Report from STSM entitled "Isolation of spermatogonia and transplantation into larvae between two sturgeon species"

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Due to complications with transport of sturgeon larvae, the aim of stay was slightly changed and instead of sturgeon we were establishing isolation and transplantion of European eel spermatogonia into zebrafish larvae in order to record development of germ cells in host. The procedure was conducted according to following protocol.

Testes was removed from two immature European eel males, washed with PBS, placed on a Petri dish and minced by scissors. The suspension was placed into a tube with 0.2 % trypsin, and 40 µg mL-1 DNAse (AppliChem A3778) in PBS (approx. 10 ml of medium per 0.1 g of tissue). Incubation run for 2 h, with gently shaking, in room temperature. The obtained suspension was filtered with 50 µm filter (Partec, Germany). Final concentration 1% BSA (Sigma-Aldrich A7511) was added to protect cells against trypsin. The suspension was loaded onto 30% percoll suspension in 15ml tubes and centrifuged at 400 x g for 30 min. The cell suspension was collected from the percoll layer, mixed with PBS and centrifuge again. The pellet is resuspended in approx. 0.3 mL of PBS. The cells was briefly examined under a microscope. Cells were loaded into a glass needle, and injected near the presumptive genital ridge (body cavity) of the host, zebrafish, using a micromanipulator and microinjector. The cells were labeled using the PKH26 Red Fluorescent Cell Linker Kit for general cell membrane labeling according to the manufacturer's protocol. The cells are loaded into a glass needle, and injected near the presumptive genital ridge (body cavity) of the host, zebrafish, using a micromanipulator and microinjector.

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