ISOLATION OF STURGEON PRIMORDIAL GONOCYTES AND SPERMATOGONIA AS MATERIAL FOR BIOTECHNOLOGY

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Introduction:

Most of sturgeons belong to endangered or critical endangered species (The IUCN Red List of Threatened Species) mostly due to caviar and complications with their reproduction characteristics such as late maturation. Sturgeon gametes are unique in term of their morphology and physiology. Sturgeon eggs covered with very thick layers are perforated by tens of micropyles at animal pole, while their spermatozoa are equipped with acrosomes, which fish spermatozoa usually lack. It was suggested that the sturgeon acrosome serves like an anchor and a spear [1]. Genetic and cytogenetic manipulations with sturgeons are very difficult. Therefore it would be very suitable to develop a more efficient system of sturgeon reproduction by means of biotechnology and micromanipulations with germ line cells and embryos. Primordial germ cells (PGCs) or subsequently spermatogonia are the only cells in developing embryos with potential to transmit genetic information to the next generation. Therefore they have a potential to be of value for gene banking and cryopreservation, particularly via the production of donor gametes with germ–line chimeras [2]. Nevertheless the first step for successful transplantation of these germ line cells is their visualization and isolation.

Methods:

Visualization of PGCs is usually done *in vivo* according to Saito et al. [3] using injection of GFP-zebrafish nos1 3'UTR mRNA to the area of egg with PGC precursor localization.

Results:

In our study electron microscopic observation revealed that only the vegetal pole of sturgeon egg contains a germ plasm-like structure, nuage (Fig. 1), which are suggested to be the precursors of germinal granules, therefore an injection of the fluorescent probe for PGCs visualization was applied into the vegetal pole in 1-4 cell stage embryo, while the embryos were incubated at 14°C. The PGCs were then localized using fluorescence stereomicroscope between gut and

Fig. 1 shows sturgeon egg envelope (E), mitochondria (M), cortical granule (C), yolk (Y) and nuage (arrow).

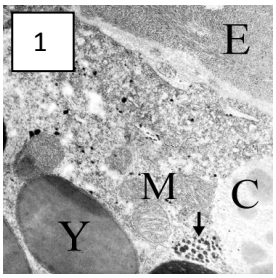


Fig. 2 shows PGCs (arrow) in sturgeon embryo after hatching.

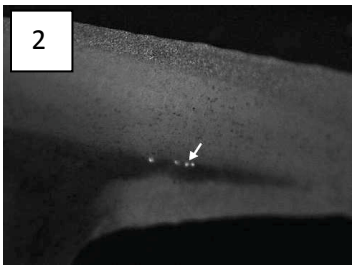
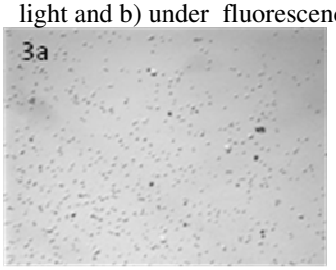


Fig. 3 shows isolated spermatogonia from layers of 10-30 % percoll, stained with antispermatogonia antigen 1, a) under light and b) under fluorescence.



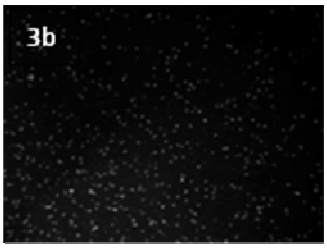
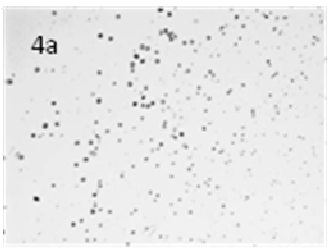
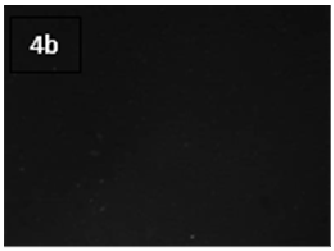


Fig. 4 shows cells from layers of 30-50 % percoll, a) under light and b) under fluorescence.





pronephric duct in the base of yolk extension region at 5th day after fertilization. The cluster of PGCs then migrated to the posterior region of yolk extension. The PGCs were later spread along the all yolk extension from 6th to 13th day (Fig. 2).

Spermatogonia could be an alternative of PGCs for biotechnology. They were isolated from immature testes of 2-4 years old sturgeon males, which did not produce any spermatozoa. The testes was cut into small pieces and incubated in 0.2% collagenase for 2 hours. The obtained homogeneous suspension was filtered through 50 µm filter. The cell suspension was then sorted by percoll gradient (10, 15, 20, 25, 30, 35, 40, 50 %) and stained with antispermatogonia-specific antigen 1 originally made for Japanese eel by Kobayashi et al. [4] combined with Anti-Rabbit IgG–FITC antibody. The cells obtained from layer 10-30% of percoll solution showed the specific fluorescent signal and shape of spermatogonia. The layers below contained cells without almost any signal with spermatocyte-like size (Fig. 3).

The isolated germ-line cells can be then cryopreserved according to Okutsu et al. [5]. To make germ-line chimeras, the PGCs or spermatogonia are isolated from donor and transplanted into the host embryos. The hosts become germ-line chimeras if the transplanted cells successively migrate to genital primordium and differentiate into functional gametes. Donor genotypes can be then restored in the next generation. Currently, the methodology is quite well elaborated for teleost species, but almost nothing was done on sturgeon. We suggest that the long generation interval of sturgeon might be extremely shortened if species that mature earlier are used as the surrogate host and produce donor gametes.

Acknowledgements:

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