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Note

Effect of temperature on polar body formation in the edible oyster, *Crassostrea madrasensis*

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Abstract

Freshly fertilized eggs of edible oyster *Crassostrea madrasensis* were maintained at temperatures of 23,29 and 31°C and the biological developments were viewed under a light microscope to monitor the kinetics of polar body extrusion at these temperatures. Time of release of fifty percent of polar bodies at 23°C, 28°C and 31°C were 18,16 and 15 minutes post fertilization respectively, indicating the temperature dependence of this event.

Considerable emphasis has been given to the genetic manipulation of molluscs, particularly the oysters, as a method for improving the quality and productivity. (Allen *et al.* 1989; Beaumount and Fairbrother, 1991). Induction of triploidy is such a technique where the meiotic events in the egg of an oyster are manipulated by application of heat, pressure or chemical so that the egg retains rather than extrudes the polar body. The egg will then contain two sets of chromosome and sperm will contribute another, resulting in a total of three sets (Allen *et al.*, 1989)

During the anaphase I of meiosis, there is separation of dyads, of which those that are designated to occupy the PB I, move to the peripheral aster chromosomes and get separated from the zygote. Separation is mediated by the formation of a contractile ring of microfilaments, presumably containing actin located at the base of the projection where the cleavage furrow develops (Longo, 1972; Schroeder, 1975). It is believed to function much in the manner of a purse string, separating the cytoplasmic projection (the presumptive PB I) from the zygote.

Immediately following the formation of the PB I the chromosomes remaining within the zygote become organized on the metaphase plate of the second meiotic apparatus. (Raven, 1964). This takes place without an intervening telophase and prophase. At anaphase II, there is separation of the chromosomes, those that move to the peripheral aster become confined within a protrusion of cytoplasm that is eventually constricted from the zygote and develops into PB II.

Since anaphase is responsible for the halving of the chromosome complement through polar body formation,

interference with the anaphase leads to the arrest of this process and production of diploid egg. Fertilization of the diploid egg by sperms leads to incorporation of the haploid chromosome complement from the male resulting in triploid individual. (Fig.1). It has been reported by Nell and Maguire, (1996) and Allen *et al*, (1989) that the ideal time for application of shock for arresting polar body II is when 50% of first polar bodies have been extruded. Therefore, determination of the time of release of polar body becomes important for ploidy induction. Most of the reported works on kinetics of polar body and induction of ploidy have been carried out in temperate condition and shall not be applicable for tropical condition where temperature is high and the kinetics may be different. The present communication embodies the results of study on the effect of temperature on polar body formation in the edible oyster *Crassostrea madrasensis*.

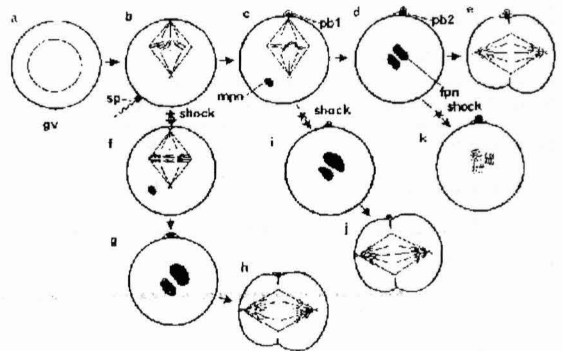

Fig. 1. *Showing cell division in a bivalve egg*

Gv = Germinal vesicle
Sp = Sperm
Mpn = Male pro nucleus
Fpn = Female pro nucleus
Pb1 = I Polar body
Pb II = II Polar body
A-E = Normal meiosis I, II and First cleavage
A = Oocyte release in water
B = Metaphase of meiosis I
C = Metaphase of meiosis II
F-H = Development of egg after treatment at meiosis I
I, J = Development of egg after treatment at meiosis II
K = Treatment of egg during I cleavage

Material and methods

Artificially fertilized gametes were used for the study. Gametes were collected by stripping the mature oysters (*C. madrasensis*) in filtered seawater having a salinity of 33ppt. The eggs were pooled, filtered through 100μ sieve to remove debris and divided into three parts and placed in three different temperatures viz. 23,29 and 31°C to monitor the biological events. Hot and cold water baths were used for the desired temperatures. Eggs and sperms were mixed at the ratio of 10:1 to facilitate artificial fertilization and to avoid polyspermy. All the events till the first cleavage of zygotes were monitored and recorded for each group in the 20-25 eggs seen under one field using 40x of microscope.

Results and discussion

The kinetics of different biological events taking place in the fertilized eggs from fertilization to first cleavage at three different incubation temperatures are presented in Table 1. As can be seen from the table that all the events like development of fertilization membrane, formation and release of polar bodies I and II, and first cleavage were at a faster pace at higher temperatures. Fifty percent of first

Table 1. *Temperature dependent biological events in fertilized egg*

Event/Temperature	23°C	29°C	31°C
Fert.memb.	10	8	6
I PB extrusion	12	10	8
50% I PB extrusion	18	16	15
II PB extrusion	23	22	21
50% II PB extrusion	38	32	28
I cleavage	43	38	34

polar bodies were released at 15, 16 and 18 minutes post fertilization at 31, 29 and 23°C respectively.

According to Lu (1986) meiosis speeds up with increasing temperature, the PB is vulnerable to manipulations sooner at higher temperatures when meiotic events of the eggs are more synchronous i.e., at higher temperatures the meiotic events transpiring in the eggs are in greater unison. Time of release of 50% of the first polar body is the critical factor for determination of the time of application of shock treatment for blocking of polar body release and induction of triploidy. Based on the time of release of 50% first polar body observed in the present study, the time for initiation of shock treatment for blocking the extrusion of polar body II have been fixed to be 18,16 and 15 minutes post fertilization at incubation temperatures of 23°C, 29°C and 31°C respectively. Allen and Downing, (1986) reported that the temperature appears to be more important than egg quality in determining the proportion of triploids induced. Since temperature affects the rate of meiosis, it follows that given the same treatment, eggs treated at different temperature gives different yields of triploids. Downing and Allen (1987) found in an extensive series of experiments that Cytochalasin-B administered at 25°C blocked the extrusion of PBII, more effectively than treatments at 18°C and 20°C, thereby producing more triploidy at faster rate.

In a tropical country like India, determination of the exact time of release 50% polar body depends upon the prevailing temperature which is a crucial factor for successful induction of triploidy, making the finding of this study relevant.

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