0.45 µm). After drying, the membrane was incubated in Licor Blocking buffer (927-40100) for 1h at room temperature with agitation. After addition of 0.2% Tween-20, the membrane was incubated with the polyclonal antibody against vitellogenin in a 1:7000 dilution (Davids Biotechnologie Regensburg) for 1 h at room temperature with agitation. After three washes with PBS-T (0.2% Tween 20) the membrane was incubated for 45 min at room temperature in secondary antibody solution (IRDye® 800CW Goat anti-Rabbit IgG (H + L),1:40.000 in Licor blocking buffer diluted 1:1 with PBS-T). After 3 washes with PBS-T (0.2% Tween 20) the vitellogenin specific signals were acquired at 800 nm using the Licor Odyssey® CLx Imaging System.

*SDS Polyacrylamide gel electrophoresis*

SDS polyacrylamide gel electrophoresis was run according to the protocols on the Licor home page (https://www.licor.com/bio/applications/quantitative_western_blots/protocol.htm) with slight modifications. In brief, one-dimensional gel electrophoresis was carried out in vertical polyacrylamide gels (10.1 x 7.3 x 0.1 cm) containing 0.1% SDS with a 4% stacking gel on top of the separating gel. Samples were diluted with 4x concentrated Orange G sample buffer for Licor blots (250 mM Tris-HCl, pH 6.8, 12% SDS, 50% Glycerol, 6% 2-mercaptoethanol, 0.2% Orange G), heated for 5 min at 95°C and subjected to electrophoresis at constant voltage (140 V) for 20 min and 190 V until the dye front has run out. Two types of one-dimensional gels were run (10% and 7.5% polyacrylamide/ 0.1% SDS gels) for the separation of proteins in the range of 30–200 kDa, with the later found to be superior for separation of the two vitellogenin forms.

*Protein extraction and digestion*

The extracted proteins were resolved on one‐dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a pre-cast gradient gel (4 - 20% polyacrylamide,

Mini-Protean Tgx, Bio-Rad Laboratories, Switzerland). The gel was stained with Coomassie Blue G-250 (Bio-Safe Coomassie Stain, Bio-Rad Laboratories, Switzerland). The visualized gel bands were then cut into 5 slices per lane and further cut up into smaller pieces. The In-gel digestion was done according to Shevchenko et al. (2006). Briefly, the gel pieces were dehydrated and washed. Disulfide bonds were reduced with 10 mM dithiothreitol, followed by an alkylation step using 55 mM iodoacetamide. The samples were digested with trypsin (Sequencing Grade modified trypsin, Promega, Switzerland) at 37°C overnight. The resulting peptides were extracted using 100 µL of 5% formic acid/ acetonitrile (1:2, v/v) followed by a second extraction step using 20 µL 1% formic acid/ acetonitrile (1:1, v/v). Both supernatants were collected into a 250 µL vial and dried to dryness with a vacuum centrifuge (GeneVac EZ-2 Plus, SP Scientific, UK). The peptide mixture was re-dissolved with 30 µL of 0.1% formic acid and analysed by liquid chromatography coupled to a tandem mass spectrometer.

*LC-MS/MS analysis*

An Agilent 1290 Infinity LC system connected to an Agilent 6540 quadrupole time-of-flight mass spectrometer (Agilent Technologies, Switzerland) was used. The peptide mixtures (12 µL) were injected onto a reversed phase C18 column (2.1 x 150 mm, 2.7 micron, 120 Å pore size, Agilent Technologies, Switzerland). The column flow was set to 400 µL/min with a mobile phase composed of solvent A (water) and B (acetonitrile with 0.1% formic acid). Initially, 2.5% of mobile phase B was held constant for 1 min, followed by a linear gradient from 2.5% – 35% B over 90 min, and a final increase over 2 minutes to 95% B where it was held for 2 min. Re-equilibration of the initial column condition was performed within 6 minutes. Column temperature was held constant at 45°C through the run. The jet stream electrospray source was operated in positive mode with following parameter settings: nebulizer pressure 35 psig, nozzle voltage 0 V, sheath gas flow 12 L/min, sheath gas temperature 250°C, drying gas flow 10 L/min, drying gas temperature 250°C, capillary voltage 4000 V and fragmentor