


Molecular analysis of ropporin 1- like protein from human liver fluke, *Opisthorchis viverrini*



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Opisthorchis viverrini and
human opisthorchiasis;
an overview

Opisthorchis viverrini

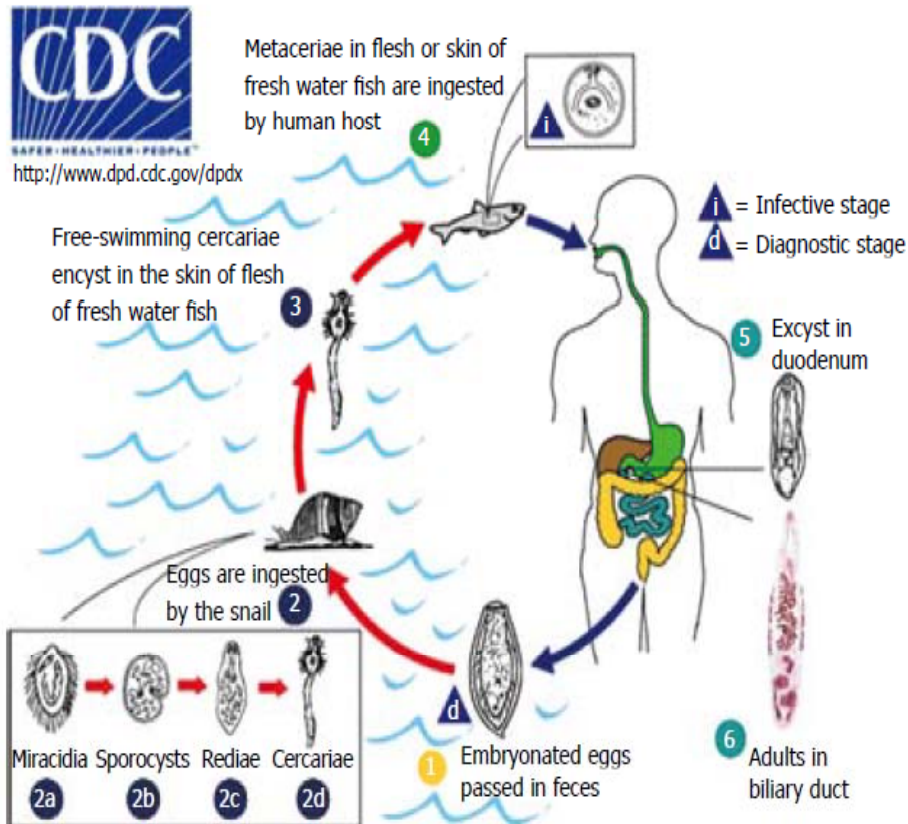


Scientific classification

- Kingdom: Animalia
- Phylum: Platyhelminthes
- Class: Trematoda
- Order: Opisthorchiida
- Family: Opisthorchiidae
- Genus: *Opisthorchis*
- Species: *O. viverrini*

Kaewpitoon et al., 2008

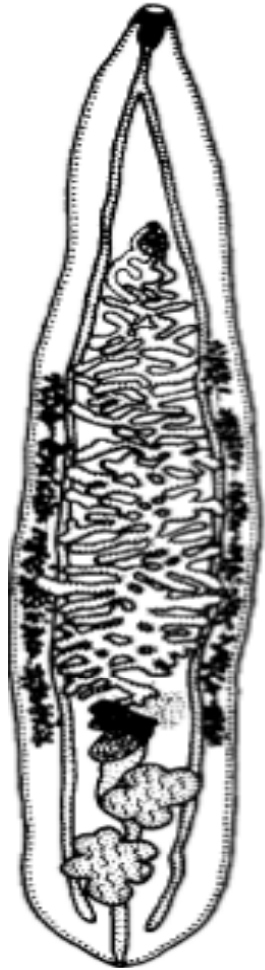
Human opisthorchiasis



Kaewpitoon et al., 2008

- Human become infected by consumption of raw or improper cooked cyprinid fish that contain infective metacercariae
- The symptom of the disease in the host is associated with inflammatory response
- Chronic infection of liver fluke might be associated with cholangiocarcinoma

Reproduction of *O. viverrini*



- The adult worm is monoecious
- Self and cross-fertilization
- Intra-uterine embryonation
- Reproductive biology at the molecular level is lacking

Ropporin 1-like protein (ROPN1L)

Ropporin 1-like protein a brief review

- A protein that participates in sperm activity, e.g. motility has been investigated
- cDNAs encoding human ropporin 1-like (ROPN1L), ropporin (ROPN), and cAMP dependent protein kinase regulatory subunit (PKA-RII α) were isolated from human testis cDNA library via yeast two-hybrid screening (Carr et al., 2001)
- Deficiency either in ROPN1L or ROPN caused a slight reduction of sperm motility (subfertility) in mice (Fiedler et al., 2013)

Ropporin 1-like protein a brief review

- The role of ROPN1L in the reproductive biology of *Opisthorchis viverrini* is still unknown
- Better understanding of the molecular biology of parasite reproduction might contribute to long term control of helminthiasis

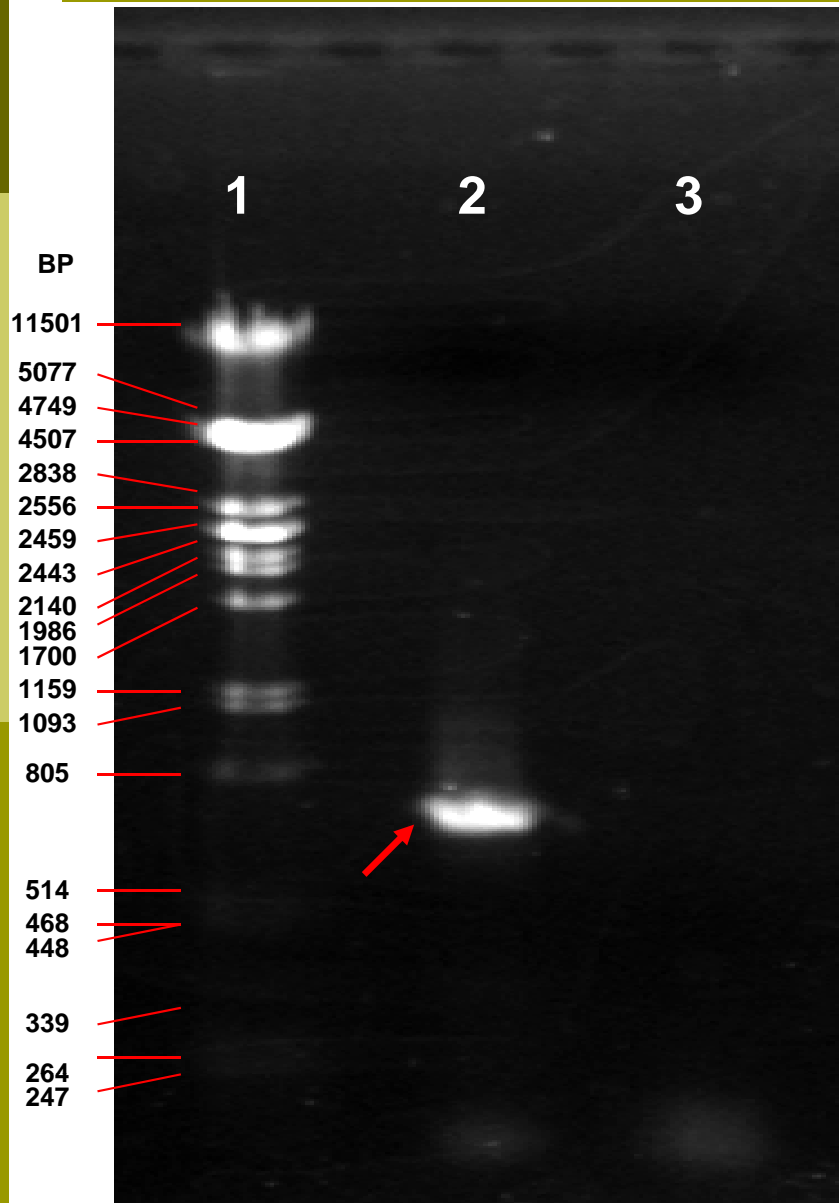
An analysis of ROPN1L in *O. viverrini* and experimental findings



Methodology

1. Molecular cloning of OvROPN1L and sequence analysis
2. Production of recombinant OvROPN1L and mouse anti-rOvROPN1L antiserum
3. Detection of native OvROPN1L in *O. viverrini* and cross-reactivity of antiserum in protein extracts of parasites and mammals
4. Analysis of OvROPN1L expression and local distribution in *O. viverrini*
5. Preliminary analysis of rOvROPN1L as antigenic molecule

Result: isolation of OvROPN1L cDNA



- Lane 1: lambda DNA / *Pst* I (marker)
- Lane 2: PCR product (670 bp)
- Lane 3: Negative control

Agarose gel electrophoresis of amplified 670 bp OvROPN1L cDNA fragment (arrow)

Result: sequence analysis

- **669 bp ORF of *OVROPN1L* cDNA => 222 amino acid residues**

Frame	from	to	Length
+1	1	668	669
-1	424	636	213
-1	223	387	165
-1	1	105	105

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ATGCCGCTTGTCAACGATCCTTATTACTGTCACGAACAGATACCTATTC
CTCCAGCACTTCCGGACATTTTGAAGCAATTTACTAAGGCCGCGATACG
AACCAGCTAAAAGATGTACTGAAGTGGTCCTACGCGTACTTTCGAGCT
TTAGCAAACAGCGAACACCTCCCGTGAAGGACCGTCTGGAGGTTCCCTA
TCAGCACACAAAAGACAGATACTGGCTTAACTCCCGGTCTCCTTCGAGT
CCTGAACAATCAGCTTGCTGTTCTGAAGACGATACCTGTAACCTTTATA
GAAGAAAATGGAAGGATTTGTCTTTGCCAATTGACCGATTTCAAGAAT
TGTGTCGTATTGAAATTTTCGTGGGCACTTGCGAATGGAGGCATTTTTT
AGCAATAGCTGCTTCAGATTTGTGTGCGACTTTAGAGGAGACACTGAAA
TTGATATGTGAGTTGCTCACAAGCGATCCAGAAGGTGGGCCAGCAAGGA
TTTCTTTCGAAACCTGGCTGGATTTCTATCGTTATTTGGGCAAGCTGGA
TGAAATTTCCGATGCGCACATCAACCACGTCATGACTTATCTCACTTTT
GATATTGCAAGCCAAGAAGGTATGATAATGCCCAGGAATTTTATGCATC
CTGAATGTCCGAAACTCAAACCACTAGACTGA
    
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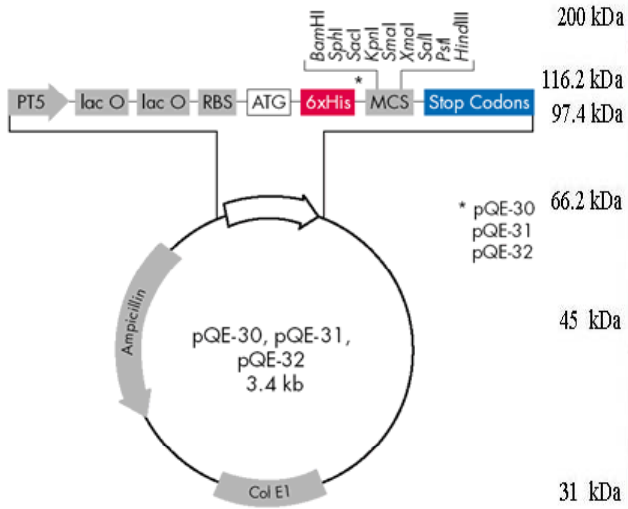
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MPLVNDPYYCHEQIP IPPALPDILKQFTKAAIRTQLKDV LKWSYAYFRALANSEPPP
VKDRLEVPISTQKTD TGLTPGLLRVLNNQLAVLKTIPVTLIEEKWKDLSLPIDRFQE
LCRIGNFVGTCEWRHFLAIAASDL CATLEETLKLICELLTSDPEGGPARI SFETWLD
FYRYLGKLDEISDAHINHVMTYLTFDIASQEGMIMPRNFMHPEC PKLKPLD
    
```

Result: sequence conservation (NCBI BLASTP)

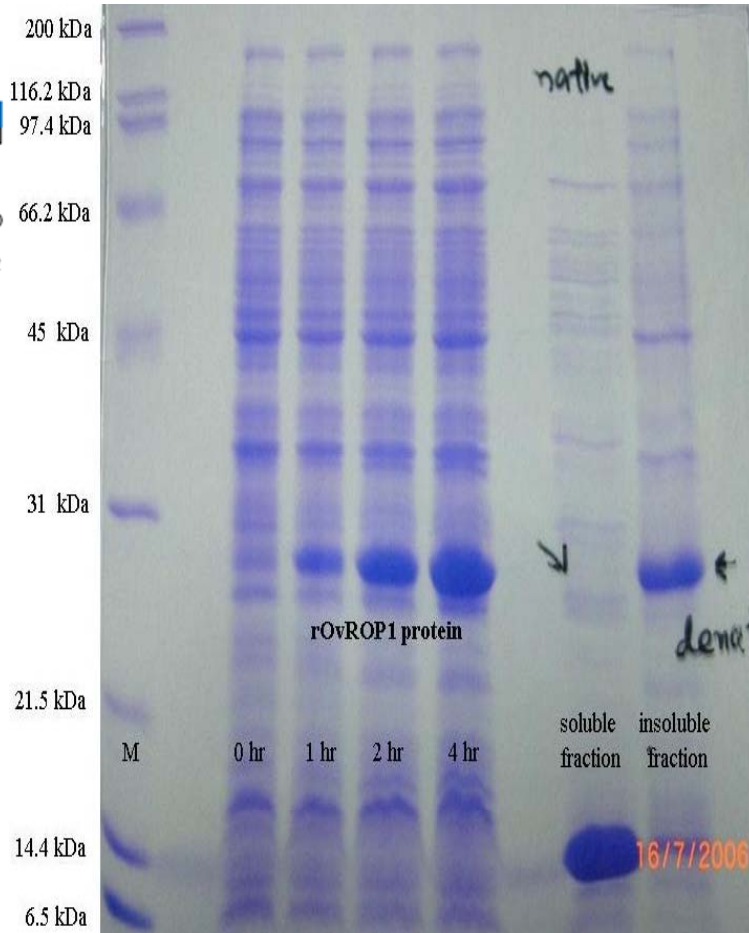
Description/ accession number	identity %	similarity %	gaps count
<i>Clonorchis sinensis</i> ROPN1L GAA55756.1	92	95	0/154
<i>Schistosoma mansoni</i> cAMP-PKA RII subunit XP_002577193.1	76	94	0/198
<i>Echinococcus granulosus</i> cAMP-PKA RII subunit CDJ18140.1	59	74	1/221
<i>Crassostrea gigas</i> ROPN1L EKC1617.1	54	73	1/215

Result: production of rOvROPN1L

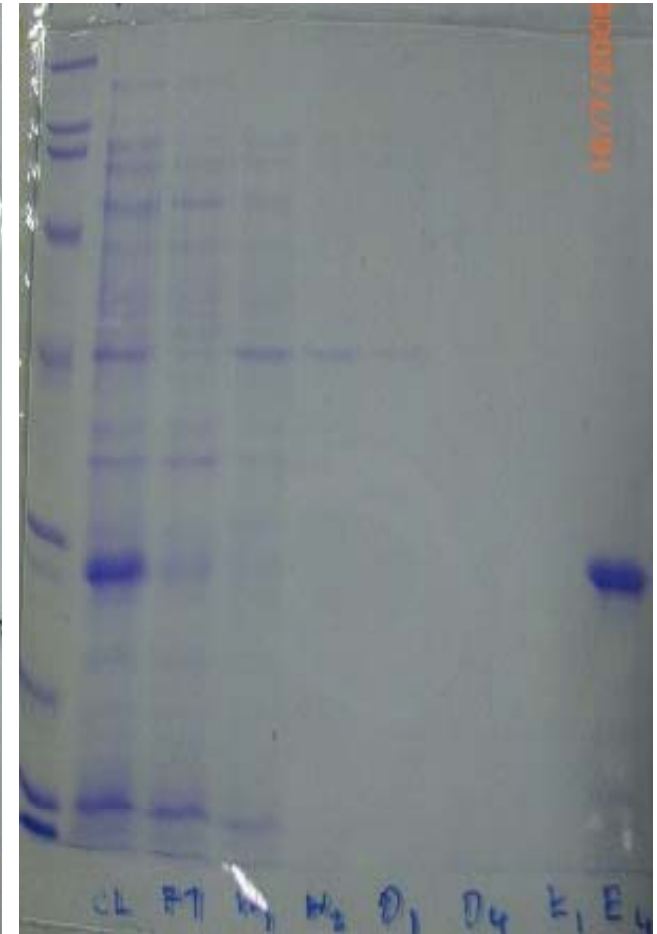


QIAGEN pQE-30, 31, 32 vectors

Bacterially expressed 28 kDa recombinant OvROPN1L was obtained

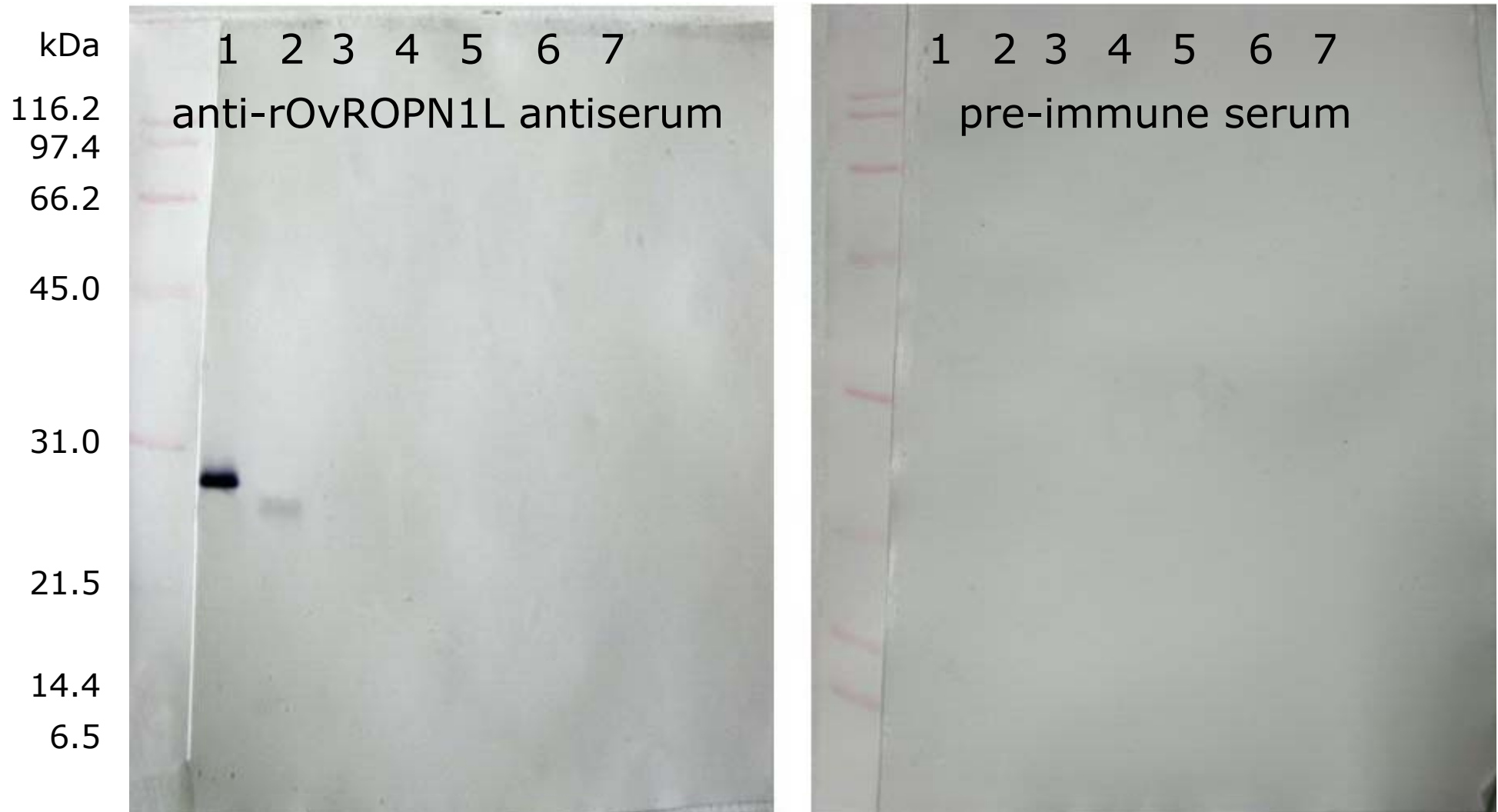


Time point expression of rOvROPN1L protein



Purification by Ni-NTA affinity chromatography

Result: Immunoblot detection of ROPN1L in *Opisthorchis viverrini* and other trematodes

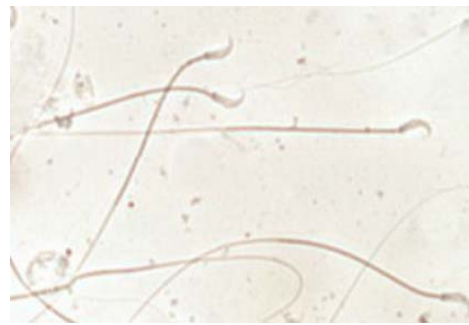


(1) rOvROPN1L, (2) Ov crude worm, (3) Ov ES product, (4) *S. mansoni*,
(5) *S. japonicum*, (6) *F. gigantica*, (7) *Paramphistomum* sp.

Result: cross reactivity of anti-rOvROPN1L antiserum to sperm proteins of mammals



Cat



Syrian hamster

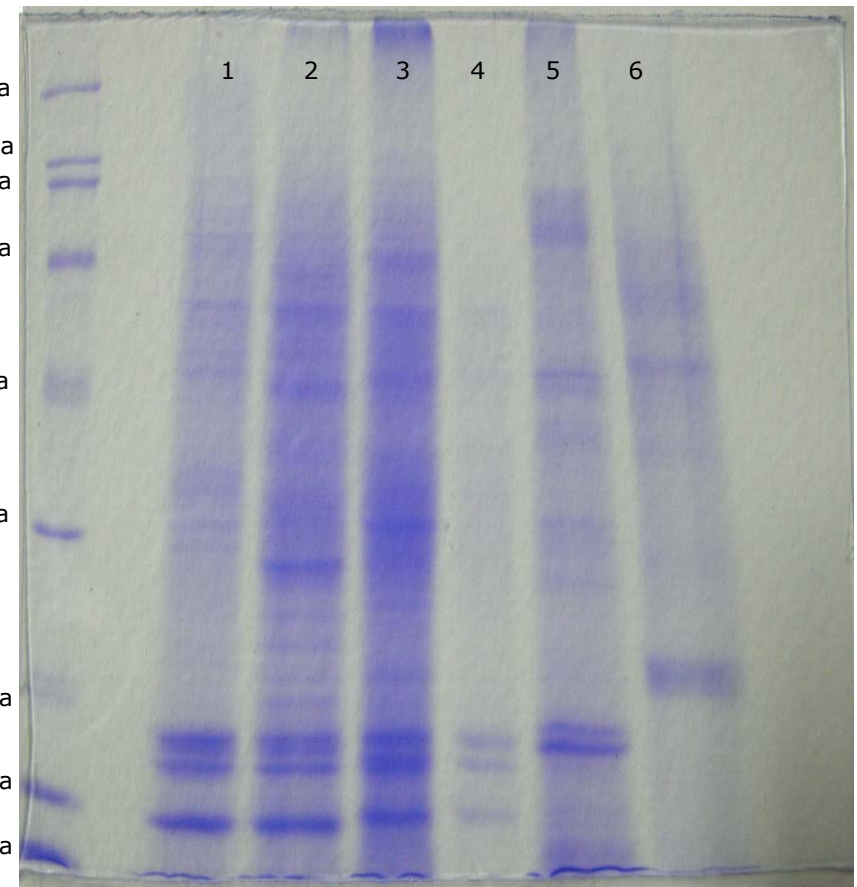


ICR mouse



Wistar rat

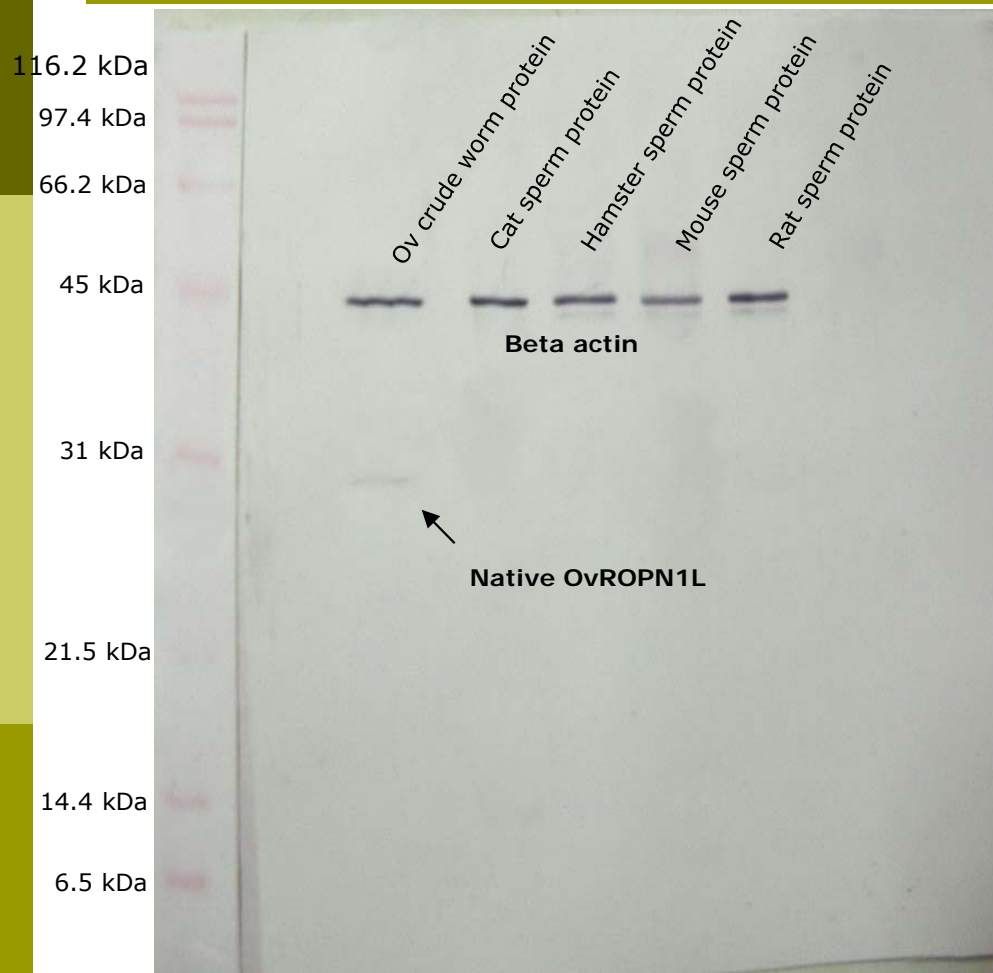
200 kDa
116.2 kDa
97.4 kDa
66.2 kDa
45 kDa
31 kDa
21.5 kDa
14.4 kDa
6.5 kDa



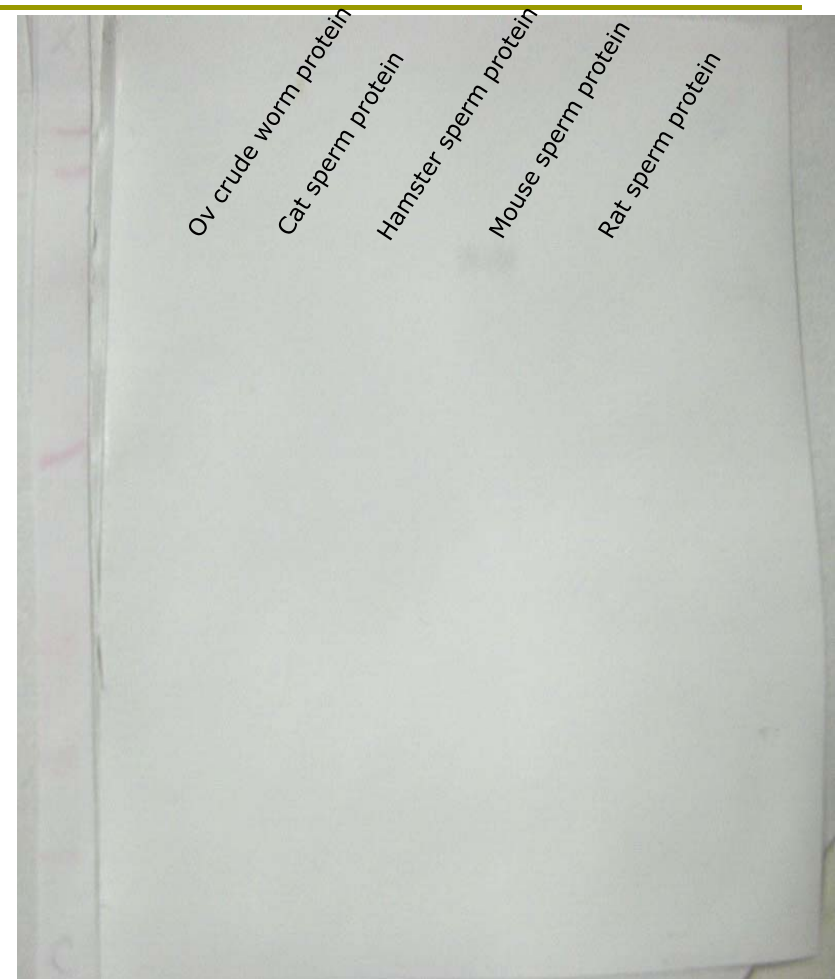
1. Ov crude worm protein
2. Cat sperm protein
3. Hamster sperm protein
4. Mouse sperm protein
5. Rat sperm protein
6. Extracted protein from hamster infected by Ov

Magnification of sperms: 400X

Result: cross reactivity of anti-rOvROPN1L antiserum to sperm proteins of mammals

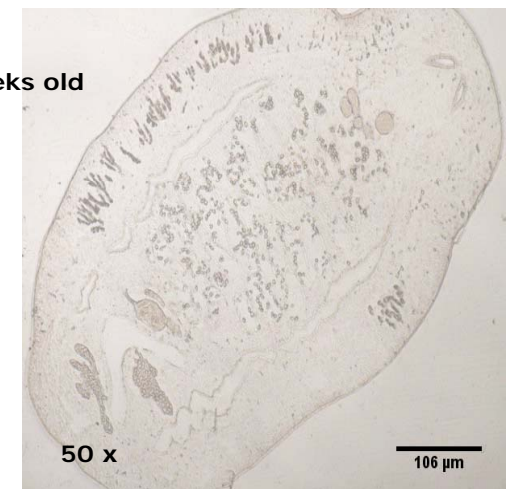
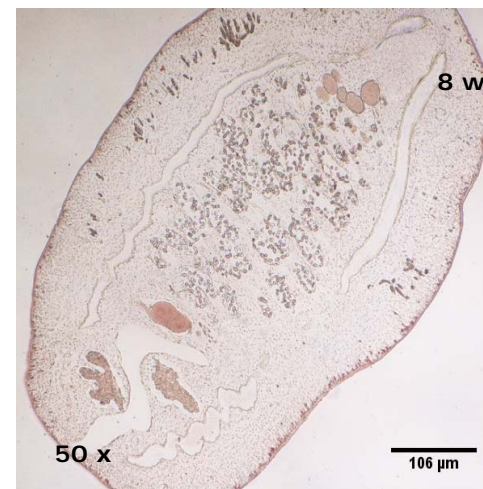
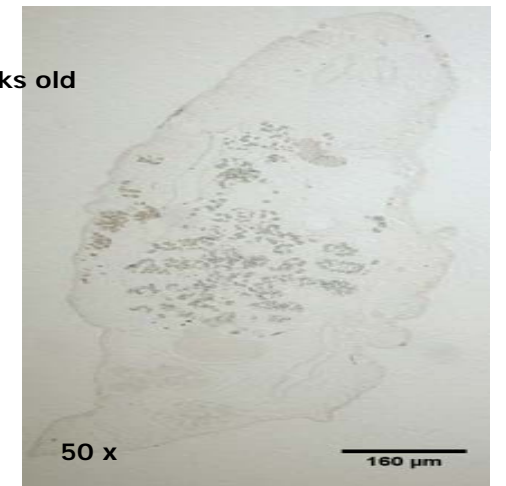
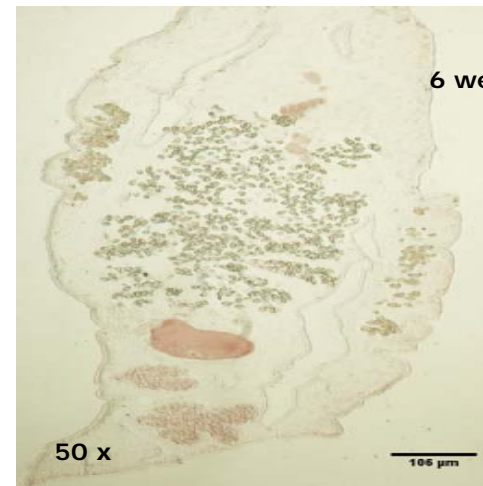
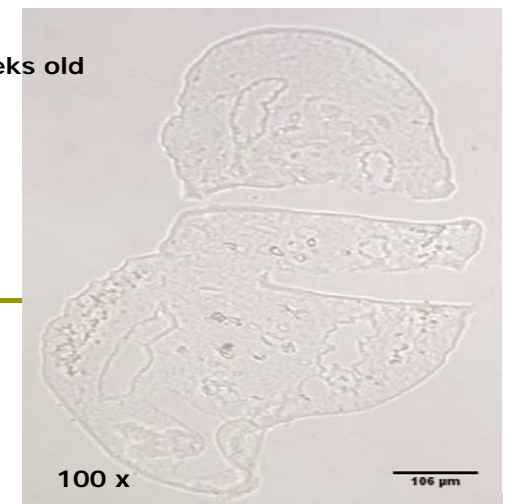
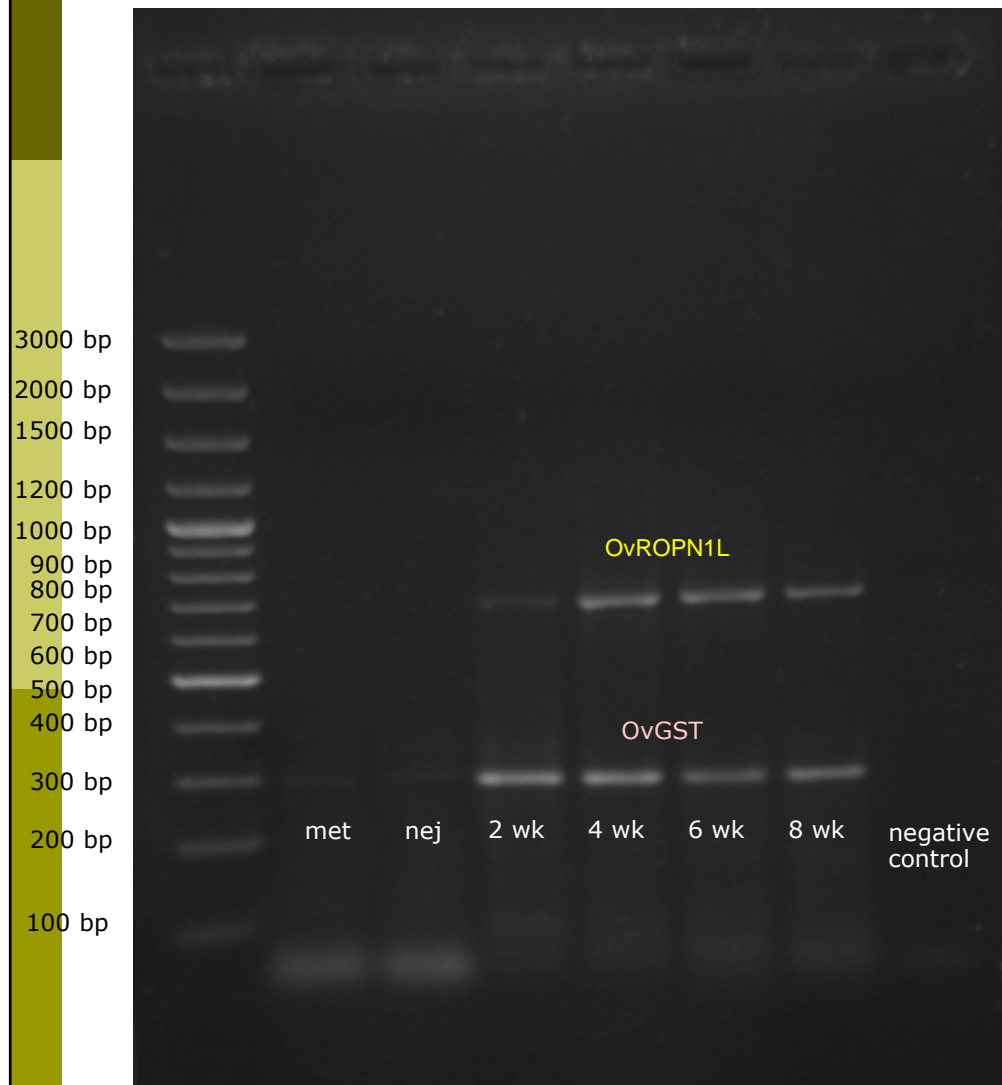


Proteins probed with mouse anti-rOvROPN1L antiserum and mouse monoclonal anti-beta actin (Sigma)



Proteins probed with mouse normal serum

Result: Analysis of native OvROPN1L expression and its local distribution

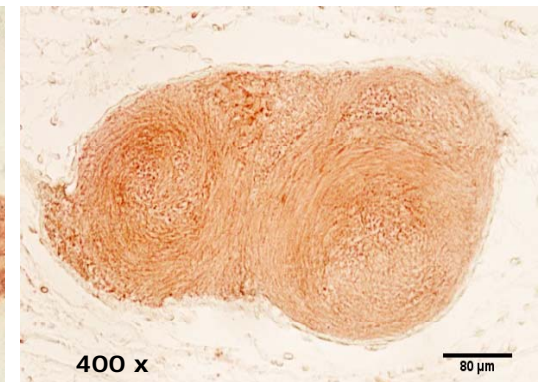
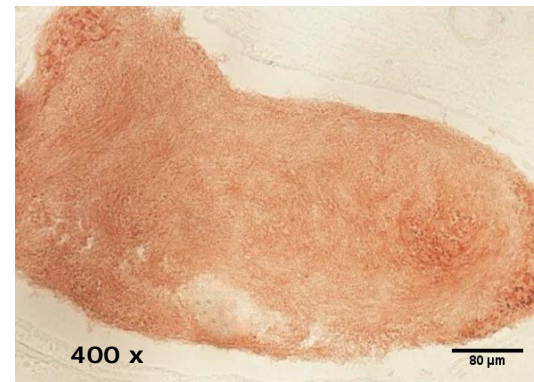
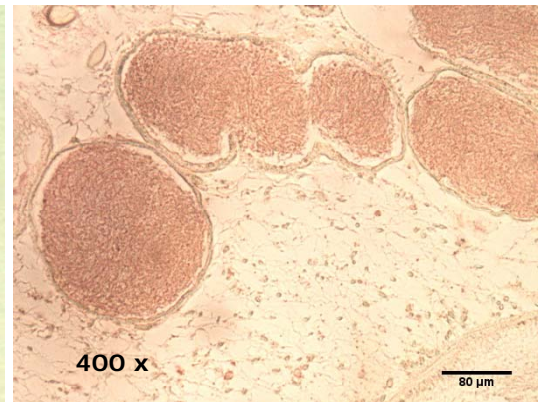
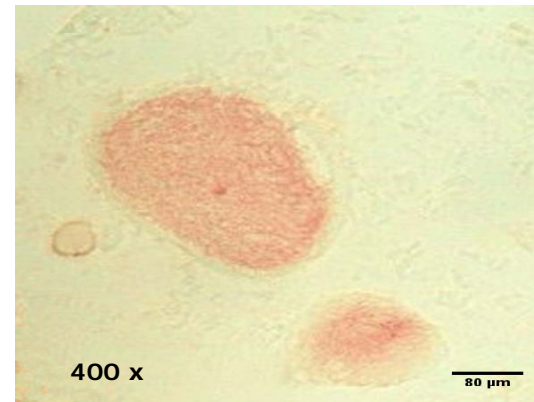
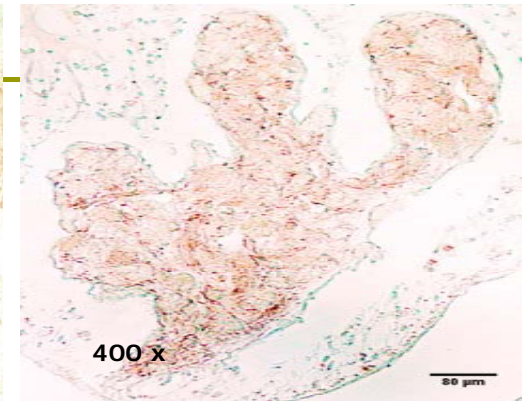
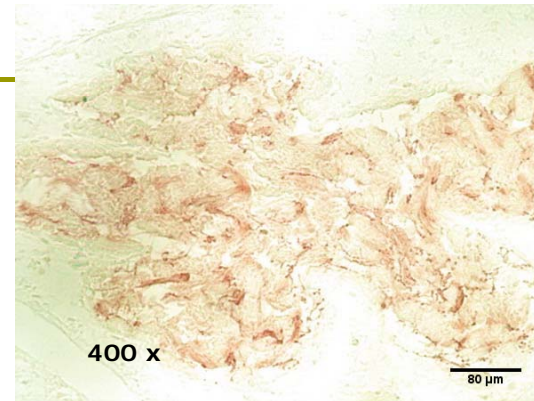
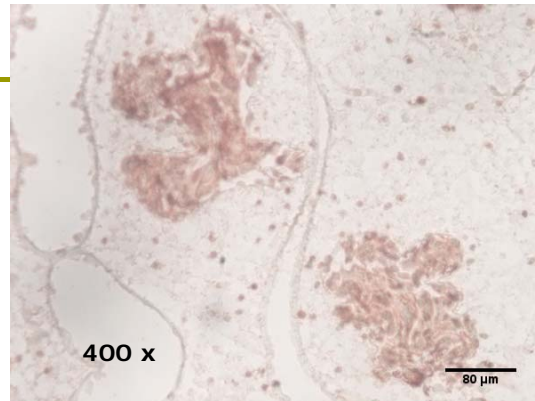


Result

2 wk

6 wk

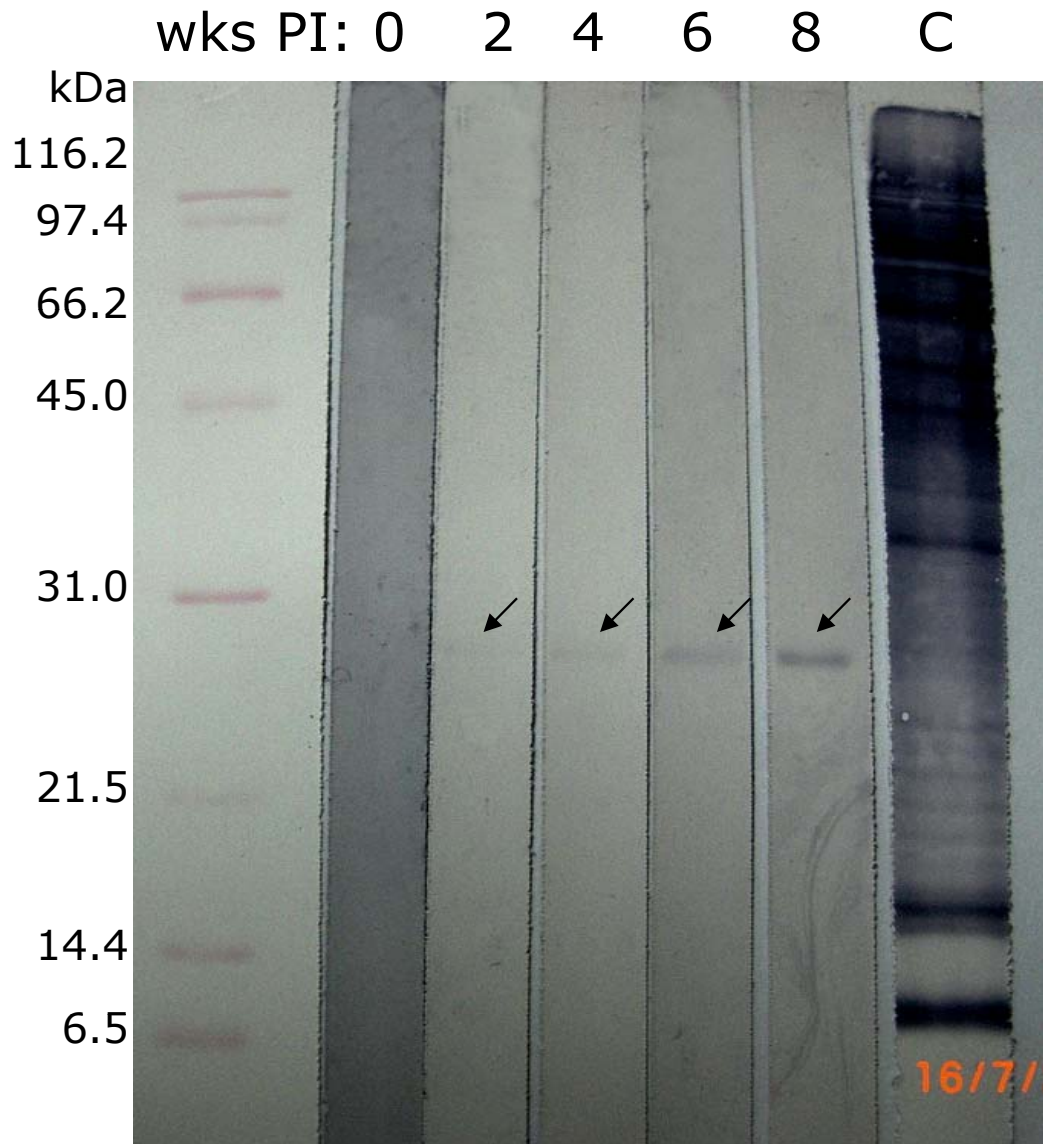
8 wk



Stage related distribution of OvROPN1L

	Testis	Seminal vesicle	Seminal receptacle
2 weeks old	X		
6 weeks old	X	X	X
8 weeks old	X	X	X

Result: preliminary analysis of rOvROPN1L protein as antigenic molecule



- Immunoblot analysis revealed the antigenicity of rOvROPN1L during during infection in hamsters
- The immune response of experimental infected Syrian hamsters to rOvROPN1L is gradually increased upon duration of *O. viverrini* infection

Discussion and conclusion

- ❑ OvROPN1L can be detected in *O. viverrini* from the juvenile to the adult stage
- ❑ OvROPN1L is restricted to the sperm cells of the parasite
- ❑ OvROPN1L might be an antigenic molecule in the host
- ❑ The results suggest that OvROPN1L contributes to the male reproductive system of *O. viverrini*, however, details of its function in the parasite are still unknown

References

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Thank you for your
attention

