Anatomy of the Ovaries of the Starfish Asterias rubens (Echinodermata)
A Histological and Ultrastructural Study

H.J.N. Schoenmakers, P.H.J.M. Colenbrander, J. Peute, and P.G.W.J. van Oordt
Laboratory of Chemical Animal Physiology and Section Comparative Endocrinology of the Zoological Laboratory, State University of Utrecht, Utrecht, The Netherlands

Summary. The ovaries of the starfish Asterias rubens were studied histologically and ultrastructurally. The reproductive system in female specimens consists of ten separate ovaries, two in each ray. Each ovary is made up of a rachis with lateral primary and secondary folds: the acini maiores and acini minores. The ovarian wall is composed of an outer and an inner part, separated by the genital coelomic sinus. The ovarian lumen contains oocytes in various phases of oogenesis, follicle cells, nurse cells, phagocytosing cells and steroid-synthesizing cells.

Oogenesis is divided into four phases: (i) multiplication phase of oogonia, (ii) initial growth phase of oocytes I, (iii) growth phase proper of oocytes I, and (iv) post-growth phase of oocytes I. The granular endoplasmic reticulum and the Golgi complex of the oocytes appear to be involved in yolk formation, while the haemal system, haemal fluid and nurse cells may also be important for vitellogenesis. The haemal system is discussed as most likely being involved in synchronizing the development of the ovaries during the annual reproductive cycle and in inducing, stimulating and regulating the function of the ovaries.

Steroid-synthesizing cells are present during vitellogenesis; a correlation between the presence of these cells and vitellogenesis is discussed.

Key words: Ovaries – Ovarian wall – Oogenesis – Steroid-synthesizing cells – Asterias rubens

Schoenmakers et al. (1976) demonstrated the presence of enzymes essential for steroid biosynthesis, particularly 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), in the gonads of the starfish Asterias rubens. Other biochemical experiments confirm the production of steroids in the ovaries and pyloric caeca of the female starfish (Schoenmakers 1977). Recently, the sites of steroidogenesis in the ovaries of Asterias rubens were localized, and the ultrastructure of cells with characteristic features of steroid-producing cells was described (Schoenmakers et al. 1977).

Send offprint requests to: Dr. H.J.N. Schoenmakers, Laboratory of Chemical Animal Physiology, State University of Utrecht, 8 Padualaan, 3508 TB Utrecht, The Netherlands

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These results led to a study of the physiological significance of ovarian steroids in *Asterias rubens*, particularly with regard to reproduction. Information on the normal structure of the reproductive system and its seasonal changes during the annual reproductive cycle is a prerequisite for such a study.

The reproductive system of many species of starfish was studied from different points of view. In *Asterias rubens* the two sexes can be distinguished, although Retzius (1911) described one specimen in which each gonad was partly male and partly female. Some aspects concerning the structure of the reproductive system of *Asterias rubens* were reported by Hoffman (1872), Ludwig (1877), Hamann (1885) and Gemmill (1914) in their studies on the anatomy and development of this animal. Recently, Walker (1974, 1975) described the morphology and histology of the gonads of *Asterias vulgaris*, with emphasis on the gonoduct and the gonad wall, based on both light microscopical and electron microscopical observations.

The present study deals with the histological and ultrastructural aspects of the ovaries of *Asterias rubens*.

**Materials and Methods**

**Animals**

The specimens of *Asterias rubens* used in this study for light-microscopical studies were collected in the Wadden Sea, east of the island of Texel (The Netherlands) at 3 weeks intervals from February 1975 to June 1976, and those used for ultrastructural studies at set intervals from February 1976 to April 1977. The animals were kept in aerated sea-water at 6°C for 3 days before being processed. Five females from each sample were studied with the light microscope; ultrastructural examination was carried out on three females per sample. Only specimens with arms varying in length between 6.0 and 10.0 cm were used in order to eliminate sexual immature (Vevers 1949; Jangoux and Vloebergh 1973) and extremely large animals. The length was measured from the center of the mouth along the oral face to the tip of the arm. Parasitized animals were discarded.

**Dissection of Animals**

The arms were cut laterally on both sides and the gonads were removed by severing the gonoduct. Sexing of the animals was carried out by squash preparation of gonadal tissue, which was checked for the possible presence of parasites.

**Histological Procedures**

Small pieces from different regions of the ovaries were fixed in Bouin's fluid, embedded in paraffin and sectioned at 7 µm. Sections were stained with Brookes' trichrome (Brookes 1968) and the periodic acid Schiff-Orange G (PAS/OG) methods.

To obtain morphometric data on the variation of oocytes, selected sections of each ovary were used. Representative areas containing at least 100 oocytes were traced with a Zeiss microscope using a camera lucida from the ovarian wall to the middle of the ovarian lumen. The thickness of the ovarian wall and the area of each of the oocytes were measured with a digitizer (Hewlett-Packard, type 9864 A) in combination with a calculator (Hewlett-Packard, type 9820). The areas, expressed in square inches, were converted into µm², and from these areas the diameters of the oocytes were calculated, assuming the oocytes to be circular in cross section.

**Electron Microscopical Procedures**

Small fragments of the ovaries were immersed in 4% glutaraldehyde in 0.3 M cacodylate buffer at pH 7.2 for 1.5 h at 0°C. The tissues were postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2 for 1 h at 0°C rinsed in the same buffer for 30 min, dehydrated in ethanol and propylene oxide, and embedded in Epon 812.