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TIMING OF OVULATION IN THE ATLANTIC WHITE SHRIMP *PENAEUS SETIFERUS* (LINNAEUS, 1767) (DECAPODA, PENAEIDEA)

BY

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ABSTRACT

The timing of ovulation in the American white shrimp, *Penaeus setiferus* (Linnaeus, 1767) was studied by sacrificing mated females and females whose spawning was interrupted for histological observation of ovarian tissue. The ovaries of the three mated females were in the cortical body stage, either pre- or post-germinal vesicle breakdown. The oocytes in all of the mated females were entirely enveloped by the follicular epithelium. Observations on the ovaries of females which stopped spawning indicated that ovulation may occur within a very short time before spawning or parallel with spawning.

RÉSUMÉ

Le déroulement de l'ovulation dans le temps a été étudiée chez la crevette américaine *Penaeus setiferus* (Linné, 1767) en sacrifiant des femelles après accouplement et des femelles dont le processus de ponte a été interrompu pour l'observation histologique des tissus ovariens. Les ovaires des femelles après accouplement étaient au stade du corps précortical, soit de la rupture de la vésicule pré- ou postgerminale. Les oocytes de toutes ces femelles étaient complètement enveloppées de l'épithélium folliculaire. Les observations sur les femelles dont le processus de ponte vait été interrompu ont montré que l'ovulation peut survenir très peu de temps avant la ponte ou parallèlement à celle-ci.

INTRODUCTION

Spawning or oviposition follow ovulation which is defined as the detachment of the oocytes from the follicular cells. Although oogenesis in penaeid shrimp has been studied and the ovarian maturation stages have been classified in several species (Anderson et al., 1984; Tan-Fermin & Pudadera, 1989), information on the events between cortical body formation and spawning is scarce. Only two reports (Anderson et al., 1984; Browdy, 1989) dealt with ovulation in *Sicyonia ingentis* (Burkenroad, 1938) and *Penaeus semisulcatus* de Haan, 1844, in which they conclude that ovulation requires less than few hours.

In this study, we report our observations on the histological events occurring in the ovary during spawning of the white shrimp *Penaeus setiferus* (Linnaeus, 1767).

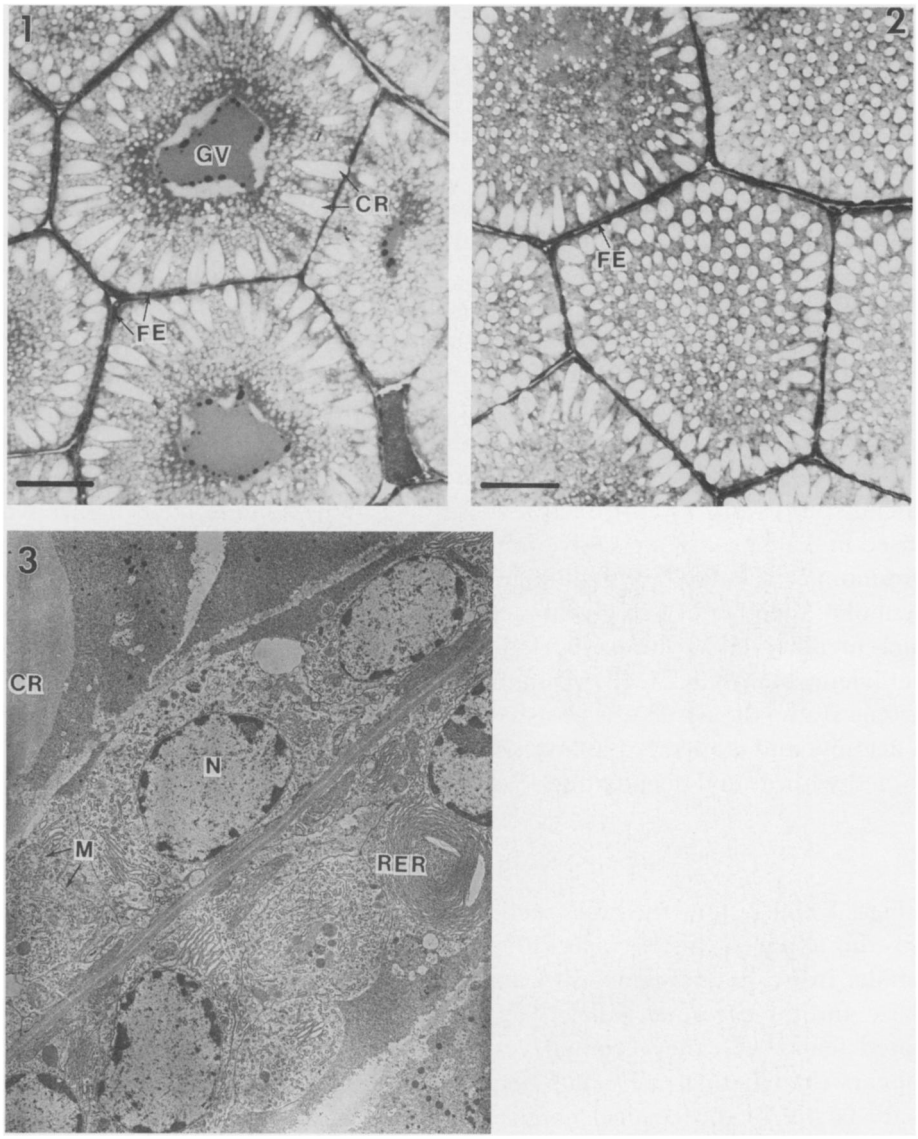
MATERIALS AND METHODS

Mature, ready to spawn females of *Penaeus setiferus* were collected by trawl in the coastal area of Charleston, South Carolina, U.S.A., from May to August, 1989. Some ovarian lobes of three mated females (A, B and C) captured in the night trawl were dissected on the boat or after transfer to the laboratory and fixed in 5% glutaradehyde - 0.07 M sodium cacodylate in artificial sea water (pH 7.4) for 4 hours. Non-mated females were transferred alive to the laboratory, artificially inseminated and kept in glass aquaria (70 liter) at 28°C and 34‰ salinity. Out of sixty three females used, thirty two spawned in captivity. Spawning of twelve females was interrupted by catching them in a dip-net, and three of them, designated D, E and F, stopped spawning. Another nine females with interruption and twenty without interruption were observed to empty their ovaries. Three ovarian lobes of the cephalothoracic region and anterior and posterior abdominal regions of the ovary were dissected from the three females (D, E and F) and fixed as described above. These fixed tissues were rinsed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 7% sucrose for a minimum of one day. For light microscopic study, the tissue was dehydrated by alcohol and embedded in glycol methacrylate (Dupont, Wilmington, DE), and thick methacrylate sections (0.5 - 1 μm) were stained with chromotrope 2R/ methylene blue (CR2/MB) (Dougherty & King, 1986). For electron microscropic study, the tissue was post-fixed in 1% $0s0_4$ for 1 to 2 hours, dehydrated in acetone and embedded in low viscosity epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate.

RESULTS

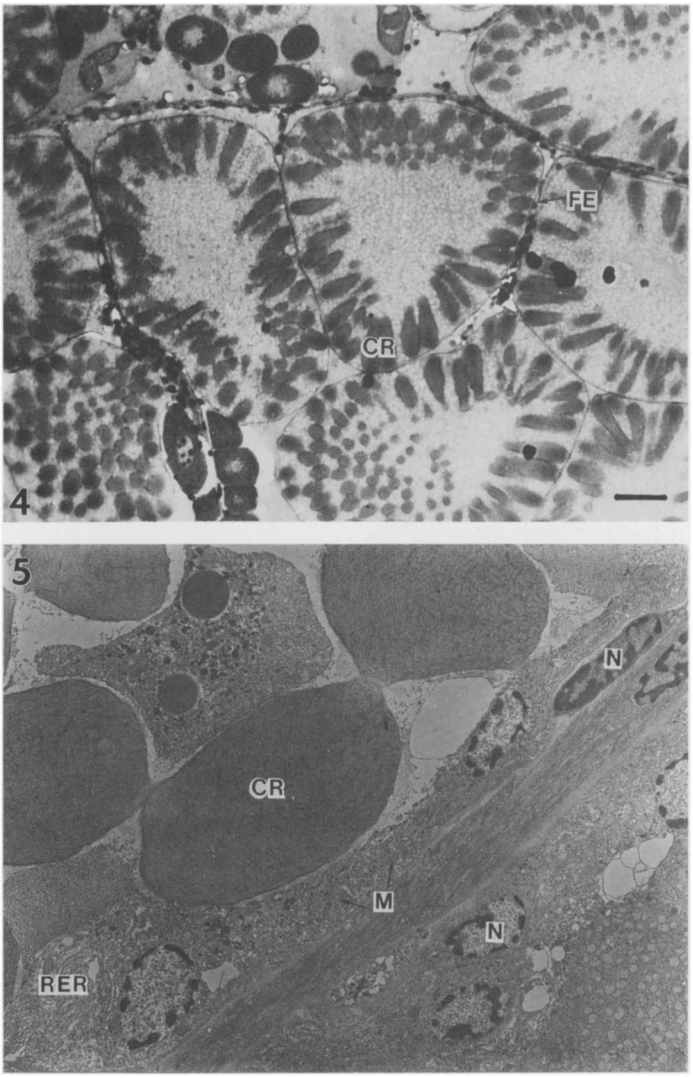
Figs. 1 and 2 show the cross sections of the ovaries of mated females. There were no apparent histological differences between the ovarian lobes within a female. In two mated females (A and B) the oocytes were in the cortical body phase and the germinal vesicle (GV) was still intact (fig. 1), while in the other mated female (C) the germinal vesicle was already broken down (fig. 2). It appears that mating can occur between a mature male and a female whose ovary is still in the cortical body phase before germinal vesicle break down (GVBD). The oocytes in all of the mated females were found to be entirely enveloped by the follicular epithelia (FE). The electron micrograph (fig. 3) shows that the follicular epithelial cells contain round or oval shaped nuclei (5 - 8 μm in diameter), abundant mitochondria (M) and rough endoplasmic reticulum (RER).

Many females in captivity spawned after midnight. Female D spawned only a few eggs and stopped thereafter. The light micrograph of the cephalothoracic region of this female's ovary indicated that many oocytes were incompletely ovulated by follicular epithelia (fig. 4). The electron micrograph showed that some of the follicular epithelia (FE) became thin and the nuclei (N) were spindle

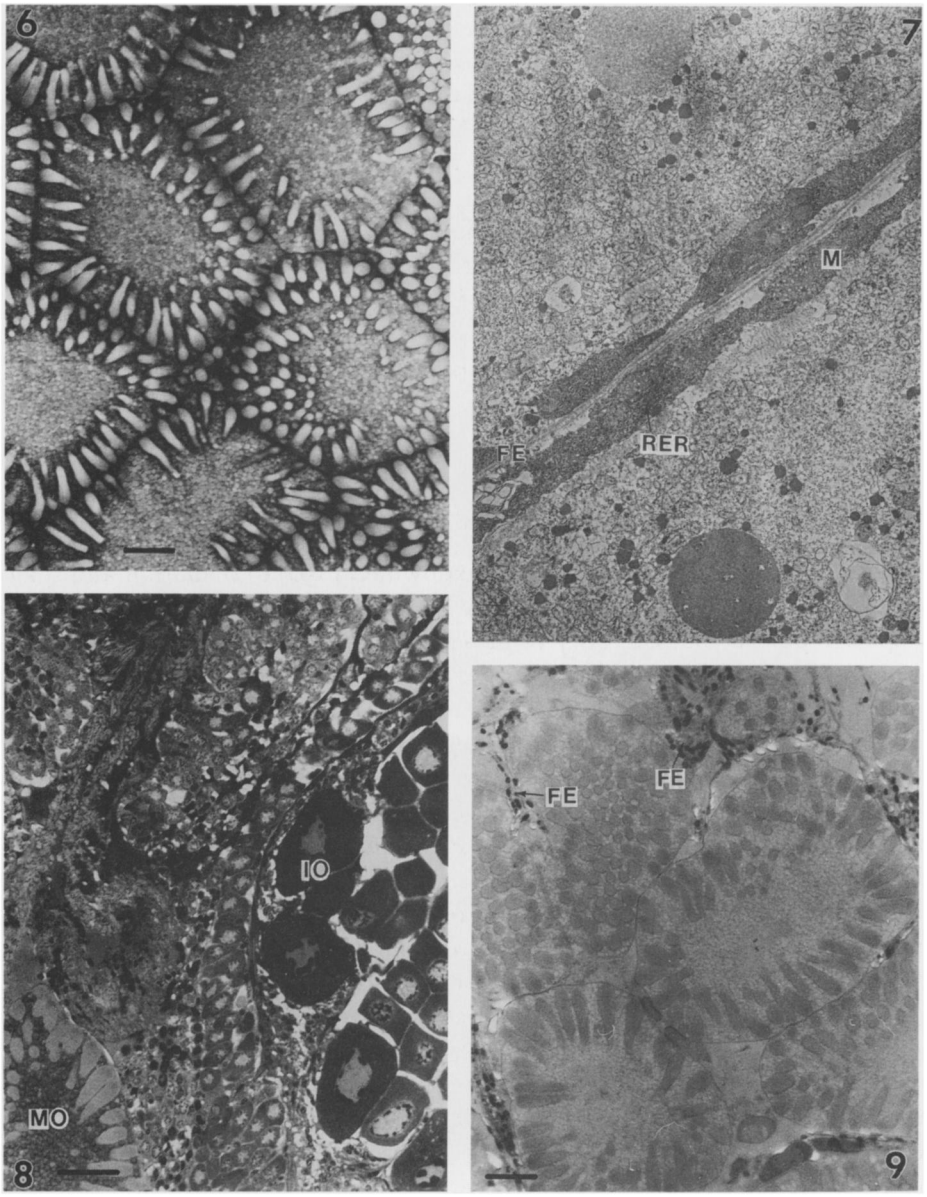


Figs. 1-3. *Penaeus setiferus* (L.). 1, light micrograph of the cephalothoracic region of the ovary of a mated female A; the oocytes which have the fully developed cortical rods (CR) and intact vesicle (GV) are entirely enveloped by the follicular epithelia (FE); bar = 30 μm. — 2, light micrograph of the cephalothoracic region of the ovary of a mated female C; germinal vesicles have broken down (GVBD); the follicular epithelia (FE) are intact; bar = 30 μm. — 3, electron micrograph of follicular epithelia in GVBD ovary of the mated female C; the cells contain oval or round nuclei (N), numerous mitochondria (M) and rough endoplasmic reticulum (RER); × 5,200.

shaped (ca. 2 × 9 μm) (fig. 5). However, in the anterior and posterior abdominal regions of the ovary of this individual, the follicular epithelia were observed intact. Female E spawned about half of the ovary in the cephalothoracic region when her spawning was interrupted. The abdominal region of the ovary looked



Figs. 4, 5. *Penaeus setiferus* (L.). 4, light micrograph of the cephalothoracic region of the ovary of female D which spawned only a few eggs and stopped thereafter; note that the envelopment by the follicular epithelia (FE) is incomplete for many oocytes indicating retreat of the follicular epithelia from the oocytes' surface; bar = 30 μm. — 5, electron micrograph of the cephalothoracic region of the ovary of female D; some of the follicular epithelia become thin and the nuclei (N) are spindle shaped; × 2,800.



Figs. 6-9. *Penaeus setiferus* (L.). 6, light micrograph of the cephalothoracic region of the ovary of female E which had evacuated about half of eggs in this region of the ovary when she stopped spawning; almost all of the oocytes in this region were found to be ovulated; bar = 30 μm. — 7, electron micrograph of the cephalothoracic region of the ovary of female E; very thin follicular epithelia are observed on a few oocytes; the follicular cells are abundant in mitochondria (M) and rough endoplasmic reticulum (RER); × 14,000. — 8, light micrograph of the completely evacuated cephalothoracic region of the ovary of female F; only a few matured oocytes (MO) were observed with many immature oocytes (IO); bar = 30 μm. — 9, light micrograph of the abdominal region of the ovary of female F; note that the follicular epithelia (FE) are retracting from the surface of the oocytes indicating that this region of the ovary was in the course of ovulation when this female stopped spawning and was sacrificed; bar = 30 μm.

intact. Almost all of the oocytes in the cephalothoracic region of the ovary were found to be ovulated, as little follicular epithelium was observed between the oocytes (fig. 6). Very thin follicular epithelium could be found on a few oocytes (fig. 7). The cell linings on the thin follicular epithelia seemed to be stretched, but contained many mitochondria (M) and rough endoplasmic reticulum (RER). Female F spawned almost all mature oocytes in the cephalothoracic region of the ovary, while the abdominal region looked intact. Many immature oocytes and only a few matured oocytes were observed in the cephalothoracic region of the ovary (fig. 8), which is representative of the spent stage as observed in the other penaeid shrimp species. Cross section of the abdominal region of the ovary indicate that this region was undergoing ovulation, as ovulated, incompletely ovulated and unovulated oocytes were observed in the same region (fig. 9).

DISCUSSION

The timing of germinal vesicle breakdown (GVBD) to ovulation and spawning may vary between penaeid species. Anderson et al. (1984) using an ovarian biopsy, reported that the oocytes of *Sicyonia ingentis* (Burkenroad) may remain surrounded by follicle cells for more than 24 hours after GVBD. Bray & Lawrence (1984) and our field survey have indicated that only a few mated females of *P. setiferus* could be caught during day time, suggesting that most of the mated females spawn in the same night, probably within 12 hours after mating. The present study provided mated females whose ovaries were still before GVBD. Therefore, oocytes of *P. setiferus* were in GVBD phase for less than 12 hours. This coincided with Browdy's (1989) observations that GVBD was more commonly observed for females of *P. semisulcatus* de Haan, 1844 sacrificed late at night, indicating a short time lag between GVBD and spawning in penaeid shrimp. Anderson et al. (1984) reported that females of *S. ingentis* having undergone GVBD but not ovulation, spawned 1, 2 and 4 hours after the biopsy examination, and conclude that ovulation required less than a few hours. Browdy (1989) reported that ovulated ovaries were only observed late at night in females of *P. semisulcatus* showing increased swimming activity, characteristic of females immediately before spawning. These studies coincide well with the present study which indicates that ovulation may be quite brief, probably requiring less than a few minutes before spawning or may occur parallel with spawning in *P. setiferus*. Furthermore, ovulation appears to proceed from the cephalothoracic region to the abdominal region of the ovary.

As observed in the present study, retracting follicular epithelia have been observed in the ovaries undergoing ovulation in another penaeid shrimp *P. semisulcatus* (see Browdy, 1989) and a freshwater palaemonid shrimp *Macrobrachium rosenbergii* (De Man, 1879) (Fauvel, 1983). Concentrations of follicular epithelia are found in the spent stage ovary of several shrimp species (King,

1948; Fauvel, 1983; Tan-Fermin & Pudadera, 1989; Browdy, 1989). Therefore, it is likely that follicular epithelia detach from the oocytes' surface and retract or migrate to certain regions of the ovary. Fauvel (1983) concluded that the retracted follicular ephithelia would be active again during the next folliculogenesis, although physiological factors and mechanisms involved in the ovulation process are not known in decapod Crustacea.

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