On the spermatophore implantation in bobtail squid *Rossia macrosoma* (Cephalopoda: Mollusca)

Meryem Akalin¹, Alp Salman¹”, Bahadır Önsoy²

¹ Department of Hydrobiology, Faculty of Fisheries, Ege University, 35100, Bornova, Izmir, TURKEY
² Department of Hydrobiology, Faculty of Fisheries, Muğla Sıtkı Koçman University, 48000, Muğla, TURKEY

*Corresponding author: alp.salman@ege.edu.tr

Abstract

In this study, mated female specimens of *Rossia macrosoma* from the Aegean Sea were examined. During mating, the male places spermatangia on the head of the female (typically around the eyes), and on the anterior mantle edge, particularly on the left side near the oviduct opening. To determine implantation structure type, deeply intradermal implanted spermatangia were dissected from mated females and their structures were investigated through histological analyses. Outline structure of the spermatangia consists of a spiral filament, sperm mass, cement body and a trailing end. The cement body is located centrally surrounded by the sperm mass from the view of the histological cross section. Spermatangia could be implanted in females’ tissue autonomously by major role of the spiral filament located on the oral part of spermatangia. Implanted spermatangia have thin, capillary-tubule shaped trailing end located aborally. In our opinion these trailing end, transfer spermatozoa obtaining fertilization of the eggs in the external environment.

Keywords: *Rossia macrosoma*, spermatangia, implantation, histology, Cephalopoda

Received: 25.07.2016, Accepted: 04.09.2016

Introduction

Rossiinae is one of the three subfamilies of Sepiolidae. They are distributed in all the world's oceans at depths between 40-2000 meters (Reid and Jereb 2005). In coleoid cephalopods, the sexes are separate and spawning is generally concentrated at the end of the animal’s life (Nesis 1995).

In males, spermatozoa are packed into spermatophores. After being transferred to the female, spermatophores go through the spermatophoric reaction and turn in to spermatangia (Marian 2012a).
Spermatangia are attached to different parts of the female’s body (Nesis 1995). For example, spermatangia are placed near the opening of the oviduct (bursa copulatrix) in sepiolids, in seminal receptacle of Heteroteuthinae (Hoving et al. 2008), on the buccal membrane within sepiids, in the mantle cavity in Illex (Ommastrephidae), and on the outer surface of the mantle in Moroteuthis ingens (Ommastrephidae) (Jackson and Jackson 2004). In octopuses, they are found within the ovary in members of Eledoninae, but in members of Octopodinae they are placed in the oviducal gland (Nesis 1995). In some species of Rossininae e.g., Neorossia caroli (Cuccu et al. 2007), Semirossia patagonica (Önsoy et al. 2008) and Rossia moelleri (Hoving et al. 2009; Laptikhovsky et al. 2008) there is no bursa copulatrix. In these species, spermatangia are implanted on different areas of the outer surface of the female’s body (Cuccu et al. 2007; Hoving et al. 2008; Salman 2011).

Rodrigues et al. (2011) stated that spermatangia implanted in bursa copulatrix of Sepiola atlantica which is another sepiolid species, might survive as long as 104 days. Middle membrane, the outer wall of the spermatangia of Rossininae (Hoving et al. 2008) has a role of supporting vital activities of spermatangia (Marian 2012b).

Even though the spermatophore implantation which is a successful egg fertilization mechanism assuming a part of the mating behavior was tried to be explained by other researchers (Hoving et al. 2010; Cuccu et al. 2011; Salman, 2011), some questions about its underlying mechanism still remains.

The aim of this study is to describe the structure of implanted spermatangia and thus to infer the fertilization process in Rossia macrosoma.

Materials and Methods

Samples were collected from the Aegean Sea monthly between April 2008 and May 2009 at depths of 350m and 550m using bottom trawls (Figure 1). Samples were stored in 10% formalin on board and separated by sex. Sexual maturity stages were determined following Laptikhovsky et al. (2008).

Four of eight females of R. macrosoma were at stage IV in 45–55 mm mantle length (ML) and other four samples were at stage V with 54–60 mm ML. For histological preparations, female tissue samples with implanted spermatangia were washed with water. After washing, samples were dehydrated in a graded ethanol series, cleared in xylol, and embedded in paraffin wax. Six-μm-thick longitudinal and transverse sections were cut with a microtome and stained with Mallory’s haematoxylin and eosin (HE). In Mallory, hematoxylin stains proteinaceous structures with blue; eosin stains cytoplasmic structures with pink. Periodic acid Schiff (PAS) was used to stain mucopolysaccharides in cement body. Sections containing implanted spermatangia were examined using
a CX-41 phase contrast microscope and photographed with an Olympus DP-20 digital camera.

Figure 1. Sampling areas

Results

Females of *R. macrosoma* lack a bursa copulatrix or seminal receptacle. Since the oviduct is located on the left side of the body, implanted spermatangia were usually located on the left ventral side of the anterior edge of the mantle or in the semi-gelatinous tissue between the muscles and the skin around the eyes. Trailing end of an implanted spermatangia stayed outside of the tissue as a thin capillary tubule (Figure 2).

Figure 2. Implanted spermatangia at the edge of the mantle with their tails shown in *Rossia macrosoma*
The length of *R. macrosoma* spermatangia was about 2.1 mm. The length of the cylindrical cement body at which the rear part of the spiral filament in oral side of the spermatangia was about 1.0 mm and its width was 0.4 mm (Figure 3A). In general, a bobtail squid spermatangia has two main parts: a head and a trailing end. The head part consists of two sides as oral and aboral. In oral part, there is a spiral filament, a cement body in the center with surrounding of sperm mass. The trailing end is located in aboral side (Figure 3B).

**Figure 3.** A: Removed spermatangia in introdermal area. B: Schematic diagram of the spermatangia section (C: Cement body, Mm: Middle membrane, Oo: Oral opening, Sm: Sperm mass, Sf: Spiral filament, t: tail).

In longitudinal histological sections

Spermatangia was surrounded by a middle membrane. The cement body and the sperm mass were located at oral part of the spermatangia (Figure 4A). At aboral part, free trailing end was located and connected with the cement body (Figure 4B).

**Figure 4.** A: Longitudinal section cement body and sperm mass in mantle tissue of *Rossia macrosoma* (HE, 10X). B: Longitudinal section of the tail inside the mantle tissue of *R. macrosoma* (PAS, 10X) (C: Cement body, Ct: Connective tissue, It: Inner tunic, Mm: Middle membrane, Oo: Oral opening of the spermatangium, Sm: sperm mass; t: tail).
In cross histological sections
The cement body was located in the center. The sperm mass surrounded the cement body and the inner tunic split the cement body and sperm mass (Figure 5).

![Figure 5](image)

**Figure 5.** Cross-sections of implanted spermatangia in female *Rossia macrosoma* (C: Cement body, Ct: Connective tissue, Mm: Middle membrane, Sm: sperm mass) (HE, 10X).

Spermatozoa in the tail could be visible in cross section of trailing end outside of the tissue (Figure 6).

![Figure 6](image)

**Figure 6.** Cross-section of a single tail canal from the exterior of *Rossia macrosoma* (C: Cement body, S: Spermatozoid) (100x).

Spermatangia were observed in semi-gelatinous tissue either in cross or longitudinal sections of mantle. There was no secretion cell observed in tissues surrounding spermatangia (Figures 4 and 5).
Discussion

Members of Heteroteuthinae and Sepiolinae have a seminal receptacle or bursa copulatrix which have similar functions (Villanueva and Sánchez 1993; Bello 1995), but members of Rossiinae lack a bursa copulatrix or seminal receptacle; in this case females carry spermatangia on different regions of the body (around the eye or in the soft tissue of the mantle) (Cuccu et al. 2007; Hoving et al. 2009; Cuccu et al. 2011; Salman 2011).

As reported by Cuccu et al. (2007) and Salman (2011) for Neorossia caroli, spermatangia were found on the left side of the body, which corresponds to the side where the oviduct is located, near the edge of the eye, on the left side of the mantle margin or on the ventrolateral left side in Rossia macrosoma.

Spermatangia implantation in R. macrosoma seemed to be “deep intradermal implantation” type as defined by Marian (2014). Similar implantation type was reported by Hoving et al. (2009) for another Rossia species, R. molleri.

There was no goblet, mucus or any other secretion cell that could immobilize spermatangia observed neither from longitudinal nor cross sections of intradermal layer surrounding spermatangia unlike in seminal receptacle of Idiosepius paradoxus reported by Sato et al. (2010).

Because there was no specialized structure for spermatangia storage found neither at the edge of the eyes nor on the mantle, one should expect that the spermatangia could not be implanted by means of a chemical agents but by mechanically with the use of the spiral filament structure at the oral part of the spermatangia. As Marian (2014) explained, the spiral filament of spermatangia has an important role in implantation of spermatangia into the intradermal tissue.

Besides we assume that these spermatangia trailing end, obtaining spermatozoa together with cement body, perform fertilization of the eggs in the external environment, not between the arms or inside the mantle cavity (Figure 6). As the evidences are limited, further studies are required to understand the fertilization process of subfamily Rossinae.

As poison ejection mechanism from glands in poisonous fish, spermatozoa might be ejected from trailing end of a spermatangia mechanically by pressure enforced by the female as either rubbing herself to hard substratum or using her arms or mantle contractions.

Further studies are required to determine the structural differences and thus characteristics of spermatangia between species and, whether these differences depend on their ecological conditions.
Acknowledgement
This study was financially supported by the EBILTEM (Science and Technology Centre of Ege University) (Project No: 2008/SUF/004) and Technological Research Council of Turkey (TUBITAK, Project No: 108Y102).

Rossia macrosoma’da (Cephalopoda: Mollusca) spermatofor implantasyonu

Öz
Bu çalışmada Ege Denizi’nden elde edilen çiftleşmiş Rossia macrosoma bireyleri kullanılmıştır. Çiftleşmiş olan dişi bireylerde spermatangialar göz çevresinde ve özellikle de ovidukt açıklığının bulunduğu mantonun sol kenarında tespit edilmiştir. Gözlenen bu implantasyon tipi yapılan histolojik analizler sonuçunda deri altı implantasyon tipi olarak tespit edilmiştir. İncelenen spermatangianın genel yapısı spiral filament, sperm kültlesi, sement ve kuyruk kısmından oluştuğu gözlenmiştir. Spermatangianın enine kesitlerinde sement merkezi konumlu olup çevresinin sperm kültlesi ile çevrilmiş olduğu görülmektedir. Spermatangianın implete edilmesinde oral bölgede bulunan spiral filamentin önemli bir rol oynadığı tahmin edilmektedir. İmplante edilmiş spermatangiada aboral kısmında ise ince kapiler bir tüp bulunmaktadır olup, bu tüpün spermatangiadan dışarıya sperm transferini sağladığını ve yumurtaların dış ortamda döllendiği tahmin edilmektedir.

Anahtar Kelimeler: Rossia macrosoma, spermatangia, implantasyon, histoloji, Cephalopoda

References


