A NEW SET OF BIOLOGICAL SAMPLES FOR MEASURING VITELLOGENIN (VTG) IN THE JAPANESE MEDAKA (*ORYZIAS LATIPES*)

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Introduction

For the assessment of endocrine disrupting chemicals (EDC), the determination of the precursor egg yolk protein vitellogenin (VTG) is widely applied (OECD TG 234 and 229). Regarding the usage of the Japanese medaka (*Oryzias latipes*), the following improvements were addressed:

- Replacement of time-consuming liver sampling
- Evaluation of epidermal mucosa as non-destructive sample matrix
- Kinetic monitoring of endocrine disrupting agents

Materials & Methods

- Semi-static exposure to 500 ng/L 17β-estradiol (E_2) for 168 h
- Gene expression analysis (RT-PCR) in different tissue samples
- SDS-PAGE of different tissues of *O. latipes*
- Western blot and immunostaining with anti-VTG antibodies
- Mass spectrometric analysis via MALDI-TOF of immunoreactive bands
- Peptide mass fingerprint analysis with MASCOT search engine
- Quantification of VTG in different tissues of males and females with a highly sensitive sandwich ELISA

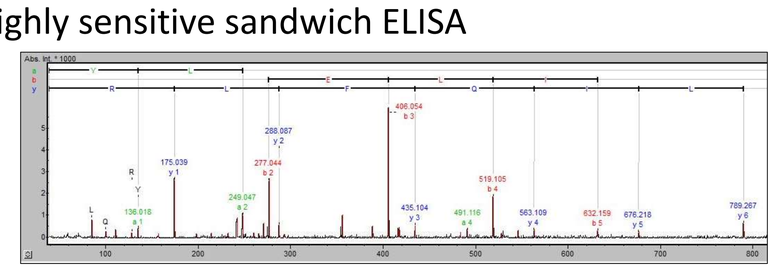

Figure 1: De novo sequencing of a VTG correlated peptide

Results & Discussions

- Immunoreactive bands were detected in samples of untreated female and exposed fish
- VTG was identified ($p<0.05$) in bands of serum and skin of exposed specimens
- Exogenously-triggered, *in situ* VTG synthesis in the brain and skin confirmed by different methodological approaches
- Distinct differences in VTG contents in several tissues of male and female medaka measured with ELISA

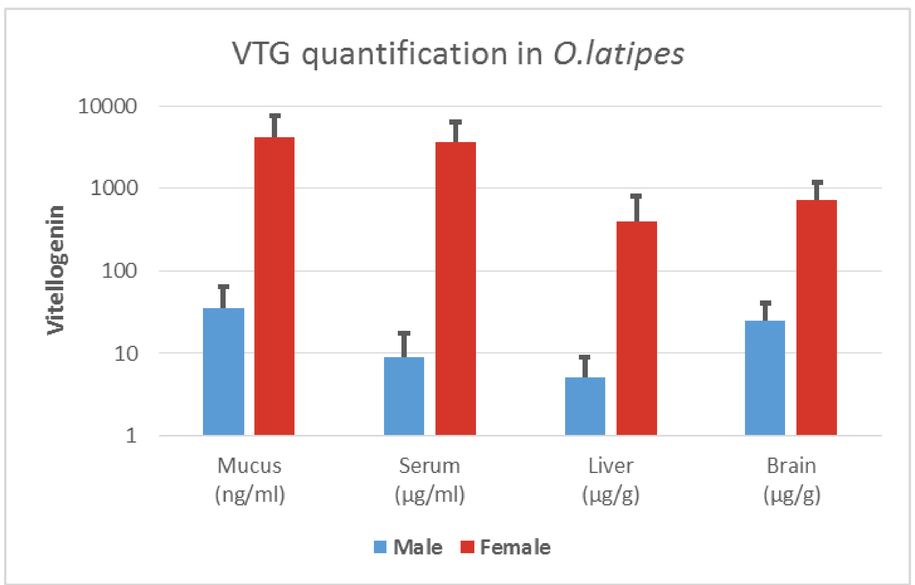

Figure 3: Quantification of VTG in different matrices of medaka

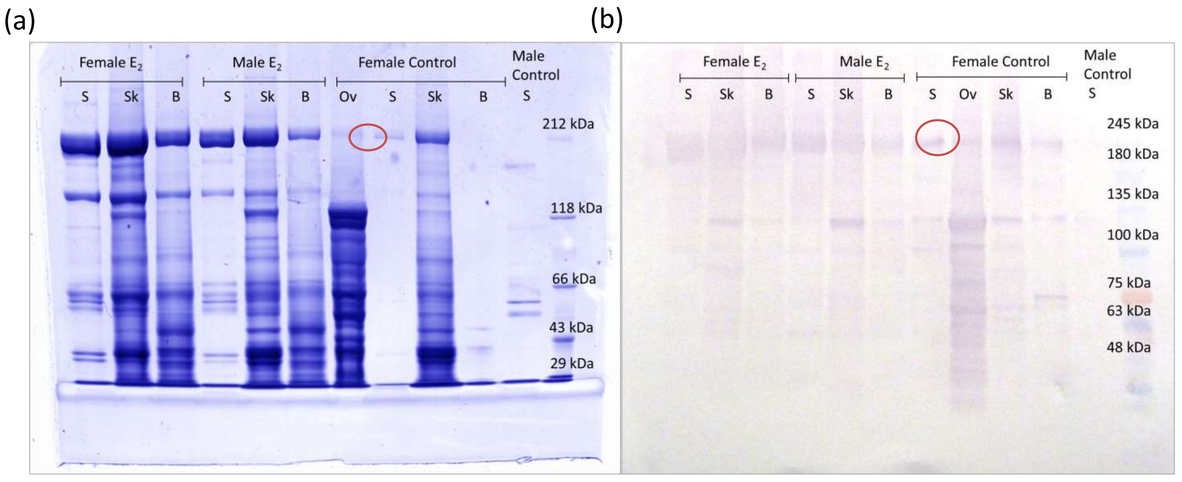

Figure 2: (a) Coomassie stained gel; (b) immune-stained Western blot

Sample	Taxonomy	Missed cleavage sites	Score	Expectation value p	Top protein identified
Female Serum	Other Actinopterygii	1	136	3.1e-08	Vitellogenin precursor 1 (Oryzias latipes)
Female Skin	Other Actinopterygii	1	79	0.015	Vitellogenin precursor 1 (Oryzias latipes)
Male Serum	Other Actinopterygii	1	68	0.19	Vitellogenin precursor 1 (Oryzias latipes)
Male Skin	Other Actinopterygii	1	85	0.0041	Vitellogenin precursor 1 (Oryzias latipes)

Table 1: Results of peptide mass fingerprint analysis with MASCOT search engine from specimen exposed to E_2. Red marker indicates a non significant match

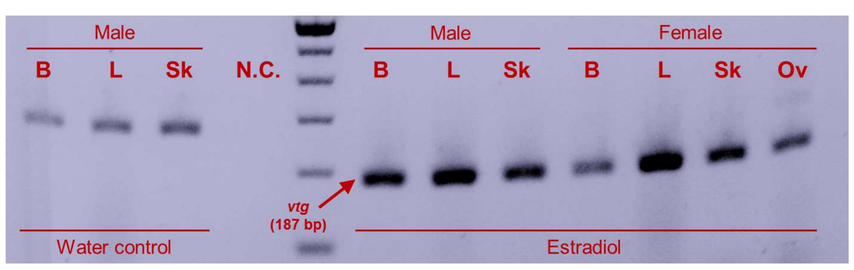

Figure 4: Detection of PCR products of expressed vtg in different tissue samples

Abbreviations of Samples:
S= Serum B= Brain;
L= Liver; Sk= Skin;
Ov= Ovary

Conclusions

- Verification of estrogen-dependent VTG in skin and brain
- Exogenously-triggered VTG production in brain and skin
- Independent identification of VTG by different methods lead to strong evidence for VTG presence.
- Skin mucus and brain are suitable biological samples for VTG measurement in medaka

Acknowledgements

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All procedures described herein were performed in full compliance with German and European Laws, with authorization numbers RTK / Anz. 1000 and RTK / 1001.