Carp (*Cyprinus carpio*) Vitellogenin: Characterization of Yolk Proteins, Development of Immunoassays and Use as Biomarker of Exposure to Environmental Estrogens

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The precursor protein of egg yolk, vitellogenin (Vg), is cleaved into three major components (lipovitellin, phosvitin and β'-component) at the time of incorporation by growing oocytes. We purified three yolk proteins (YP1, YP2 and YP3) from ovaries of the common carp (*Cyprinus carpio*) by a combined method of ammonium sulfate precipitation and column chromatography. Biochemical analyses of the purified proteins of this species suggest that YP1, YP2 and YP3 are lipovitellin, β'-component and phosvitin, respectively. A specific antiserum against purified carp YP1 (lipovitellin) was used to develop a single radial immunodiffusion (SRID) technique and an enzyme-linked immunosorbent assay (ELISA) for carp Vg. By SRID and ELISA, we measured the circulating carp Vg level to be in the ranges of 12.5–400 μg/ml and 2.0–1000 ng/ml, respectively, which cover a wide range of Vg levels. From 1997–1998, male and female carp were captured at points of effluent discharge from a sewage treatment plant connected to the Tama River, where estrogenic compounds were later detected, and the presence of Vg in their circulation was examined. Vg was detected in both male and female carp at the mg/ml level, suggesting that estrogens such as estrone and estradiol were sufficiently high to induce Vg in male carp inhabiting this area. The result of this study supports the use of carp Vg as a biomarker of fish exposure to environmental estrogens.