Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Regional morphology and mucus composition in the urogenital papilla skin of the blackbelly rosefish *Helicolenus dactylopterus* (Delaroche, 1809)

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ARTICLE INFO

Keywords:
Reproduction
Copulating organ
Glycohistochemistry
Fish
Epidermis

ABSTRACT

Blackbelly rosefish *Helicolenus dactylopterus* is a zygoparous fish whose males are equipped with the copulating organ named urogenital papilla (UP). This study deals with the morphology and the glycoconjugate pattern of the UP epidermis, which is the male tissue interacting with the female internal body during copulation. The carbohydrate content was studied by means of conventional and lectin histochemistry. The epidermis was shown to be a stratified cuboidal epithelium and to exhibit characteristic intraepithelial pits in the apical zone. The mucous cells are scattered in the epidermis. The epidermal cell layers and their thickness as well as the size of mucous cells varied along the UP. Conventional histochemistry showed that the mucous cells contained i) only neutral glycoproteins in the basal zone; ii) both neutral and acidic non-sulphated glycans as well as only acidic non-sulphated or sulphated glycoconjugates in the intermediate zone; iii) neutral and sulphated glycoconjugates in the apical zone. The mucous cells in the basal region expressed O-linked (mucin type) glycans terminating with αGalNAc, Galβ1,3GalNAc which could be α2,3-linked to sialic acid, and high mannose type N-linked glycans terminating with fucose, lactosamine, and sialic α2,6-linked to galactose/N-acetylgalactosamine; terminal Gal and terminal/internal GlcNAc were also found. The mucous cells in the intermediate zone lacked Galβ1,3GalNAc and showed less terminal α2,3-linked sialic acid, lactosamine, fucose, galactose, and internal N-acetylglucosamine residues. In the apical region, mucous cells only exhibited O-glycans terminating with GalNAc and N-acetylglucosamine. The demonstrated region-specific differences in the UP skin provide new insights into the reproductive biology of fishes with internal fertilization.

1. Introduction

The reproductive strategies adopted by fish show a marked diversity in relation to their aquatic habitats and the taxonomy. Most fish are oviparous and have external fertilization of spawned eggs. Males fish with internal fertilization are equipped with an intromittent (copulating) organ, which differs in origin, shape and size from species to species (Meisner et al., 2000; Meisner, 2005;

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https://doi.org/10.1016/j.anireprosci.2017.12.018
Received 29 September 2017; Received in revised form 22 December 2017; Accepted 22 December 2017

Fishelson et al., 2006).

Most studies describe the macroscopic morphology of the male urogenital papilla and some of them deal with the histological details which concern the separate urinary and sperm ducts. Little is known about the morphology and the histological details of the skin covering this intromitting organ (Rasotto and Shapiro, 1998) which has recently been suggested as a useful indicator of exposure to pollutants (Kruger et al., 2013).

The epidermis constitutes the uppermost layer of the skin and in fish consists of epidermal cells and various types of unicellular glands (Mittal et al., 1994a,b) such as mucous (goblet) cells and club cells. Epidermal cells are the smallest and most numerous cells and are found all over the epidermis, from basal to superficial layers. Mucous cells are round cells with a peripheral flattened nucleus, located on the apical region of the epithelium. These cells secrete the glycoproteins constituting a gel-like and viscous mucus that covers the epithelial surface of the skin. Skin surface mucus protects the fish against mechanical damage and biological stress such as microbes, viruses, or proteolytic degradation, as well as playing a role in skin lubrication and ionic regulation (Shephard, 1994; Raj et al., 2011; Jin et al., 2015). Lastly, club cells secrete substances associated with distinct functions (Ralphs and Benjamin, 1992), including pheromonal (Zaccone et al., 1990), antipathogenic (Suzuki and Kaneko, 1986), or phagocytic activities (Lufty, 1964).

The composition and structure of glycoconjugates can be decoded using several techniques. Lectins, which are glycan binding proteins, are particularly well suited for discriminating between glycoconjugates because of their specificity and ability to distinguish sugar isomers as well as branching, linkage, and terminal modifications of complex glycans (Sharon and Lis, 2004). Lectins have been used for skin analysis in several vertebrates such as mammals (Virtanen et al., 1986; Desantis et al., 2003), amphibians (Kaltenbach et al., 2004), and fishes (Burkhardt-Holm, 1997; Al-Banaw et al., 2010; Jin et al., 2015).

Despite the epidermis of genital papilla representing the outermost male tissue directly interacting with internal female surfaces when the copulation occurs, reports concerning its histological structure, including the presence of mucous cells, are scarce (Rasotto and Shapiro, 1998). To the best of our knowledge, no study has yet been reported describing the glycan pattern of the intromittent organ surface in fish species. The surface glycoconjugates of the genital papilla could act as a lubricant able to protect the epithelial cells from physical or chemical injury. Consequently, they could have some effects on the internal female surfaces during the copulation as well as on sperm survival. On this basis, the aim of this study was to investigate the structural features as well as the glycoconjugate composition by means of conventional and lectin histochemistry of the urogenital papilla epidermis of the male blackbelly rosefish *Helicolenus dactylopterus*. This teleost is a zygoparous species with internal fertilization (White et al., 1998; oz et al., 1999, 2002;) which has received special attention in recent years because it represents an important component of commercial fishing in both Atlantic waters and the Mediterranean Sea in which this species is highly widespread (for review see D'Onghia et al., 2015).

2. Materials and methods

2.1. Tissue preparation

Adult *H. dactylopterus* males were collected, at 480 m in depth, during an experimental fishing survey carried out in the Santa Maria di Leuca cold-water coral province (Central Mediterranean) during September–October 2010 within the framework of CoralFISH EU_7FP project. In particular, for this study six males were analyzed (total length ranging from 235 to 380 mm). Urogenital papillae were extracted, cut either in sagittal or transverse sections and fixed by immersion in Bouin's fluid for 24 h at room temperature (RT) (n = 4).

The Bouin fixed tissues were dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Serial sections (4 μm thick) were cut and, after de-waxing with xylene and hydration in an ethanol series of descending concentrations, were stained with Haematoxylin-Eosin for morphological studies and, by means of conventional histochemical procedures or lectin histochemistry according to Desantis et al. (2011) for glycoconjugate characterization.

2.2. Conventional histochemistry

Sections were treated with: 1) periodic acid-Schiff (PAS) reaction for neutral glycoconjugates (Mc Manus, 1948); 2) Alcian blue 8GX at pH 2.5 (AB 2.5) for testing sulphate esters and carboxyl groups in glycoconjugates; 3) Alcian blue 8GX at pH 1.0 (AB 1.0) for the characterization of sulphated glycoconjugates (Pearse, 1968; Spicer et al., 1967). In order to reveal cellular combinations of both acidic and neutral glycoconjugates, the AB 2.5/PAS and AB 1.0/PAS staining sequences were performed.

2.3. Lectin histochemistry

The lectins used are listed in Table 1. The horseradish peroxidase (HRP)-conjugated lectins (Con A, GSA II, LTA, RCA_{120}, and PNA) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA), whereas biotinylated lectins (succinylated WGA, PSA, MAL II, SNA, and GSA I-B_4) were purchased from Vector Laboratories Inc. (Burlingame, CA, USA).

De-waxed and re-hydrated tissue sections were immersed in 3% hydrogen peroxide for 10 min to suppress the endogenous peroxidase activity. Tissue sections treated with HRP-conjugated lectins were rinsed in 0.05 M Tris-HCl buffered saline (TBS) pH 7.4, and incubated in lectin solution at appropriate dilutions (Table 1) for 1 h at RT. After 3 rinses in TBS, the peroxidase activity was visualised by incubation in a solution containing 0.05% 3,3′-diaminobenzidine (DAB) and 0.003% hydrogen peroxide in 0.05 M TBS (pH 7.6) for 10 min at RT. Tissue sections incubated in biotinylated lectins for 1 h at RT (MAL II, SNA, DBA, and GSA II) were rinsed 3