Repore for Sea Urchin Practical.

1. Morphology upon fertilisation.

We took the pre-collected urchin eggs from female 1 and noted its appearance. (See Fig:1).

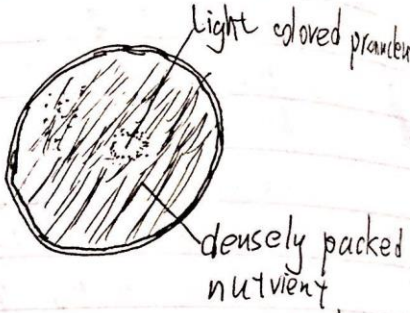


Fig.1 Schematic unfertilised egg.

Sperms were precollected from male 1, ~~and~~ 5μL of stock was diluted in 20mL sea water.

Upon inspection, about 50% of the sperms are actively moving while the rest are stationary. The movement is apparent only under x40 objective.

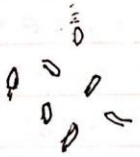


Fig.2. Schematic Sperm.

~~Additionally, there are eggs~~
~~Since 5~~

We add 10 drops of diluted sperms to the eggs at 15:00. Fertilisation membrane arises in some of the eggs. Among the eggs we count, 23/29 shows such elevation. (79.3%). ~~No nucleus is appearant~~ ~~appres~~ Nucleus is not apparent in those fertilised eggs.

Fig.3. Schematic fertilised egg.

Additionally. Both before and after fertilisation, there are transparent egg-size structure in the same dish as egg, ~~pass~~ some of show disrupted

membranes and leaked content. They are possibly damaged eggs, or undeveloped ~~oocytes~~ progenitors of oocytes.

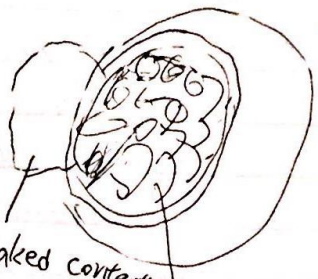


Fig.4 A. Damaged egg

2. First Cleavages.

Although elevation of fertilisation membranes indicate successful sperm entry, none of ~~the~~ these zygotes divide up to 16:30. This is possibly a delayed cleavage.

In the replacement embryos ~~observed~~ where cleavages took place, it was 3 hr (16:40) after its fertilisation (13:40), although they might have entered 2-cell stage a while ago. We thus made observation of the ~~last~~ latter.

~~We~~ Among the 13 ~~two~~ embryos examined, 7 show two distinct cells, 2 show incomplete furrowing and 4 are apparently 1-cell. For those at 2-cell stage, the two cells are of equal size. Interestingly, most 2-cell embryos retain a spherical envelope, though. one of them shows ~~a narrowed envelope~~. an envelope narrowed at the equatorial plane.

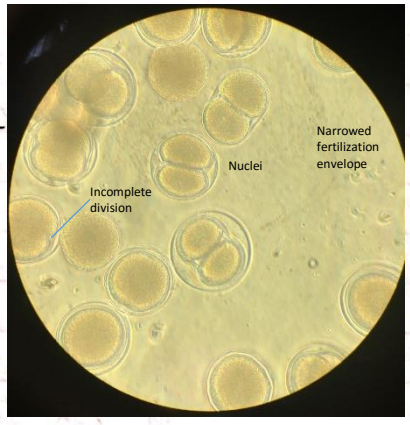


Fig.5 2-cell stage embryos

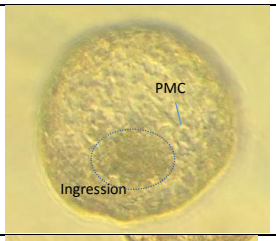
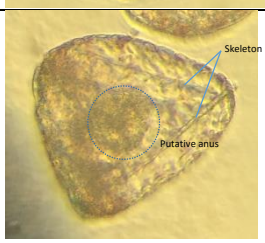
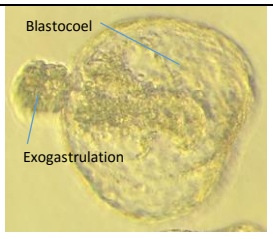
As to the timing, we weren't able to note the first appearance of

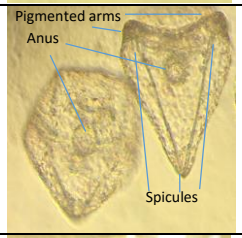
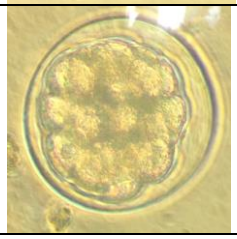
cleavage in the replacement sample. At the time of our observation (16:40, 3hr after fertilisation), some embryos are already in 4-cell stage, while most of them are still in 2-cell stage. ~~Advance~~ which is delayed compared to ~~Progress in development varies between~~ the literature prediction of 2-cell stage. ~~from~~ from 2.5hr to 3hr. This delay is possibly due to ~~the frequent~~ observations ~~that~~ require lighting thus heating the embryo to a suboptimal temperature, ~~under~~ which slows its development.

No picture is available for 4-cell stage.

The nuclei are apparent in some embryos, while the resolution is not high enough to distinguish aster.

3. Embryos at different stages

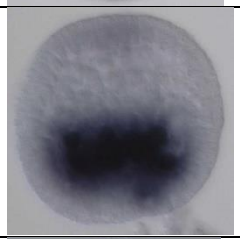
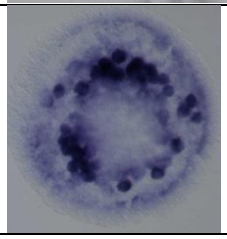
Culture No.	Appearance	Note	Estimated Stage/Treatment
A		Embryos have hatched and rotate vigorously. Primary mesenchyme cells (PMCs) have already ingressed and localise around the ingression. In some embryos, invagination have formed an early archenteron	Mesenchymal blastula, Early gastrula (24hpf)
B		Two pieces of skeleton joins at the point (aboral) end of the prism-shaped embryo. There are heavy pigmentation at the round (oral) end. The archenteron possibly hides behind the green pigmentation zone in the middle, which will become anus. The spicules are not apparent due to focus and pigmentation. The embryos are not moving thus possibly dead. A perturbation is also possible since the embryos are “fatter” than usual (see Appendix).	Prism (56hpf), possibly perturbed with Nickel.
C		Embryos have hatched. The blastocoel is evident while the cell movement is aberrant. The invagination and formation of archenteron is evidently perturbed. This phenotype is heterogonous with some embryos exogastrulating and others gastrulating both inwards and outwards. (See Appendix) The embryo is not moving thus possibly dead.	Primary invagination (30-35hpf), Perturbed. Possible vegetalised with LiCl

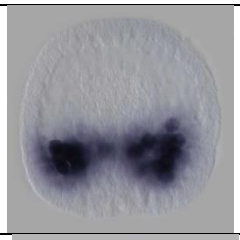
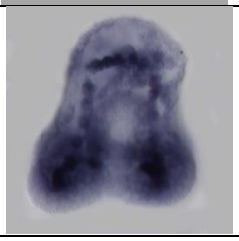
D		Hollow blastocoel is surrounded by a layer of cell. The embryo has hatched the fertilisation envelope. Morphology is normal.	Mid-blastula (15hpf)
E		Embryos have developed well-defined arms, skeletons and anus. Occasional movement is observed thus evidencing they are alive. The spicules are not distinct due to focusing, but there are roughly 3 of them in each embryo.	Early Pluteus (72hpf)
F		Eight cell ring is evident. The embryo has not hatched yet. It is hard to count the cells that are not on the focus. The morphology is normal.	32 cell (8-9hpf)

Comment:

Within each sample population, there exist embryos at multiple stages, reflecting a variable rate of development. For the perturbed embryos, the phenotype ranges from weak perturbed to strongly perturbed, again reflecting the varying nature of development of individual.

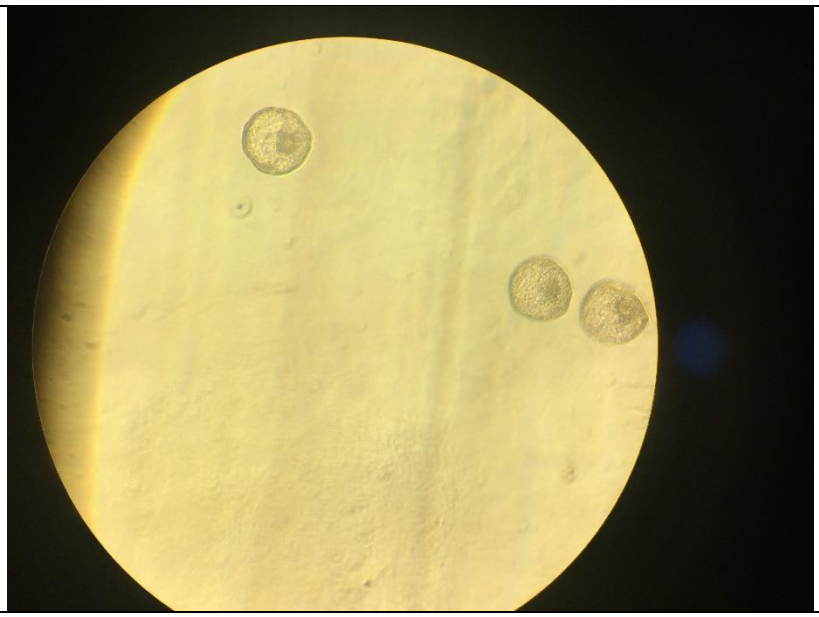
4. Expression pattern of a gene

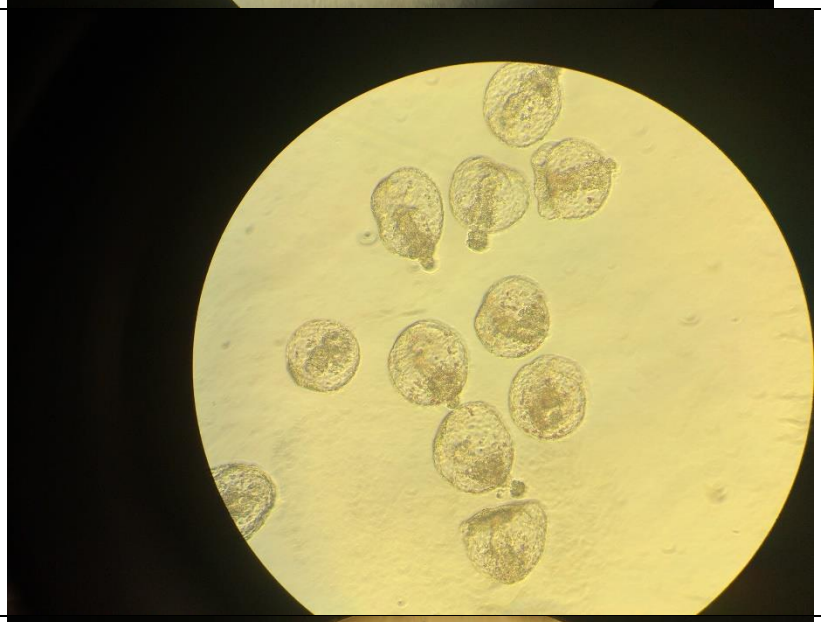
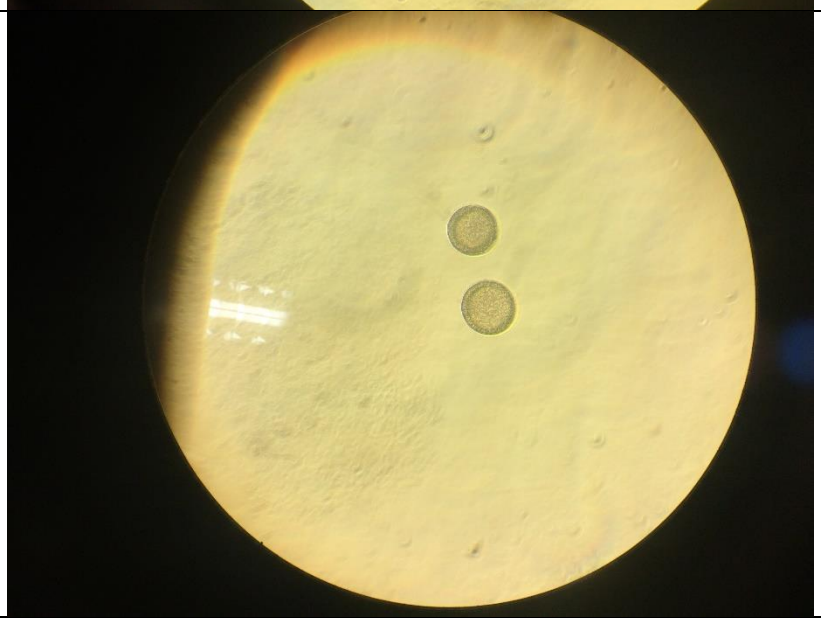
No	stage	Picture	Expression pattern
F2	Early blastula (vegetal view) to early gastrula		Expressed in micromere progeny at the vegetal pole. It is not clear whether ingression takes place yet.
F4	Late blastula to early gastrula		Expressed in ingressed PMC. It is clear that cells have ingressed but not invaginated yet.
F3	Major invagination to late gastrula (vegetal view)		Expressed primarily in PMC with weak expression around periphery. First spicules have arisen and skeletongenesis started.

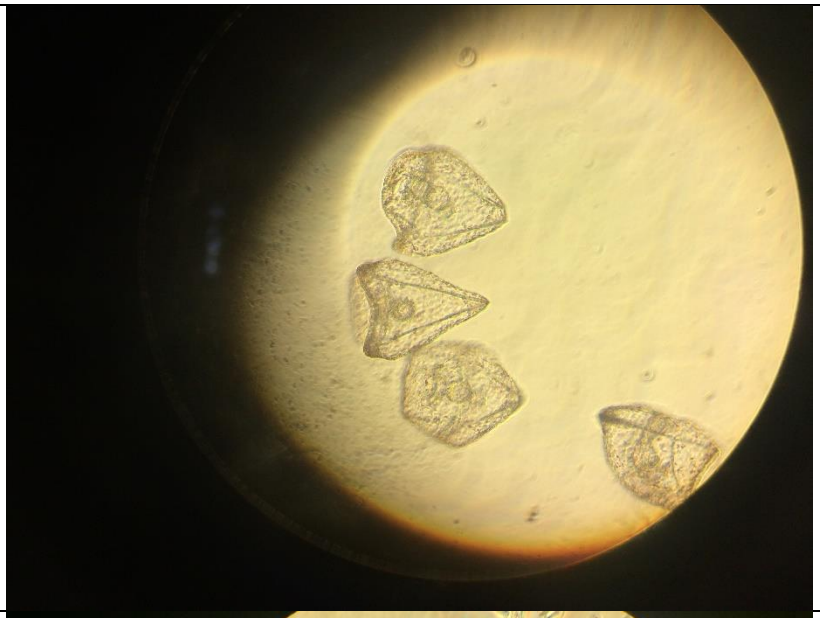
F5	major invagination (early archenteron evident)		Primarily in PMC and not in the neighbouring SMC
F1	Early-Prism		Primarily in skeleton and diffusely in developing arm.

Taken together, this gene is expressed in the micromere-PMC-skeleton cell lineage, possibly HesC.

Appendix-Raw images for embryos from numbered culture

A		

B		
C		
D		

E		
F