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A Histological Study of the Seminal Vesicle of the Armoured Catfish *Corydoras aeneus*

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With 9 figures and 1 table

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Summary

Most species of *Corydoras* exhibited a reproductive behaviour called 'T-position', and exhibited an accessory gland in the male genital tract, called the seminal vesicle. It appeared that both the structure and the composition of the fluid varied considerably between the species investigated. Consequently, different opinions were proposed regarding the possible role of seminal vesicle on this particular reproductive behaviour. Male adults of *Corydoras aeneus* were collected, anaesthetized, and samples of seminal vesicle were fixed in Bouin's solution. The sections were stained with haematoxylin-eosin and periodic acid Schiff. The seminal vesicle showed a system of anastomosed secretory tubules, forming a vesicular collective network, which gave rise to the vesicular ducts. The latter fused with the testicular efferent ducts and formed the spermatic ducts. Considering this fusion, when the sperm cells reached the spermatic ducts, the fluid produced at the seminal vesicle covered them. Histochemical studies evidenced the presence of neutral and acid glycosaminoglycans in the seminal fluid. Considering the reproductive behaviour of *C. aeneus*, it is believed that the protection associated with the immobilization of the sperm cells assures the sperm integrity during the passage through female's intestine until fertilization.

Introduction

Among the catfish species, the genus *Corydoras* (Callichthyidae) consists of more than 120 freshwater teleostei species occurring in South America (Matsuzaka, 1993). These species are popular among aquarists, and their reproductive behaviours have been observed in aquaria by many authors (Sakurai et al., 1985; Burgess, 1987, 1992; Matsuzaka, 1993; Kohda et al., 1995).

Most species of *Corydoras* exhibit a unique reproductive behaviour called 'T-position', where the female attaches her mouth to the anal region of the male (Sakurai et al., 1985; Burgess, 1987, 1992; Matsuzaka, 1993). In this particular reproductive behaviour, the sperm passes through the female's digestive tract, without losing their fertilization capability, as eggs are inseminated at high rates, about 87.6% (Kohda et al., 1995).

The siluriformes, differing from most of freshwater teleost, exhibit an accessory gland in the male genital tract, called the seminal vesicle (Nayyar and Sundararaj, 1970; Singh and Joy, 1999; Meisner et al., 2000; Chowdhury and Joy, 2001; Mansour et al., 2002). Based on the light microscopical studies, the seminal vesicle in teleosts has been described by different authors (Van den Hurk et al., 1987; Loir et al., 1989; Meisner et al., 2000; Mansour et al., 2002; Guimarães-Cruz and Santos, 2004). It appeared that both the structure and the composition of the secretion varied considerably between the species investigated (Van den Hurk et al., 1987; Legendre et al., 1996; Meisner et al., 2000). Consequently, this gave rise to different opinions regarding the function of the seminal vesicle and its secretory product. Various substances and functions are related to the secretion produced by the seminal vesicles of teleosts. They can store sperm cells, secrete glycoproteins, steroids and pheromones, increase semen volume, and also influence fertilization and female attraction (Van den Hurk et al., 1987; Lahnsteiner et al., 1990, 1992; Singh and Joy, 1998, 1999; Guimarães-Cruz and Santos, 2004). Thus, the present investigation deals with a histological and histochemical study of the seminal vesicle of *C. aeneus*, to obtain information about its structure and possible role on the particular reproductive behaviour of *C. aeneus*.

Material and Methods

Male adults of *C. aeneus* were collected in Tiete river, Itápolis, São Paulo State, Brazil. A total of 60 specimens (7.12 cm ± 0.27) were anaesthetized (benzocaine, 1 g/15 l water) and killed.

Anatomical procedures

Ten specimens were fixed in 10% Formol solution and dissected in Petri dishes. The material was analysed in Olympus stereomicroscope (Olympus, Japan).

Histological and histochemical procedures

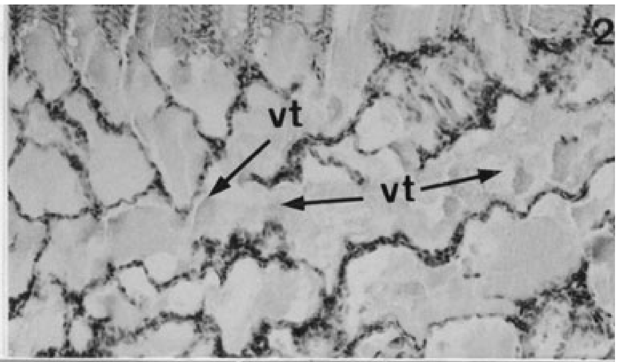
Samples of seminal vesicle were removed and fixed in Bouin's solution. Paraplast-embedded tissue blocks were cut to a thickness of 5 μm, and the sections were stained with haematoxylin-eosin, Heidenhain-Schleicher and Masson's triple stain, according to Bancroft and Gamble (2002). The presence of carbohydrates was examined by use of periodic acid Schiff (PAS) reaction with and without pre-treatment with 0.5%

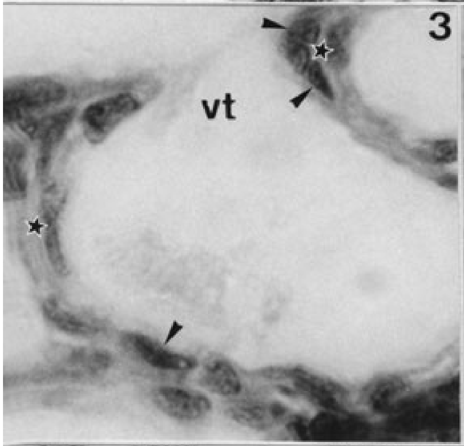
diastase digestion stage (Bancroft and Gamble, 2002). The presence of acidic radicals was tested with alcian blue at pH 0.5 and 2.5 (Bancroft and Gamble, 2002). The material was analysed and photodocumented with the Image Analysis Program – Image pro plus, model 4.1 (Media Cybernetics, Silver Spring, MD, USA) coupled to Olympus BX50 light microscope (Olympus, Japan).

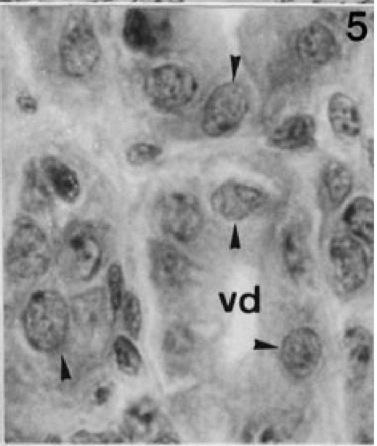
Results

The seminal vesicle of *C. aeneus* was identified as a single, rounded structure, and was situated caudal to the testis (Fig. 1). Microscopic analysis of this gland showed a system of secretory tubules (Fig. 2), lined by a simple squamous to cubic epithelium. The cells presented an elongated nucleus with intense basophile condensed chromatin (Fig. 3). The lining epithelium of the adjacent secretory tubules lay a basal membrane with a discreet intervening interstitial tissue (Fig. 3), with sparse fibroblasts and blood vessels.

The secretory tubules filled with seminal fluid were anastomosed, forming a vesicular collective network, which gave rise to the vesicular ducts (Fig. 4)\. These ducts were lined by a simple cubic epithelium. The epithelial cells showed







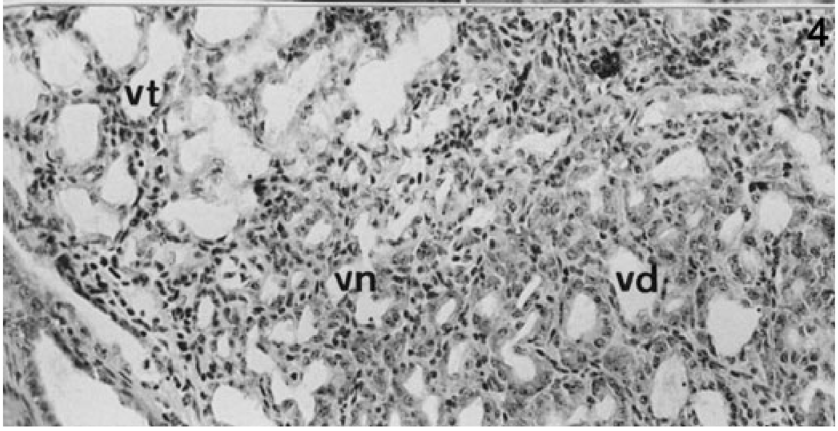


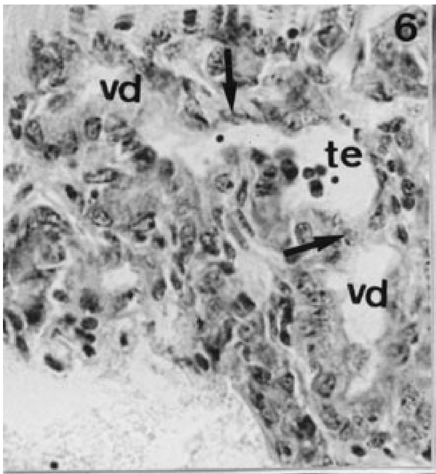
Fig. 1. Diagram of the testes (arrowheads) and the seminal vesicle (arrows) of *Corydoras aeneus*.

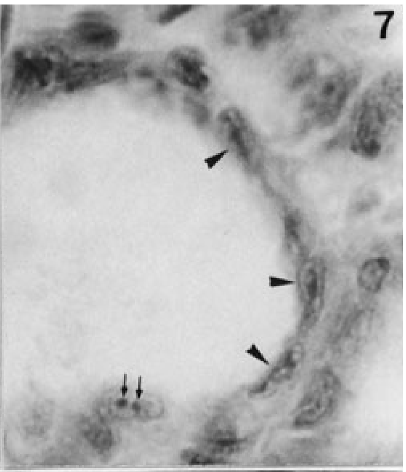
Fig. 2. Seminal vesicle with a system of vesicular secretory tubules (vt). H/E, ×100.

Fig. 3. The squamous epithelium of the vesicular secretory tubules (vt) presenting cells with an elongated nucleus (arrowheads). Note a discreet intervening interstitial tissue (stars). H/E, ×1000.

Fig. 4. The anastomosed vesicular secretory tubules (vt) forming a vesicular collecting network (vn), which gave rise to the vesicular ducts (vd). H/E, ×200.

Fig. 5. The simple cubic epithelium of the vesicular ducts (vd) showing homogenous cytoplasm and a round nucleus (arrowheads). H/E, ×1000.





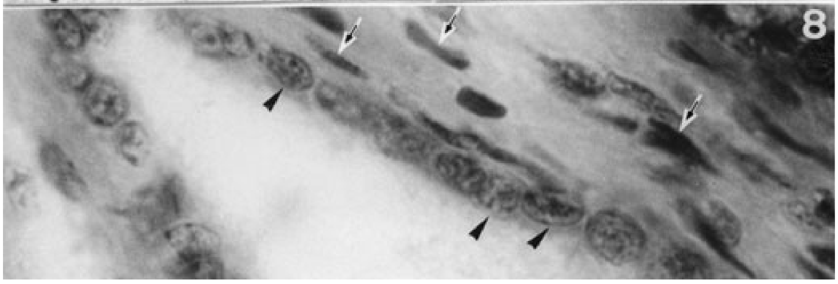


Fig. 6. The fusion (arrows) of the vesicular ducts (vd) with the testicular efferent ducts (te). H/E, ×400.

Fig. 7. The simple squamous epithelium of the testicular efferent ducts showing cells with an elongated nucleus (arrowheads) and one or two nucleolus (arrows). H/E, ×1000.

Fig. 8. The spermatic duct lined by a simple cubic epithelium (arrowheads), continuous with a loose connective tissue, with sparse collagenous fibres and fibroblasts (arrows). H/E, ×1000.

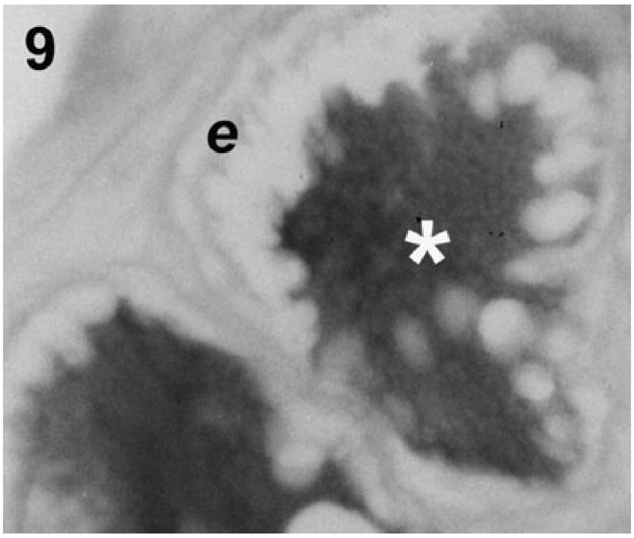


Fig. 9. The seminal fluid (*) and the epithelium (e) of the vesicular tubules stained with periodic acid Schiff (PAS). PAS, ×1000.

homogenous cytoplasm with a round nucleus and a visible and generally eccentric nucleolus (Fig. 5).

The vesicular ducts fused with the testicular efferent ducts (Fig. 6). The latter were lined by a simple squamous epithelium, whose cells showed an elongated nucleus with one or two nucleolus (Fig. 7). The fusion of the testicular efferent ducts with the vesicular ducts (Fig. 6) formed the right and left spermatic ducts. The spermatic duct was lined by a simple cubic epithelium, supported by the basal membrane, continuous with a loose connective tissue, composed of sparse collagenous fibres and fibroblasts (Fig. 8). The fusion of the right and left spermatic ducts resulted in the main spermatic duct. The main spermatic duct emptied into the urogenital papilla. So, the sperm in the seminiferous tubules were drained into the testicular efferent ducts and after that to the right and left spermatic ducts. Considering the fusion of the vesicular ducts with testicular efferent ducts, when the sperm reached the right and left spermatic ducts, the fluid produced at the seminal vesicle covered them.

Histochemical studies evidenced the presence of neutral and acid glycosaminoglycans in the fluid produced by the seminal vesicle. The fluid and the epithelium of the vesicular tubules stained strongly with PAS. The fluid always stained stronger than the epithelium (Fig. 9). Polysaccharides, thus, were the main components of the epithelium and the fluid of the seminal tubules (Table 1). The epithelium also stained weakly with AB pH 0.5 and pH 2.5. This pointed to the presence of both strongly acidic, sulphate groups and weakly acidic, carboxyl radicals. The fluid of the seminal vesicle stained somewhat stronger than the secretory epithelium with AB pH 0.5 and 2.5 (Table 1). Detection of neutral and acid polysaccharides was analysed by the reaction of PAS + AB pH 2.5. The fluid of the seminal vesicle stained somewhat stronger than the seminal vesicle secretory epithelium, indicating the existence of mixed chains of polysaccharides (Table 1).

Table 1. Results of the histochemical tests on the seminal vesicle of *Corydoras aeneus*

Reactions	Vesicular tubules	
	Secretory epithelium	Fluid
Periodic acid Schiff (PAS)	+ +	+ + +
PAS + Amilase	–	–
AB pH 0.5	+	+ +
AB pH 2.5	+	+ +
PAS + AB pH 2.5	+ +	+ + +

+ + +, strong; + +, moderate; +, weak; –, absent.

Discussion

The seminal vesicle of *C. aeneus* consisted of a single structure at the caudal end of the testes. Yet, for other siluriformes, this gland was described as a system composed of one to many pairs of seminal lobes (Nayyar and Sundararaj, 1970; Van den Hurk et al., 1987; Singh and Joy, 1999). Microscopically, the seminal vesicle of *C. aeneus* presented almost the same characteristics described for *Clarias gariepinus* (Van den Hurk et al., 1987). Both species exhibited secretory tubules lined by simple epithelium. In *C. aeneus*, the epithelial cells were squamous to cubic, but in *C. gariepinus* they were cylindrical (Van den Hurk et al., 1987).

Observations concerning the involvement of the seminal vesicle with the testes of *C. aeneus* differed in part to what was described for *C. gariepinus* (Van den Hurk et al., 1987). For *C. aeneus*, the testicular efferent ducts fused with the vesicular ducts and, afterwards, opened in the spermatic ducts. However, for *C. gariepinus* the testicular efferent ducts and the seminal vesicle tubule discharged, respectively, sperm and secretion directly into the unpaired part of the spermatic duct (Van den Hurk et al., 1987). In both cases, the spermatic duct was continuous to the urogenital papilla (Van den Hurk et al., 1987).

The histochemical analysis of the seminal vesicle in *C. aeneus* showed that the secretory fluid was similar to what was found for other teleosts (Nayyar and Sundararaj, 1970; Van den Hurk et al., 1987; Lahnsteiner and Patzner, 1990; Lahnsteiner et al., 1992; Singh and Joy, 1998). The organic components of the seminal vesicle fluid might serve as nutrient source for sperm cells and to prolong the period of sperm viability (Lahnsteiner et al., 1994). In addition, sperm cells might be immobile in both the seminiferous tubules and seminal vesicle fluid as in *C. gariepinus* (Van den Hurk et al., 1987). According to Mansour et al. (2002), in *C. gariepinus*, the seminal vesicle fluid did not improve sperm viability (motility which could be activated with water). This fluid presented high viscosity that completely inhibited sperm motility. When the seminal vesicle, fluid was diluted in the water the viscosity decreased and subsequently the motility suppressing effect was neutralized (Mansour et al., 2002). So, according to the authors, during spawning seminal vesicle fluid would not be released together with the semen and played no role in improving the sperm and egg contact. In *C. aeneus*, the histochemical studies proposed that, besides the other functions of the seminal vesicle, the fluid of this gland would also assume the role of protecting the sperm cells, thus forming a gelatinous environment for the transport of the sperm cells through the female's digestive tract. So, the gelatinous environment would assure the protection of these cells until the moment of fertilization, besides the motility suppressing effect during the digestive transit.

After the transit through the digestive tract, the female discharges the sperm through the anus and makes a 'shell-shaped' pouch with her ventral fins to discharge the ova and consequently to promote the insemination (Kohda et al., 1995). The sperm cells are activated by the dilution of the remaining seminal vesicular fluid with water. According to Kohda et al. (1995), this reproductive behaviour occurs every 5 min during 3 h. *Corydoras aeneus* females must drink moderate quantities of semen during the T-position to avoid dilution of the sperm by additional water swallowed. This behaviour resulted in an optimal density of sperm in the pouch, securing fertilization of the eggs (Kohda et al., 1995). *Corydoras* species have short intestines, pelvic fins beside the anal region and frequently swallow air for intestinal breathing (Burgess, 1989; Podkowa and Goniakowaska-Witalinska, 2002). These characteristics contribute to this reproductive behaviour, and are widespread among other small catfishes (Burgess, 1989), suggesting that this mode of insemination may not be restricted to the *Corydoras* genus (Kohda et al., 1995). Indeed, the T-position is observed in some small catfishes other than *Corydoras* and other than Callichthyidae (Burgess, 1989; Kanda and Minoguchi, 1990). Therefore, considering this reproductive behaviour, it is believed that the seminal vesicle fluid of *C. aeneus* assumes several roles including immobilization, protection and probably nutrition. The fundamental role is the protection associated with the immobilization of the sperm cells. These functions assure sperm integrity during the passage through the female's intestine, besides keeping the sperm aggregated and immobile until the moment of fertilization. It is important to emphasize that the function of the seminal vesicle fluid is not yet established for the teleost fishes.

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