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Sperm storage in *Hemidactylus mabouia*: Morphological and ultrastructural aspects of a reproductive strategy

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ABSTRACT

Sperm storage is a common phenomenon in most female reptiles. Evidence of sperm storage is based on the observation that female fertilization occurs even when females are separated from males, as well as the presence of agglomerates of spermatozoa in specific regions of the oviducts. Lizards are capable of storing sperm in the uterine tube, vagina, or in both regions. However, representatives of the Gekkonidae family commonly store spermatozoa in the uterine tube, which is considered an ancestral character state for Squamates. Using comparative techniques of light, transmission and scanning electron microscopy, we observed stored sperm organized in compact bundles with their heads facing the bottom of the crypts of the uterine tube, indicating chemotactic attraction. The alignment and packing of spermatozoa in *Hemidactylus mabouia* indicates that the process of evacuation of the crypts for fertilization may be related to the passage of the egg that exerts mechanical pressure against the walls of the uterine tube, causing its distension and the release of spermatozoa. We conclude that the sperm storage region and the morphological organization of the crypts in the uterine tube of *H. mabouia* is similar to other previously studied species of lizards, supporting the notion that sperm storage is a common reproductive strategy among female reptiles.

1. Introduction

Sperm storage in the oviduct during the reproductive cycle is a common phenomenon amongst female reptiles, where insemination and ovulation/fertilization occur in distinct periods (Schuett, 1992; Sever and Hamlett, 2002). In 1943, Ludwig and Hahn described sperm storage in the oviducts of *Crotalus viridis viridis* females. Saint Girons (1962) and Fox (1963) reported the first studies of such sperm storage in lizards in the 1960s.

Evidence for sperm storage is based on the observation that female fertilization occurs even when the females are separated from males and is related to the presence of agglomerates of spermatozoa in specific regions of the oviducts (Adams and Cooper, 1988). However, the existence of these spermatozoa agglomerates does not guarantee sperm storage. Phagocytosis sites are observed in the reptilian oviduct where a large amount of spermatozoa could be present. Additionally, phagocytosis occurs within the storage sites themselves in lizards (Sever and Hopkins, 2004; Siegel and Server, 2007; Nogueira et al.,

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2011a,b). Several spermatozoa have been observed in the vagina of *Eumeces egregius* lizards (Schafer and Roeding, 1973); however, these findings do not indicate this region as a storage site due to the absence of structures specialized for this function, such as crypts. Similarly, in *Hemidactylus mabouia*, intense phagocytosis of spermatozoa by vaginal epithelial cells has been observed, despite the fact that no structures specialized in storage were observed; thus, this oviduct region is characterized as an exclusively phagocytic site (Nogueira et al., 2011a,b).

Lizards store spermatozoa in the uterine tube, vagina or both of these regions (Sever and Hopkins, 2004; Siegel and Server, 2007). Conversely, members of the Gekkonidae family generally store sperm in the uterine tube (Cuellar, 1966; Murphy-Walker and Haley, 1996; Girling et al., 1997), and this feature is considered to be the ancestral state for Squamates according to the hypothesis of phylogeny based on spermatozoa storage tubules proposed by Sever and Hamlett in 2002.

Various studies have described and compared the structural and ultrastructural aspects of storage sites in lizards (Cuellar, 1966; Conner and Crews, 1980; Bou-Resli et al., 1981; Girling et al., 1997, 1998; Sever and Hamlett, 2002; Sever and Hopkins, 2004). Here, we describe for the first time the uterine tube sperm storage process in *H. mabouia*, thus providing new insights into the understanding of this important reproductive strategy in a member of the Squamata.

2. Materials and methods

2.1. Animals

Ten female *H. mabouia* lizards measuring from 52.9 to 59.3 mm were collected under license number 10504-1 of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). The carcasses were fixed in a buffered formalin solution (Carson et al., 1973) for 24 h at room temperature and deposited at the João Moojen Animal Science Museum of the Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil. The experiment was conducted in accordance with the ethical principles for the utilization of laboratory animals published in the Colégio Brasileiro de Experimentação Animal (COBEA). Identification and collection of the reproductive organs was performed *in situ* via a median longitudinal incision in the ventral region of the animals. The collected material was deposited in vials containing saline solution.

2.2. Optical microscopy

Uterine tube fragments were fixed in a buffered formalin solution (Carson et al., 1973) for 24 h at room temperature and dehydrated in increasing concentrations of ethanol. The fragments were embedded in glycol methacrylate (Historesin®, Leica) and sectioned at 2 μm in an automatic microtome (Reichert-Jung, Germany). The obtained sections were stained with 1% toluidine blue/sodium borate and mounted on slides with Entellan® (Merck) for analysis with an Olympus BX-60 microscope.

2.3. Transmission electron microscopy

Uterine tube fragments (approximately 1 mm^2) were fixed in Karnovsky's solution (Sheehan and Hrapchak, 1980) for 4 h. The material was then washed with 0.1 M sodium cacodylate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide for 2 h. The material was subsequently dehydrated in increasing concentrations of acetone and embedded in Epon resin. Ultrathin sections were obtained in an ultramicrotome and contrast stained with 2% uranyl acetate and 0.2% lead citrate. The material was examined and photographed using an EM 109-Zeiss transmission electron microscope in the Nucleus of Microscopy and Microanalysis at Federal University of Viçosa, Brazil.

3. Results and discussion

The uterine tube of *H. mabouia* is composed of four distinct regions that are named as follows from the anterior to the posterior side: infundibulum, uterine tube, uterus and vagina (Nogueira et al., 2011a,b). The uterine tube of *H. mabouia* is internally coated with a simple epithelium containing secretory non-ciliated cells and ciliated cells (Fig. 1A and B). Crypts, which are branched epithelial invaginations formed from the epithelial inner lining, are observed inside the uterine tube. The walls of the crypts contain both ciliated and non-ciliated cells; however, very few ciliated cells are present at the bottom. This organization is similar to previous descriptions of crypts in other lizard species (Bou-Resli et al., 1981; Murphy-Walker and Haley, 1996; Girling et al., 1998; Sever and Hamlett, 2002; Yamamoto and Ota, 2006; Eckstut et al., 2009). Sever and Hopkins (2004) and Bou-Resli et al. (1981) described numerous ciliated cells in the neck of the crypts but not at the bottom of *Acanthodactylus scutellatus* (Gekkonidae) and *Scincella laterale* uterine tubes. In the uterine tube of *Anolis sagrei*, the distal portion of the crypt is not specialized; however, the proximal portion exhibits a similar proportion of ciliated and non-ciliated cells (Sever and Hamlett, 2002). Indeed, no apparent pattern regarding the proportion of ciliated and non-ciliated cells is noted among previously studied lizard species.

The non-ciliated cells present in the uterine tube of *H. mabouia* produce a metachromatic secretion when stained with toluidine blue. The secretion produced by non-ciliated epithelial cells located at the bottom of the crypts may be related to a variety of functions, such as nutrition (Cuellar, 1966; Bou-Resli et al., 1981), defense (Bou-Resli et al., 1981), chemotaxis (Saint Girons, 1973; Adams and Cooper, 1988) and maintenance of an aqueous environment, which is appropriate for spermatozoa survival. However, further investigation must be performed to clarify the role of the non-ciliated cell secretions.

Images obtained using scanning electron microscopy (Fig. 2) demonstrate that the bottom of the crypts in the uterine tube of *H. mabouia* exhibit a smooth surface due to the presence of non-ciliated cells. The rough endoplasmic reticulum of the non-ciliated cells is highly developed, and the nucleoli are generally clearly visible and exhibit loose chromatin. Some mitochondria and numerous vesicles dispersed in the cytoplasm can be observed (Figs. 3 and 4).