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Oviducal Glands in Chondrichthyans

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10.1 INTRODUCTION

Oviducal glands (OG), also referred to as shell or nidamental glands (Prasad 1945, 1948; Knight et al. 1993) are discrete, specialized regions of the anterior portion of the oviduct in cartilaginous fishes. Oviducal glands produce the components of the egg jelly that surrounds the fertilized egg in the early stages of embryogenesis. They also produce the tertiary egg envelopes including the rigid egg capsule of oviparous species, the thin pliable transient egg candy case of yolk sac species and the thin plaited egg envelope in placental sharks (Hamlett et al. 1998a, 1999; Hamlett and Koob 1999). OG have also been implicated in sperm storage (Metten 1939; Prasad 1945; Pratt 1993; Hamlett et al. 2002a, b, 2003). Historically the terms shell, nidamental and oviducal gland have been used imprecisely and interchangeably. The region of the oviduct that produces a tough egg case that is deposited to the exterior in oviparous species is correctly termed the shell gland since this term denotes its function. A shell is defined as a hard, outer covering, hence the designation. The term nidamental gland is best used to refer to the gland that secretes the thin egg coverings of some viviparous species. The term nidamental is derived from *nidus* L. for nest. In many placental species each embryo is surrounded by its own egg covering and the embryo and its coverings develop in its own uterine compartment, hence the nest. Neither of the

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previous terms can be correctly applied to the gland when referring to the region of the oviduct in some rays where no egg covering is produced. To establish a consistent terminology, we have chosen not to use the terms shell or nidamental but to use only the term OG to refer to any of the aforementioned glands since they are all derived from the oviduct.

Early descriptions of OG in elasmobranchs were given by Borcea (1904, 1906), Widakowich (1906), Filhol and Garrault (1938), Nalini (1940), Prasad (1945, 1948). The vast majority of work elucidating the structure and function of elasmobranch OG has centered on the oviparous dogfish, Scyliorhinus canicula. Metten (1939) described the structure of the gland and several authors considered its histochemical characteristics (Threadgold, 1957; Rusauënn 1976). Krishnan (1959) added information on the histochemistry of the OG in Chiloscyllium griseus. The formation and nature of the components of the egg capsule of S. canicula has been well studied (Rusauënn et al. 1976; Rusauënn-Innocent 1985, 1990; Hunt, 1985; Peng and Knight, 1992, 1994a, b; Knight et al., 1993; Knight and Peng, 1992, 1994a, b; Hepworth et al., 1994; Knight et al. 1996). Thomason et al. (1994) investigated the antifouling properties of the egg case and Koob and Cox (1993) reported on tanning of the egg capsule in the little skate, Leucoraja erinacea. Pratt (1993) discussed sperm storage in the OG of some elasmobranchs. OG structure and function were surveyed in a variety of elasmobranchs (Hamlett et al. 1998a) and the fundamental zones discussed (Hamlett et al. 1999). A comprehensive treatment of reproductive biology in female elasmobranchs, including a consideration of the OG, was recently published (Hamlett and Koob 1999).

Older literature variously referred to the OG zones as albumen, mucous and shell secreting (Metten 1939; Prasad 1945) and in Scyliorhinus canicula terminology was based on histochemistry and zonation was classed A zone, B zone, B1 zone, B2 zone, etc. (Rusauënn 1976; Knight et al. 1996). Recently, terminology relating to the zonation in elasmobranch OG has been reconsidered (Hamlett et al. 1998a, 1999). These authors noted morphological features common in species of various reproductive modes. They described zones from anterior to posterior as club, papillary, baffle and terminal. The terminology is based on light microscopy (LM) of frontally sectioned glands. Club and papillary refer to the profile of the surface layer when viewed via LM. Baffle refers to the baffle plates that form the lips of the extrusion dies in the region that produces the various types of tertiary egg envelopes. Terminal refers to its caudad position. Terminology based on morphology allows reliable comparisons to be made across reproductive modes, whereas the histochemical composition of OG cells varies during gestation, reflecting the synthetic and secretory activity of the cells, hence are not reliable. Older descriptions generally refer to the club zone as the albumen zone (despite the lack of evidence for albumen secretion in this region), the papillary zone as the mucous zone and the baffle zone as oblique plates (Metten 1939) and tufts (Prasad 1945). Previous descriptions of the zonation in S. canicula have reported five to
nine zones depending on the author and the means of analysis, i.e., histochemistry, morphology via scanning electron microscopy, etc. (Threadgold, 1957; Rusaouen 1976).

Species that produce an egg case, candie case or egg envelope all share general design characteristics of the OG. The club and papillary zones produce the various types of egg jelly that initially surround the egg. The baffle zone produces the tertiary egg envelope and has a highly conserved structure (Fig. 10.1A, B). Secretory cells in simple tubular glands produce nascent egg envelope material that moves to the secretory duct by ciliary movement and secretion pressure exerted within the tubule from new secretion. From the secretory duct egg envelope material is passed to the spinneret which is composed of paired baffle plates that help to manipulate and orient the material as it passes to transverse grooves that extend the full width of the OG. Both Urobatis halieri (Babel 1967) and U. jamaicensis lack a discrete functional baffle zone and consequently does not produce an egg envelope. Members of the family Nectarinidae, the numbfishes, are reported to have no oviducal glands at all (Prasad 1945).

10.2 CLUB ZONE

The nature of the secretions of the club zone has been examined by histochemical means in Scyliorhinus canicula. Threadgold (1957) noted periodic acid-Schiff positive (PAS+) staining as well as positive staining with tussulofullidine blue. Stains for proteins were weak or absent. He concluded that the secretory product was a carbohydrate, possibly a neutral mucopolysaccharide and not albumen as it was believed to be by Fihlof and Garrault (1938). Rusaouen (1976) noted that secretory materials of the club zone of S. canicula stained alcian blue positive (AB+) at acid pH, indicating strong polyanions. Toluidine blue staining yielded a strong metachromatic reaction suggesting that the products might be sulfated polysaccharides. She concluded that secretions of the club zone were neutral mucopolysaccharides and acidic polysaccharides not bound to protein.

10.3 PAPILLARY ZONE

Staining properties suggested to Nalini (1940) that the product of the papillary zone in Chiloscyllium griseum was a type of mucin, the purported function was to separate the fluid component of the club zone from the capsule materials and to serve as a lubricant between the fluid and the forming capsule during encapsulation. In S. canicula, Threadgold (1957) noted metachromatic staining and concluded that the products contained carbohydrate. Rusaouen (1976), however, indicated that the zone was a strongly sulfated mucopolysaccharide. Feng and Knight (1992) observed AB+ staining and suggested that the material was a sulfated glycosaminoglycan. In Iago omanensis (Hamlett et al. 2002b) the papillary zone is PAS+, indicating glycoprotein or any mucous substance containing
neutral sugars. The caudal-most papillary lamella is AB+ at pH 2.5 indicating the presence of sulfated and unsulfated acid glycosaminoglycans and sialoglycoproteins.

Even though the exact chemical composition of both the club and papillary zones is unknown, it is clear that this region produces the more-or-less fluid jelly compartment surrounding eggs contained within the tertiary egg envelopes of all elasmobranchs that encapsulate their eggs. *Urospis jamaicensis* does not have a club or papillary zone and no egg jelly is produced (Hamlett unpublished).

Koob and Straus (1998) concluded that egg jelly in the little skate, *Raja erinacea*, functions as a structural device to hydrodynamically support the egg and developing embryo. Diversity in the nature of the jelly investment also exists in species with differing reproductive modes. In some sharks and rays, the egg and jelly fill the entire envelope; the developing embryos break out of the envelope to complete development free in the uterus. In other viviparous species the jelly occupies only a small proportion of the envelope. In placental sharks the modest jelly component surrounds the fertilized egg only during the early stages of gestation.

### 10.4 BAFFLE ZONE

The baffles zone produces tertiary egg envelopes. Simple tubular gland secretory cells produce a liquid crystalline material (Knight *et al.* 1996). It emerges from the secretory duct and is manipulated by the divergent die and baffle plates of the spinneret that function at extrusion. The liquid crystal material is assembled into a highly ordered fibrillar component of each subsequent layer within transverse grooves of the gland. Although the morphology of the baffle zone is similar in all species thus far examined, that produce a tertiary egg envelope, diversity exists in the nature of the egg covering, hence diversity exists in the composition and function of the egg coverings formed (Hamlett *et al.* 1998a; Hamlett and Koob 1999). *Urospis jamaicensis* lacks baffle plates and cells of its highly modified OG is non-secretory, hence no egg envelope is formed (Hamlett unpublished).

### 10.5 TERMINAL ZONE

The last zone of the oviducal gland is the terminal zone which is the site of formation of surface hairs that adorn the exterior of the capsule in Fig. 10.1 A. Composite line diagram of a "generic" oviducal gland showing club, papillary, baffles and terminal zones. B. Baffle zone unit consisting of secretory gland tubule, secretory duct, spinneret with baffle plates and transverse groove between plateau projections. Figs. 10.1A-B from Hamlett, W. C., Knight, D. P., Koob, T., Jeziorn, M., Luong, Rozycki, T., Brunette, N. and Hysell, M. 1998a. Journal of Experimental Zoology 282: 399-420. Fig. 1. C. Eggcase of *Scylliorhinus canicula*. l = tendrils, arrow = marginal rib. Original.
species that have hairs and sperm storage. A feature of some oviparous
OG is the secretion of hair filaments from the terminal zone. In the
holocephalan Callorhynchus mili (Smith et al. 2004) and the batoid Raja
eglanteria (Hamlett et al. 1999), the terminal zone differentiates into a
region of hair production and a region of sperm storage. The hair region
has mucoid tubules near the lumen and the base of the same tubules
contains secretory cells that resemble bladder zone secretory cells. Initially,
the basal portion of the tubules produce a secretion that extrudes up the
tubule by secretion pressure from below. As the emerging hair filament
passes cells of the luminal mucoid region, it is coated with secretions.
There are similar observations of two cell types in the same tubule in the
holocephalan Hydrolagus colliel (Prasad 1945; Stanley 1963). The dorsal
surface of the egg case in C. mili has abundant hairs that aid adhesion of
sand to the egg case. The ventral surface of the egg case is smooth with
lateral flanges that are concave. This formation results in a suction cup
arrangement that helps to attach the egg case to the muddy ocean floor.
Hence the egg case is attached to the mud and the sticky dorsal surface
with hairs is rapidly coated with sand thereby providing camouflage.
Similar arrangements occur in skates where the egg case is smooth but
elongate. Spiral tendrils extend from the lateral margin and ends of the
egg case. They are sticky and serve the same purpose of attaching sand for
camouflage. Additionally the tendrils coil with sea grass to attach and
support the egg case.

10.6 DIVERSITY OF OVIDUCAL GLAND MORPHOLOGY AND
TERTIARY EGG ENVELOPE

In oviparous species including Scyllorhinus canicula (Fig. 10.1C), Raja
eglanteria (Fig. 10.2C) and the holocephalan Callorhynchus mili (Fig. 10.3D)
a tough firm egg case is formed. Oviducal glands in oviparous species are
the largest in chondrichthians. In S. canicula the egg case has tendrils to
attach the capsule to a substrate, frequently sea plants and marginal ribs for
stability. Raja eglanteria lacks tendrils. Callorhynchus mili has a convex
surface covered with sticky hairs that attach sand thereby camouflaging the
case. The opposite side is smooth and acts as a suction cup to keep the
capsule planted in the sand.

Yolk sac viviparous species have thin transient candle cases. In the
spiny dogfish, Squalus acantbias, (Figs. 10.2A, B) and common sawshark,
Pristiophorus cirrtus (Fig. 10.2D) the OG is short and barrel shaped (Stevens
2002). The candle case encloses multiple eggs in S. acantbias (Fig. 10.2B)
but each candle contains a single egg in P. cirrtus (Fig. 10.2D). In both
cases a small volume of egg jelly initially coats the egg inside the candle.
In both species the candle dissolves and the embryo completes
development free in the uterus.

Species that have uterine compartments have a thin egg envelope that
is initially pleated but unfolds as the embryo grows (Fig. 10.3B). The
Fig. 10.2  A. Oviducal gland of *Squalus acanthias*. o = ovary, og = oviducal gland, od = oviduct. Original. B. Candle case of *Squalus acanthias* with two embryos. Arrows = egg jelly inside candle. C. Egg case of *Raja eglanteria*. D. Reproductive tract of *Pristiophorus cirratus* containing candle case with one egg. og = oviducal gland, od = oviduct, u = uterus, arrow = egg jelly inside candle. Original.
Fig. 10.3 A. Oviducal glands of *Mustelus canis*. Upper oviducal gland (og) has coiled lateral extensions (asterisks). The lower gland has been sectioned. od = oviduct. B. Partly unfolded plaited egg envelope of *Mustelus canis*. C. LM of longitudinally sectioned OG of *M. canis* similar to the bottom gland in Fig. 10.3A. c = club zone, p = papillary zone, b = baffle zone, t = terminal zone. D. Egg case of *Callorhinus milii* showing central oval portion that houses the embryo and lateral flanges that anchor the capsule to the sandy bottom. Original.
placental smooth dogfish shark *Mustelus canis* and gummy shark *M. antarcticus* that has a yolk sac (Storrie 2004) both have OG that are virtually identical at the gross level (Fig. 10.3A) and microscopically (Fig. 10.3C). To produce a wide egg envelope, the gland does not extend laterally but coils like a ram’s horn. This allows for the production of a large, plaited envelope while restricting the overall size of the gland. Microscopically the club, papillary, baffle and terminal zones are evident (Fig. 10.3C). In both *M. canis* and *M. antarcticus* the terminal zone sweeps laterally and anteriorly such that sperm storage tubules are located below baffle zone tubules.

### 10.7 HOLOCEPHALON OVIDUCAL GLANDS

Observation of the OG of holoccephalans is limited, but there are early reports of sperm in the upper oviduct of a *Chimaera* (Dean 1895, 1906) and a description of the morphology of the egg capsules (Dean 1912). An early description of the OG in *Hydrolagus coliei* includes an anterior ‘albumen zone’ that secretes into cranial transverse bands and a few mucous lamellae between the ‘albumen’ and shell zones (Prasad 1948). The shell zone was divided into a cranial region with baffle plates and a caudal shell zone with a few mucous tubules. Our results for *Callorhynchos milii* verify and extend these observations, but we adopt current terminology based on the morphological characteristics of the zones (Hamlett *et al.* 1998a) rather than the purported nature of the secretions. As there are presently no biochemical studies of the composition of the secretions, the terminology of albumen and mucus is not warranted and the types of secretions may not be consistent across the reproductive modes. Subtle variations in the type of secretions undoubtedly occur in the production of different types of egg coverings, whereas the basic morphological zonation persists. Our club zone is synonymous with the earlier described ‘albumen’ zone, our papillary zone with the earlier middle mucous zone, our baffle zone with the earlier cranial shell zone and our terminal zone with the earlier caudal shell zone.

In *Callorhynchos milii*, the club and papillary zones elaborate egg jelly that coats the fertilized egg, initially filling the lumen of the egg capsule. It has been suggested that its function must be critical to the developing embryo for at least the early stages of its development (Hamlett *et al.* 1998a). The egg jelly in *Leucoraja erinacea* functions as a structural device that hydrodynamically supports the egg and developing embryo, and the jelly from various regions have differing carbohydrate compositions. The egg jelly supports the embryo during the fragile period of embryogenesis and while it has external gill filaments. The jelly is progressively liquefied by secretory activity of eclosion glands located on the rostrum of the embryo (Hamlett unpublished). There is currently no evidence that the jelly layers are nutritive for the embryo (Koob and Straus 1998).

Secretions produced by the club and papillary zones have been examined histochemically adequately for only one species, the oviparous
catshark Scylliorhinus canicula. Secretions of the club zone were initially reported to be as PAS+ carbohydrate (Threadgold 1957), but the secretions were subsequently reported as both PAS+, AB+ polysaccharide (Rusanun 1976) while the club zone secretions in Callorhinus milii stained both PAS+, AB+ (Smith et al. 2004).

Secretory material of the papillary zone in Callorhinus milii is AB+ with more intense staining in the caudal-most papillary tubules. The exact chemical composition of papillary secretions has yet to be determined, but one suggestion is that the material is a type of mucin, functioning to separate and lubricate the region between the egg and the forthcoming capsule (Naini 1940). Other suggestions include the secretion of the caudal tubules functions to bind the perimeter of egg jelly to the egg capsule (Knight et al. 1996; Hamlett et al. 1998a). In Scylliorhinus canicula papillary secretions contain carbohydrate and stained metachromatically (Threadgold 1957). Feng and Knight (1992) identified the material as a sulfated glycosaminoglycan as it stained AB+. While we cannot assume that the material secreted in C. milii is the same as that of oviparous elasmobranchs, its function of surrounding the egg and filling the egg capsule is thought to be similar.

10.8 STRUCTURE AND FUNCTION OF THE EGG CASE OF SCYLLIORHINUS CANICULA

The Selachian egg case serves mainly as a shock absorber to protect the embryo. The wall of the case is a highly cross-linked collagenous composite material with a complex, multi-lamellar, highly hierarchical construction. In S. canicula much of the thickness of the case wall is formed from about 20 lamellae. Each lamella is built from a single layer of flattened collagenous ribbons. These overlap within the lamellae like tiles on a roof. Ribbons are held together by small quantities of amorphous matrix. The ribbons are continuous throughout the length of the egg case. Each ribbon is formed from numerous transversely banded collagen fibrils arranged in a remarkably regular way that varies somewhat in different regions of the egg case. Each collagen fibril is practically crystalline being constructed from a tetragonal array of collagen molecules (Knight and Hunt 1974) regularly kinked approximately midway between their ends and with the kinked segments running at approximately 20° to the long axis of the fibril. Bands of low protein density within the axial periodic structure of the fibril may allow the egg case wall to act as a semi-permeable membrane, highly permeable to oxygen, carbon dioxide and nitrogenous waste (Knight and Feng 1994b; Knight, Feng et al. 1996). The collagen molecules of the egg case are held together by extensive covalent cross-linking (Luong et al. 1997), which contributes to the material’s mechanical and thermal stability, its extreme insolubility and resistance to enzymatic degradation. The detailed structure of the egg case collagen fibril and its relation to similar fibrils of type VI collagen has been analyzed.

10.8.1 Properties of the Egg Case of Scyliorhinus Canicula

The mechanical properties of wet longitudinal strips of the Scyliorhinus canicula egg case wall have been investigated (Hepworth et al. 1994). The strips are remarkably strong (tensile strength 11.9 ± 0.7 MPa, n = 8) and extensible (strain at fracture 0.39 ± 0.03, n = 8). The combination of high tensile strength and extensibility gives them a high toughness (6 x 10³ J m⁻² or 6 kJ kg⁻¹ for a specimen measuring 4 x 1 x 10 mm). This toughness is in the range of that of steels. Three mechanisms of energy storage and dissipation probably help to account for the material's toughness. Firstly an increase in axial periodicity in moderately strained material demonstrated by low angle X-ray diffraction and TEM suggests that the kinks in the collagen molecules straighten out progressively as the material is strained and spring back when released. Recovery is substantially complete up to a global strain of up to 24%. This suggests an entropic mechanism of energy storage. A less marked kinking in mammalian tendon collagen molecules may have a similar function. Secondly as the material is progressively strained, the composite construction allows different layers of fibrils to rotate and fail successively depending on their initial orientation. Energy is thought to be dissipated by deformation of the matrix between the collagen fibrils as they rotate and by the formation of numerous cracks as matrix and fibrils fail. Thirdly, filler particles are present in high concentrations in the outer layers of the case (Knight and Vollrath 2001). Ultrastructural evidence (Knight and Feng 1994) suggests these are bound into the meshwork of collagen fibrils and stretch when the egg case is strained probably helping to store and/or dissipate energy.

10.8.2 Extrusion of the Egg Case in Scyliorhinus Canicula

The elasmobranch egg case is secreted as a continuous extrusion from the OG (Knight et al. 1996a). The pressure required for extrusion must be fairly small as the OG is only surrounded by a relatively thin connective tissue sheath and is minimally reinforced internally with connective tissue. The extrusion pressure is thought to be derived from the secretion and swelling of a jelly-like material into the lumen of the oviducal gland and from the transport of the large ovum into it (Knight et al. 1996a). Although the rate of protein secretion is extremely high (an estimated 140 mg of dry collagen per gland per day) (Knight et al. 1996a; Knight and Vollrath 2001) the linear extrusion rate appears to be quite small. As the egg case wall (60 mm long) and tendrils have a combined length of approximately 1 m and as the fastest observed rate of egg case production is approximately one egg case per gland every 60 hours, the average extrusion rate (0.3 mm min⁻¹) is very slow (Knight and Vollrath 2001). This calculation, however, assumes that there is no pause for rest and that the case wall is extruded at the same rate as the tendrils. Slow secretion of the case wall is further suggested by the
observation that it is not difficult to catch mature fish on their breeding grounds in the act of secreting this structure.

The bulk of the thickness of the OG is constructed from numerous narrow (100 μm diameter) and very long (up to 18 mm) tubular glands. The epithelium of each tubular gland is constructed from ciliated cells and gland cells. The gland cells synthesize the collagen and store it, together with accessory proteins, in secretory vesicles measuring about 2 μm in diameter (Knight et al. 1993).

The ultrastructure of storage and secretion has been studied by transmission electron microscopy (Knight et al. 1993). Within the rough endoplasmic reticulum cisternae the collagen appears isotropic but becomes assembled into a smetic A or laminar phase in the Golgi cisternae. This phase persists in early secretory granules, where it is found in conjunction with a micellar phase. As these granules mature, the collagen appears to pass through a cholesteric mesophase before adopting a hexagonal columnar arrangement. On merocrine secretion the granule contents revert rapidly to the smectic A-lamellar and micellar phases. As it passes along the OG gland tubules, the collagen is present as a lamellar phase before assembling within the transverse grooves into the final fibrils that constitute the egg capsule. These ultrastructural changes can be divided into distinct phases: (I) collagen assembly within the trans Golgi (Figs 10.4A, B), (II) formation of storage granules at the trans face of the Golgi (Figs 10.4C), (III) maturation of storage granules (Figs. 10.4D, E: 10.5A-F; 10.6A), (IV) merocrine secretion of the mature granules and their coalescence to produce a strand of secreted material within the lumen of the gland (Figs 10.6B, C), (V) transport of the strand to the transverse grooves and initiation of fibrillogenesis (Figs. 10.7A-D) and

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Fig. 10.4 A. Ultrathin section of material stained for acid phosphatase. Banded material (phase II; small arrows) with a D periodicity of approximately 34 nm is revealed by a negative staining effect in some regions of the trans Golgi cisternae. Tangentially oriented material with a similar periodicity is seen in the periphery of a developing granule (broad arrow) close to the trans face. Scale bar 250 nm. B. Material similar to that seen in Fig. 10.4A is demonstrated in a trans Golgi cistern any a positive staining effect. Uranyl acetate and lead citrate. Scale bar 100 nm. C. A large number of developing granules are seen close to the trans face of the Golgi. Tangentially oriented banded phase II material (small arrows) is seen in the periphery of these granules. The bulk of the granule appears to contain phase I material together with some narrow, short and irregular oriented segments of phase II material. The amorphous material at the periphery of the granule may represent transversely sectioned phase II material. A scalped pit (broad arrow) is seen in the limiting membrane of the granule. Uranyl acetate and lead citrate. Scale bar 500 nm. D. Part of a developing storage granule. Much of the material (phase I) in the developing storage granule appears to be constructed from rather evenly spaced granules approximately 15 nm in diameter. Tangentially oriented phase II material (small arrow) is seen at the periphery of the granule. A small segment of this material (broad arrow) is seen in the center of the granule. Uranyl acetate and lead citrate. Scale bar 200 nm. E. Portion of a hexagonal columnar phase V) seen in a mature granule. A fine dot can be seen at the center of the clear area in some of the hexagons (small arrow). Uranyl acetate and lead citrate. Scale bar 100 nm.
Fig. 10.5 Contd. ...
and their coalescence to produce a strand of secreted material within the lumen of the gland (Figs. 10.6B, C), (V) transport of the strand to the transverse grooves and initiation of fibrillogenesis (Figs. 10.7A-D) and (VI) formation of the white capsule and its transformation into the final clear capsule (Figs. 10.7E-F). A summary diagram depicting ultrastructural changes occurring during collagen assembly and secretion is shown in Fig. 10.8) (Knight et al. 1993).

Each tubular gland is connected to its own extrusion die (Fig. 10.1A-B) (Knight et al. 1997). The latter are arranged in a precisely defined way, overlapping like tiles in a single row at the base of each transverse groove. The lumen of the extrusion die has a circular cross-section (approximately 10 mm in diameter) at its input face (connected to a tubular gland) but rapidly expands in one plane so the output face is an elongated slit measuring approximately 10 x 150 to 425 μm (Knight et al. 1996c; Knight et al. 1997), the actual size depending on location in the oviducal gland. The rapid divergence of the die can be described by a hyperbolic function (Knight and Vollrath 2001). The external lips of each die are formed by two flattened roughly semi-circular plate-like structures that protrude into the base of the transverse groove. Each of these plates is formed from two

Fig. 10.5 A. A granule which appears to be almost entirely packed with phase II material. This is thought to represent a transitional stage between the developing granule (Fig. 10.4C and D) and the mature granule with the double twisted cholesteric mesophase arrangement (Fig. 10.5C; phase III). Uranyl acetate and lead citrate. Scale bar 0.5 μm. B. A granule similar to that seen in Fig. 10.5A which appears to be largely filled with phase II material. A concentric pattern of rather evenly spaced light and dark bands is seen similar to that in Fig. 10.5C but with the banding seen in phase II material. The pale bands appear to be practically amorphous at this magnification are thought to represent transversely and obliquely sectioned ribbons of phase II material. The granule is thought to represent a transition stage between the developing and the mature granule. Uranyl acetate and lead citrate. Scale bar 0.5 μm. C. A mature granule largely filled with phase III material but with small quantities of what appears to be phase IV material. The pattern of dark and light bands is similar to that seen in Fig. 10.5B, but the constituent material is not D periodic and is seen to be composed of fine protofibrils. Uranyl acetate, phosphotungstic acid and lead citrate. Scale bar 250 μm. D. A mature granule containing both phase III (broad arrow) and columnar hexagonal phase IV material (narrow arrow). The hexagonal columnar material is found within a lattice defect in phase III material. Uranyl acetate, phosphotungstic acid and lead citrate. Scale bar 250 μm. E. A mature granule which appears to be largely filled with phase IV material but with a small quantity of phase III material visible at the periphery of the granule on the right hand side. The bulk of the contents appear as two hexagonal crystallites with a lattice dislocation between them. Uranyl acetate, phosphotungstic acid and lead citrate. Scale bar 0.5 μm. F. When phosphotungstic acid is omitted the phase IV pseudocell has the same spacing as in material stained with phosphotungstic acid but appears as a dark hexagon with a light center. What appears to be a view down the 001 plane (arrow) and several lattice effects can be seen. Uranyl acetate and lead citrate. Scale bar 0.5 μm. Original.
epithelial sheets arranged back to back. This epithelium, like that of the transverse groove contains numerous ciliated cells and some gland cells. The latter are thought to secrete the adhesive that holds individual ribbons together (see below).

The extrusion apparatus is thought to function in the following way (Knight and Vollrath 2001). The dope contains two major components: the collagen as a highly concentrated lamellar liquid crystalline phase and, suspended within the collagen solution, droplets that eventually form the filler particles. Ciliary action probably helps to transport the dope to the extrusion dies. The relatively low pH (approximately 6.6) of the lumen of the tubular gland (Feng and Knight 1994b) is probably beneath that of the isoelectric point of the collagen and therefore maintains a positive repulsive charge on the collagen molecules. This opposes weak attractive forces between the collagen molecules, maintaining a liquid crystalline state and preventing premature aggregation and hydrogen bonding of the molecules. On reaching the extrusion die, the rapid divergence of the lumen causes the dope to be subjected to an elongational flow in the hoop (transverse) direction. Here, the liquid crystallinity of short rod-shaped collagen molecules or molecular aggregates causes them to orient rapidly and efficiently in the flow field. This gives rise to a nested arc pattern of molecular orientations (Figs. 10.9A-B). These can be readily demonstrated by polarising microscopy in aldehyde-fixed dope withdrawn from the transverse grooves and in fully-formed ribbons peeled from the egg case wall. The fluid droplets present in the dope also elongate in the flow field, taking up the same orientation as the surrounding collagen. This can be seen in Nomarski differential interference micrographs of aldehyde-fixed dope withdrawn from the dies (Fig. 10.9A). Thus elongational flow in the hoop direction results in the extrusion of ribbons containing rather precisely defined collagen with similarly orientated elongated droplets of tyrosine-rich protein. As the resulting ribbons pass out from between the

Fig. 10.6 A. Part of a mature granule showing phase III material which has the appearance of a cholesteric mesophase. Uranyl acetate, phospholipidic acid and lead citrate. Scale bar 100 nm. B. Two granules (arrowed) at the apical surface of the cell which appear to have been fixed in the act of merocrine secretion apparently contain only phase II material. The vast majority of granules found in the apex of the cell are mature granules containing a mixture of phase III and IV material. The simplest hypothesis to account for this is that the contents of mature granules show a rapid transition to phase II as soon as the limiting membrane is breached. Uranyl acetate and lead citrate. Scale bar 500 nm. C. A section through the lumen of the lower (proximal) part of a tubule shows the coalesced strand of material (B) and the contents of a secreted granule (A) which has not yet coalesced with B. The granule appears to be largely filled with phase II material closely resembling that seen in the grating section of the coalesced secretion. The transverse order in this material does not appear to be as good as in phase VI. The lumen contains numerous myelin figures. In two instances these appear to adhere to the membrane of a cilium (arrowed). Uranyl acetate and lead citrate. Scale bar 100 nm. Original.
Fig. 10.7 Contd. ...
curved lips of the extrusion dies they adhere together to give lamellae. Similarly, as the lamellae flow out from the transverse grooves into the main lumen of the gland they adhere together. Adhesion probably results from pulling the ribbons and lamellae together by pumping out water from the transverse grooves and from secretion of an adhesive from numerous gland cells on the plate-like lips of the die and walls of the transverse grooves.

Further processing occurs as the lamellae of extruded collagen leave the transverse grooves and travel into the main lumen of the gland. Here, the pH is thought to increase from about 6.6 to about 8.1 (Feng and Knight 1994b). It is not known how this is achieved but the epithelium may transport hydrogen or bicarbonate ions or the large ovum may produce ammonia. This increase in pH is thought to have two important consequences (Knight et al. 1996a): First, it removes repulsive charges and allows the collagen molecules to approach one another closely and hydrogen bond together to form the crystalline fibrils described above. This locks in place the molecular orientations defined by the extrusion dies. Second, an increase in pH brings inactive peroxidase and phenoloxidase previously secreted into the dope, to their pH optima enabling them to form covalent cross-link, thus stabilizing both the collagen and the tyrosine-rich protein. A thin layer of water lubricates the dope as it passes through the die.

Fig. 10.7 A. Grazing section of coalesced material in the lumen as in Fig. 10.6C. showing two different types of destilation (arrowed) Uronyl acetate and lead citrate. Scale bar 200μm B. Tangentially oriented material (broad arrow) lies at the surface of the coalesced material in the proximal part of the tubular gland while the deeper material shows some phase I material and small irregularly oriented segments of phase II material (narrow arrow). Uronyl acetate and lead citrate. Scale bar 200μm C. High magnification micrograph of an ultrathin section of columnar phase material stained with uranyl acetate and lead citrate. The dense hexagonal ring appears to be constructed from six subunits (arrows). Scale bar 50μm D. Proximal part of the tubule. Secreted material (M) which forms the coalesced strand appears to be constructed largely from phase I material and is closely similar to the contents of the granule (G) whose limiting membrane (arrowed) is thought to have recently ruptured. Microvilli, cilia and numerous myelin figures are seen in the lumen. Uronyl acetate and lead citrate. Scale bar 1 μm E. White egg capsule. The material appears to consist of two phases. Fibres similar to those seen in the final egg case (phase VI; broad black arrow) and micelles (phase V) in between which appear to consist of a dense ring with a light center in which a dense central dot (tip of white arrows) is sometimes seen. The high degree of transverse order in the fibril can be seen on the right of the black arrow. The dense transverse bands of the fibrils appear to run continuously from fibril to fibril. Uronyl acetate, phosphotungstic acid and lead citrate. Scale bar 100μm F. Final egg capsule. This appears to be constructed almost entirely from phase VI fibrils. These appear longitudinally sectioned in most of the micrograph but are obliquely sectioned in a narrow strip at the bottom. Material fixed in a solution containing 1% glutaraldehyde and 3% paraformaldehyde and 1% tannic acid. Uronyl acetate, phosphotungstic acid and lead citrate. Scale bar 10μm Original.
Fig. 10.8 Contd. ...
10.8.3 What Holds the Ribbons and Lamellae of the Egg Case Together?

The question of what sticks each flattened ribbon from a row of spinnerets into a lamina and each lamina together to form the capsule wall, rib or tendril has received little attention in the literature. A simple observation suggests that there is an additional adhesive over and above the collagenous material that forms the bulk of the egg case. Soaking the egg case for 24 hours in fairly concentrated acetic acid produced only slight swelling of the ribbons and lamellae but enabled them to be easily separated from one another (Knight et al. 1996) suggesting the extraction of an adhesive secreted in the last part of the secretory pathway, probably from the surface of the baffle plates (to stick the ribbons together) and transverse grooves of the OG (to stick the laminae together). At least in one location, the middle laminae of the marginal rib of the egg case, there is good evidence that the baffle plates and transverse grooves do secrete an additional material that coats the ribbons and laminae. In laser confocal images the surfaces of the individual ribbons are seen to be coated with a thin layer of autofluorescent granules while the laminae are coated by a slightly thicker layer of the same material (Knight et al. 1996b).

In an attempt to locate gland cells which might be responsible for the secretion of an adhesive we have recently examined the epithelium of the baffle plates and transverse grooves in both SEM and TEM. A low power SEM of the surface of the baffle plates and the wall of the transverse groove reveals the presence of a high density of glandular cells almost entirely lacking the numerous cilia seen in the adjacent ciliated cells (Fig. 10.10A). The gland cells are distributed fairly randomly between the columnar ciliated cells and are particularly numerous in the more basal parts of the transverse grooves. A closer view of the anterior edges of the baffle plates shows that the apical surface of these glandular cells is relatively smooth at least in inactive cells except for a thin band of microvilli outlining the junction between the apical and lateral surfaces of the cell. Low bumps with a diameter of about 0.3 μm occur on the apical surface of the gland cells may represent secretory granules which are seen

Fig. 10.8 Diagramatic drawing of a part of a secretory cell in the proximal part of the baffle zone. Secretory tubule to illustrate the scheme for the sequence of changes that occur during storage and secretion of the (pro)collagen. Nucleus (N). The ER contains the isotropic phase. The Golgi cisternae contain some phase II material: 1. Developing granules containing phases I and II. 2. Developing granule containing a higher proportion of phase II. 3. Granule containing spirally oriented phase II. 4. Double twisted cholesteric hexagonal columnar phase (IV) and some phase II material. 5. Granule containing both cholesteric (phase III) and phase IV material. 6. Granule containing mainly phase IV material. 7. and 8. Granules undergoing merocrine secretion with consequent reversion to phases I and II. Figs. 10.4A-10.8 from Knight, D. P., Feng, D., Stewart, M., and King, E. 1993. Philosophical Transactions of the Royal Society London B 341: 419436, Figs. 3-16.
Fig. 10.9 A. Nomarski photomicrograph of a single row of spinneret casts. An overlapping row of ribbons fuses together to form a single lamella. The superposition of the semicircular arced pattern in successive ribbons can be seen in the lamella. The overlapping of 5 ribbons at each point in the lamella gives rise to a plywood-like construction. B. Highly simplified diagram showing how a novel plywood-like construction is produced by the superposition of ribbons. The stepped rotation of the fibers in this construction can be seen in the center of the diagram, where 5 ribbons each containing nested arcs of fibers overlap. The width of each ribbon is approximately 425 μm. Figs. 10.9A-B from Knight, D. P., Hu, X. W., Newton, R. H., Cipollone, M., Gathercole, L. J. and Koob, T. 1996. Journal of Biomimetics. 4: 105-120, Figs. 3, 6.
Fig. 10.10 A. SEM of baffle plates of Scyllorhinus canicula showing ciliated cells and secretory cells (asterisks). 3,068X. B. TEM of baffle plates of Scyllorhinus canicula with ciliated cells, secretory cells (asterisks) and nascent egg envelope. 2,000X. C. TEM of secretory cell with secretory vesicles containing adhesive material in baffle plates of S. canicula. 10,000X. Original.
in TEM sections to slightly protrude onto the cell surface in some regions (Fig. 10.10B). A high magnification TEM of the apical part of active gland cells (Fig. 10.10C) reveals numerous secretory vesicles with a diameter of about 0.35 μm containing material with a range of densities perhaps representing secretory material at different packing densities. Where the material within the secretory vesicles is less dense it is seen to be nanofibrillar and resembles the nanofibrillar material lying between the apical surface of the cell and densely staining lamina of egg case material. In active gland cells some of the microvilli appear to adhere to the lamina of egg case material, possibly helping to ensure that concentrated adhesive material from the secretory granules is transferred directly to the lamina.

These observations suggest that gland cells on the baffle plates and transverse grooves secrete an adhesive, possibly a glycoprotein soluble in acetic acid, which helps to stick the ribbons and laminae of the egg case together. Removal of water from the space between the baffle plates and transverse grooves probably helps to bring the ribbons and laminae together. The water removal may be facilitated by the action of a Na⁺K⁺-dependent ATPase apparently present in high concentrations between the highly infolded lateral surfaces of the ciliated cells (Knight and Feng unpublished). The epithelium also contains numerous small vesicles which stain vitally with neutral red indicating that a proton pump might also be involved in water transport from the transport groove (Knight and Feng unpublished). Finally it is likely that the adhesion of the laminae is assisted by pressing them together once they reach the main lumen of the gland and that the pressure for this is derived mainly from the swelling of the "jelly" secreted into the lumen of the gland by the A-zone. Thus the sticking together of the ribbons and laminae is likely to be a fairly complex process in which different parts of the oviducal gland play a part. The formation of the layered structure of the egg case shows some resemblance to industrial processes for the formation of glued laminates but is more ingenious in that it is a continuous extrusion process.

10.9 OVIDUCAL GLAND IN RAJA EGLANTERIA

The general shape and microscopic organization of the OG of Raja eglanteria is fundamentally similar to that of Scyllorhinus canicula, Leucoraja erinacea and the holoccephalan Callorhinus milii. Examination of the baffle zone in OG actively elaborating egg case material in R. eglanteria reveals the extrusion process (Figs. 10.11A, B; 10.12A). Nascent egg case material emerges from a single secretory tubule and passes between baffle plates to emerge in the transverse groove as an individual thread. Adjacent threads adhere to produce broad ribbons. As ribbons widen they form sheets of the egg case (Figs. 10.11A, B). When an actively secreting gland is sectioned longitudinally the extrusion process is revealed. Threads of secretory material pass between baffle plates fuse as ribbons and eventually produce sheets. Figure 10.12A shows how luminal threads (yellow) are transformed
Fig. 10.11 A. SEM of *Raja eglanteria* baffle zone showing egg envelope material emerging from transverse grooves alternating with plateau projections (p). In the more anterior levels egg envelope initially emerges and thin threads (l) that coalesce as flat ribbons (r) that them blend to form laminar sheets (s). 50X. B. Baffle zone of *R. eglanteria* showing egg envelope threads (l), ribbons (r) and a completed sheet (s). 75X. Original.
Fig. 10.12 A. SEM of longitudinally sectioned baffle zone of *Raja eglanteria* showing how secretory material from each transverse groove contributed to the laminar organization of the egg case. bp = baffle plates, p = plateau projections. 150X. B. SEM of razor blade section of egg case in *Raja eglanteria* to show laminar organization of the egg case and fibrils that constitute the layers. 750X. Original.
into sheets that contribute to the lamellae of the egg case. Examination of a portion of the egg case that has been fractured reveals the lamellar organization of the case and that each lamella is composed of fibrils that generates a plywood-like arrangement (Fig. 10.12B).

**10.10 HAIR FORMATION IN THE TERMINAL ZONE**

The terminal zone of oviparous species is broad and extensive. Instead of possessing lamellae, simple tubular glands are scattered throughout the terminal zone where they perform two functions, sperm storage and the production of fine hairs that decorate the outside of the egg capsule in species that form hairs. The deepest region of the hair forming tubules in *R. eglanteria* have secretory cells that are virtually identical microscopically to capsule forming cells of the baffle zone (Fig. 10.13A). Cells close to the opening of the tubules into the main lumen of the OG are mucous. The apparent difference in the secretory product of the baffle zone tubules and hair forming tubules of the terminal zone is that the lamellar architecture of the baffle zone results in a continuous sheet of egg capsule being formed. Secretion of each tubule emerges as a liquid crystal polymer that then blends with secretions of adjacent tubules to form a complete sheet (Knight *et al.* 1996). In the hair forming tubules of the terminal zone the hairs never merge but remain separate. We suggest the surface mucous segment allows the hairs to remain separate as they emerge. The chemical basis of this phenomenon remains unresolved.

**10.11 SPERM STORAGE IN THE TERMINAL ZONE**

Metten (1939) described sperm storage in *Scyllarhinus canicula* as occurring in the shell secreting tubules. This is contrary to observations of Hamlett *et al.* (1998a, 1999). Metten examined the OG of the oviparous shark *S. canicula* from animals in various stages of secretion of the tertiary egg envelope. He cited Hobson's (1930) work in the skate and stated that ova were found in the upper oviducts, between the ostium and OG, whilst egg capsules were three-quarters completed. In his own observations in *S. canicula*, Metten reported that in fish with ova in the coelom or upper oviduct, the egg capsule was half secreted or less. He believed that fertilization and egg capsule secretion occur simultaneously and that the shell secreting tubules provided some nutrient material for the spermatozoa in the capsule substance. He noted that some sperm were incorporated into the egg capsule substance and that it hardened immediately upon leaving the glands. He also claimed that sperm in the bottom of shell secreting tubules actively secreting egg capsule material were in the process of “turning around” to exit the glands along with the egg capsule material. In studies of the same animal, Knight *et al.* (1996a) examined the structure of the shell secreting tubules of the OG and concluded that fertilization must occur in the upper oviduct or abdominal cavity. They noted sperm in the baffle zone tubules in animals secreting...
Fig. 10.13 A. LM of hair forming terminal zone tubule in *Raja eglanteria*. The base of the gland has secretory gland cells (gc) virtually identical to baffle zone secretory cells that elaborate egg envelope material (ee) The neck of the tubule has mucous cells (m). 600X. B. Luminal aspect of hair forming tubules in terminal zone of *R. eglanteria*. The egg envelope material remains as discrete elements (asterisks) and does not fuse into sheets as in the baffle zone. Presumably the mucous coat contributes to this phenomenon. 200X. C. Diagram of OG in *R. eglanteria*, zone sizes not to scale. Original.
egg capsule but did not notice sperm in the caudal segment of the OG corresponding to the terminal zone.

Metten (1939) did not recognize the terminal zone but pictured a broad caudal region of the OG that he indicated had short mucous glands. This corresponds to the terminal zone. The histological organization of the OG of two other oviparous chondrichthyans have been studied. In the skate Raja eglanteria (Hamlett et al. 1999) and the elephantfish Callorhinchus milli (Smith et al. 2004) terminal zone tubules are short, broadly dispersed and do not form lamellae. Their surface area is large due to the width of the terminal zone, not the depth of each gland, as in viviparous species. In both Raja and Callorhinchus sperm have been observed in the terminal zone and no sperm were seen in the baffle zone. Metten’s results show incidental sperm occurrence in baffle zone tubules and sperm being purged from the tubules with secretion of egg capsule. The refilling of gland tubules may be the result of repeated inseminations.

The organization and distribution of terminal zone tubules in Raja eglanteria, Leucoraja erinacea (Hamlett et al. 1998a) and Scyliorhinus canicula (Knight et al. 1996) are all very similar. The terminal zone tubules in Callorhinchus milli strongly resemble the tubules of these oviparous elasmobranchs and perform similar functions.

The organization and distribution of terminal zone tubules in three triakid sharks is in sharp contrast to terminal zone tubules in oviparous Chondrichthians (Hamlett et al. 2003). In Mustelus canis (Hamlett et al. 2002a), M. antarcticus (Storrie 2004) and iago omanensis (Hamlett et al. 2002b) terminal zone tubules sweep laterally from the OG lumen and form laterally situated dilated recesses that harbor sperm year round. Recently, Conrath and Musick (2002) studied various aspects of the reproductive biology in M. canis and reported observations on sperm storage. They made transverse histological sections of the caudal one-third of the OG from samples collected throughout the year and consistently found sperm in the OG, specifically the terminal zone.

The fate of spermatozoa deposited within the female reproductive tract reproductive tract has been described in the smooth hound, Mustelus canis (Hamlett et al. 2002a, 2003). Evidence of sperm-uterine association is presented as well as documentation of sperm storage specifically in the terminal zone of the OG. Immediately postpartum placental-uterine attachment sites, now termed uterine or placental scars, begin to remodel to a mucous epithelium for the next gestational cycle. Sperm become embedded in the uterine epithelium adjacent to placental scars. Fertilization is presumed to occur in the anterior oviduct above the OG. Hamlett et al. (2002a) suggested uterine embedded sperm may be in the process of being activated and capacitated as in mammals. This does not explain why it would be necessary to activate sperm before storage in the terminal zone. Alternatively uterine sperm may be phagocyted by uterine epithelium. The physiological mechanisms that mediate sperm-uterus attachment, release, storage in the terminal zone of the OG are
Fig. 10.14 A. Diagram of OG in *lago omanensis* to show the sperm storage tubules beneath baffle zone tubules. Zone size not to scale. B. TEM of sperm storage tubule in *lago omanensis* with luminal sperm (s). 6,000X. C. Ciliated epithelium of sperm storage tubules of *lago omanensis* showing sperm (s) surrounded by luminal matrix (asterisk). 600X. Figs. 10.14A-C from Hamlett, W. C., Fishelson, L., Baranes, A., Hysell, C. K. and Sever, D. M. 2002c. Marine and Freshwater Research 53: 601-613, Figs. 23, 24.
currently under investigation. Various workers have commented on uterine sperm, generally immediately after insemination. Metten (1944) reported uterine digestion of sperm in Scylliorhinus canicula. Our observations in M. canis do not confirm Metten's (1944) conclusions. Leesa et al. (1986) observed sperm in the uterus and OG of Rhinobatos borkelli from Brazil after birth and subsequent copulation. The published micrograph of sperm in the OG does not allow determination of what precise zone of the OG was involved but it appears that the sperm are in the gland lumen. Fishelson and Baranes (1998) described the folded endometrium of gravid placental Lago omanensis as forming simple tubular glands at the bases of the folds. They saw aggregations of sperm in the tubules but did not report sperm being embedded in the uterus.

Storrie (2004) reported on sperm in the OG in Mustelus antarcticus during different periods of gestation. She evidence demonstrated sperm storage exclusively in the terminal zone, although transient occurrence of sperm was noted in other gland tubules in animals not actively secreting jelly or egg envelope. Noteworthy is the fact that terminal zone sperm were found in both mature (pregnant, non-pregnant and postpartum) and immature (prior to first ovulation) animals throughout the year. Efforts are being directed at elucidating whether terminal zone sperm are being stored for prolonged periods from a single mating event with one or more males or result from multiple matings throughout the year. Feldheim et al. (2001) have recently applied genetic analysis using DNA microsatellite loci developed for lemon sharks, Negaprion brevirostris, to investigate the possibility of multiple paternity. Their results demonstrated that at least three males sired a single litter. Additionally, using other molecular methods for genetic analysis Saville et al. (2002) that at least four fathers contributed to a brood of 32 pups in the nurse shark, Ginglymostoma cirratum. Histological examination of the OG from either species was not performed.

Although viviparity is advantageous for nourishment and growth of the offspring, it involves an immunological risk for the sperm containing paternal antigens. The placental smoothhound dogfish, Mustelus canis has sperm storage in the terminal zone of the oviducal gland. Sperm storage tubules (SST) in the terminal zone were examined using immunohistochemistry to investigate the tissue expression of the cytokine interleukin IL-1 receptor type I (IL-1R I) and macrophage migration inhibitory factor (MIF) (Paulse et al. 2003). Immunostaining of the SST were positive for both MIF and IL-1 receptor. The present findings on the expression of the interleukin system and MIF in SST suggest that these cytokines are involved in evolutionarily conserved immunological mechanisms that anticipate the presence of paternal antigens in maternal tissues, specifically the terminal zone.

10.12 CONCLUSIONS

The OG of chondrichthyan fishes is a unique structure that is found in most species where it functions to: 1) produce egg jelly, 2) form the tertiary
egg envelope, 3) store sperm, 3) protect sperm from immunological attack by the mother, 4) nourish sperm. There is diversity in the morphology of the tertiary egg envelopes depending on the method of reproduction: oviparous species produce egg cases, yolk sac viviparous species produce transient candle cases and placental species produce an egg envelope. Few species do not encapsulate eggs with jelly and egg envelope, notably Urolophus halleri (Babel 1967) and U. jamacensis (Hamlett et al. 1998a, 1999). It is rare that the OG is missing as in Narcine (Prasad 1945).

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10.14 LITERATURE CITED


Oviducal Glands in Chondrichthyans


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