Does gametogenesis occur naturally in pearl oyster mantle tissue?

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Importance of mantle tissue

In marine and freshwater bivalves, mantle tissue is responsible for a number of specialized functions, such as sensorial capacity, nutrient storage, direction of feeding currents, and synthesis of substances that participate in shell mineralization and pearl formation (Addadi and Weiner 1997; Checa 2000; Barik et al. 2004; Acosta-Salmón and Southgate 2005). Furthermore, mantle tissue plays another important role in Mytilidae as the main sustaining means for gametogenesis (see Lowe et al. 1981; Bayne et al. 1982). So far, Mytilidae is the only known bivalve family in which gonadal development occurs naturally in the mantle tissue at the expense of stem cells and stored reserves (Mourazos et al. 2001). Several studies have characterized the microscopic anatomy of *Mytilus edulis* mantle tissue, and have reported the presence of cellular elements such as amoebocytes with glycogen that provide energy for gametogenesis (Lozada and Reyes 1981; Gabbott and Peek 1991). In the same species, other kinds of storage cells, such as adipogranular (ADG) cells and vesicular connective-tissue (VCT) cells have been reported to participate during gametogenesis, not only as sources of energy, but also as precursors of neurosecretions (Peek and Gabbott 1989; Lubet and Mathieu 1990; Mathieu and Lubet 1993; Mathieu et al. 1991). Some of these precursors are proteins called mantle connective tissue polypeptides (MCTPs), located in the ADG cells of the mantle, and whose expression directly relates to the mantle connective tissue volume and inversely relates to gonad acini volume (Mikhailov et al. 1996).

In pearl oysters, studies of histological, histochemical, biochemical, and ultrastructural composition of mantle tissue have focused more on aspects of shell mineralization and pearl formation than on reproduction. This is true for *Pinctada maxima* (Dix 1972, 1973; Dong 1999), *P. margaritifera* (Jabbour et al. 1992), *P. fucata* (Wada 1973), and *P. mazatlanica* (García-Gasca et al. 1994; Vite-García 2005).

First report of gonad development in *Pinctada mazatlanica* mantle tissue

As part of a broad project studying reproduction of the pearl oyster *P. mazatlanica* (Hanley, 1856), we report the first evidence of gonad-in-mantle tissue. Out of 180 samples analyzed during an annual cycle, 1.1% (two oysters) showed incidence of female acini in the marginal zone, where a diffuse connective tissue matrix with abundant packages of longitudinal and radial muscle fibers were evident (Fig. 1). In these samples, collected in January when the water temperature was 20.5° C (corresponding to spent or undifferentiated sexual stages), the integrity and development of female gametes appeared normal, and several groups of stem cells, oogonias, previtellogenic (small, immature gametes lacking yolk), vitellogenic (still immature, but growing peduncle-shaped cells), and postvitellogenic (fully ripe, free polygonal-shaped) oocytes could easily be observed lining up or filling acini (Fig. 2).

To our knowledge, this is the first report, worldwide, of a marine bivalve other than Mytilidae showing gametogenic activity in the mantle tissue. However, since male or female acini do not occur naturally in the mantle of Pteriidae, we are uncertain of the origin and expression mechanism of this phenomenon. Unlike the mantle tissue of Mytilidae, where the presence of stem cells and other types of storage cellular elements (amoebocytes and VCT and ADG cells) and active substances such as MCTPs has already been confirmed (Lozada and Reyes 1981; Peek and Gabbott 1989; Lubet and Mathieu 1990; Gabbott and Peek 1991; Mathieu and Lubet 1993; Mathieu et al. 1991; Mikhailov et al. 1996), we can only speculate at this point on the following: If the mantle tissue in Mytilidae favors the occurrence of gametogenesis at the expense of its own stem cells and reserves, the mantle of other bivalves, such as pearl oysters, may have similar cellular and reservoir elements or genetically non-expressed stem cells that become active under particular circumstances, such as culture conditions (e.g. density) or the oyster's reproductive, physiological, and/or epidemiologic condition (e.g. sex reversal, stress, disease or parasite infections). Although this hypothesis cannot be confirmed yet, previous observations in mantle tissue of *P. mazatlanica* support this likelihood. For example, García-Gasca et al. (1994) identified different cell-types in the marginal and paleal area of mantle tissue that not only synthesize enzymes related to the mechanism of shell mineralization, but also store glycogen, protein, and lipid reserves that may, under appropriate conditions, activate and sustain gametogenesis. In pearl culture opera-

Figure 1. Photomicrograph of a longitudinal section (4X) of the marginal zone of *Pinctada mazatlanica* mantle tissue, showing evidence of female acini (FA). CT = mantle connective tissue matrix; PZ = paleal zone; IE = internal epithelium; MF = radial and longitudinal muscle fibers. Scale bar = 100 µm.

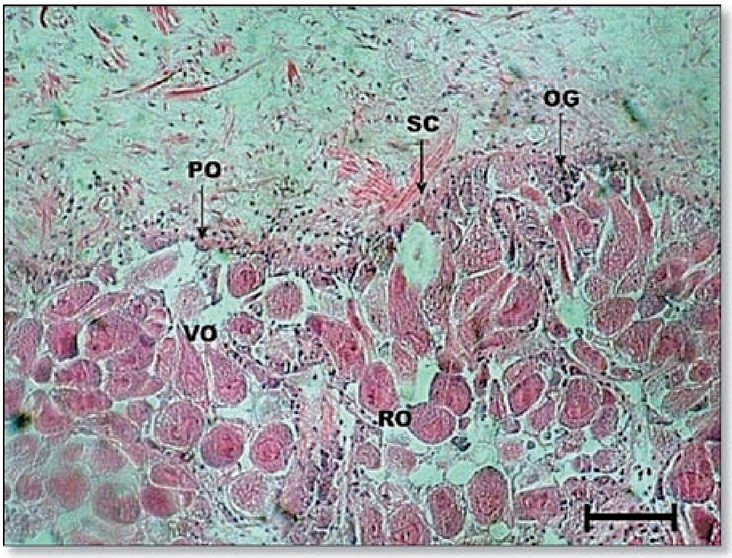

Figure 2. Photomicrograph of longitudinal section (20X) of the marginal zone of *Pinctada mazatlanica* mantle tissue, evidencing development of female gametes. SC = stem cells; OG = oogonias; PO = previtellogenic oocytes; VO = vitellogenic oocytes; RO = postvitellogenic (ripe) oocytes. Scale bar = 100 µm.

tions, Saucedo et al. (2001) observed that oysters inserted in the gonad with a mantle allograft, as part of the procedure to induce round pearl formation, were in better reproductive condition (greater gonadal development and more and larger postvitellogenic oocytes) and showed significantly different biochemical composition (higher protein, lipid, and triglyceride content in the gonad and less protein reserves in the muscle) compared with ungrafted oysters. The biochemical trend observed in treated oysters indicates redirection of energy flows from adductor muscle to acini to favors an active proliferation of gametes and rapid maturation of the gonad (Saucedo et al. 2001). In contrast, recent studies of seasonal changes in biochemical composition of mantle tissue suggest that internal storage reserves are not used during gametogenesis (Vite-García 2005).

Implications for mariculture

Previous results obtained by Saucedo et al. (2001), together with our gonad-in-mantle finding, may have a potential application for commercial hatchery production of spat, particularly to ensure gonadal maturation without depending on traditional broodstock conditioning under "controlled" conditions. In other words, these preliminary results may set the basis for a biotechnology that uses mantle tissue from donor oysters to induce gonadal maturation in receiver oysters. Although this perspective requires further validation, its application is relevant given that traditional gonadal conditioning usually faces serious problems, such as: 1) maintaining broodstock for long periods of time (average of 30–50 days), 2) supplying large quantities of microalgae diets, which is laborious and expensive, and 3) providing diets with suitable levels of lipids and highly unsaturated fatty acids, which is a necessity for producing high quality gametes, viable larvae, and continuous and sufficient yields of spat under hatchery conditions.

So far, our digital images represent the first evidence of gonadal development in mantle tissue of a pearl oyster species. Further investigations of the effects of mantle allografts on cycles of energy reserves, endocrine regulation of gametogenesis, physiological response and energy balance of grafted and ungrafted specimens should confirm our hypothesis and provide elements for validating a biotechnology to induce broodstock maturation through mantle allograft. Such a technology may sustain programmes for reproducing subtropical and temperate mollusc species requiring conditioning at times other than the short natural season for reproduction. This approach applies for most shellfish species inhabiting the shallow waters of the Gulf of California.

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