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# Ultrastructural organization of seminal receptacle and sperm storage in *Porcellio laevis* Latreille (Crustacea: Isopoda Oniscidea)

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**Abstract.** The seminal receptacle of *Porcellio laevis* is a specialized region of the genital tract placed at the confluence of the oviduct with the ovary. In virgin sexually mature females the seminal receptacle wall consists of a monolayered epithelium lying on a thin basal lamina and delimiting a narrow and anfractuous lumen. The cells are joined by cell junctions only in their apical portion and do not show marked secretory activity; numerous cells appear to undergo a partial or complete process of autophagy that preludes a remodelling of the seminal receptacle allowing it to receive and store a conspicuous number of spermatozoa. In mated females epithelial cells are characterized by wide intracellular spaces in their basal region, by an extensive development of smooth reticulum and by the release into the lumen of an abundant lipidic secretion. Some spermatozoa stored in the lumen are captured by pseudopodial-like protrusions produced by the epithelial cell surface and then drawn into cavities that form within the epithelial cells themselves. The spermatozoa stored in the seminal receptacle appeared to be well conserved and apparently able to fertilize even several months after insemination.

**Keywords:** Oniscidea, ultrastructure, seminal receptacle, sperm storage

## Introduction

The oniscidean isopods are the only suborder of crustaceans to have become completely terrestrial and capable of colonizing all parts of the globe, excluding the poles and highest peaks. The importance of this endeavour is exemplified by the approximately 300 genera, grouped into 33 families, and more than 4000 documented species. This achievement is certainly due to the considerable genetic, morphological, biochemical and physiological 'plasticity' that characterizes the oniscidean isopods and which has led to their extraordinary adaptive wide spread occurrence. Such plasticity is also a feature of their reproductive biology, as represented that is, by the intriguing phenomenon of monogeny (Juchault & Legrand, 1989) by which some females give rise to entirely female broods (the thelygenous females of Vandel, 1964) or entirely male broods (arrhenogenous females). Moreover, some females switch from a unisex brood to a bisex one, or vice versa (Warburg, 1993).

Many species of oniscidean isopods also exhibit a more or less high degree of spanandry. Some species, such as *Platyarthrus aiasensis*, even have populations characterized by high spanandry in addition to populations in which the sex ratio is almost 1:1, as well as exclusively parthenogenetic populations (Caruso, 1968).

These characteristics explain, at least in part, why the oniscidean isopods have been widely able to effect a long-term sperm conservation within the female reproductive system. This has allowed, with a single mating, the fertilization of eggs, as in *Armadillo officinalis* (Vandel, 1941), that have matured over a period of 17 months without the female having to resort to further inseminations.

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After some initial controversy about the identification of the area of the female reproductive system involved in sperm storage (Vandel, 1925, 1937, 1941), it has now been clearly established that in the majority of documented species this is a specialized area of the reproductive tract located in the region in which the oviduct inserts into the ovary and it is known as the 'entonnoir' (Besse, 1976) or seminal receptacle (De Luca et al., 1987).

However, in other oniscidean species the site for sperm storage would appear to be located elsewhere. Vandel (1937) reported in *Trichoniscus pusillus provisorius* the presence of a spermatheca, having the form of a small sac-shaped diverticulum produced by the oviduct wall, which is suitable for storing part of the spermatozoa received during mating.

Our preliminary observations (Longo et al., 1994, 1995) concerning some species of the family Oniscidae, i.e. *Halophiloscia couchi*, *H. hirsuta* and *Stenophiloscia zosterae*, revealed a different arrangement of the organization of the female reproductive system, which entails a reduction in the length of the ovary in order to leave room for the formation of a large sacciform diverticulum suitable for storing a considerable quantity of sperm.

Despite these stimulating indications, research has so far provided a negligible contribution to the knowledge of the morphology and ultrastructure of the female reproductive system and, in particular, of the structures for sperm storage and survival. The present study thus aims to contribute to knowledge of the ultrastructural organization of the seminal receptacle of *Porcellio laevis* Latreille in order to understand the conditions on which the long-term conservation of the sperm stored therein is based.

## Materials and methods

Investigations were carried out on specimens of a population of *Porcellio laevis* bred in the rearing rooms of the Department of Animal Biology, University of Catania.

In this species the annual breeding season lasts from spring to late autumn, during which the females generally breed twice; the first parturition takes place in spring-summer and the second in summer-autumn. Occasionally, some females can breed three times a year, as has been observed in rearing conditions.

In order to evaluate the morphological and ultrastructural variations taking place within the seminal receptacle during reproductive activity, we used: (a) virgin sexually mature females, and (b) mated sexually mature females, at various stages of the reproductive cycle.

### Histological and histochemical methods

The reproductive system was collected by dissection of the animals in Ringer solution modified by Legrand (Besse, 1976) for terrestrial isopods; some samples were observed and photographed in toto without preliminary fixation or briefly fixed, others were immediately transferred to the chosen fixative.

For histological investigations, specimens were fixed in Carnoy or in 4% formaldehyde in 2% calcium acetate, dehydrated in the alcohol series and then embedded in Histowax (Jung). For the general histological observations, samples were serial sectioned at 6–8 μm and stained with haematoxylin–eosin.

The following histochemical methods were used for preliminary histochemical characterization of the secretory product in the seminal receptacle: (a) standard PAS test (Lillie, 1965); (b) Alcian blue, pH 2.5; (c) Alcian blue, pH 1.0; (d) high iron diamine test (Spicer, 1965); (e) Hg-bromphenol blue method after Bonhag (Pearse, 1978).

Sections obtained by cryostat were treated with Sudan black B for lipid detection. The same method was applied to in toto samples of reproductive systems that were either mildly fixed in 4% formaldehyde in 0.1 M phosphate buffer at pH 7.2, or not fixed at all.

### Transmission electron microscopy

For transmission electron microscopy (TEM) observations, the seminal receptacles were fixed in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer at pH 7.4 for 4 h at room temperature. Specimens were then repeatedly rinsed in the same buffer, postfixed in similarly buffered 2% osmium tetroxide for 1 h at room temperature, dehydrated and embedded in Embed 812 (Taab).

Ultrathin sections were cut with an LKB III ultramicrotome equipped with a diamond blade, mounted on rhodium-copper grids and stained with uranyl acetate and lead citrate. To detect lysosomal activity in the cells of the seminal receptacle, some samples were subjected to the ultrastructural localization of acid phosphatase technique based on cerium (Robinson & Karnovsky, 1983).

A Philips CM 10 electron microscope was used for observations.

## Results

### General organization of female reproductive system

As in other previously examined species of oniscidean isopods (Besse, 1976), the female reproductive system of *Porcellio laevis* consists of two separate ovaries located dorsolaterally to the intestine. In sexually mature females, the ovaries extend from the second to seventh segment of the pereion. At the level of the fifth segment, a short oviduct arises from each ovary (Fig. 1) and opens into a gonopore located at the base of the fifth pereiopod.

Each ovary is connected to the lateral wall of the body by four suspensory ligaments.

The ovary has the form of a small dorsoventrally flattened sac delimited by a thick basal lamina. Isolated, thin muscle fibres can be observed on the outside of the basal lamina. The germigen, having primary oogonia and follicle

cells (Fig. 2), runs along the entire lateral margin of the ovary and is interrupted only at the point of emergence of the oviduct. Medial to the germigen, the ovarian lumen is occupied by the developing oocytes (Fig. 2), the diameter of which reaches about 700 μm at maturity.

At the site in which the oviduct inserts, the ovary wall forms a slight reniform dilatation – the so-called 'entonnoir' of Vandel (1925) and Besse (1976) who interpreted it as the first zone of the female genital tract. The wall of this region is formed by a layer of narrow elongated epithelial cells lying on a thin basal lamina surrounded by small muscle fibres oriented towards the oviduct. In mated females, numerous spermatozoa are present in the lumen of the seminal receptacle; they are clustered together to form a sort of skein (Fig. 3) that often takes the shape of an 8.

This region thus corresponds to a true seminal receptacle (De Luca et al., 1987) in which the long-term conservation of sperm is effected after mating.

**Ultrastructural aspects of the seminal receptacle in virgin females**

In virgin females, the wall of the seminal receptacle is formed by a monolayer of narrow elongated cells (Fig. 4), all distributed around a small and very anfractuous central lumen. These cells lie on a homogeneous basal lamina that is slightly more than 1 μm thick and is constituted of a finely fibrillar material. The cells extend some short protrusions into the basal lamina (Fig. 5).

The delimitation between the seminal receptacle and the ovary is easily identified by the different appearance the basal lamina acquires around the ovary: it thickens abruptly, becoming two-layered and very sinuous. Scattered fat body cells are present external to the basal lamina together with rare thin muscle fibres.

The epithelial cells have a large nucleus that is generally ovoidal or pyriform and is located in the basal cell region (Figs 4, 5) where the course of the plasma membrane is regular and straight and where cell junctions are very rare. In contrast, the apical part of the epithelial cells is much narrower and forms a broad system of interdigitations (Fig. 6) where zonulae adhaerentes and extensive septate junctions occur (Fig. 7). The appearance of the cytoplasm is variable: irregularly distributed groups of cells with light cytoplasm are in fact alternated with others having dark cytoplasm (Fig. 8). The presence and distribution of the cytoplasmic organelles do not seem to differ substantially in these two cell types. Both present several mitochondria dispersed below and above the nucleus, rare ergastoplasmic cisterns, scattered ribosomes and microtubules that are generally arranged along the longitudinal axis of the cell. Small multivesicular bodies and elements of the smooth endoplasmic reticulum are often observable in the apical cell region whereas small Golgi complexes are only rarely seen.

Despite the scarce presence of the endocellular organelles involved in secretory activity, an abundant secretion, in form of tubular structures of 50 nm in diameter, is present in the lumen of the seminal receptacle of the virgin females (Fig. 12). This secretion gives a negative response to Alcian blue, pH 1.0 and to the high iron diamine test while it stains weakly after Alcian blue, pH 2.5. On the other hand, its response to the Hg-BPB method and to the PAS standard test is clearly positive, thus giving evidence of its glycoproteic nature.

Numerous cells appear to undergo a degenerative process which involves either only a part of the cytoplasm or, in some cases, the whole cell. The cytoplasm appears inhomogeneous and highly vacuolate (Fig. 9) and abounds with lamellar bodies and complex vacuoles (Fig. 10). The forementioned structures showed a strongly positive reaction (Fig. 11) to the method used for acid phosphatase localization.

**Ultrastructural aspects of the seminal receptacle in mated females**

Immediately after mating, the seminal receptacle undergoes a series of evident changes that seem to be correlated to the storage and long-term conservation of the sperm received during insemination.

The first step in this rearrangement consists in a pronounced increase in the size of the seminal receptacle which protrudes conspicuously from the ovary wall; consequently, the epithelial cells flatten considerably and in their basal region large intracellular spaces filled with an electron-transparent material are now present (Fig. 13).

The nucleus of the epithelial cells is located in an even more basal position, below which the cytoplasm exhibits mitochondria, rare RER cisterns and vesicles with heterogeneous electron-dense content (Fig. 14). The basal surface of the cells becomes more irregular due to the presence of numerous thin expansions which penetrate into the basal lamina (Fig. 14).

The most significant changes, however, concern the supranuclear cell region in which the smooth reticulum develops considerably (Fig. 15), accompanied by an increase in the number of microtubules, oriented towards the cell apex (Fig. 16), and the presence of numerous vesicles of variable size containing an electron-transparent material (Fig. 15). From the apical cell surface, numerous, more or less irregular protrusions extend towards the centre of the lumen which thus appears subdivided into numerous cavities containing a conspicuous number of spermatozoa (Fig. 17). In the lumen, the tubular structures observed in the seminal receptacle of the virgins are sometimes still discernible, although they are scarcer and fragmented (Fig. 18).

When the spermatozoa come into contact with the general surface of the epithelial cells or with a protrusion, the cell membrane forms one or more pseudopodial-like extensions that rise around the spermatozoon (Fig. 19) and surround it with a multiple envelopment similar to a myelinic figure (Fig. 20). The sperm tails are more frequently captured whereas the sperm head is rarely

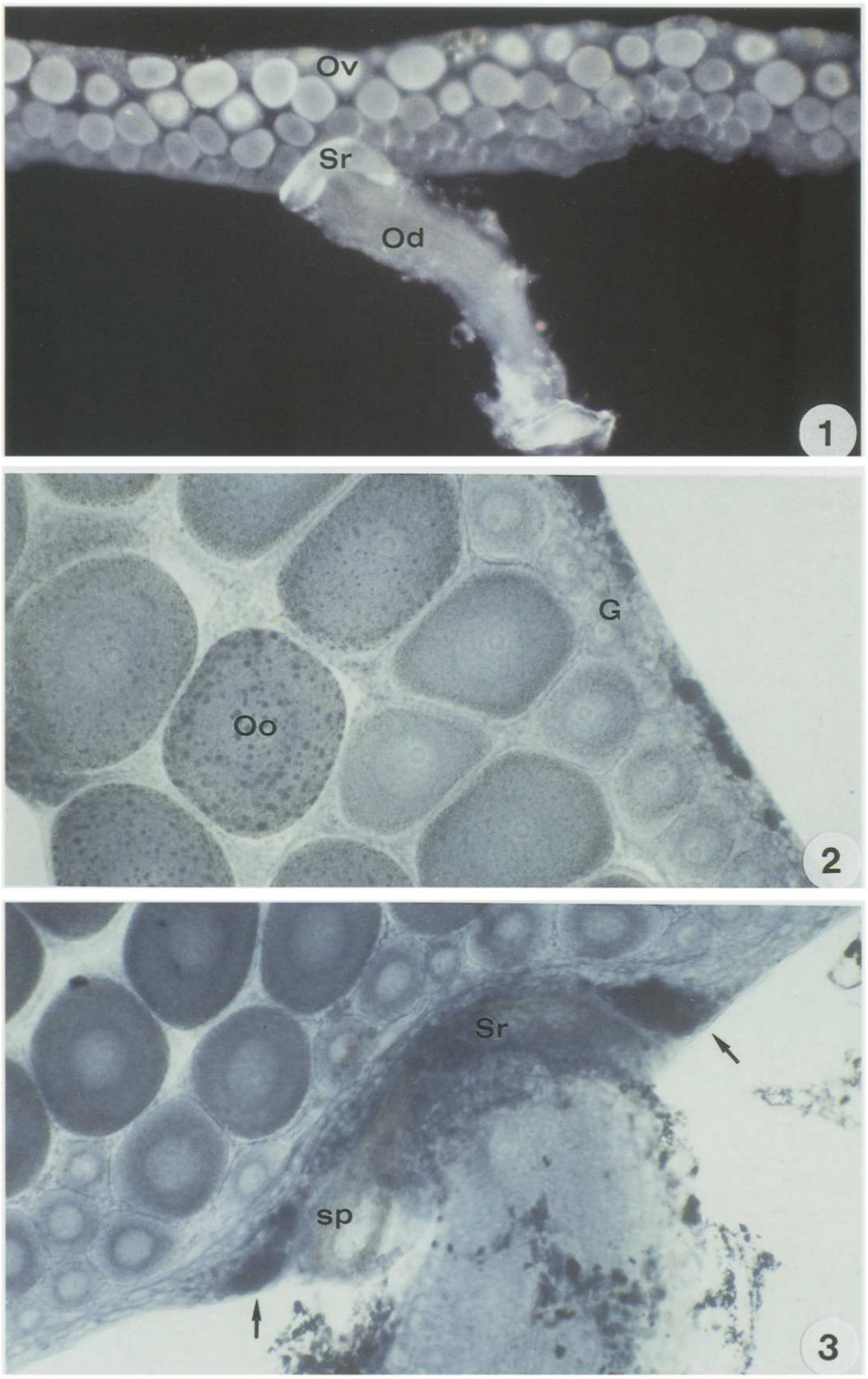
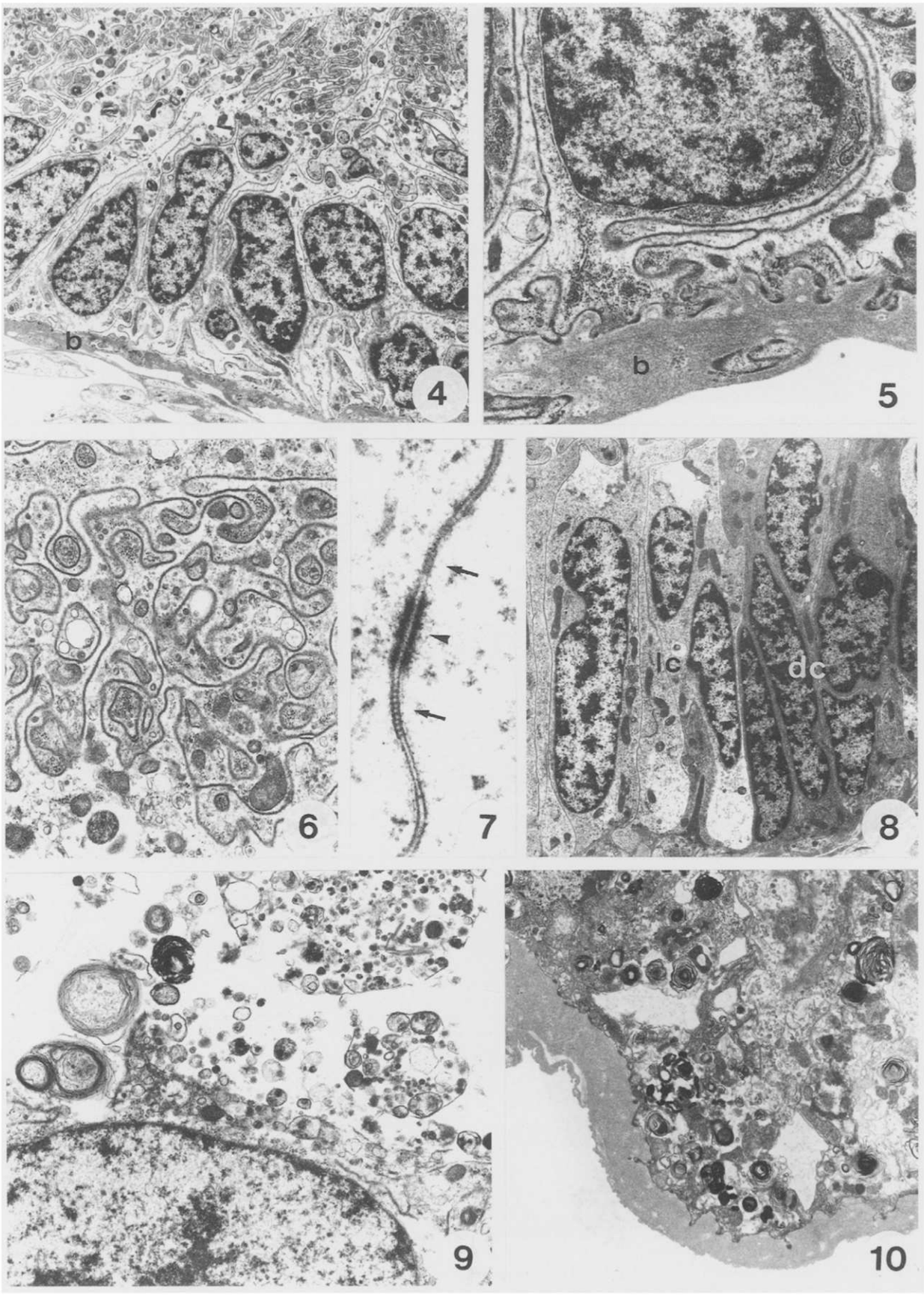



**Fig. 1** Genital apparatus of mated female, briefly fixed and not stained. Ov, ovary; Sr, seminal receptacle filled with spermatozoa; Od, oviduct. × 36.

**Fig. 2** Ovary of mated female stained in toto with Sudan black B. Oo, growing oocytes; G, germigen. × 160.

**Fig. 3** Ovary of mated female stained in toto with Sudan black B. The seminal receptacle (Sr) contains a bundle of spermatozoa (sp) and numerous large drops of lipidic secretion (arrows). × 100.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**Figs 4–12** Ultrastructural aspects of seminal receptacle in virgin females.

**Fig. 4** Seminal receptacle wall. b, basal lamina. × 3000.

**Fig. 5** Basal region of the epithelial cells. b, basal lamina. × 11 500.

**Fig. 6** At their apical region the epithelial cells form a broad system of interdigitations. × 11 500.

**Fig. 7** Zonulae adhaerentes (arrowhead) and extensive septate junctions (arrows) are present in the apical region of the epithelial cells. × 52 000.

**Fig. 8** The presence of irregularly distributed groups of light (lc) and dark (dc) cells is a peculiar feature of the seminal receptacle epithelium. × 3900.

**Fig. 9** Some cells undergoing a degenerative process show a high degree of cytoplasmic vacuolization. × 11 500.

**Fig. 10** In the cytoplasm of degenerating cells numerous lamellar bodies and complex vacuoles are extensively present. × 6600.

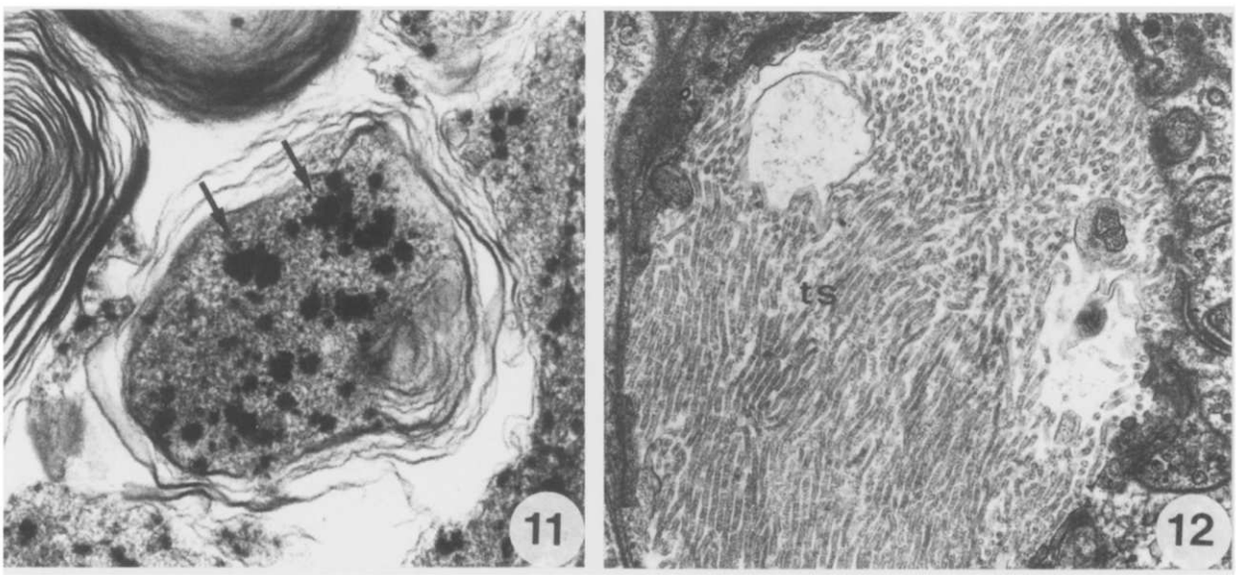



**Fig. 11** Acid phosphatase reaction product (arrows) associated with a lamellar body. × 46 000.

**Fig. 12** In the virgin females the lumen of the seminal receptacle is narrow and filled with abundant thin tubular structures (ts) representing a peculiar aspect of a glycoproteic secretion. × 15 000.

enclosed (Fig. 22). A discrete space is always present between the surface of the spermatozoon and the plasma membrane of the epithelial cells (Figs 21, 23). The captured spermatozoa are then drawn into thin tubular cavities that form within the epithelial cells (Figs 21, 23). These cavities can go as far as the proximity of the basal lamina (Fig. 24).

Some cells in the seminal receptacle of the mated females also present evidence of the cytological profiles interpreted as phenomena of partial or total cytolysis. Within these cells, lamellar bodies and very large complex vacuoles (Fig. 25) are particularly abundant and identified – once again using the cerium technique – as secondary lysosomes which are a prelude to complete cell disorganization.

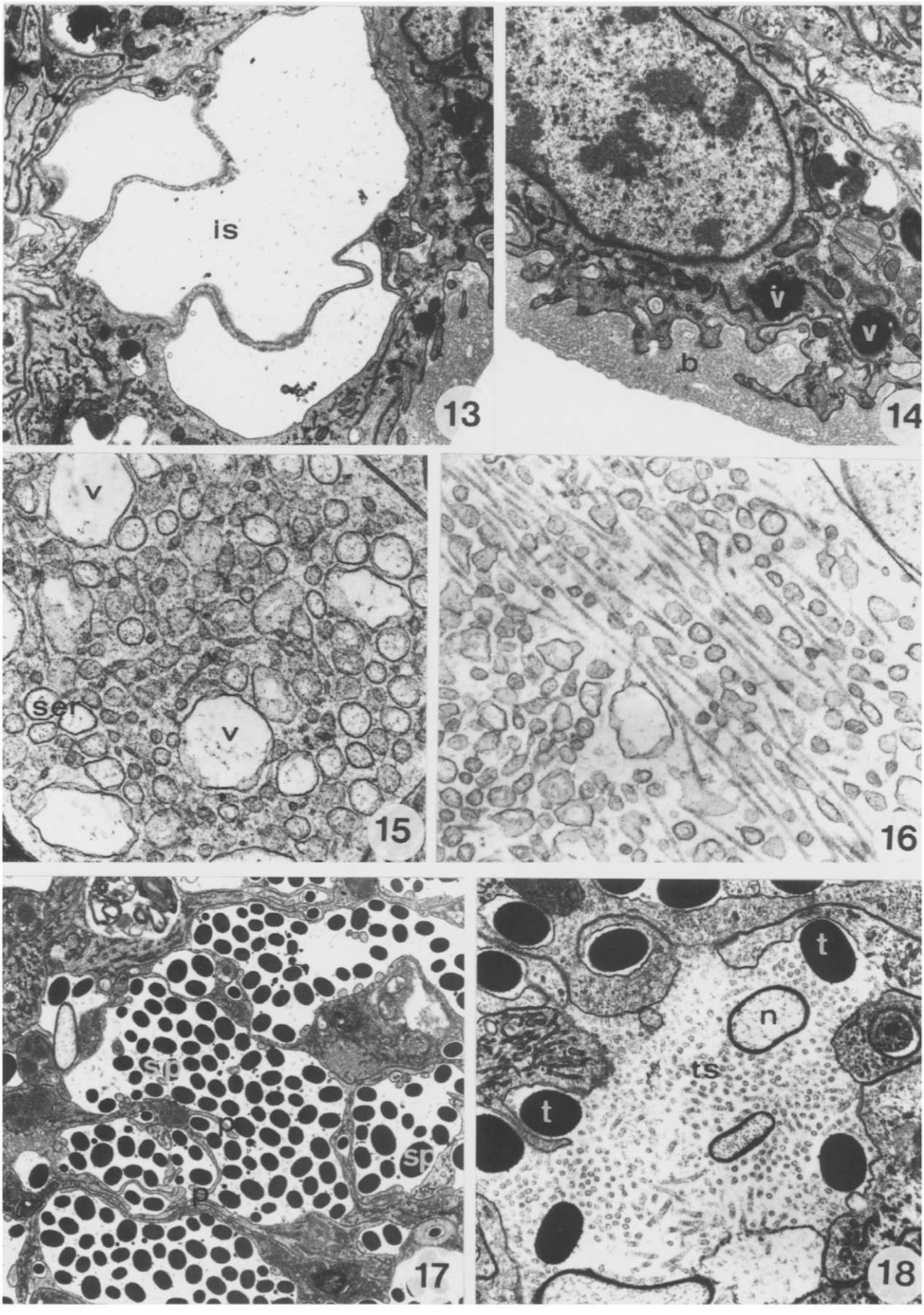
The extracellular presence of large homogeneous spheroidal drops (Fig. 26) was observed in various regions of the seminal receptacle. The Sudan black method, applied to cryostat sections or to the in toto genital apparatus, clearly establishes their lipidic nature (Fig. 3).

## Discussion

In her review of the features of sperm storage in the female genital tract of vertebrates and invertebrates, Selmi (1992) reported that, excluding mammals, the regions suited for sperm storage are always characterized by a basically similar structural organization. Their wall consists of a muscular sheath, a basal lamina and a monolayered epithelium. The epithelial cells have cilia or microvilli on the lumen surface and usually exhibit intense secretory activity.

The abovementioned features are also applicable to the seminal receptacle of *Porcellio laevis* in which the wall is composed of a monolayered epithelium lying on a thin basal lamina. However, the seminal receptacle of *P. laevis* lacks a well-defined muscular sheath and sparse isolated muscle fibres are observable only in the vicinity of the oviduct.

One intriguing aspect of the seminal receptacle of *P. laevis* is the presence of 'light' and 'dark' cells in the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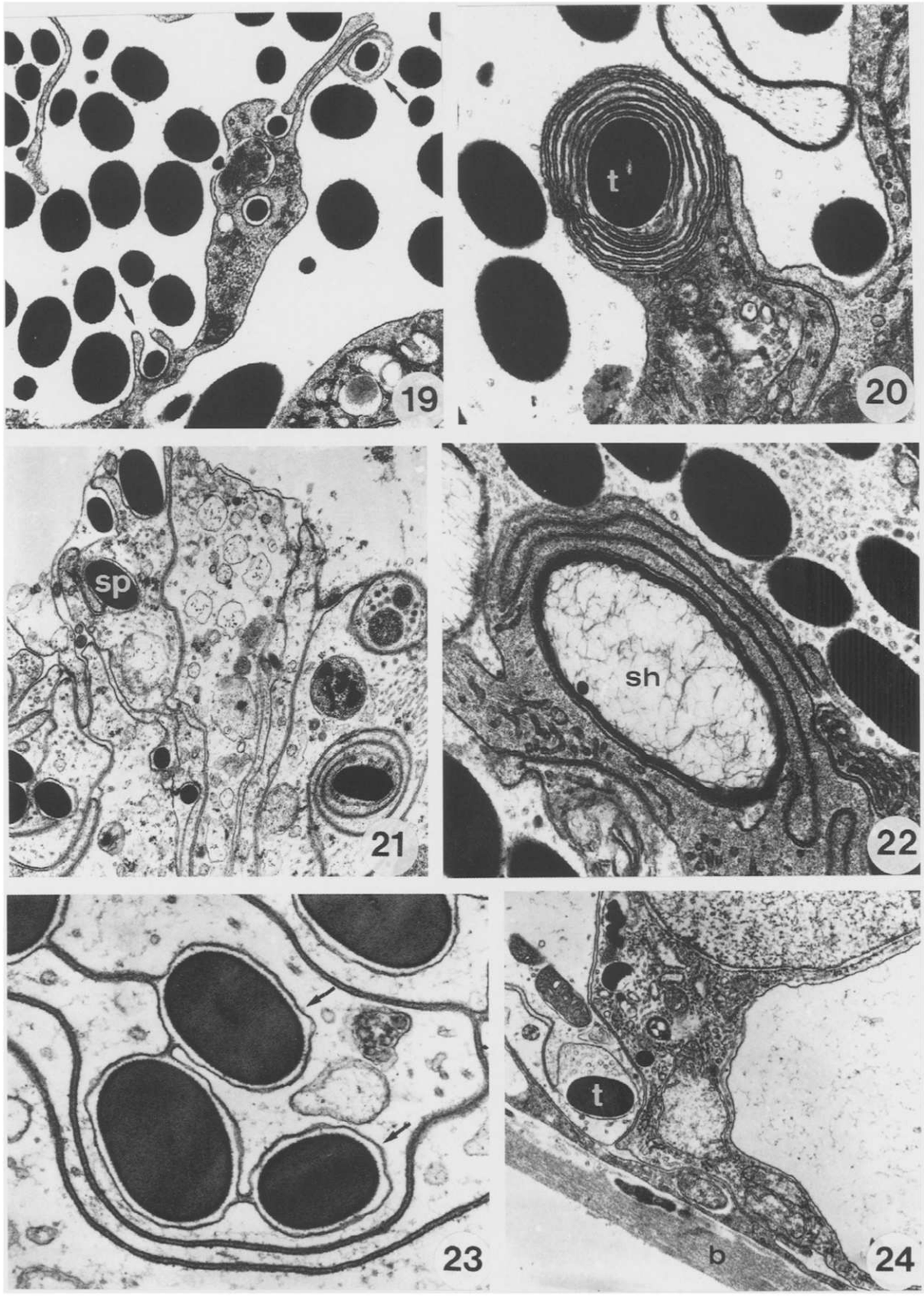
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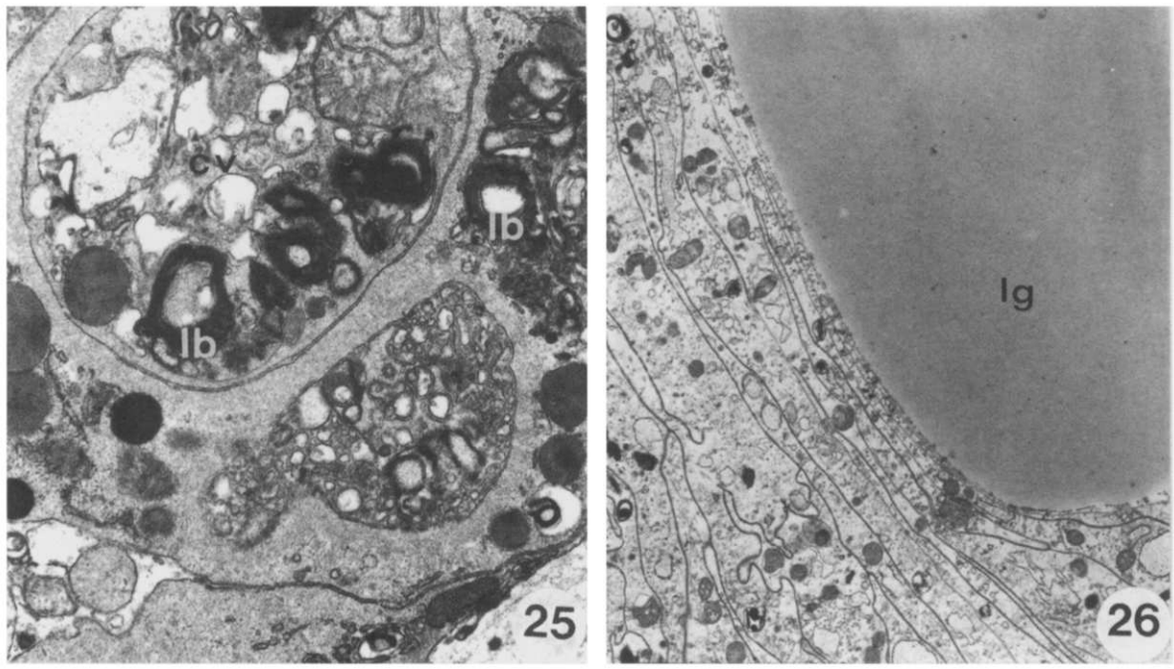


**Figs 13–26** Ultrastructural aspects of the seminal receptacle in mated females.

**Fig. 13** After mating the seminal receptacle undergoes a rearrangement, one aspect of which is the formation in the basal region of the epithelium of large intracellular spaces (is) containing electron-transparent material. × 8900.

**Fig. 14** Basal region of the epithelial cells. Below the large nucleus the cytoplasm contains mitochondria, scattered RER profiles and numerous vesicles (v) with heterogeneous electron-dense content. b, basal lamina. × 11 500.

**Fig. 15** In mated females the apical region of the epithelial cells shows an extensive smooth endoplasmic reticulum (ser) and numerous vesicles with electron-transparent content (v). × 18 000.

**Fig. 16** In the apical region, the epithelial cells always show numerous isolated microtubules directed towards the cell apex. × 27 500.

**Fig. 17** Numerous irregular protrusions (p) of the cell surface extend toward the lumen of the seminal receptacle which thus appears divided into numerous cavities filled with spermatozoa (sp). × 8900.

**Fig. 18** The thin tubular structures (ts) observed in the seminal receptacle of the virgin females are still present in some scattered cavities of the seminal receptacle of the mated females. t, sperm tails; n, sperm nuclei. × 15 500.

**Fig. 19** Some spermatozoa present in the lumen are captured by pseudopodial-like extensions (arrows) of the epithelial cell surface. × 21 000.

**Fig. 20** Frequently the pseudopodial-like extensions form around the sperm tail (t), a multiple membranous envelopment resembling a myelinic sheath. × 28 500.

**Fig. 21** Some of the captured spermatozoa (sp) are drawn into tubular cavities that form within the epithelial cells. × 15 500.

**Fig. 22** Sperm head (sh) captured by a pseudopodial-like extension of the epithelial cell; the two cell membranes seem to come into close contact at some part of their extension. × 28 500.

**Fig. 23** A discrete space (arrows) is always present between the sperm tail surface and the epithelial cell membrane. × 46 000.

**Fig. 24** The thin tubular cavities containing the sperm tails (t) sometimes nearly reach the basal lamina (b). × 18 000.

**Fig. 25** In the cells undergoing autolysis the cytoplasm contains numerous large complex vacuoles (cv) containing lamellar bodies (lb) and degenerating organelles. × 8900.

**Fig. 26** A lipidic secretion in the form of large globules (lg) fills the lumen of the seminal receptacle (see also Fig. 3). × 7000.

epithelium. This feature is not entirely a novelty since the presence of light and dark cells has been repeatedly documented in various normal or pathological tissues in numerous animal species; this aspect is attributed to the different states of hydration of the cytoplasm of a single cell type (Dohrmann, 1970).

Our ultrastructural observations did not reveal any substantial differences between the cytoplasmic organization of the two cell types that would allow their diversity in appearance and hydration to be correlated to different stages of secretory activity.

Another noteworthy aspect is the occurrence, within the seminal receptacle, of cells showing a series of involutional profiles – represented by the presence in the cytoplasm of numerous lamellar bodies, vesicles containing an heterogeneous material and large complex vacuoles – that is

followed by progressive and extensive vacuolization of the cytoplasm and, sometimes, the involution of the nucleus. Most of the abovementioned cytoplasmic structures reacted positively to the method for the localization of acid phosphatase activity (Robinson & Karnovsky, 1983) which identified them as secondary lysosomes. The fact that the number of cells involved in this phenomenon is distinctly higher in the virgins than in the mated females suggests that it is mainly related to a remodelling process of the organ. Nonetheless, the possibility that this activity may also represent a mechanism aimed at ensuring trophic supply to sperm stored within the seminal receptacle cannot be ruled out.

The secretory activity of the seminal receptacle seems to be modulated to the reproductive cycle. In virgin females, in fact, the secretory product is represented by thin tubular structures of glycoproteic nature, as shown by its positive response to the Hg-BPB method and to the PAS standard test; this type of secretion is not unusual because it has already been found in the male and female genital tract of some other species of invertebrates (Bairati, 1966; Perotti, 1971; Leopold, 1980; Dallai et al., 1993).

On the other hand, in the mated females the secretory activity leads to the release of an abundant lipidic material that accumulates as large globules of variable size in the lumen of the seminal receptacle. The lipidic nature of the secretion is demonstrated by the strongly positive response to Sudan black, and by the ultrastructural aspects of the epithelial cells which show, in mated females, pronounced development of smooth endoplasmic reticulum whereas they present a scarcity of rough endoplasmic reticulum and Golgi complexes.

The appearance of the lipidic globules observed in the seminal receptacle of *P. laevis* partly recalls the so-called 'oleospheres' reported by Vogt and Strus (1992) for the hepatopancreas of the decapod crustacean *Troglocaris schmidtii*. According to these authors, the oleospheres could represent a unique mode of extracellular lipid storage that can be interpreted as an adaptation to the extreme environmental conditions in which the species lives.

Another interesting feature is the relationship that is established between the spermatozoa and the seminal receptacle wall: while some of the sperm are free within the lumen of the seminal receptacle, others adhere more or less extensively to the surface of the epithelial cells. At the point of contact, the plasma membrane of the epithelial cells rises to form pseudopodial-like extensions that repeatedly encircle the sperm and thus resemble myelinic-type figures. This process usually involves the peculiar immobile rigid sperm tail of isopod spermatozoa (Fain-Maurel, 1970; Cotelli et al., 1976). The sperm head, containing a sacciform nucleus, nearly always remains in the lumen and is only rarely 'captured'. The sperm tails are thus drawn into cavities that form within the epithelial cells themselves and which extend inwards as far as the basal lamina.

No particular type of interaction seems to exist between the surface of the epithelial cells and the sperm surface. Similarly, we found no evidence of interstitial material such as to suggest a possible exchange of substances between the two cell types, as has been observed in other animal groups (see review of Selmi, 1992).

The type of interaction that we observed between the wall of the seminal receptacle and the sperm present in the lumen of *P. laevis* is largely comparable to that which occurs in the spermatheca of some species of Oligochaeta, as reported by Richards & Fleming (1982) who interpreted the capture and subsequent internalization of sperm within the epithelial cells as essentially a mechanism for removing and eliminating aged sperm.

Spermiophagy has also been reported in an annelid polychaete, *Pisione remota*, for which Westheide (1988) described spermiophagic activity operated by the cells of the seminal receptacle on 'female' spermatozoa, which he interpreted as a means of supplying supplementary nutrition to the developing oocytes. In *P. laevis*, however, we did not observe unequivocal elements suggesting a spermiophagic phenomenon within the seminal receptacle. Moreover, the sperm stored therein appeared to be well conserved and apparently able to fertilize even several months after insemination; all this is inconsistent with the observation of Cotelli et al. (1976) who, only in *P. laevis*, found a marked change in sperm head morphology after transfer of the sperm into the genital tract.

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