# Hoechst 33342によるキンギョの雌性前核の顕在化


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**Short paper**

# Visualization of Female Pronucleus in the Goldfish *Carassius auratus* using Fluorescent Dye, Hoechst 33342

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Recently, nuclear and gene transfer techniques have been widely applied to animal eggs for embryological and genetic studies. Fish eggs, however, have been seldom used because of their tough and opaque chorion. There are many particles of various size in the ooplasm of the goldfish and it is very hard to distinguish pronuclei, even after removal of the chorion.[1]

Hoechst 33342 is a nontoxic vital stain specific to DNA.[3] It has been used to visualize pronuclei or to distinguish the paternal nucleus from the maternal one in bovine and porcine eggs with dense cytoplasm similar to that of goldfish.[2,3] In this paper we report the visualization of the female pronucleus in goldfish using this dye.

*Procedure*

(1) Stock solution (1 mg/m*l*) of Hoechst 33342 (Sigma) was diluted with modified Woynarovich's solution (0.4% urea and 0.5% NaCl) at 10 $\mu$g/m*l*.

(2) Stripped eggs from ovulated goldfish were activated in this solution and left for 10 min.

(3) Activated eggs were dechorionated by treatment with 0.1% trypsin-0.4% urea-Ringer's solution (pH 7)[1] for about 10 min.

(4) Denuded eggs were rinsed two times with Ringer's solution and observed under a fluorescence microscope (Olympus model BH-RFL). Excitation was induced at 334 or 365 nm.

There were a few flucrescent particles of various size on the surface of the denuded eggs just after dechorionation. It was not clear whether or not these particles were polar bodies released from the eggs. In the cytoplasm, however, there were no fluorescent objects just under the fluorescent particles on the egg surface. We could not specify pronuclear location without doubt in eggs which did not form their blastodisk just after dechorionation.

Forty minutes after activation, a fluorescent particle was clearly recognized in the germ disk of one-cell eggs. As only one particle was found in each germ disk, it was assumed that this particle was the female pronucleus, which was not detectable under a light microscope (compare *a* and *b*, or *c* and *d* in fig. 1). Occasionally, the polar body was observed on the egg surface (Fig. 1-*c* and -*d*).

The pronucleus and the polar body were not distinguishable in eggs with chorion, because of opaqueness and fluorescence originating from the perivitelline space.

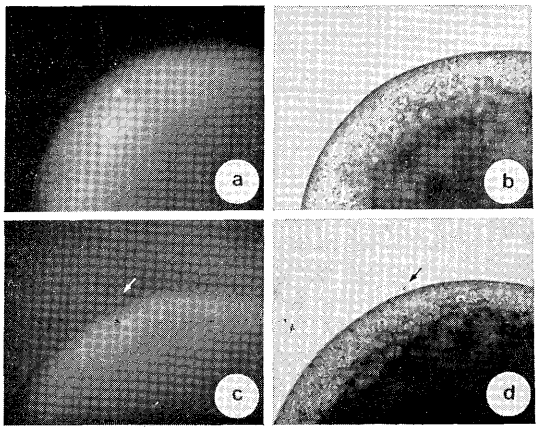


**Fig. 1.** Visualized female pronuclei with Hoechst 33342 (*a* and *c*). Light microscopic appearances (*b* and *d*, corresponding to *a* and *c*, respectively). Arrows indicate the polar body existing on the egg surface in *c* and *d*.

It has not been confirmed whether this staining and exposure to ultraviolet light are harmful for further development of normally fertilized eggs. It was suggested that ultraviolet excitation after exposing to Hoechst 33342 detracted from the continued development of murine embryos.[2] Microinjection of DNA into the pronucleus of fish eggs may become easier by this technique. Detailed studies, however, will be required to clarify the effect of the fluorescent dye and the ultraviolet rays on the development of fertilized eggs in fish. In the case of pronucleus removal for nuclear transplantation experiments, such ultraviolet effect may be of little consequence.

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