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Advantages

Adult zebrafish are small in size and breed readily

Embryos can be genetically manipulated

Easy to house and care

The eggs are externally fertilised and the embryos develop quickly

In laboratory

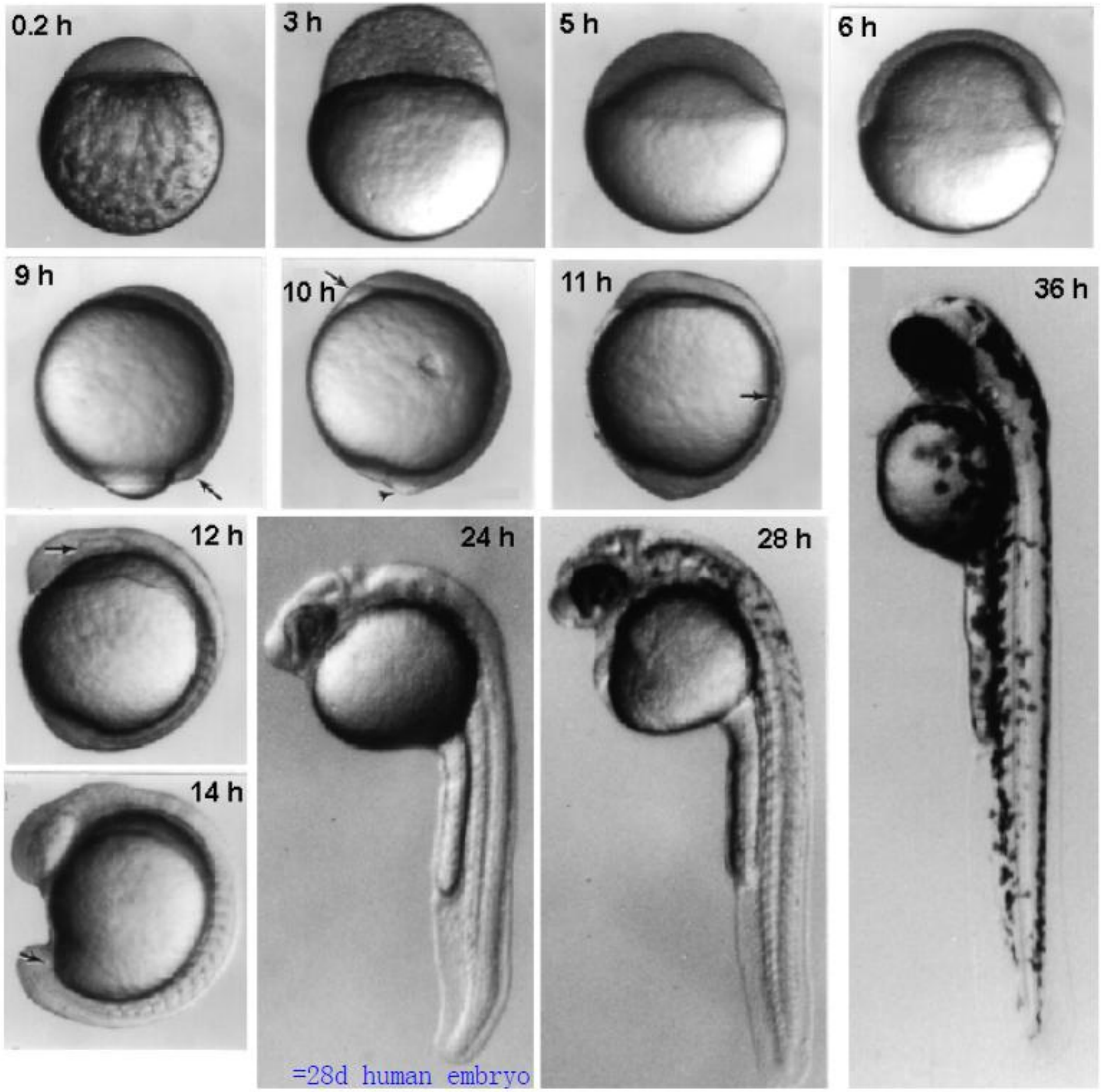
It is small and robust

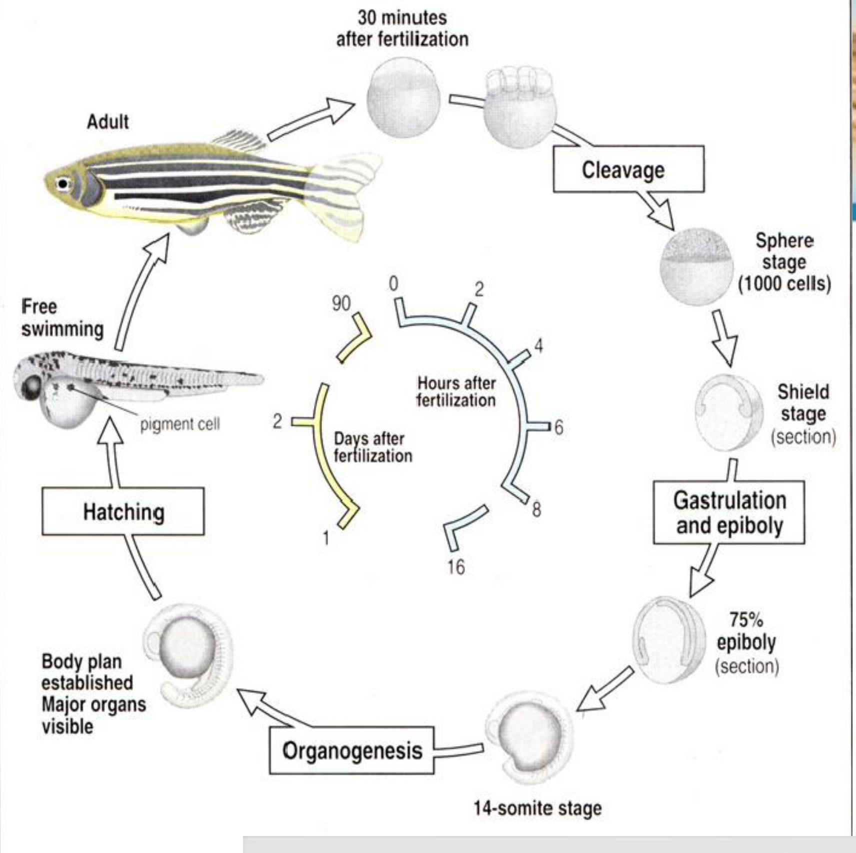
Low cost

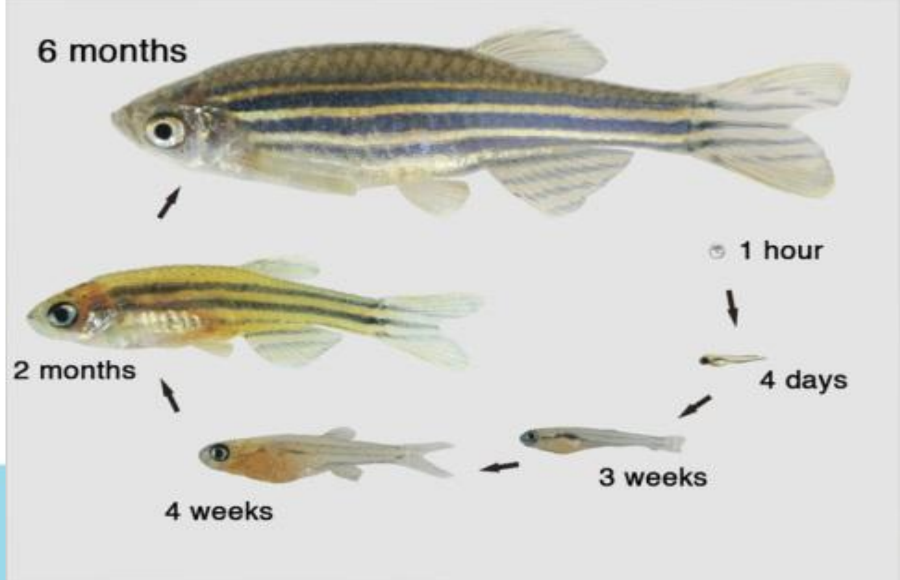
Transgenic lines

Larger number of offspring in each generation

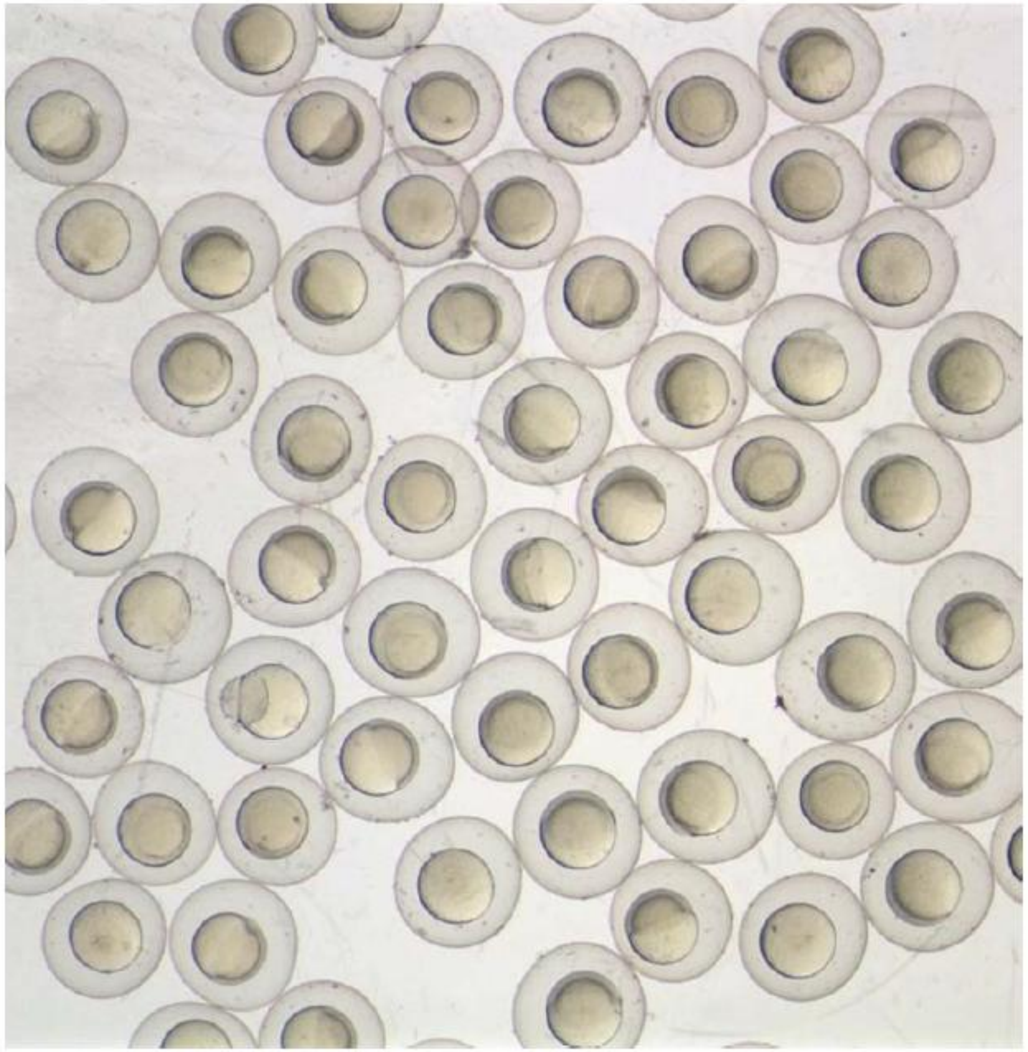
1. Rapid development







2. High reproductivity

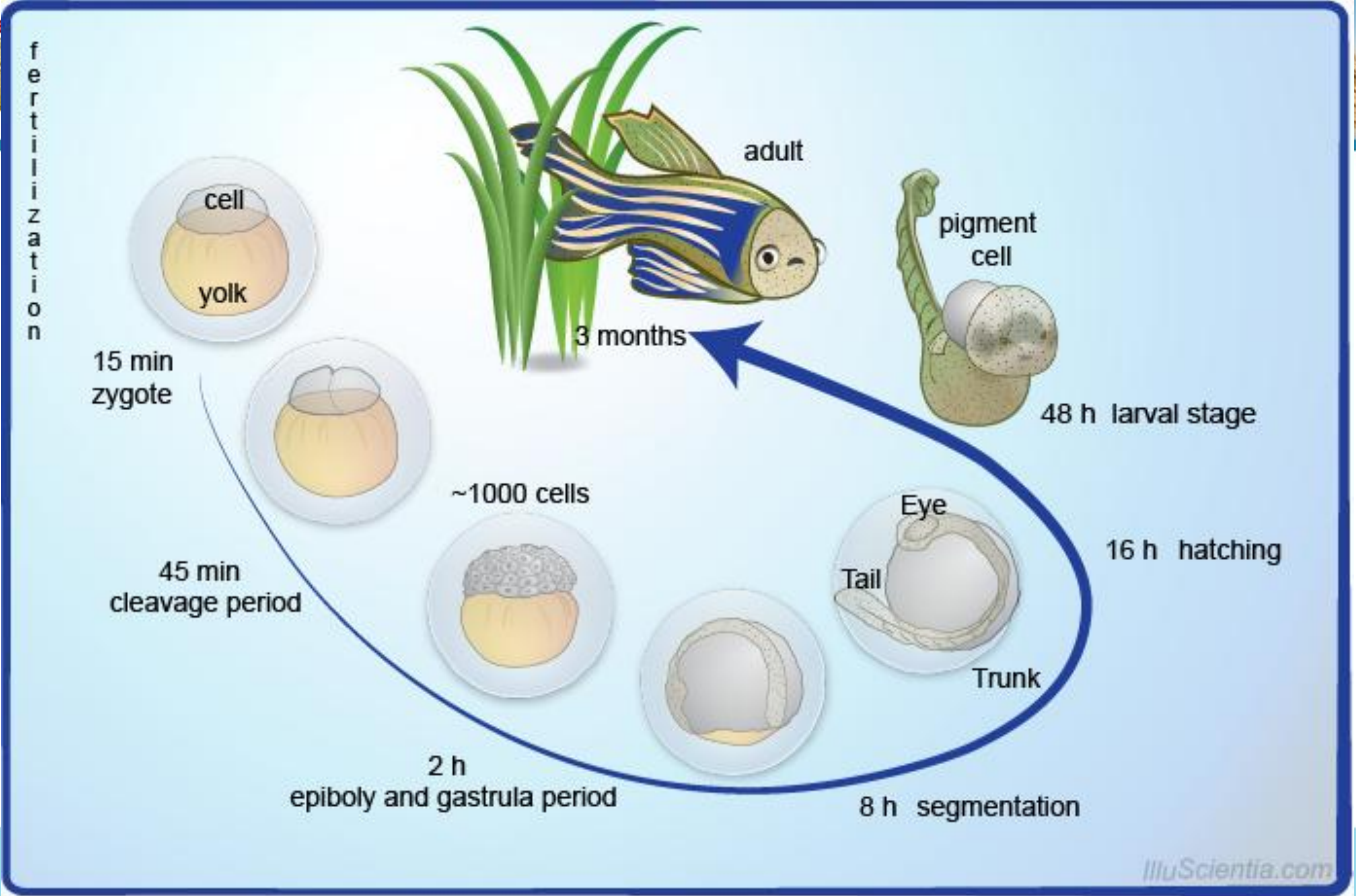


- A few hundreds of eggs per female
- Laying weakly
- Controllable laying time
- External fertilization and development
- Transparent embryos for easy observation

3. Small size and easy raising

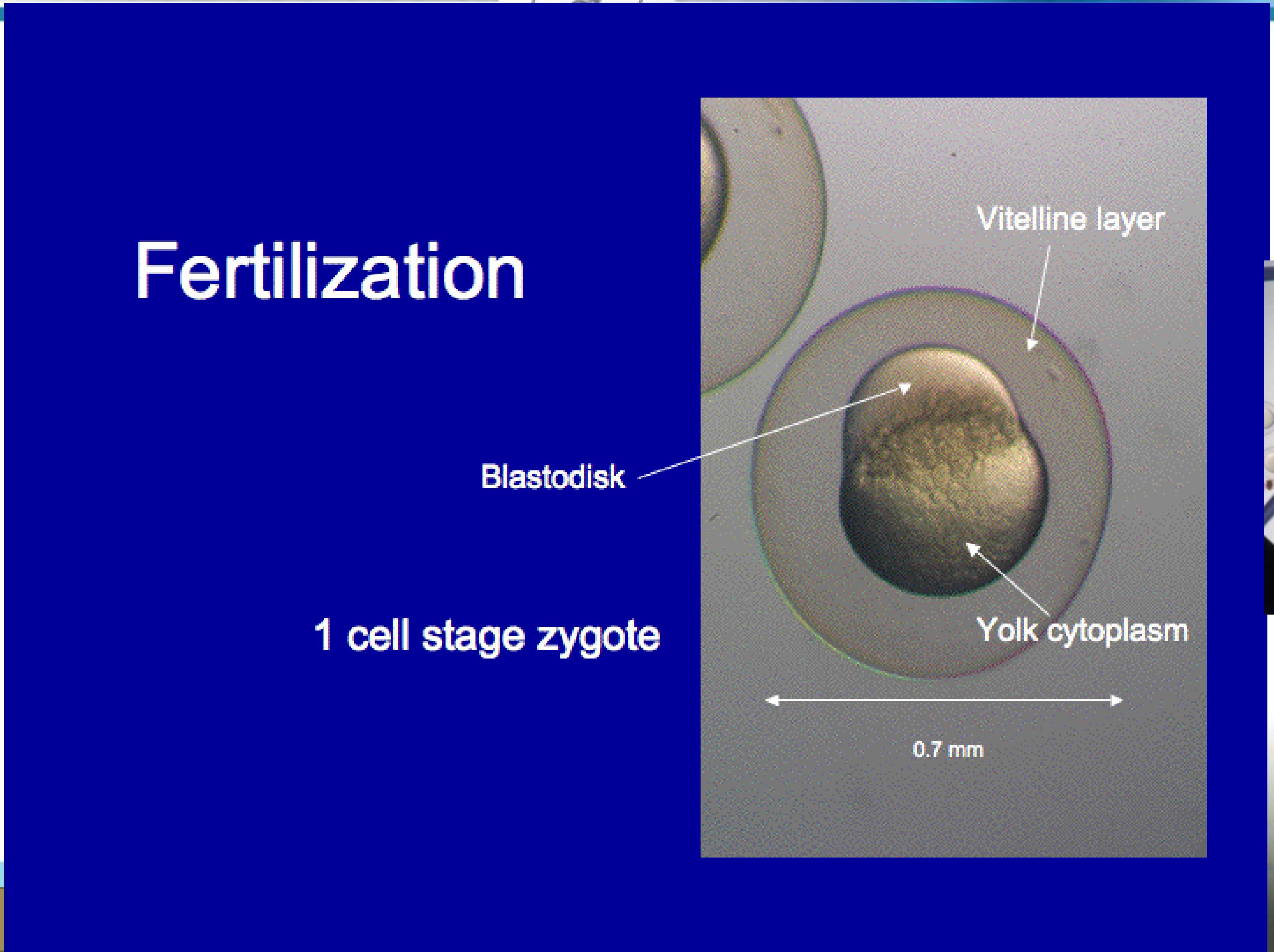
Zebrafish life cycle

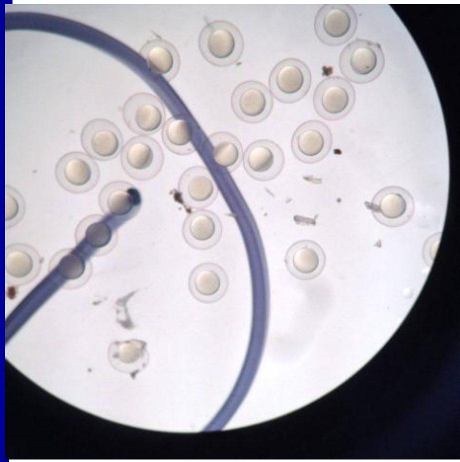
https://www.jove.com/video/4196/cura-e-manutenzione-periodiche-di-zebrafish?language=Italian


fertilization
cell
yolk
15 min
zygote
45 min
cleavage period
~1000 cells
2 h
epiboly and gastrula period
8 h segmentation
Eye
Tail
Trunk
16 h hatching
pigment
cell
48 h larval stage
3 months
adult
IlluScientia.com

Fertilization

40 min after the fertilization in the zebra embryo start the first cell division





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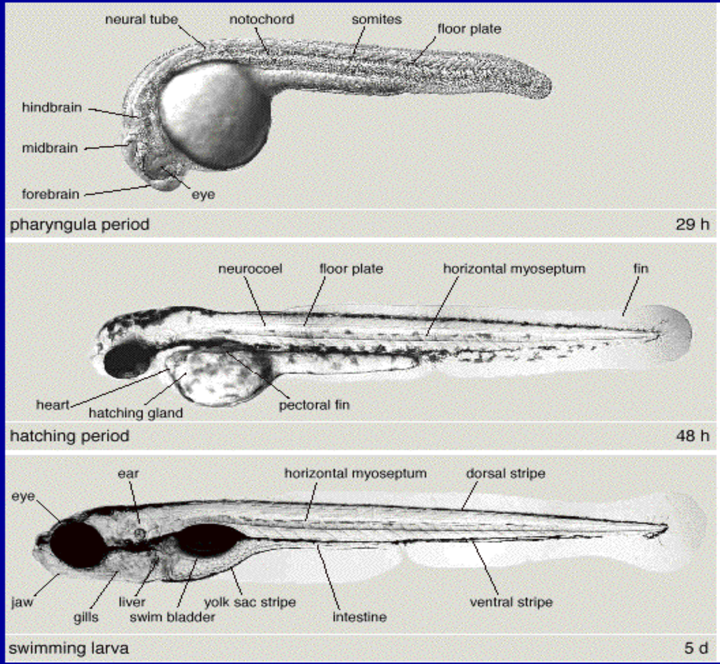
Developmental Timetable

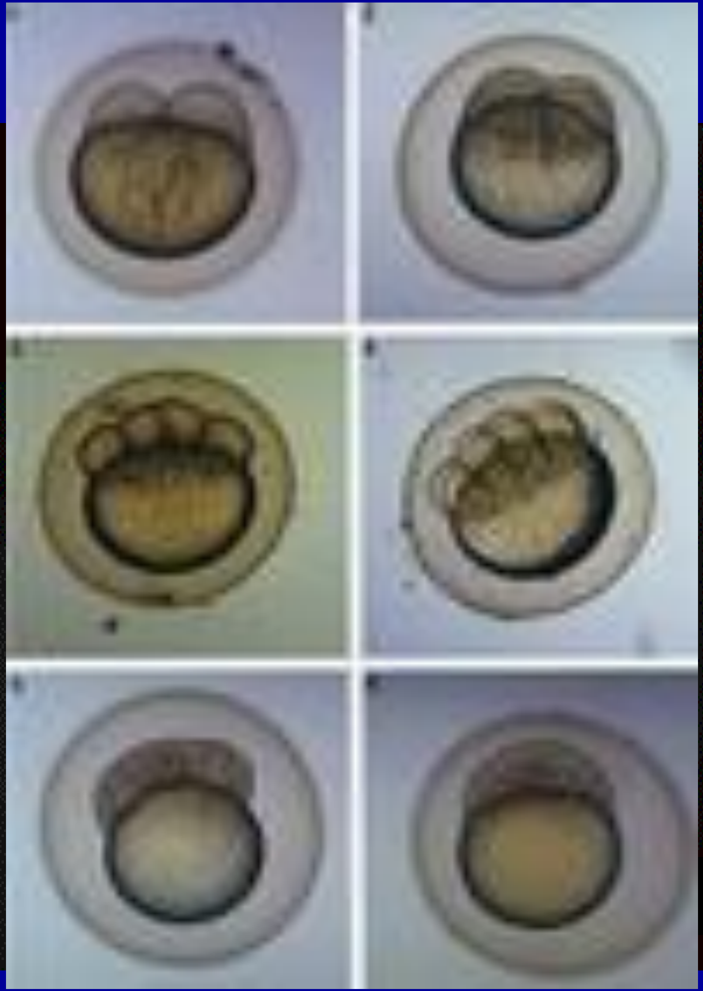
Zygote	0-0.75 hr
Cleavage	0.75-2.25 hr
Blastula	2.25-5.25 hr
Gastrula	5.25-10 hr
Segmentation	10-24 hr
Pharyngula	24-48 hr
Hatchling	48-72 hr
Larval Fish	72 hr

haryngula

Hatchling

Larva





17 Hours of Development

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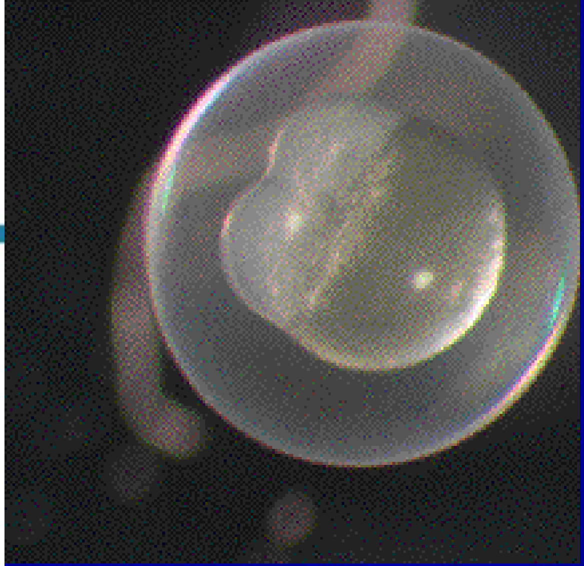
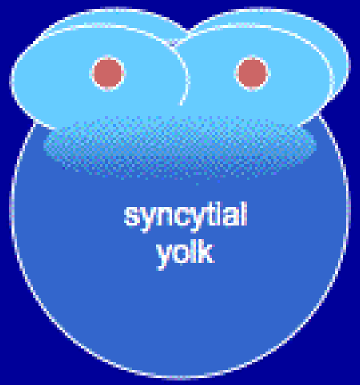
2





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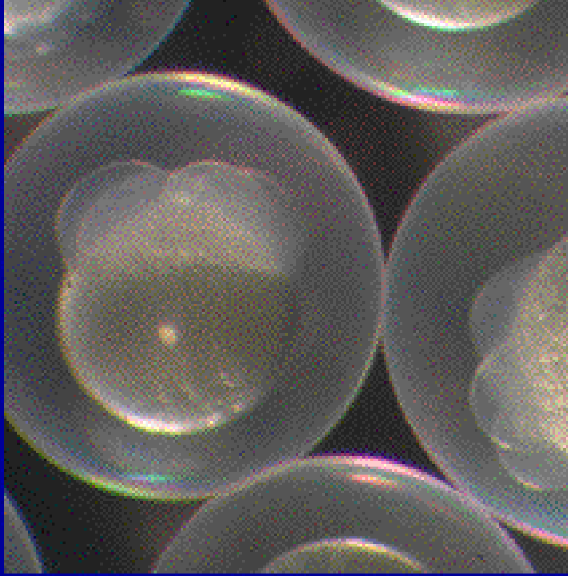
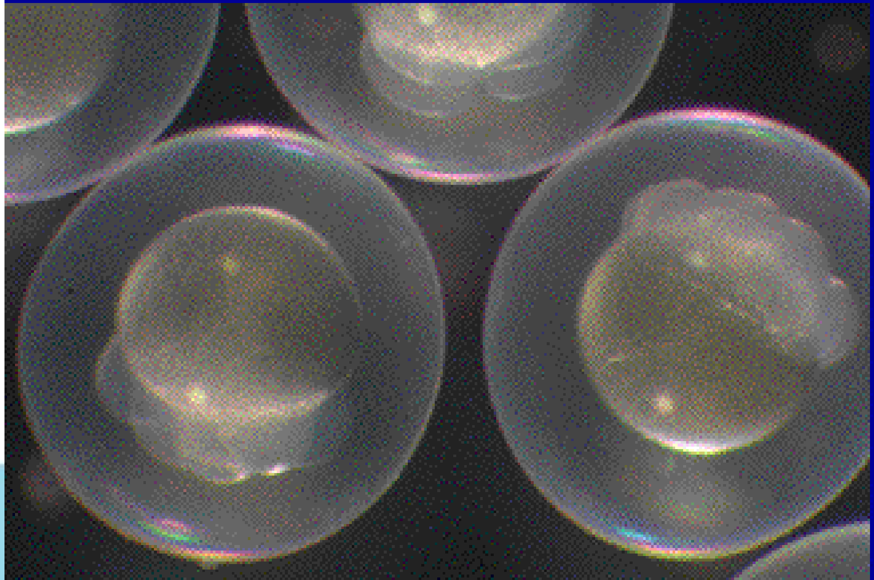


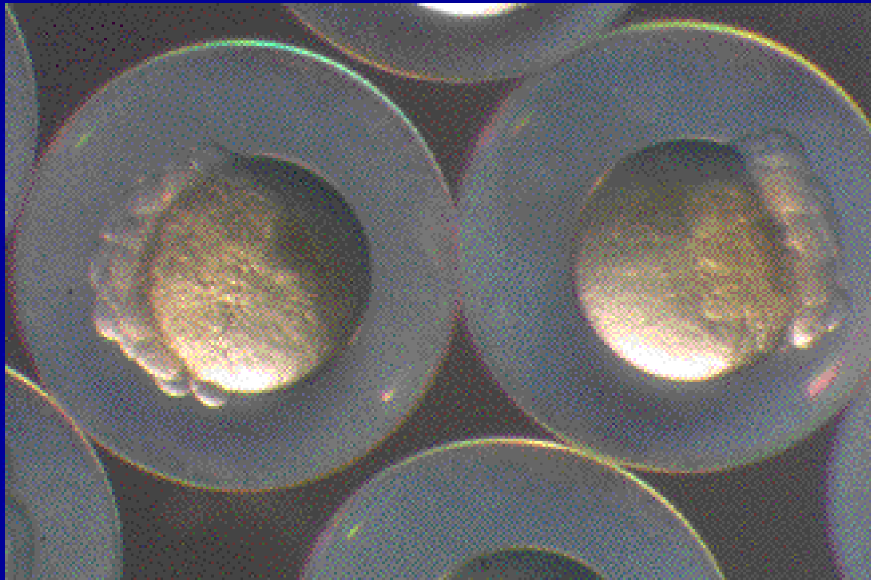
Cleavage

of the Blastodisk Cytoplasm

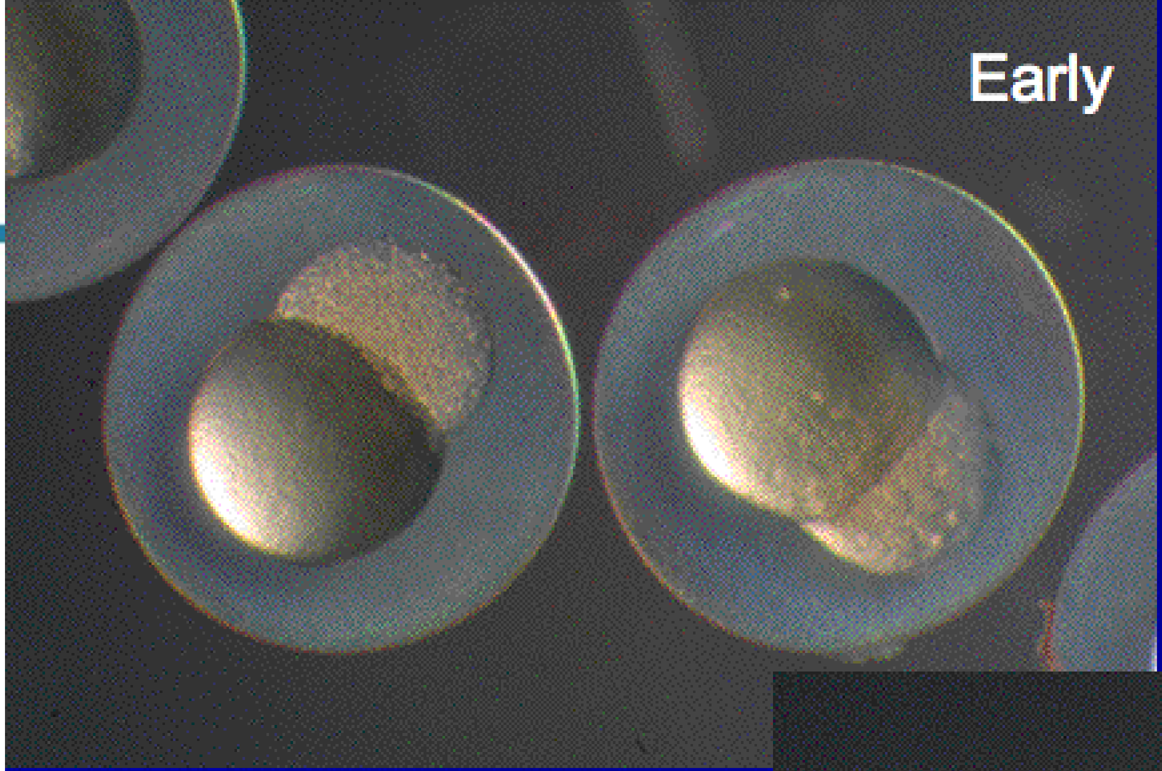
8

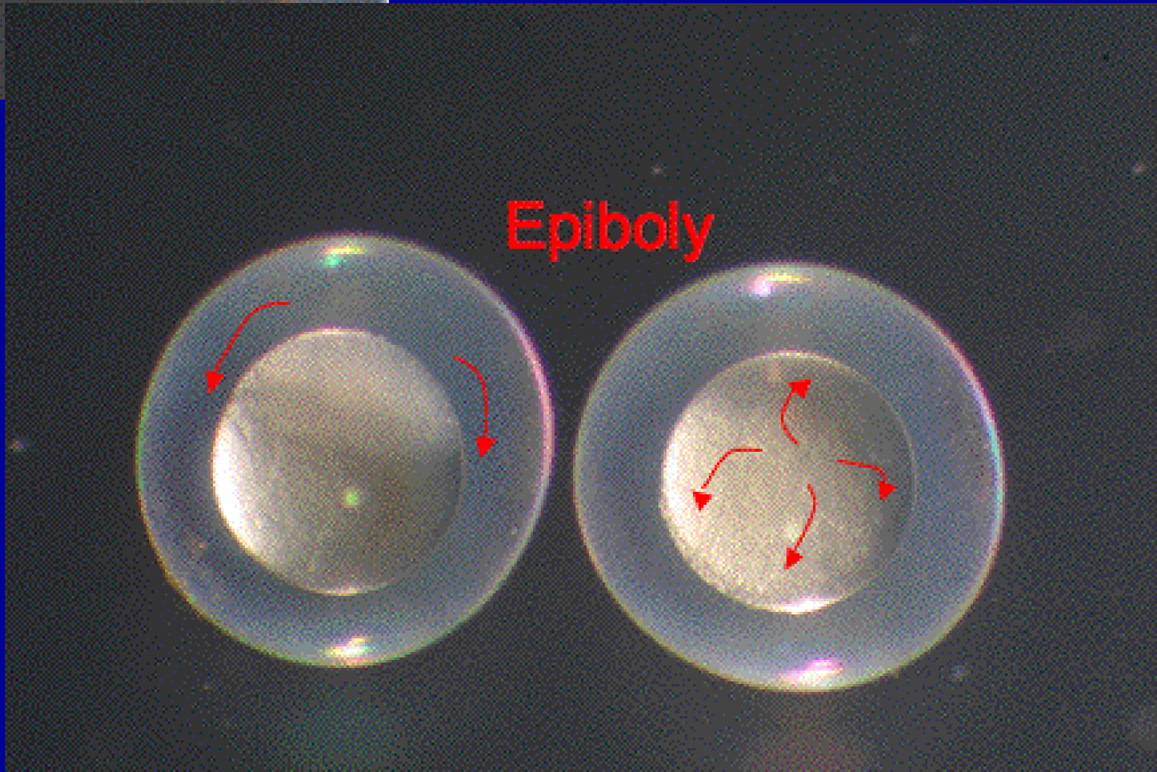


32



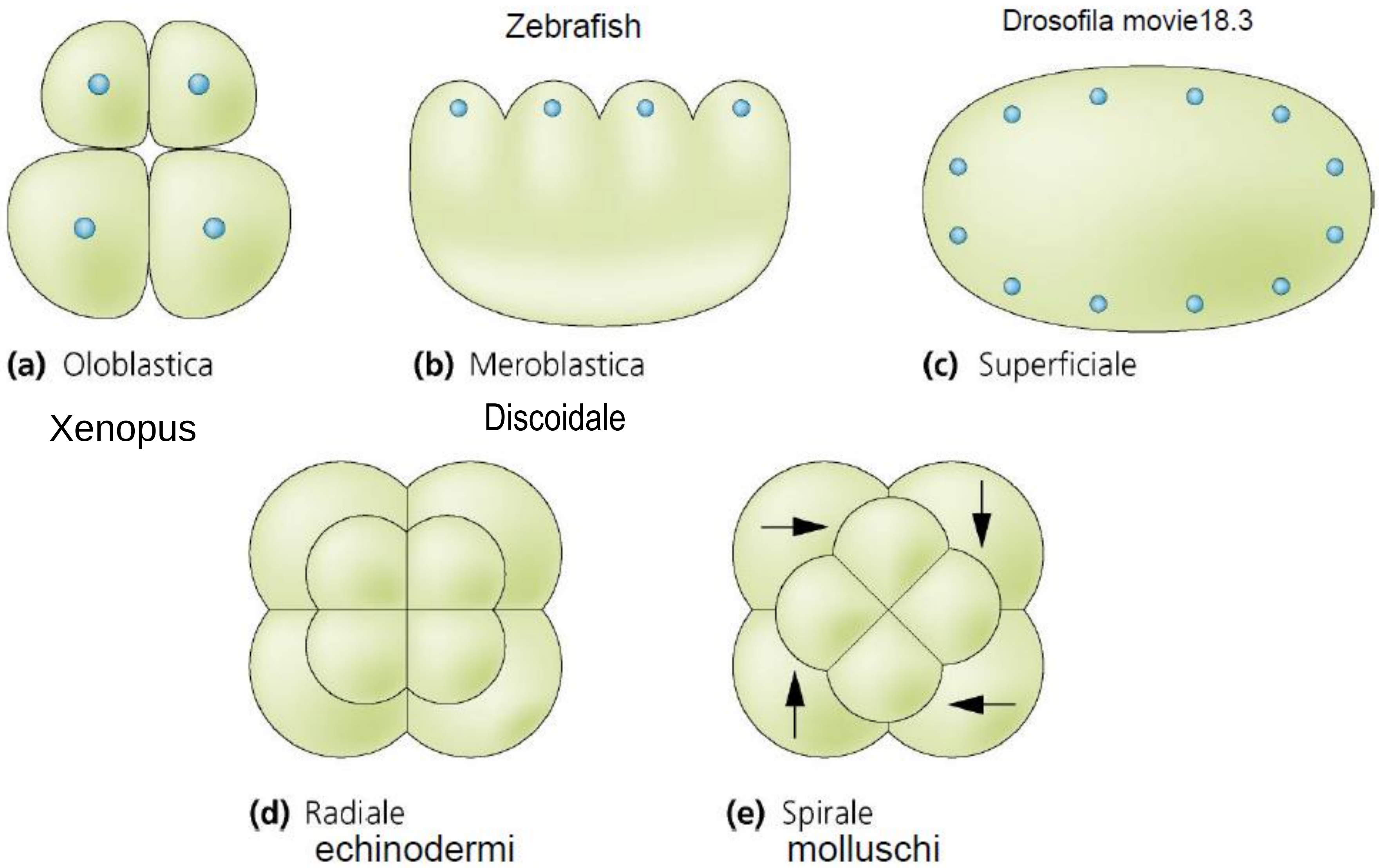
Blastula





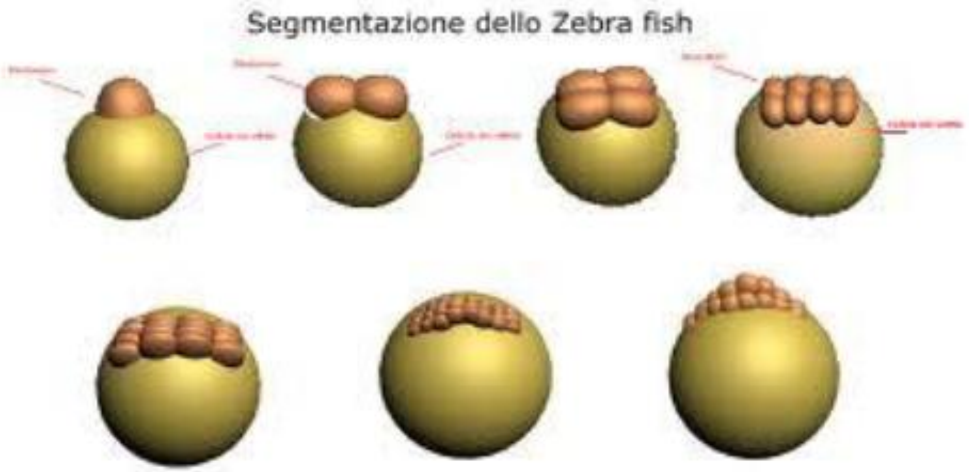
Zebrafish egg development over 24 hours.mp4

Segmentazione

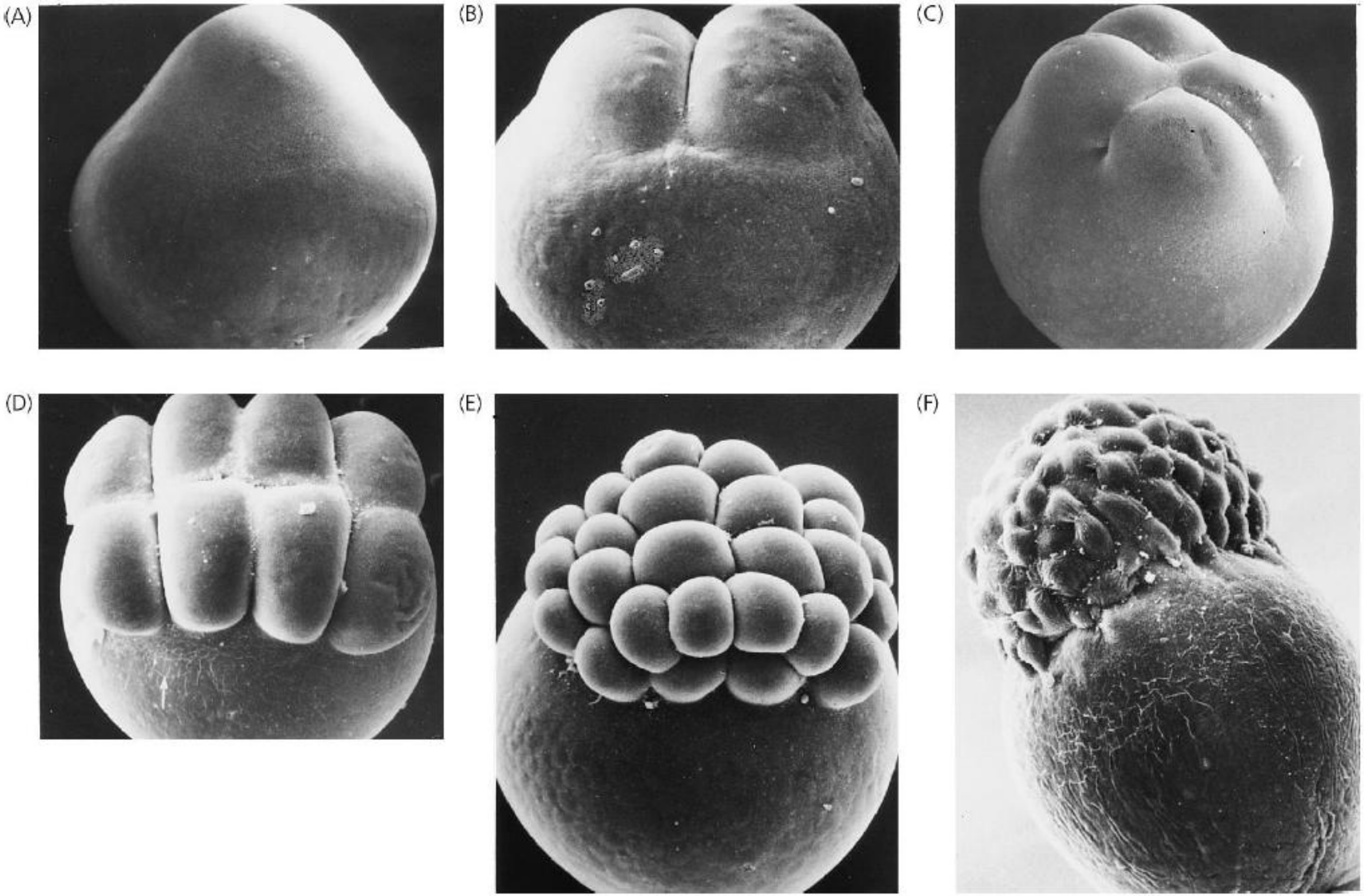


Segmentazione in Zebrafish

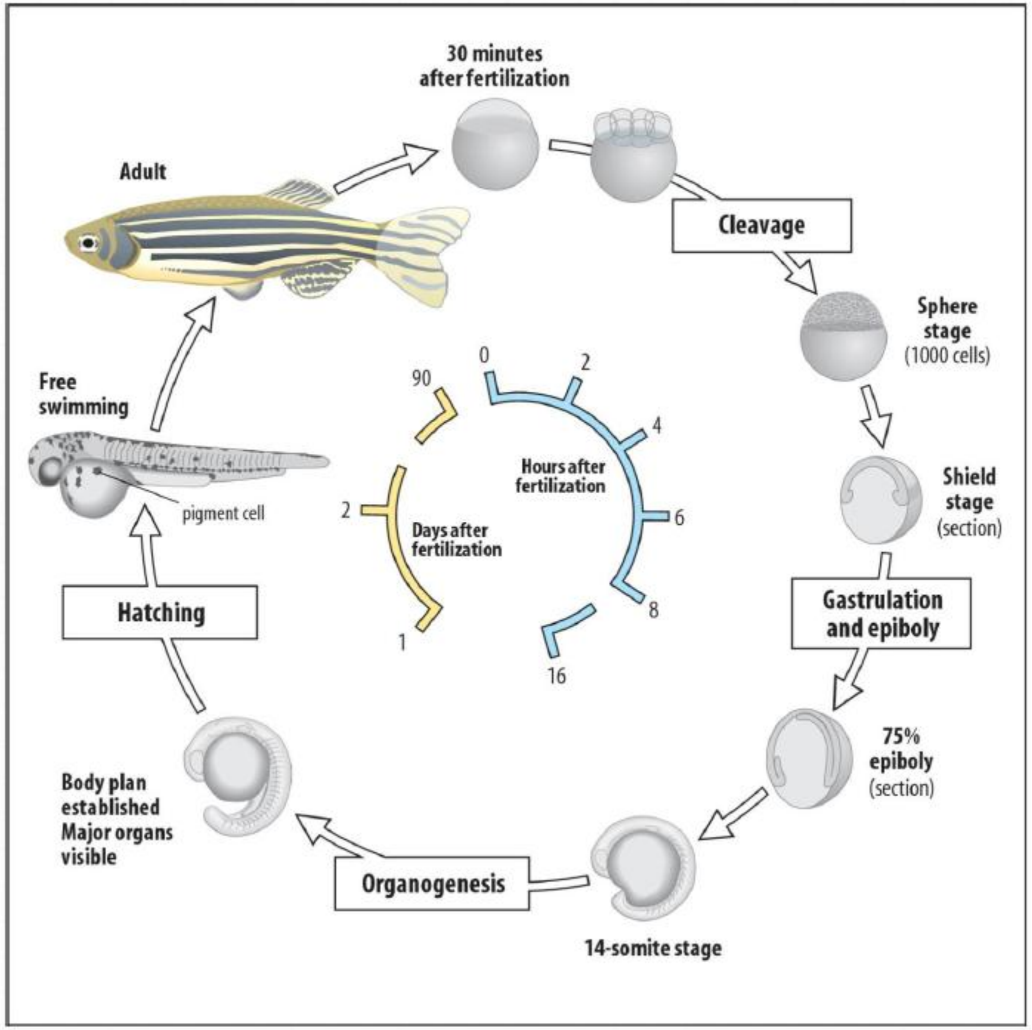
Uovo Telolecitico → **Segmentazione Meroblastica Discoidale**

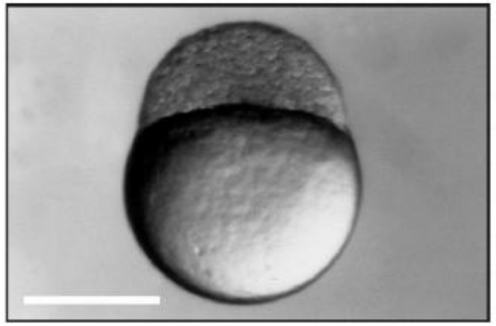


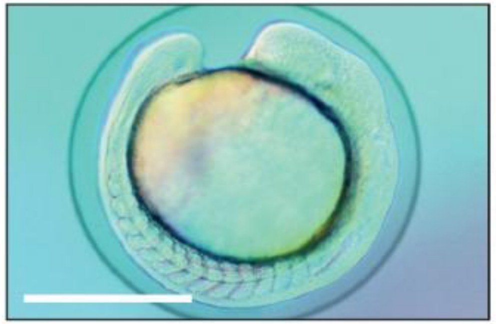
Segmentazione meroblastica discoidale nell'uovo di pesce zebra

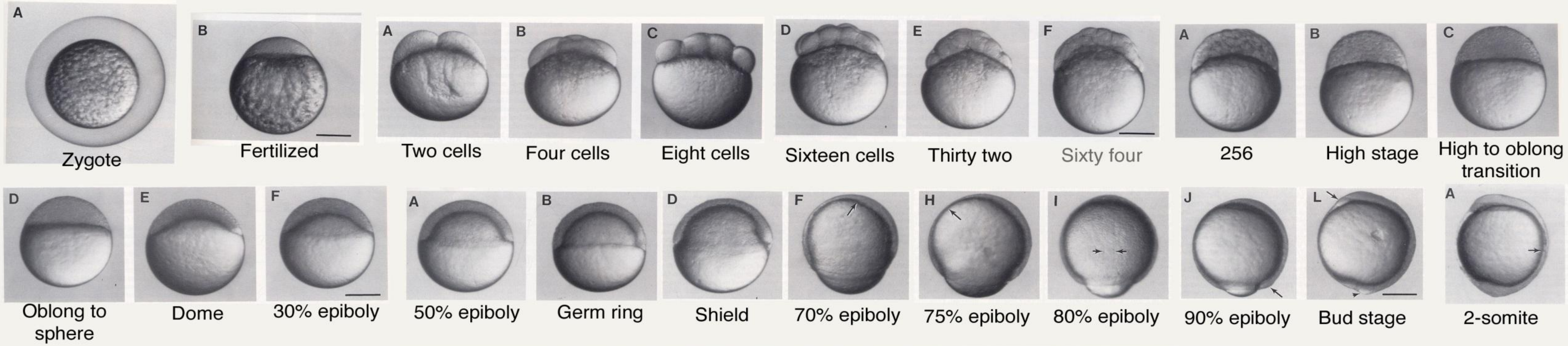


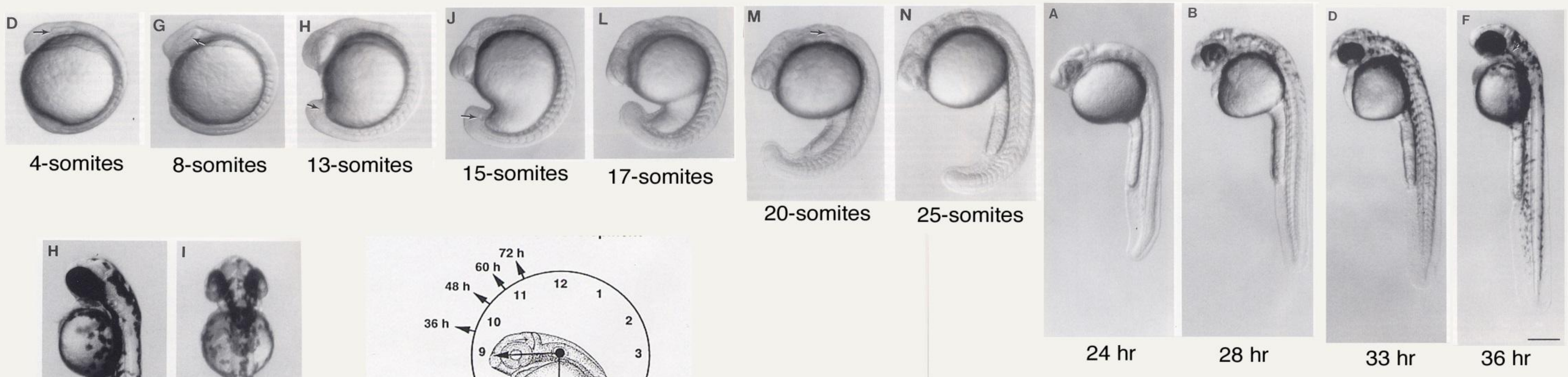
Sviluppo di Zebrafish

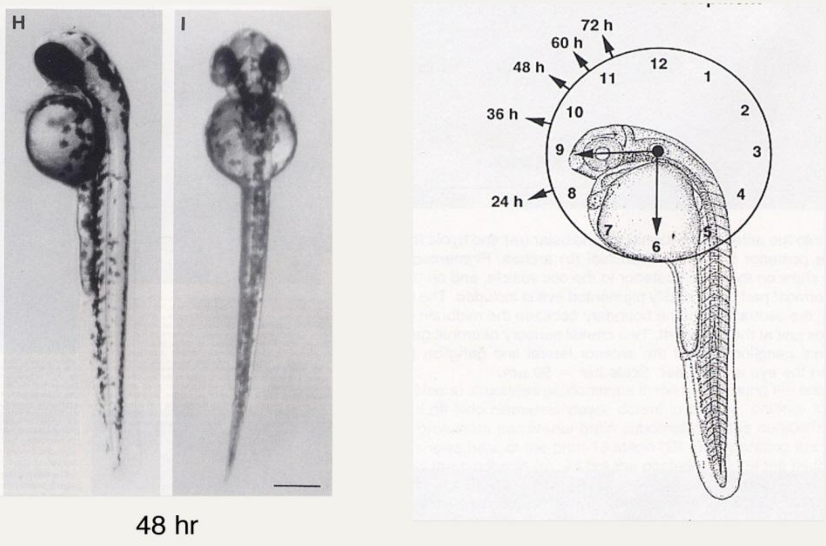








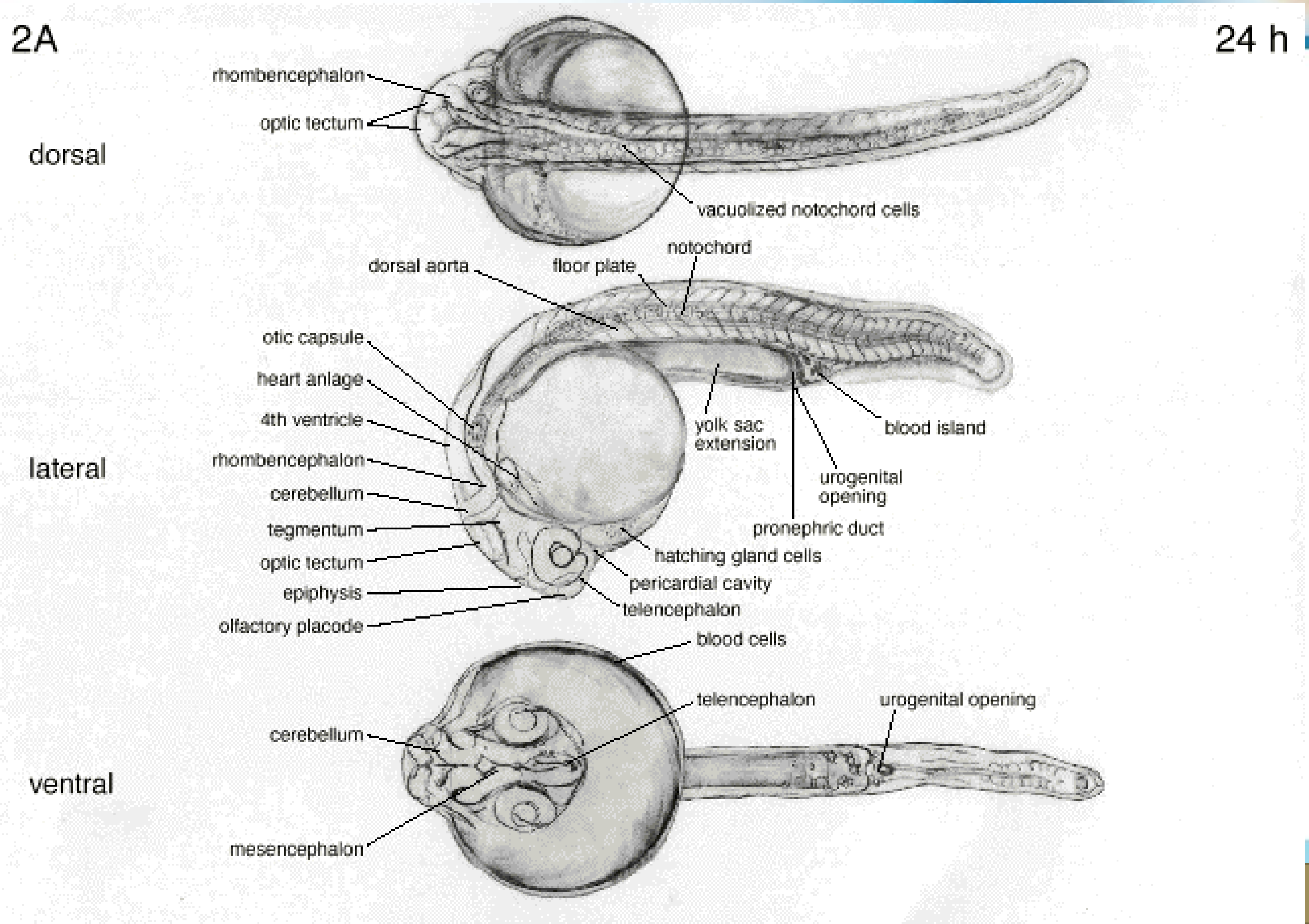




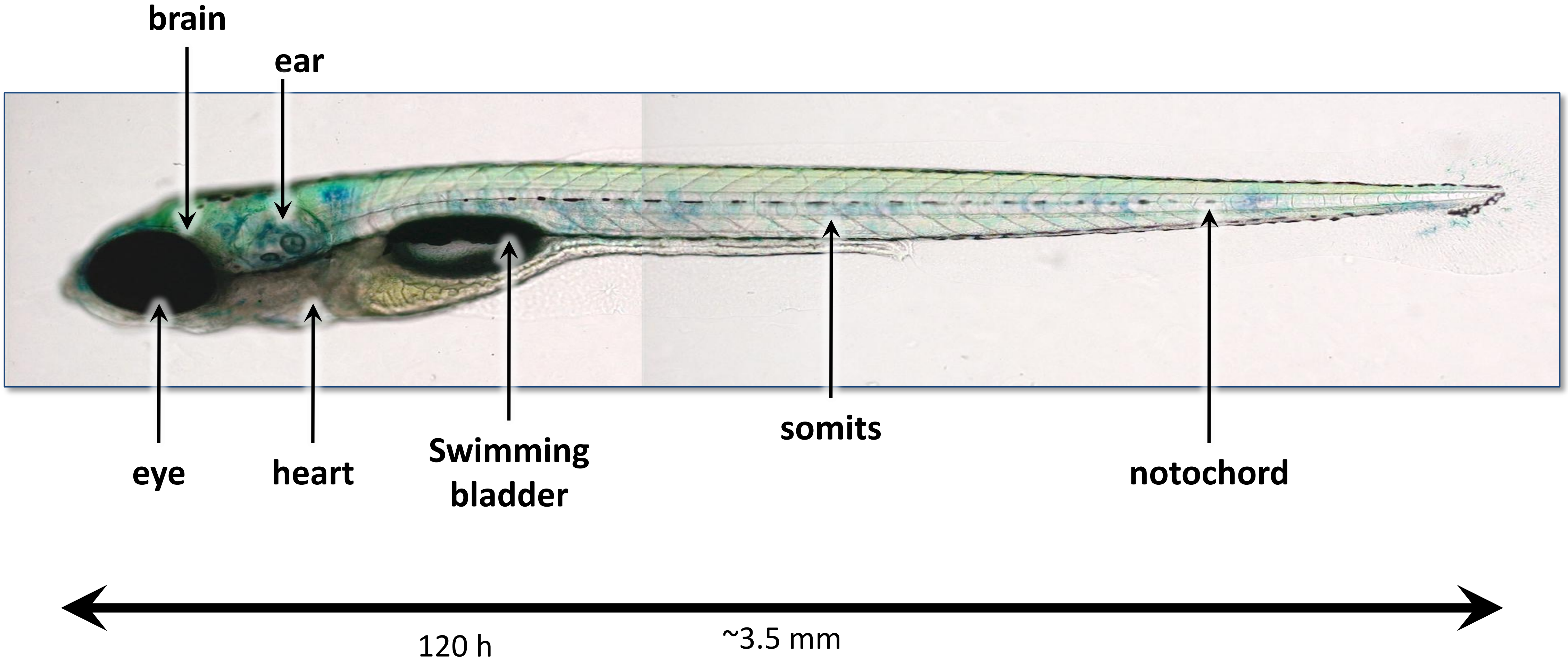
From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

Primi Stadi di Sviluppo di Zebrafish

Organs


2A
24 h
dorsal
rhombencephalon
optic tectum
vacuolized notochord cells
lateral
dorsal aorta
floor plate
notochord
otic capsule
heart anlage
4th ventricle
rhombencephalon
cerebellum
tegmentum
optic tectum
epiphysis
olfactory placode
yolk sac extension
blood island
urogenital opening
pronephric duct
hatching gland cells
pericardial cavity
telencephalon
ventral
blood cells
telencephalon
urogenital opening
cerebellum
mesencephalon

Zebrafish larvae



Absent: stomach, lung, prostate, mammary tissue

Why zebrafish as model organism?

Definition of Model Organisms

A **model organism** is a non-human species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made in the organism model will provide insight into the workings of other organisms. Extensively studied in research laboratories for understanding of cellular function, development and diseases.

What makes a good model organism?

Size : 6 tons 250kg food eaten every 100kg of elephant dung/day

Gestation : 23 months Females give birth to single offspring every five years

Sexual maturity at age 12

Gas: 2000 liters of methane gas released/day!

Size : 1 mm in length Live on a diet of bacteria

Gestation : 500,000 offspring in 1 week from single organism

Sexual maturity in 3 days

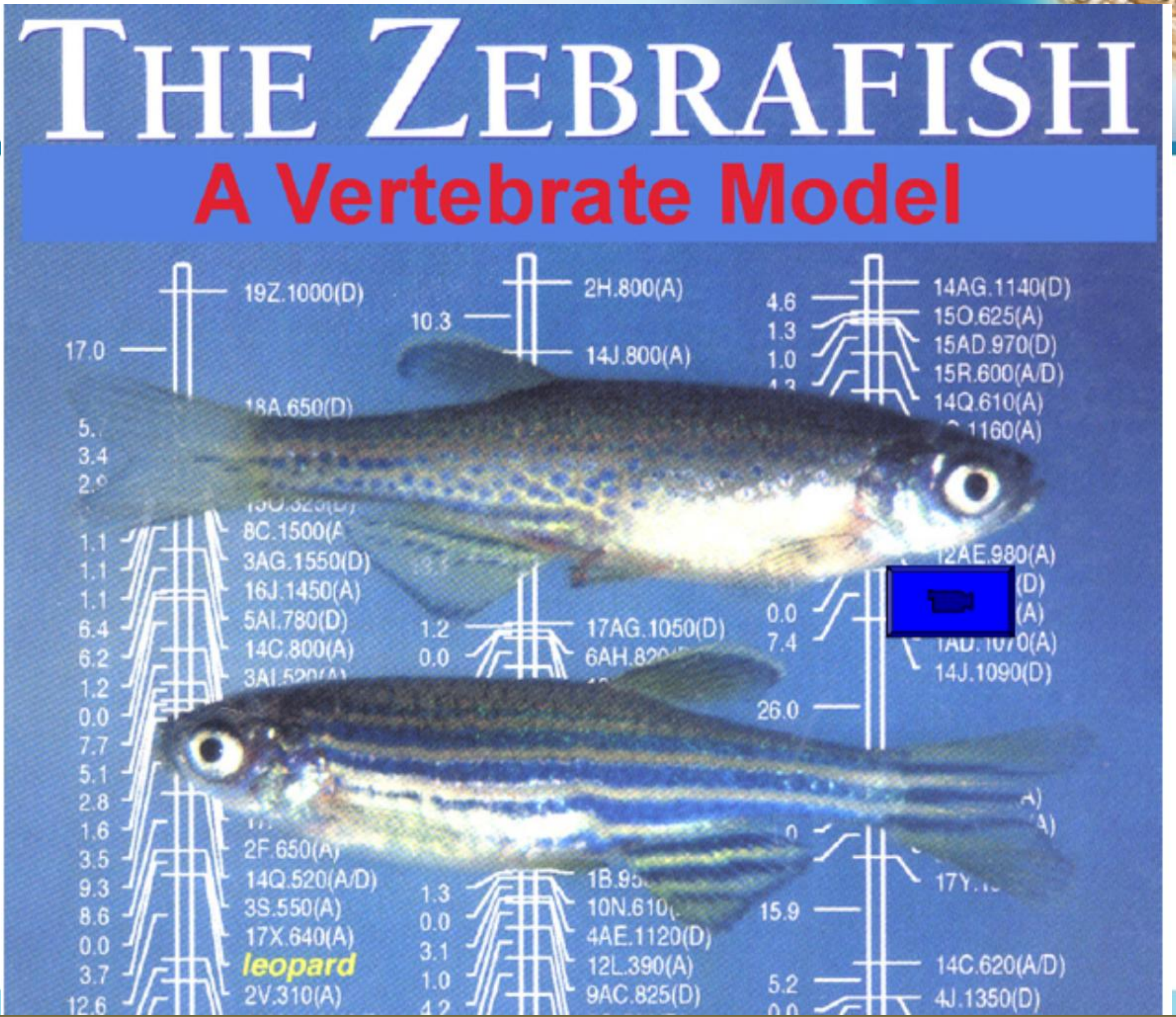
Genome : Sequenced!

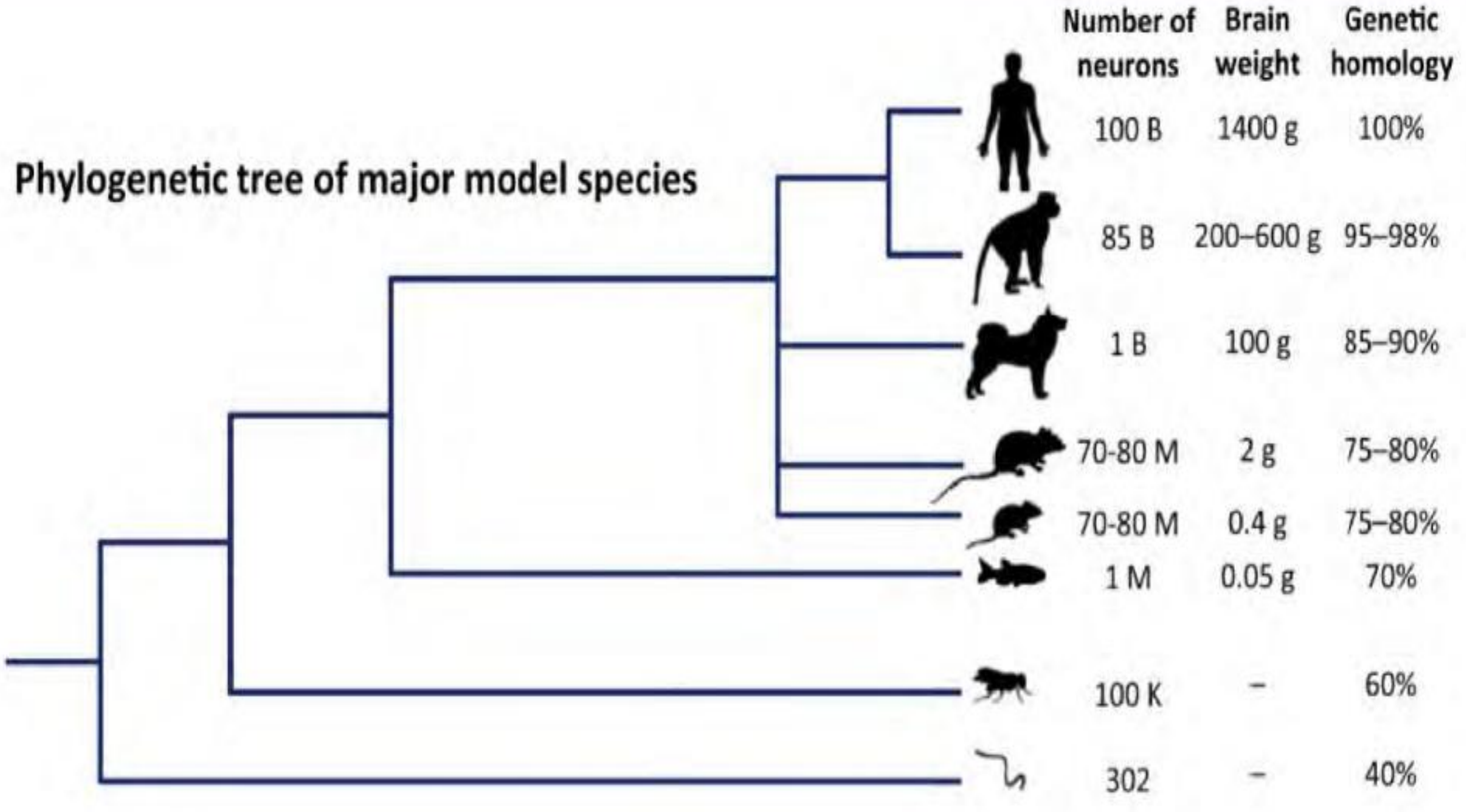
Current Models

- Drosophila
- Xenopus
- Zebrafish
- Mouse
- C. elegans
- Yeast
- E. coli
- Arabidopsis

Model organisms

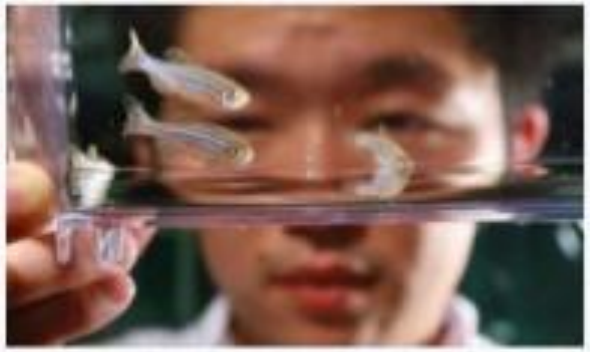
Genetic model organisms	Experimental model organisms	Genomic model organisms
Good candidates for genetic analysis.	Good candidates for research into developmental biology.	Good candidates for genome research.
Breed in large numbers. Have s... rge scale c... severa...	Proc... n be easil...	Easy to manage genomes e.g. small ge... re... G...

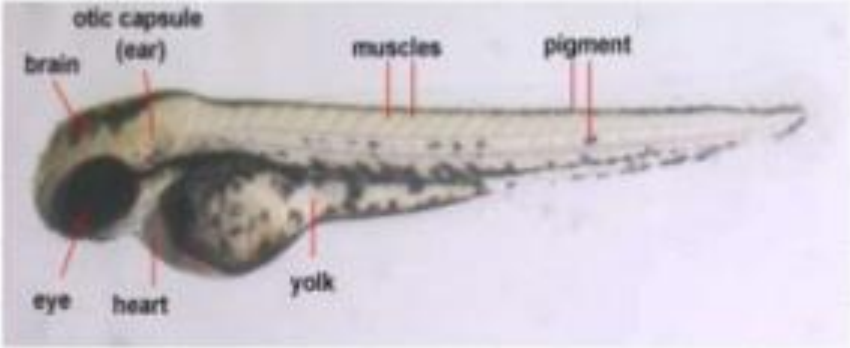

THE ZEBRAFISH
A Vertebrate Model
19Z.1000(D)
2H.800(A)
14J.800(A)
14AG.1140(D)
15O.625(A)
15AD.970(D)
15R.600(A/D)
14Q.610(A)
8C.1500(A
3AG.1550(D)
16J.1450(A)
5AI.780(D)
14C.800(A)
17AG.1050(D)
12AE.980(A)
14J.1090(D)
2F.650(A)
14Q.520(A/D)
3S.550(A)
17X.640(A)
leopard
2V.310(A)
10N.610
4AE.1120(D)
12L.390(A)
9AC.825(D)
14C.620(A/D)
4J.1350(D)


Phylogenetic tree of major model species
Number of neurons
Brain weight
Genetic homology
100 B 1400 g 100%
85 B 200–600 g 95–98%
1 B 100 g 85–90%
70-80 M 2 g 75–80%
70-80 M 0.4 g 75–80%
1 M 0.05 g 70%
100 K – 60%
302 – 40%

Zebrafish a model system

- Small size
- Short life cycle & generation time
- Good reproduction captivity
- External fertilization
- **Optically transparent embryo**
- Rapid embryonic development



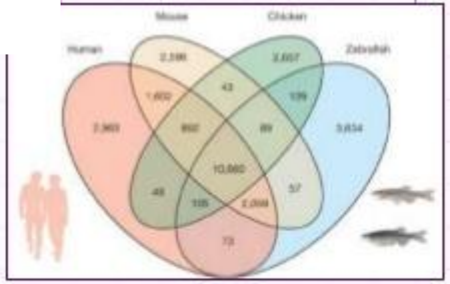


Powerful model organism

- Genetics
- Developmental biology
- **Toxicology**
- Pharmacology
- DNA repair
- Cancer

Because

Orthologue genes between
Danio rerio & humans



- High similarity in cellular structure, signaling & physiology with other high-order vertebrate
- Drug metabolising CYPs (1A, 3A) & phase II enzymes (e.g. GST, sulfotransferases)

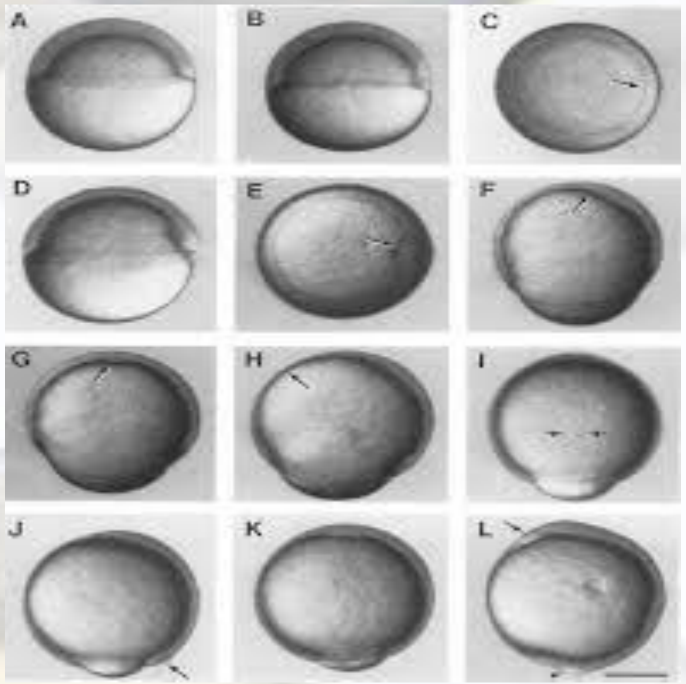
(Goldstone *et al.*, 2010)

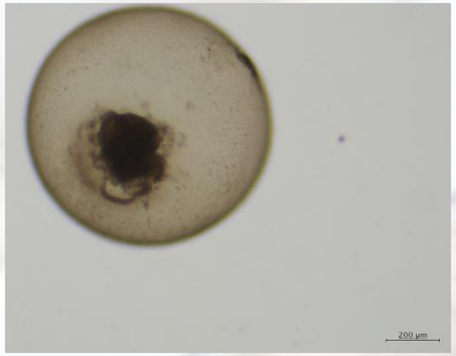
Model for toxicological test

Transparency of embryos allowes to evaluate*:*

- Morphology of different organs;
- Necrotic processes or other alterations;
- Blood circulation

Alterations (edema)





Fish acute toxixity tests (FET tests)

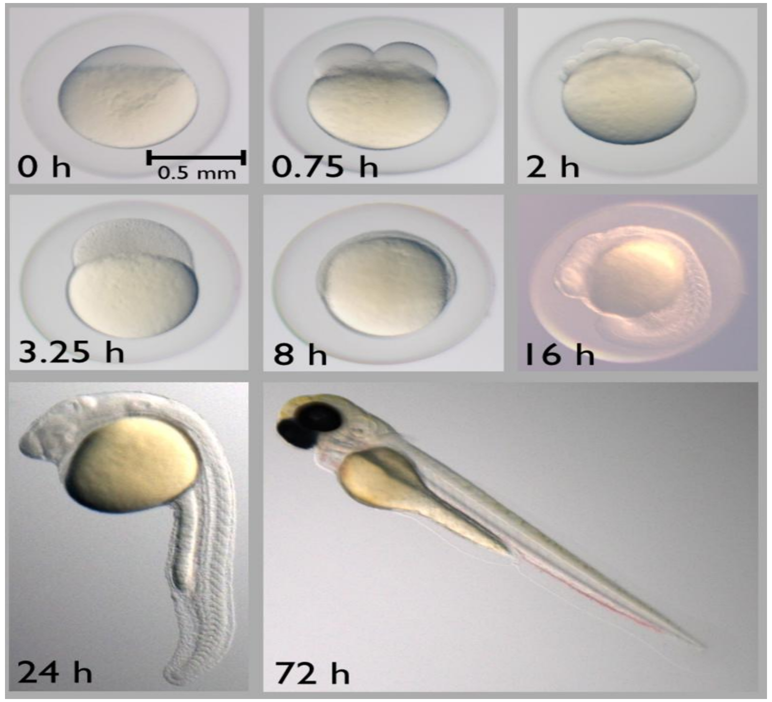
Current OECD guidelines

- OECD TG 236: Fish Embryo Acute Toxicity (FET) Test
- OECD TG 203: Fish, Acute Toxicity Test
- OECD 204: Fish, Prolonged Toxicity Test: 14-day Study
- OECD 215: Fish, Juvenile Growth Test

It is possible to perform toxicological tests using

Adults (>3 months)

Embryos (< 5 dpf)



Larvae (>5dpf)

Standard method for acute fish toxicity

Test guideline	OECD TG 203, Fish, acute toxicity test
Species	rainbow trout, bluegill sunfish, common carp, guppy and others

Fish embryo acute toxicity test (OECD TG 23

Background

§ Included into OECD TG work plan in 2004; lead country: Germany

§ 2006 draft TG & supportive background document submitted

• draft TG based on "fish egg test" (DIN 38415-6, ISO 15088) for effluents testing;
zebrafish; 48h exposure

§ 2006 OECD ad hoc expert group FET created to address WNT comments

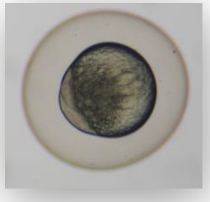
§ 2008 – 2012 validation study to assess the reproducibility (within- and between laboratories) of the FET using zebrafish embryos (ZFET)

§ 2012 – 2013 Finalisation of TG (incl WNT commenting rounds)

§ 2013 adoption by OECD

MATERIALS AND METHODS

FET Test - procedure



≤ 1 hpf

Spawning unit

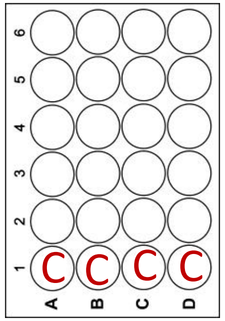
Danio rerio wild tipe AB strain

collecting , washing
pretreatment

control of fertilization ≤ 1 hpf
malformations and alterations

≤ 3 hpf

20 embryos concentration / C + C-, 4 embryos C.I.



96 h, T: 26± 1 °C

Endpoints:

- Coagulation
- Tail no detached
- No somite formation
- No heart-beat

Procedure

Preconditioning of glass vessel, 24-well plates

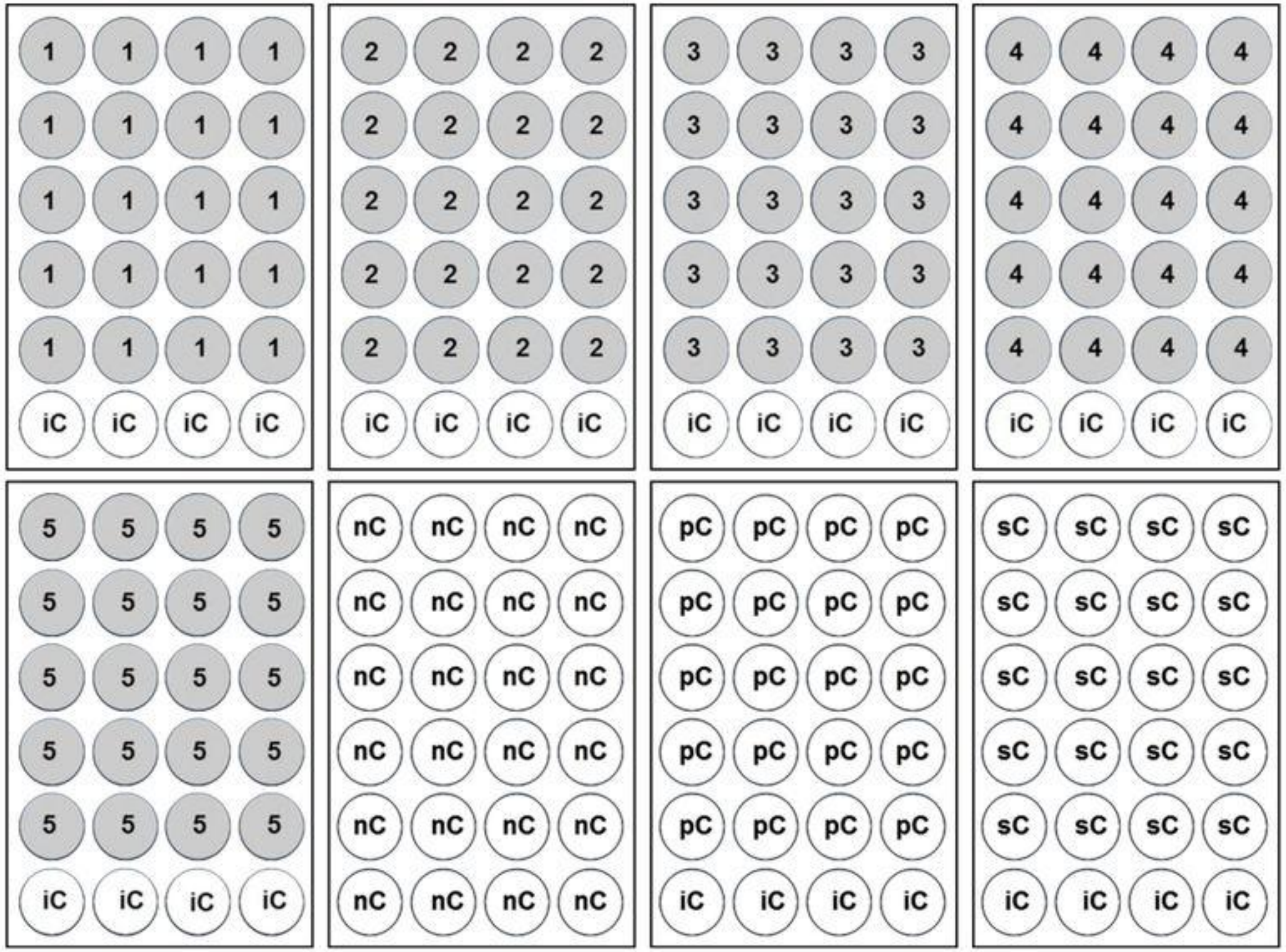
- Newly fertilised zebrafish embryos
- 20 embryos/concentration/control
- 5 test concentrations
- 2 ml/well; 26±1°C & light cycle
- 96h exposure; daily renewal of the test concentrations
- 4 endpoints for acute lethality (24, 48, 72, 96h):

coagulation, lack of heart beat, lack of somites, tail bud not detached

- LC50 calculation at 48 and 96h *OECD TG*

ANNEX 4

Fig. 1: **Layout of 24-well plates**



1-5 = five test concentrations / chemical; nC = negative control (dilution water); iC = internal plate control (dilution water); pC = positive control (3,4-DCA 4mg/L); sC = solvent control

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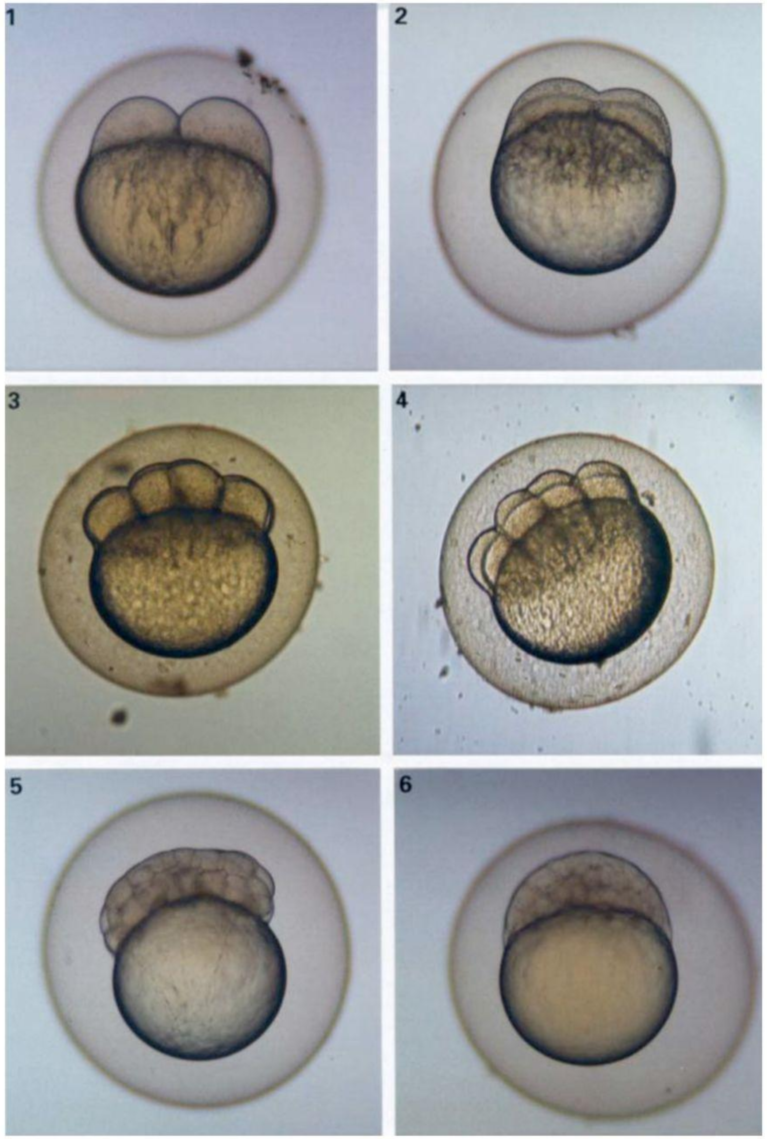
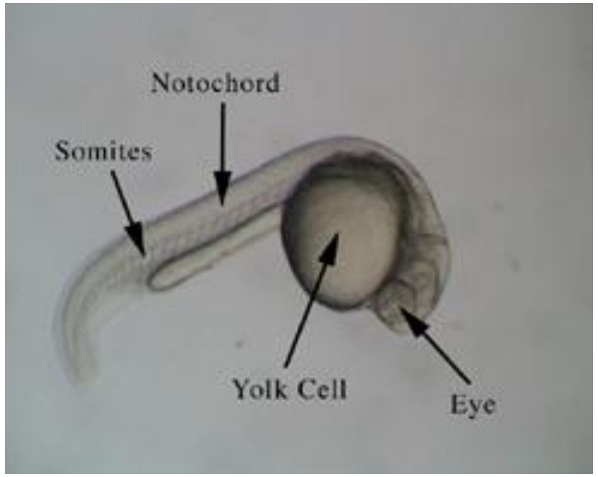


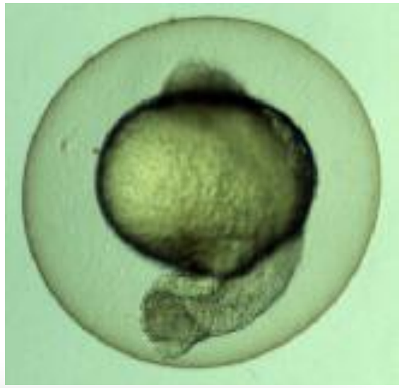
Fig. 3: **Normal development of zebrafish (Danio rerio) embryos**: (1) 0.75 hrs, 2-cell stage; (2) 1 hr, 4-cell stage; (3) 1.2 hrs, 8-cell stage; (4) 1.5 hrs, 16-cell stage; (5) 4.7 hrs, beginning epiboly; (6) 5.3 hrs, approx. 50 % epiboly (from Braunbeck & Lammer 2006 (40)).



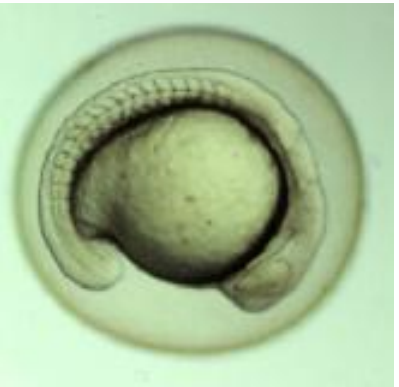
FET Test - endpoints

- Coagulation

- Lack of somites

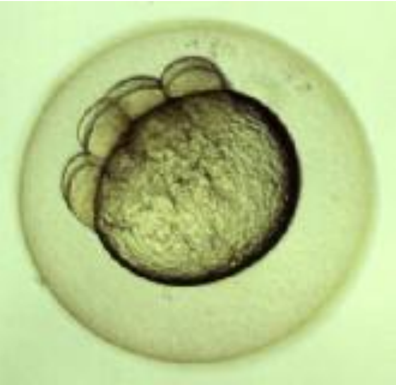


- Tail bud not detached



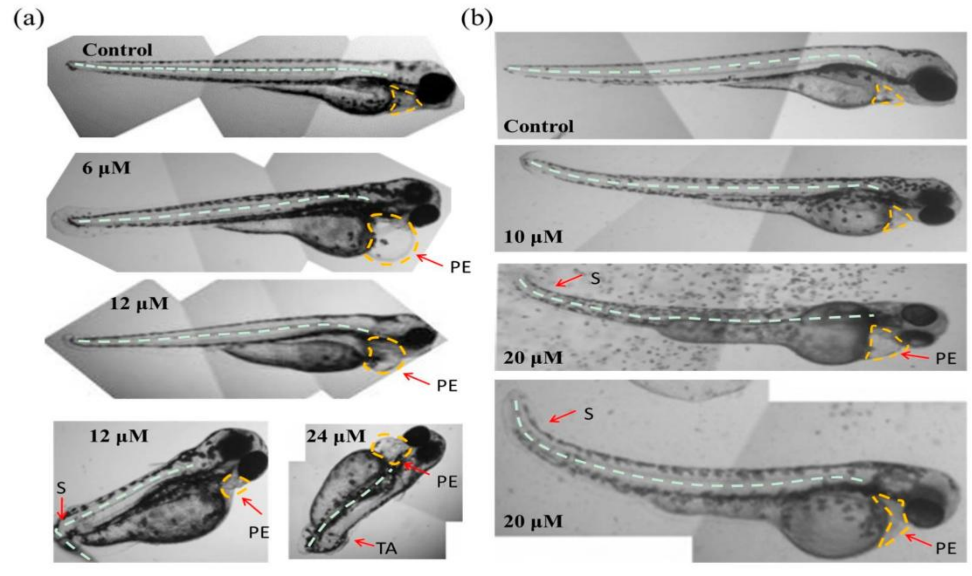
- Lack of heart beat

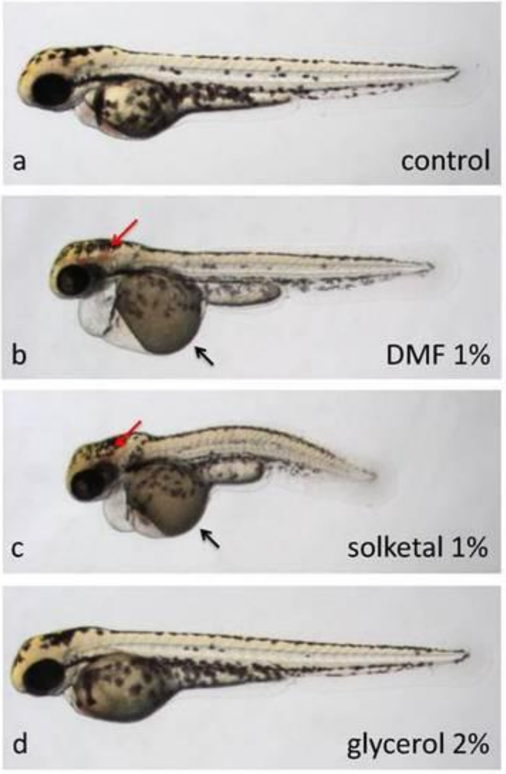
NORMAL



Sublethal parameters

- Reduction of heartbeat
- No spontaneous movement
- Deformed head
- Missing eyes
- Reduction or absence of blood circulation
- Deformed tail/somites
- Bent tail (scoliosis/lordosis)
- Yolk edema
- Pericardial edema
- Alteration of pigmentation
- General malformation
- General underdevelopment





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FET Exposure time (hpf) : ______ Date ______

	1	2	3	4	5	6(NK)	notes
A							
B							
C							
D							

	1	2	3	4	5	6(NK)	
A							
B							
C							
D							

	1	2	3	4	5	6(NK)	
A							
B							
C							
D							

	1	2	3	4	5	6(NK)	
A							
B							
C							
D							

	1	2	3	4	5	6(NK)	
A							
B							
C							
D							

	1	2	3	4	5	6(NK)	
A							
B							
C							
D							

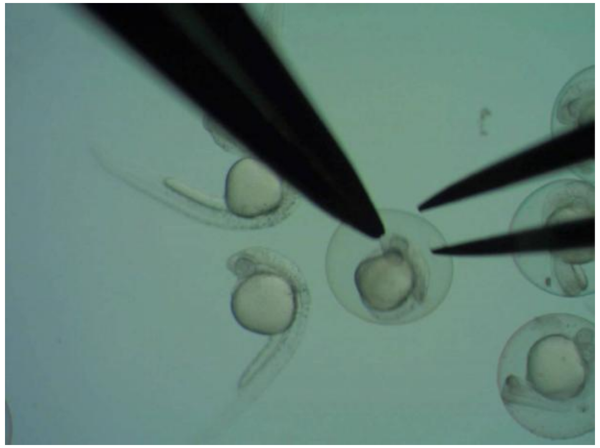
	1	2	3	4	5	6(NK)	
A							
B							
C							
D							

Dechorionating zebrafish embryos

Why ?

https://www.youtube.com/watch?v=3LbYTEu1Fo8

Limited permeability of the chorion for some compounds
embryos can be dechorionated at 24h post-fertilization (hpf). The positive control test substance, 3,4-dichloroaniline, should be replaced by acetone, since 3,4-dichloroaniline exerts its effects during the first 24h of development



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GRAZIE DELL'ATTENZIONE