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A Report on the Fertilisation and Early Development in the Sea Urchin

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ABSTRACT

The primary objective of this study was to investigate fertilisation, cleavage and gastrulation in sea urchin embryos. It was found that a difference between room and 4°C stored samples were obtained, with only 55% of the embryos reaching the plateus larvae stage. After 72h, cell death occurred in the 4°C stored samples. The parthenogenic embryos died, and didn't pass the blastula stage of early gastrulation. This paper addresses 4 main topics of sea urchin embryo development, including parthenogenesis and the interference of fertilisation.

KEYWORDS: Development, embryos, cell division, gastrulation, parthenogenesis, fertilisation, death

INTRODUCTION

One of the easiest ways to understand fertilisation, cleavage processes and gastrulation in developmental biology, is to understand these processes by observing sea urchin embryos (Singh, 2019). Through the process of spawning, i.e. the induction of egg and sperm production, in sea urchins, one is able to obtain a wider view on these processes, by understanding what happens during early development in the sea urchin (Singh, 2019). This can be achieved by studying sea urchin embryo cleavage patterns across 3 days, or 72 hours, period. Some basic stages that are observed under ambient temperature conditions are: the fertilisation envelope, cell division, blastula, gatsula, morula, and the plateus larva (UKZN, 2019). However, cell death and slight changes after 24 hours can be obtained under room temperature, as well as, cold temperature viz. at 4°C (Singh, 2019, UKZN, 2019).

MATERIALS AND METHODS

a) Fertilisation
An aliquot of 15 ml of sea urchin eggs and sperm was collected. A drop of each was placed onto a glass slide. Thereafter the droplet was covered with a cover slip and observed under the light (compound) microscope at 4X. The difference between mature and immature eggs were noted. Then, a drop of egg and sperm were placed apart from each other, and thereafter a cover slip was placed on the drops to allow them to mix. The efficiency of fertilisation was calculated using the formula: Fertilisation efficiency = fertilised eggs / total number of eggs observed. Then it was determined if there were eggs whereby the fertilisation envelope rose but did not reach completion. The question of whether sperm continues to be attracted to the egg once the fertilisation envelope is raised was asked, as well as the fate of the immature eggs. The egg was then observed at 40X in order to observe protrusion of the sperm, or the acrosomal process (UKZN, 2019).

b) Cleavage and gastrulation
Sterile pasteur pipettes were used to transfer 2 large drops of diluted sperm and egg. 15 ml sterile sea water was used to mix the drops in a container. This was duplicated so that room and cold room samples were obtained. These served as the sterile cultures that were onserved across 3 days. The results were thereafter tabulated, and a number of questions were answered (UKZN, 2019).

c) Interference with fertilisation
Some eggs were exposed to sea water that had a pH of 5. The eggs were swirled in the water for 2 minutes and then transferred to normal sea water. Thereafter, sperm was used to fertilise the eggs. Whether fertilisation occurred or not was observed (UKZN, 2019).

d) Parthenogenesis
The eggs were placed in a hypertonic solution of sea water for 5 – 10 minutes. Whether there a fertilisation envelope observed, as well as why a blastula stage isn't observed often with parthenogenic embryos, was answered (UKZN, 2019).

RESULTS AND DISCUSSION

The sea urchin is an ideal organism for studying fertilisation and early development (Singh, 2019). In this study, it was observed that the mature eggs were the ones with a distinct vitelline membrane and a small nucleus, while the immature eggs were the ones with a large nucleus and a thin outer membrane (Singh, 2019). The fertilisation efficiency was 50%. Some of the eggs had fertilisation envelopes that reached completion. This was because all the eggs were either unfertilised or fertilised. Those that had fertilisation envelopes that didn't reach completion, could have been due to pathogenesis, or even partial activation by the sperms, due to damaged eggs (read UKZN, 2019; Parisi *et al*., 1978). Once the fertilisation envelopes are raised, the sperms bounce off it in order to avoid polyspermy. Due to the negatively charged vitelline membrane, because of the increase in voltage potential caused by the influx of sodium ions, the sperms repel the eggs (Singh, 2019). The immature eggs, on the other hand, due, since they aren't able to become fertilised by the sperms. At 40X, no acrosomal processes were observed since the sperms were too small to observe. However, some acrosomal processes were observed at 100X objective (Singh, 2019). The cleavage and gastrulation results at room and 4^{o} C temperature are tabulated below:

Table 1: Cleavage and gastrulation of sea urchin embryos at 4^{o}C and room temperature

Hours of Development	Date / Time	Stage / Appearance	
		Room temperature	4^{o} C
0 Hrs	14:00	Eggs and sperm (60%)	No observation (100%)
1 Hr	15:00	Dividing cells (60%)	Eggs and inactive sperm (70%)
2 Hrs	16:00	Blastula (55%)	No change (60%)
24 Hrs	15:00	Morula / Gatsula (55%)	Cell division (60%)
72 Hrs	15:00	Plateus larva (40%) / Cell division (15%)	Cell death (50%)

It's evident that at room temperature, some of the sea urchin embryos reached the plateus larvae stage, while some of them died at 3 days. There was stagnant cell division after 24h for the cold room sample, followed by cell death 3 days later. After 2 hours, the cold room sample had no change, while for the room temperature sample, the blastula was obtained. For the room sample, it was expected that cell division would occur. The table also shows that percentage of eggs what survived at each stage for example, 40% of the 50% of eggs from the 24hrs room temperature sample, reached the plateus stage, whereas 15% of the 55%, were killed. At 4^{o}C, 50% of the 60% of eggs in the 24h sample experienced cell death. These results were expected. In order to obtain results representative of the ocean, the pH, water temperature as well as saline conditions can be adjusted in the laboratory. Another factor that can be adjusted is the light intensity since the process of development in the sea urchin is complex, if the sperm did not reach the egg, fertilisation wouldn't have occurred (Singh,

2019). If the male and female pronuclei did not fuse, then cleavage wouldn't occur. There would be no cell division, and thus, no cleavage if DNA synthesis did not occur and if the centrosome did not align the chromosomes properly at metaphase (Singh, 2019; read Moorhouse *et al*., 2015; Parisi *et al*., 1978). The larva would not develop if gastrulation went out instead of in, and if the skeleton failed to from the plateus larvae (Singh, 2019).

When the eggs are immersed in pH5 sea water and then placed in normal water, fertilisation occurs with the sperms, because pH seldom affects fertilisation (Zhang et al., 2017). Furthermore, it's possible for fertilisation to be delayed, but not stopped. Since the ambient conditions under which fertilisation would have occurred is similar to be often ocean environment with normal sea water being a solution used to dilute the sea water can be expected that fertilisation would occur (Zhang et al., 2017). In the hypertonic solution experiment, some of the eggs were fertilised, while some remained unfertilised. Therefore, some fertilisation envelopes were observed. Exposures to the hypertonic solution promote cell division, because this environment is similar to the sperm ocean environment that the sea urchin lives in (Singh, 2019). However, some parthenogenesis involves the egg development without fertilisation, i.e. asexual fertilisation, the lack of sperm relates to the fact that the embryos can't reach the blastula stage (UKZN, 2019). These embryos often die, but growth up to the blastula stage, is initiated by activation of the PIP cycle when the eggs are exposed to the hypertonic solution (UKZN, 2019).

CONCLUSION

Sea urchin eggs and sperm has a distinct structure. One of the best methods of studying sea urchin early development is through the use of microscopy, namely light microscopy. There are mature and immature eggs, and their fertilisation efficiency depends on the environment, and environmental conditions, available for fertilisation to occur in the lab. Although pH affects fertilisation, sometimes this is not the case. At room temperature, all the stages of embryonic development were observed, including the Plateus larva. In addition, cell death was observed after 3 days for both the room temperature and cold stored samples. The parthenogenic embryos eventually died, with a few reaching the blastula stage. Due to asexual fertilisation, or the lack of sperms, these embryos can't form plateus larvae.

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