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A COMPARATIVE STUDY OF THE OVARIAN HISTOLOGY OF EYESTALK ABLATED AND UNABLATED *Farfantepenaeus paulensis* AFTER SPAWNING

[Estudo comparativo da histologia ovariana de *Farfantepenaeus paulensis* com e sem ablação do pedúnculo ocular após a desova]

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ABSTRACT

The effect of unilateral eyestalk ablation on ovarian histology of *Farfantepenaeus paulensis* after spawning was evaluated in the present study. Wild females were captured in deep-sea waters of southern Brazil (27° S) and randomly divided in two groups: unilaterally eyestalk ablated and unablated (intact) females. A total of six eyestalk ablated and five unablated females were sampled after spawning in separated tanks. Morphological variables were recorded and ovaries were histologically evaluated according to oocyte type and diameter. In the ovarian tissue of unablated females, basophilic oocytes (48.8 ± 18.7 µm) were dominant (99.7 ± 0.6%), with presence significantly lower in the ablated females (93.5 ± 13.8%). Larger acidophilic oocytes (114.9 ± 16.9 µm), with yolk granules in the cytoplasm, were detected only in the ovaries of ablated females. The significantly higher occurrence of atretic oocytes (4.8 ± 10.3%) is another distinguishable feature in the ovaries of ablated females compared with the unablated ones. The presence of advanced yolky oocytes in ablated females just a few hours after spawning, may indicate the effect of eyestalk ablation on the precocious rematuration of the ovary. The higher number of atretic oocytes in the ovary of ablated females could be relevant to their reproductive performance, as they represent a percentage of oocytes that were not released in the spawning process. The present results of the histology of the ovaries reveal the existence of substantial differences in the cell arrangement of ablated and unablated females after spawning. The results also demonstrate the effects of unilateral eyestalk ablation in the reproductive cycle of *F. paulensis* under laboratory conditions.

Key words: eyestalk ablation; gonad; histology; reproduction; *Farfantepenaeus paulensis*

RESUMO

O presente estudo avalia o efeito da ablação do pedúnculo ocular na histologia gonadal de *Farfantepenaeus paulensis* após a desova. Fêmeas selvagens foram capturadas em águas profundas na região sul do Brasil (27° S), sendo divididas aleatoriamente em dois grupos: com ablação unilateral do pedúnculo ocular e sem ablação (intactas). Um total de seis fêmeas sem pedúnculo e cinco intactas foram amostradas após suas desovas individualizadas. Após avaliação dos parâmetros morfológicos, os ovários foram histologicamente analisados quanto ao tipo e diâmetro dos ovócitos. Nos ovários das fêmeas intactas, os ovócitos basófilos (48,8 ± 18,7 µm) foram dominantes (99,7 ± 0,6%), sendo sua presença significativamente inferior (93,5 ± 13,8%) em fêmeas sem pedúnculo. Ovócitos acidófilos de maior tamanho (114,9 ± 16,9 µm), com citoplasma granuloso, foram observados apenas nos ovários das fêmeas sem pedúnculo. A ocorrência elevada de ovócitos em atresia (4,8 ± 10,3%) foi outra característica dos ovários das fêmeas sem pedúnculo, quando comparados aos das fêmeas não-abladas. A presença de ovócitos em desenvolvimento mais avançado em fêmeas sem pedúnculo, algumas horas após a desova, parece indicar o efeito da ablação do pedúnculo ocular na maturação precoce dos ovários. A alta incidência de ovócitos atrésicos em fêmeas sem pedúnculo pode ser relevante para seu desempenho reprodutivo, já que representam uma porcentagem de ovócitos não liberados no momento da desova. Pode-se concluir que a histologia dos ovários permite revelar diferenças significativas no arranjo celular de fêmeas sem pedúnculo e intactas. Os resultados demonstram o efeito da ablação do pedúnculo ocular no desempenho reprodutivo de *F. paulensis* em laboratório.

Palavras-chave: ablação do pedúnculo ocular; gônada; histologia; reprodução; *Farfantepenaeus paulensis*

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Introduction

Unilateral eyestalk ablation is the commonest technique and the most effective way to induce the ovarian maturation and spawning of many species of penaeid shrimp in captivity (BROWDY, 1992). With this technique, the endocrine system is directly affected by reducing the inhibitory control over reproduction (DALL *et al.*, 1990). Although faster maturation and a decrease in the latency period between spawnings are commonly observed, conflicting results have been reported about the effect of the eyestalk ablation on spawning quality (BEARD and WICKINS, 1980; EMMERSON, 1980; BROWDY and SAMOCHA, 1985; TAN-FERMIN, 1991). Histological analysis in the ovaries of ablated and unablated females showed significant differences in the size and type of oocytes (VOGT; QUINITIO; PASCUAL, 1989; TAN-FERMIN, 1991) as well as in the frequency of atretic cells (KELEMEC and SMITH, 1984), which have been associated with reproductive performance.

Although the ovarian histology of wild *Farfantepenaeus paulensis* has been described previously (WORSMANN *et al.*, 1971; WORSMANN and SESSO, 1977) and eyestalk ablation has been successfully applied in broodstock from various sources (MARCHIORI and BOFF, 1983; MARCHIORI and CAVALLI, 1993; CAVALLI; SCARDUA; WASIELESKY, 1997; PEIXOTO *et al.*, 2002), no information on ovarian histology of eyestalk ablated females is currently available. In the present study, we evaluated whether unilateral eyestalk ablation affects the ovarian histological characteristics and oocyte type in recently spawned *F. paulensis* females.

Material and Methods

Female sampling

Wild females of *F. paulensis* were captured in deep-sea waters from southern Brazil (27º S) and maintained in maturation tanks (5,000 L) under standardized environmental conditions (CAVALLI; SCARDUA; WASIELESKY, 1997). After one week of acclimation, the animals were randomly isolated in two groups: (1) unablated (intact) females and (2) unilaterally ablated females by cutting and cauterizing eyestalk. Maturation tanks were daily inspected and any female with ripe ovaries was transferred to separate 200 L spawning tanks filled with 90 L of sea water. Only completely spawned females were selected in the next morning, otherwise they were returned to the maturation tank. A total of five unablated and six ablated females were sampled for histological ovarian analysis. After the measurements of carapace length (distance from the postorbital margin to the mid-dorsal posterior edge of the carapace, CL), body length (distance between the tip of the rostrum and tip of the telson, BL).

A comparative study of the ovarian histology of eyestalk ablated and unablated *F. paulensis* and body wet weight (BW), the ovary was dissected and weighted (OW). The gonadosomatic index (GSI) was calculated as a percentage of the OW relative to the BW.

Ovarian histology and data analysis

Middle portions of the ovaries (2-3 mm) were fixed in Davidson solution for 24 h, embedded in paraffin, sectioned (6 mm) and stained with hematoxylin-eosin (BELL and LIGHTNER, 1988). Images of histological sections (100x) were captured by video with a camera (CSC-730, color NTSC) connected to a microscope (Nikon SC). Ten randomly digitized images from different fields of each ovary were analyzed using the software ImageTool version 2.0 for Windows (The University of Texas Health Science Center in San Antonio, USA).

The oocytes were categorized according to the histological features described by WORSMANN *et al.* (1971). The frequency of each oocyte type and degenerating oocyte (atresia) was taken as a percentage of the total number of oocytes counted in each field. The largest diameter of 30 oocytes or the total number available per category was measured in each image. Only oocytes showing nuclei sectioned approximately at the equatorial plane were measured.

Student t-test was used to detect differences, in the analyzed characteristics, between unablated and ablated females at the 5% significance level. Prior to the analysis, percentage data (e.g. GSI and frequency of oocytes) were arcsine transformed, but only original values are presented.

Results

There were no significant differences (P>0.05) in CL, BL, BW, OW or GSI between unablated and ablated females (Table 1). However, histological analysis of the ovaries revealed significant differences (P<0.05) in the frequency of oocytes and occurrence of atresia between the two groups (Table 2).

In the ovarian tissue of unablated females, basophilic oocytes (BO) represented nearly 100% of the cell population (Table 2; Figure 1A). These oocytes had a nucleus larger than the cytoplasm size and several nucleoli dispersed in the interior (Figure 1C). The cell diameter ranged from 25 to 100 µm with smaller oocytes lying in the center of the ovarian lobe. Despite the predominance of BO in the ovaries of ablated females, their presence was significantly lower than among unablated spawners (Table 2).

Acidophilic oocytes with yolk granules (YO) in the cytoplasm were detected in the periphery of the ovarian lobe of ablated females (Figure 1B), but these oocytes were not observed in the ovaries of unablated ones. The nucleoli of the YO are organized on the periphery of the nucleus. A considerable increase in cytoplasm is observed, with cell diameter ranging from 100 to 220 µm (Figure 1C).

The significantly high occurrence of atretic oocytes (AO) in the eyestalk ablated females is another distinguishable feature in the cell arrangement of the ovaries of the females of the two groups (Figure 1B).

Table 1. Mean (± SD) carapace length (CL), body length (BL), body weight (BW), ovarian weight (OW) and gonadosomatic index (GSI) of eyestalk ablated and unablated females of *F. paulensis* (No significant differences were found. P>0.05).

	Eyestalk unablated	Eyestalk ablated
CL (cm)	4.7 ± 0.4 ^{a}	4.8 ± 0.5 ^{a}
BL (cm)	19.2 ± 0.5 ^{a}	18.6 ± 0.8 ^{a}
BW (g)	61.9 ± 9.4 ^{a}	64.8 ± 15.7 ^{a}
OW (g)	1.32 ± 0.24 ^{a}	1.27 ± 0.12 ^{a}
GSI (%)	2.15 ± 0.35 ^{a}	2.15 ± 0.27 ^{a}

Table 2. Frequency of occurrence and diameter (mean ± SD) of basophilic oocytes (BO), yolky oocytes (YO) and atretic oocytes (AO) in the ovarian tissue of eyestalk ablated and unablated females of *F. paulensis*. (Different superscripts within rows indicate significant differences. P<0.05).

	Eyestalk unablated	Eyestalk ablated
BO (%)	99.7 ± 0.6 ^{a}	93.5 ± 13.8 ^{b}
YO (%)	0 ^{a}	1.7 ± 2.6 ^{b}
AO (%)	0.3 ± 0.56 ^{a}	4.8 ± 10.3 ^{b}
BO (µm)	48.8 ± 18.7 ^{a}	47.8 ± 19.6 ^{a}
YO (µm)	NA	114.9 ± 16.9

NA = not available

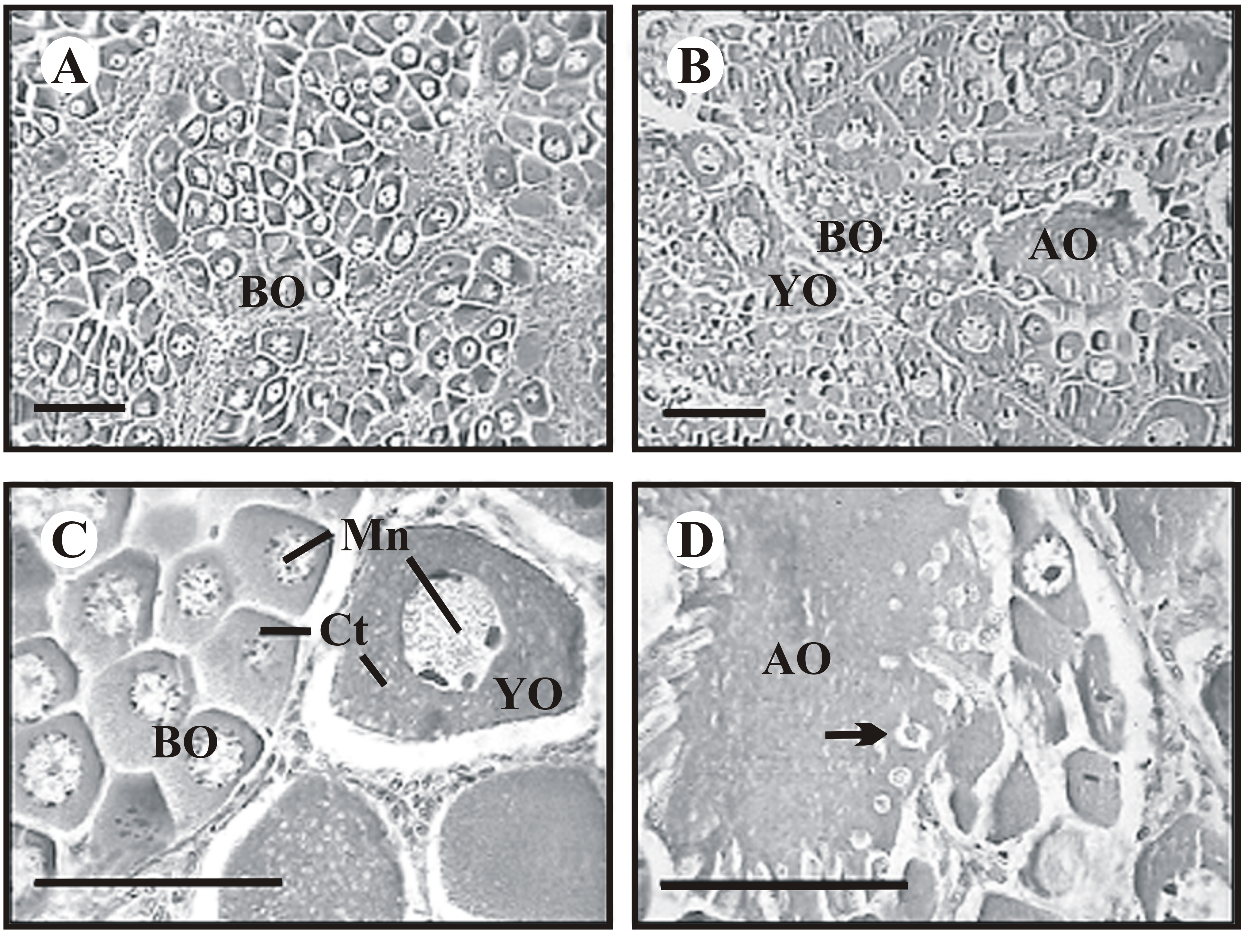

Figure 1. Histological sections of ovaries from eyestalk unablated (A) and ablated (B-D) females of *F. paulensis* after spawning. A) Basophilic oocytes (BO) are absolutely dominant in the ovaries of unablated females (100x); B) Besides basophilic oocytes (BO), yolky oocytes (YO) and atretic oocytes (AO) are also observed in the ovarian tissue of ablated female (100x); C) Enlarged view (400x) of the ovary of an ablated female showing basophilic oocytes (BO) with nucleus (Nu) larger than its cytoplasm (Ct) and acidophilic yolky oocytes (YO) with a considerable increase in cytoplasm size (Ct); D) Enlarged view (400x) of an atretic oocyte (AO) with cortical rods (arrow) in the ovarian tissue of an ablated female. Scale bars: 100 µm.

Discussion

Histological techniques have been widely applied to describe the ovarian maturation process of several penaeids (KING, 1948; TAN-FERMIN and PUDADERA, 1989; TAN-FERMIN, 1991; MEDINA *et al.*, 1996; QUINTERO and GARCIA, 1998), including *F. paulensis* (WORSMANN *et al.*, 1971; WORSMANN and SESSO, 1977). Despite some discrepancies on the nomenclature of the three basic oocyte types described in these studies, their results generally agree that basophilic oocytes are observed throughout ovarian development though they are predominant in the immature and spent (after spawning) stages. Yolky acidophilic oocytes occur during intermediary development and acidophilic oocytes with cortical rods are only found in the pre-spawning stage.

In our study, the presence of advanced yolky oocytes (YO) in the ovaries of ablated females just a few hours after spawning may indicate a relatively faster rate of ovarian maturation in these females. Furthermore, the frequency of BO was comparatively lower in the ovaries of ablated females, which is probably the result of the development of a certain number of BO into YO. In accordance, VOGT; QUINITIO; PASCUAL (1989) reported that an ablated *Penaeus monodon* female exhibited oocytes in all stages of maturation 2h after spawning, while the unablated ones contained only immature oocytes. These findings were later confirmed by TAN-FERMIN (1991), who also concluded that the precocious rematuration of the ovary soon after spawning was a direct result of eyestalk ablation.

The endocrinological processes controlling gonad maturation in penaeids is not yet fully understood, but eyestalk ablation presumably accelerates this process as it reduces the synthesis and release of the gonadal inhibitory hormone (GIH) from the neurosecretory complex located in the eyestalk (DALL *et al*., 1990; BROWDY, 1992). KULKARNI and NAGABHUSHANAM (1980) found that during spawning eyestalk unablated *Parapenaeopsis hardwickii* females had comparatively higher GIH levels which could retard the next cycle of ovarian maturation. In contrast, the eyestalk ablated ones were able to start a new maturation cycle immediately as they had a lower concentration of GIH in the hemolymph. This hypothesis is in line with the histological features of ovaries of eyestalk unablated and ablated *F. paulensis* observed in the present study.

Atretic oocytes have been reported in various stages of reabsorption in the ovary of unablated penaeids after spawning (KING, 1948; MARTOSUBROTO, 1974; QUINTERO and GARCIA, 1998), including *F. paulensis* studied by WORSMANN *et al*. (1971). The reabsorption of mature oocytes in the ovary (atresia) could have a significant effect on the reproductive performance, as they represent oocytes that were not released in the spawning process (KING, 1948). Nevertheless, the effects of eyestalk ablation either on the incidence of atretic oocytes (TAN-FERMIN, 1991; KELEMEC and SMITH, 1984, VOGT; QUINITIO; PASCUAL, 1989) or on the number of eggs released (EMMERSON, 1980; BROWDY AND SAMOCHA, 1985) are still conflicting. According to our results, the higher frequency of atretic oocytes suggests that eyestalk ablated *F. paulensis* females may have a decrease in the number of eggs released per spawning. However, further studies correlating ovarian histology and spawning events must be undertaken, in order to have conclusive results.

The present results of the histology of the ovaries reveal the existence of substantial differences in the cell arrangement of eyestalk ablated and unablated females after spawning. The results also clearly demonstrate the effects of unilateral eyestalk ablation in the reproductive cycle of *F. paulensis* under laboratory conditions. Eyestalk ablation of *F. paulensis* females remains necessary in commercial hatcheries, aiming to produce large amounts of nauplii, due to the faster maturation and higher spawning frequency (PEIXOTO *et al*., 1998). However, it might be advantageous to collect the first spawns of wild females as soon as they are captured, since this probably maximize the production cycle of the broodstock.

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