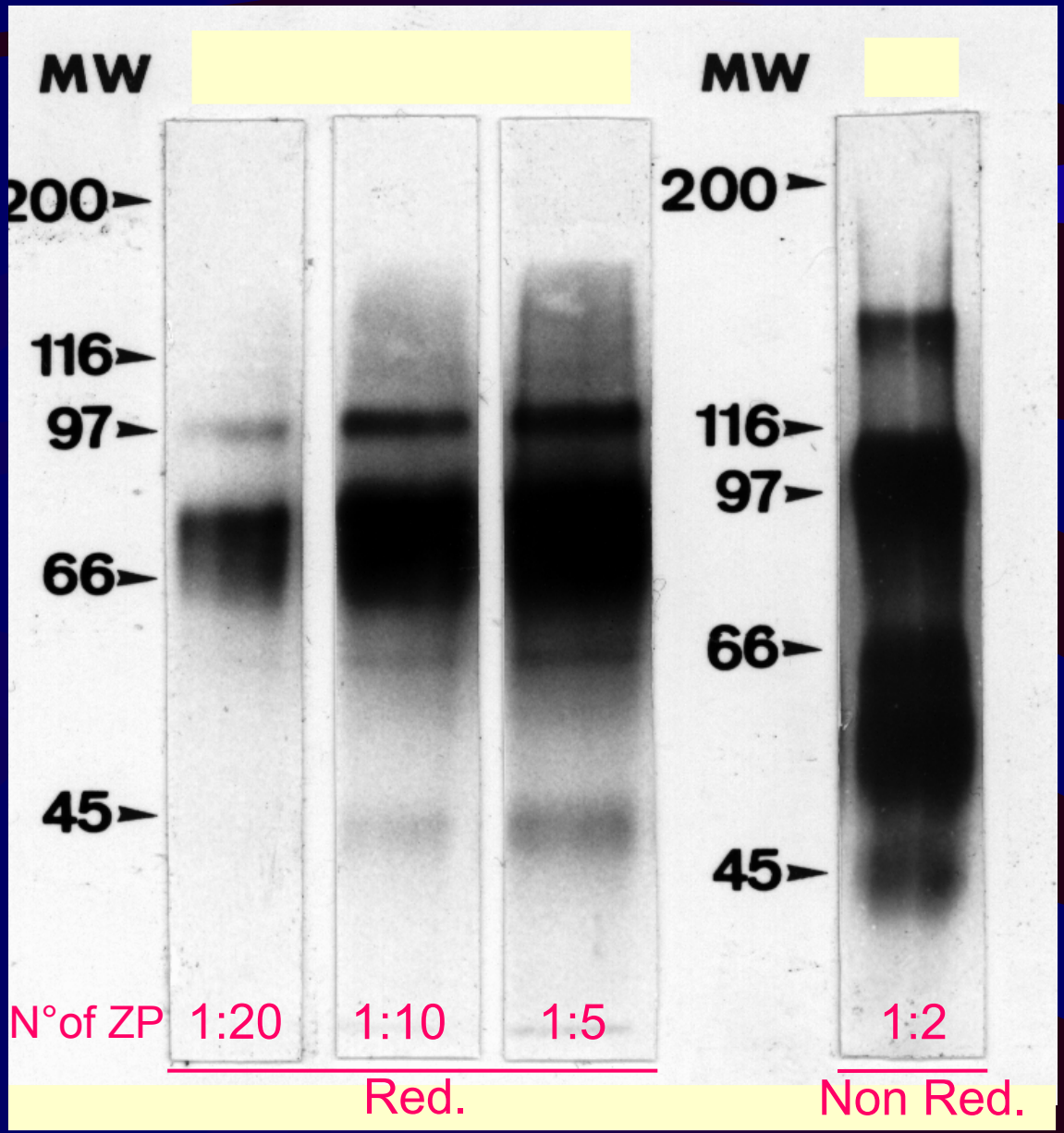
Sensitivity of 1D-SDS-7%PAGE :

Biotinylated ZP obtained from oocytes which failed to fertilize

ZP1 : 140 kDa

ZP2 : 110 kDa

ZP3 : 55-70 kDa



THIS VERY SENSITIVE METHOD OF ELECTROPHORESIS :

- 1/ Allows to detect the ZP1 glycoprotein

- 2/ Allows to study slight modifications of ZP glycoproteins consecutive to fertilization, and the oligosaccharides of native ZP

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ZP glycoprotein modified by fertilization :

Biotinylated-ZP obtained from oocytes which failed to fertilize or from embryos.

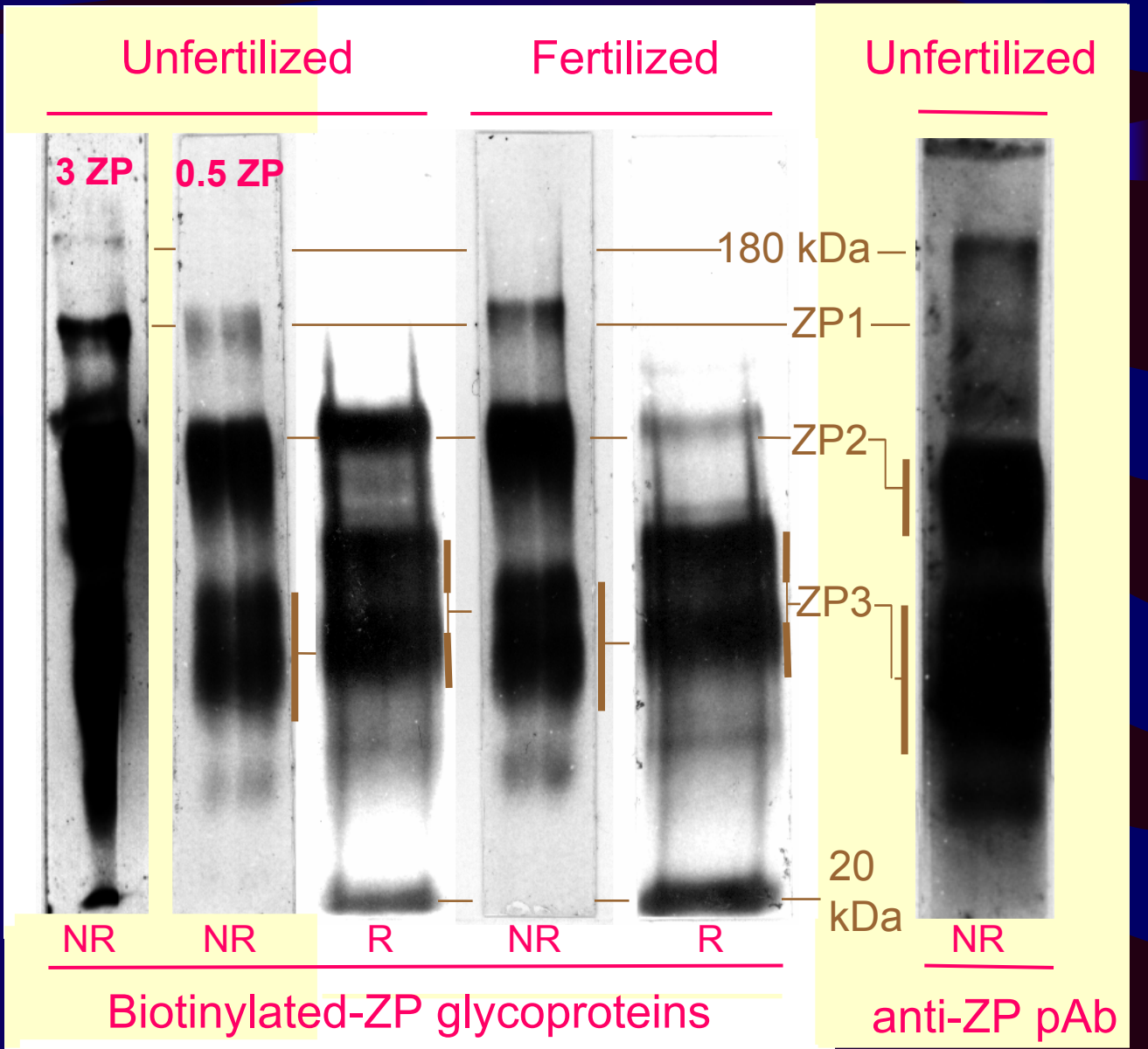
ZP1 : 140 kDa

ZP2 : 110 kDa

ZP3 : 55-70 kDa

20 kDa

180 kDa



ZP1 :

Is a glycoprotein of 140 kDa not modified by fertilization (non reducing conditions)

Is probably a dimere as described in the mouse (reducing conditions)

180 kDa band:

Is a new described band which could correspond to a polymere

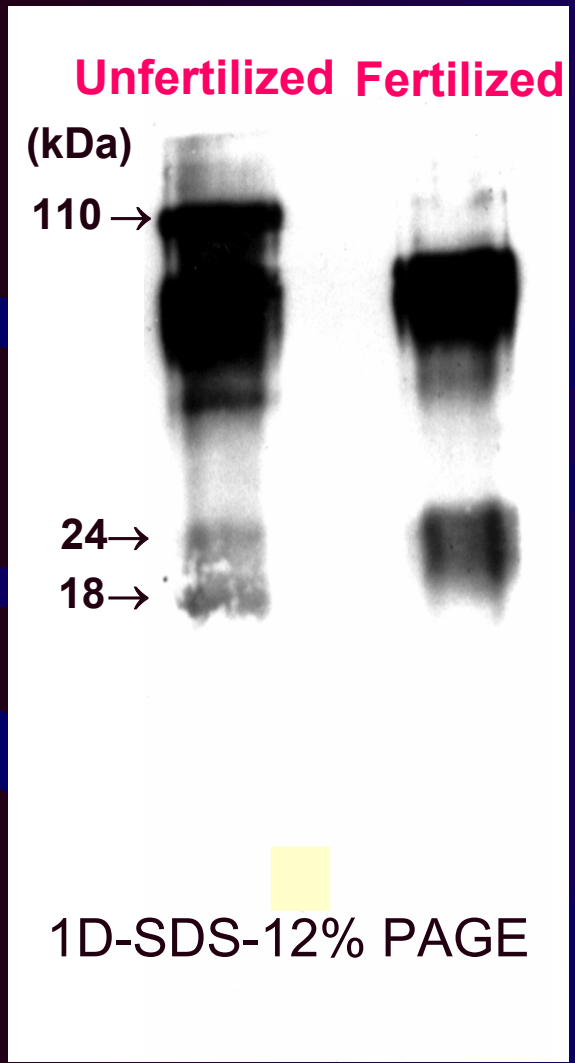
Is a minor ZP glycoprotein, about 1%

It oligosaccharidic content will be further studied

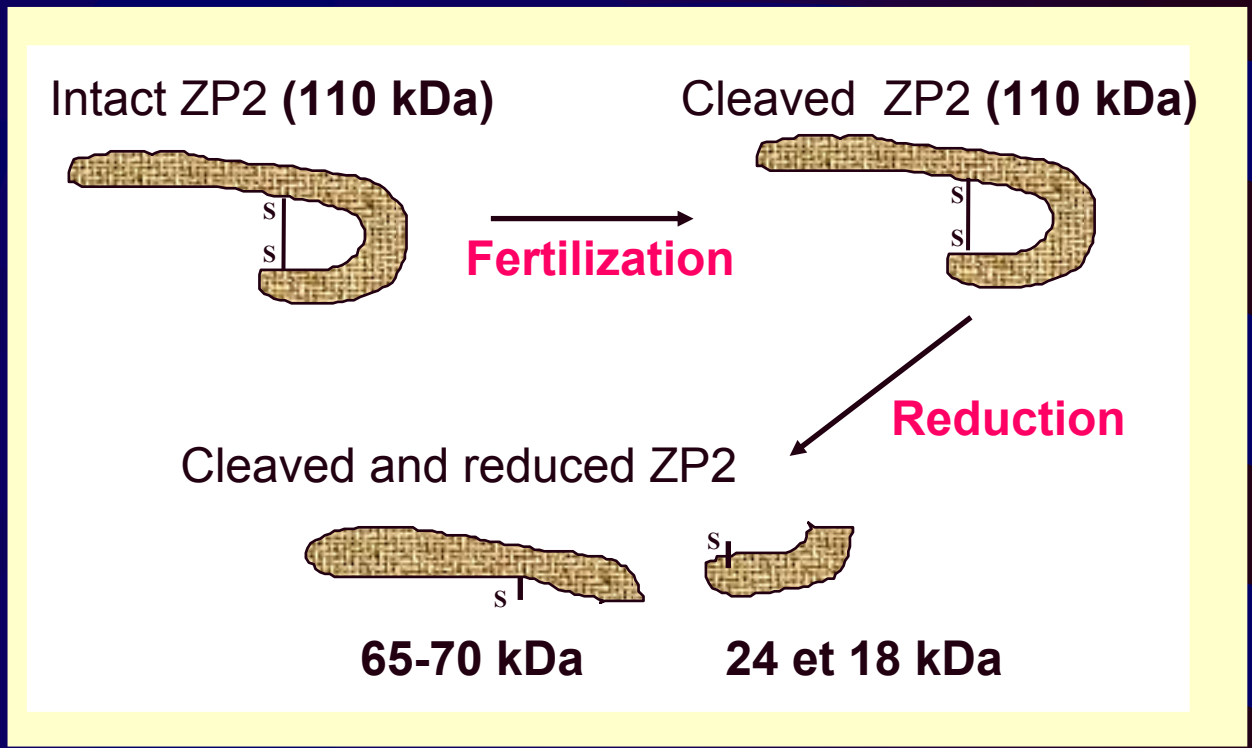
ZP3 :

Seems not to be modified by fertilization

ZP2, 20 kDa :



ZP2 shift as revealed by 1D-SDS-12%PAGE



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CYTOCHEMISTRY WITH LECTINS ON HUMAN ZP :

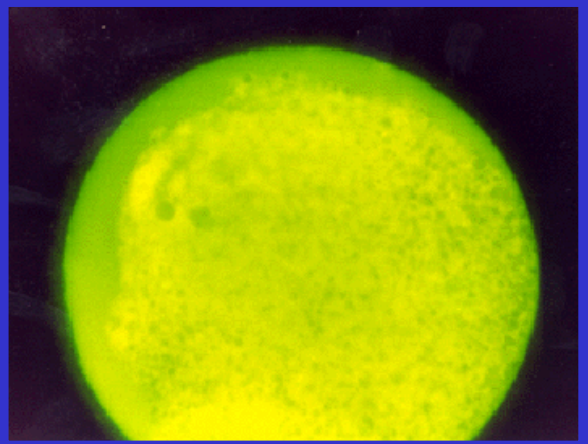
Ulex europaeus (UEA-1)

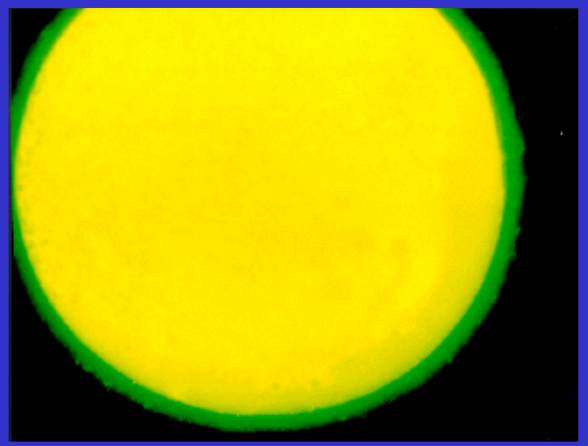
Aleuria aurantia (AAL)

Lotus tetragonolobus (LTA)

Pisum sativum (PSA)

Wheat germ agglutinin (WGA)





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Lectin-based assay on human ZP glycoproteins :

AAL : *Aleuria aurantia*

PSA : *Pisum sativum*

WGA : *Wheat germ agglutinin*

LTA : *Lotus tetragonolobus*

180 kDa : LTA and PSA

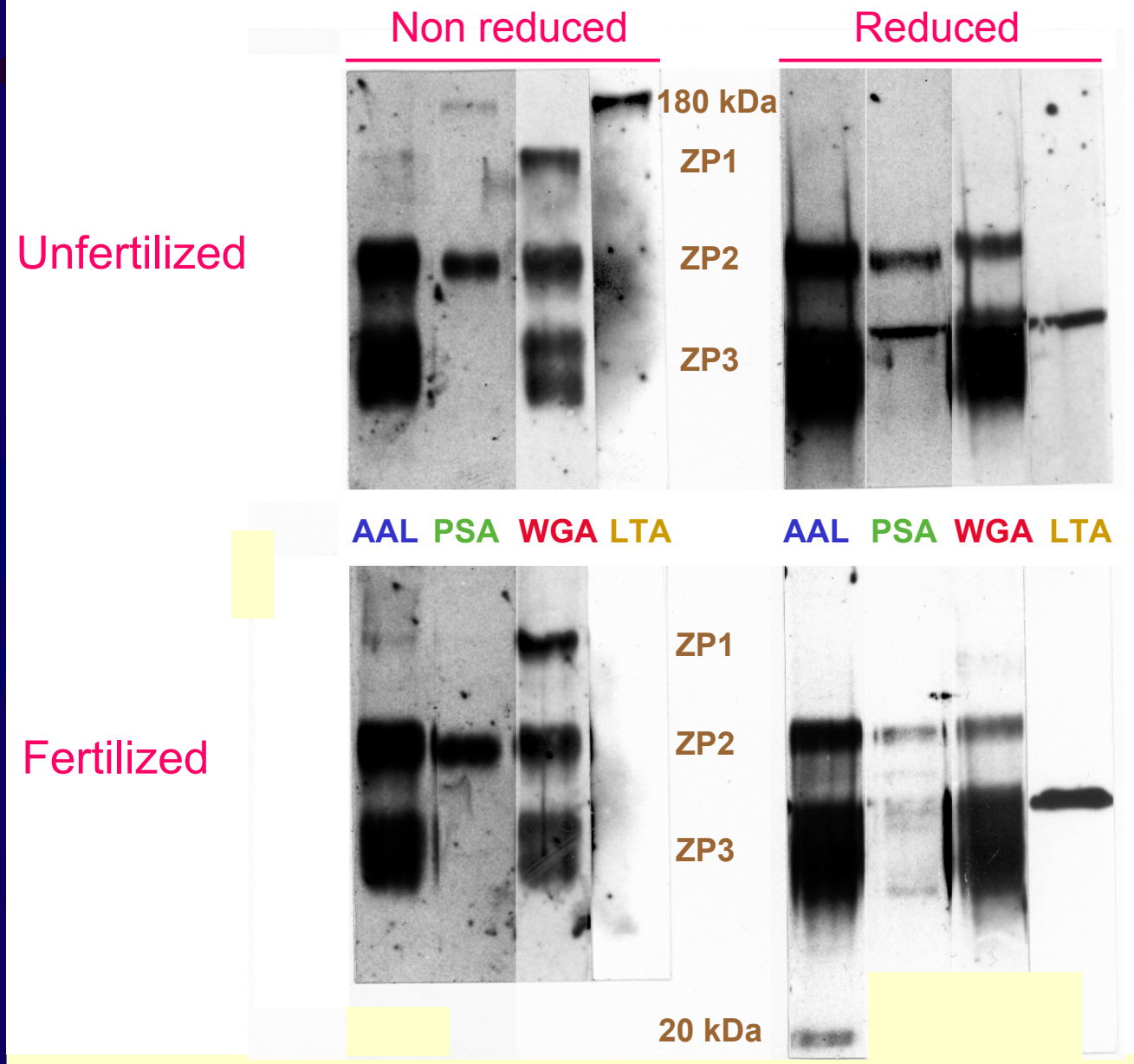
140 kDa : AAL and WGA

100 kDa : AAL, PSA and WGA

50-70 kDa : AAL and WGA

20 kDa : AAL

1D-SDS-7%PAGE



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Western blot with fucosylated anti-oligosaccharides mAb on human ZP glycoproteins :

siaLe^a : Ac anti-sialyl-Lewis a

Le^b : Ac anti-Lewis b

siaLe^x : Ac anti-sialyl-Lewis x

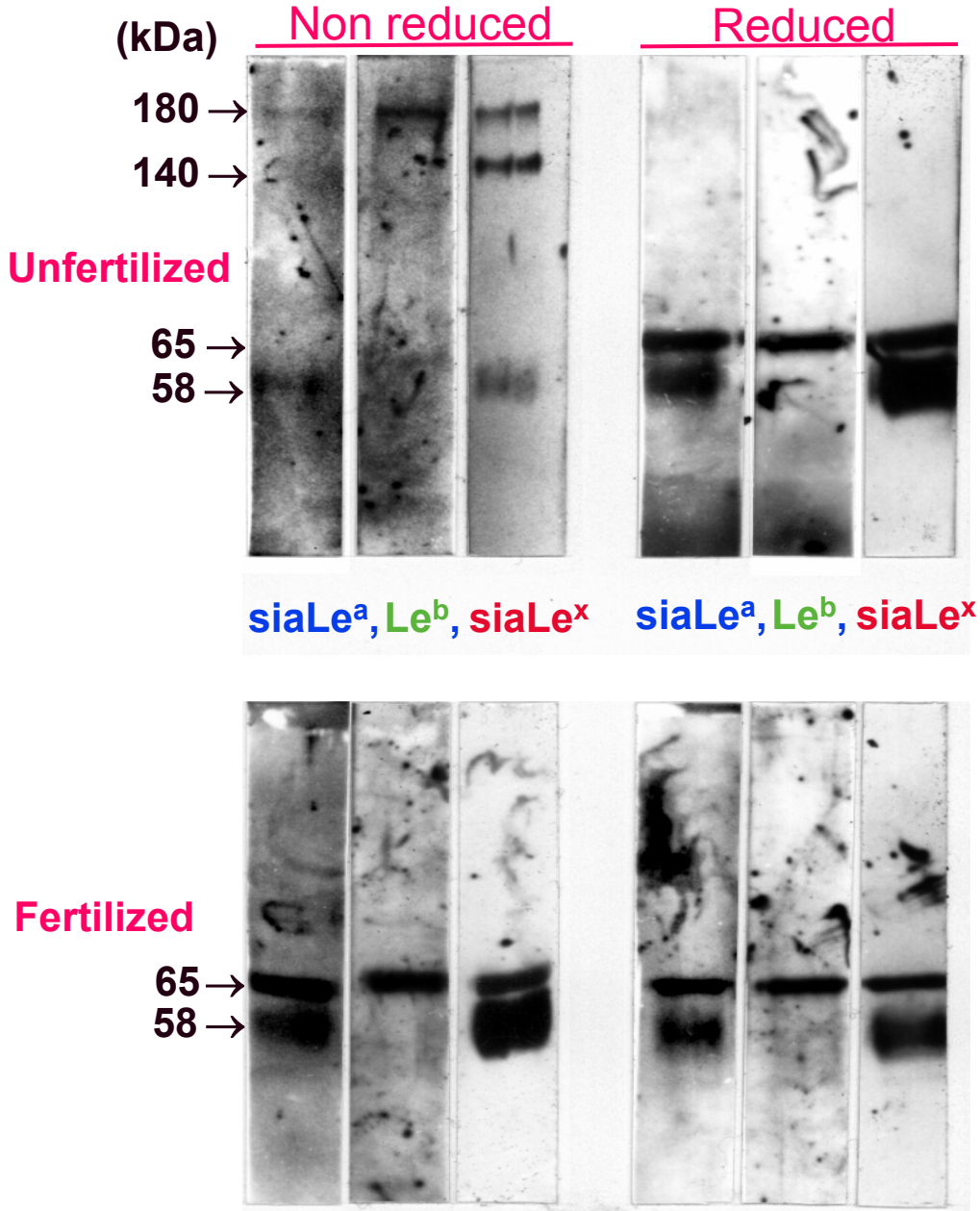
180 kDa : siaLe^a, Le^b , siaLe^x

ZP1 : siaLe^a and siaLe^x

ZP2 kDa : no labelling

65 kDa : siaLe^a, Le^b , siaLe^x

58 kDa : siaLe^a and siaLe^x



1D-SDS-7%PAGE

180 kDa :

Is a multimeric glycoprotein which contains fucosylated residues which are probably required for a high affinity binding to sperm as described in the mouse (Johnston *et al*., *J. Biol. Chem.* 1998),

Could correspond to an heteropolymere of ZP3 and ZP2, as previously described in pig (ZPB-ZPC) (Yurewicz *et al*., *J. Biol. Chem.* 1998),

Consequently , this complexe seems to contribute to the generation of conformational ligands important for sperm-zona interactions in pig.

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Perspectives :

Functionnal studies are currently performed to elucidate the importance of fucosylated oligosaccharides in human sperm-zona binding.

Preliminary results indicate that it exists a lectin-like sperm receptor which displays high affinity for identified ZP-Lewis oligosaccharides.

This study will going on...

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