Chapter 11

Hormones and Reproduction in Chondrichthyan Fishes

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SUMMARY

Chondrichthyans represent a diverse and successful group of fishes that occupy a critical position in the evolution of vertebrate animals. The evolutionary success of these fishes is partly attributed to their many reproductive adaptations, and an understanding of reproductive endocrinology in this group can provide insights into hormonal function in all vertebrates. This chapter summarizes the current knowledge of the role of hormones in the reproductive physiology of chondrichthyan fishes, and identifies important areas for future research. The roles of peptide and steroid hormones in both males and females are discussed in relation to the brain–pituitary–gonadal axis, regulation of the reproductive tract and gametogenesis, sexual maturation, mating behavior, and environmental influences on reproduction. Recent studies on chondrichthyan endocrinology have provided important information on how hormones regulate reproduction in this diverse group of fishes, and demonstrate that many regulatory mechanisms are conserved through vertebrate evolution.

1. REPRODUCTION IN CHONDRICHTHYAN FISHES

The cartilaginous fishes (class Chondrichthyes) include the well-known subclass Elasmobranchii (sharks, skates, and rays) and the smaller, less understood, Holocephali (chimaeras, ratfishes, and rabbitfishes). Chondrichthyan fishes have a long evolutionary history that goes back over 400 million years. Part of their evolutionary success is attributed to their diverse reproductive modes and adaptations related to reproductive physiology. For example, all chondrichthyan fishes have internal fertilization, where the male mates with the female and uses one of his paired claspers (intromittent copulatory organs) to transfer sperm to her reproductive tract for egg fertilization. This internal fertilization, coupled with efficient maternal nourishment of embryos and the birth of large, well-developed offspring, contributes to the enormous success of these fishes. Internal fertilization and the many complex reproductive modes have led to the notion that cartilaginous fishes are reproductively more similar to reptiles, birds, and mammals than to other fishes. This great diversity in reproductive adaptations also requires a complex hormonal system that regulates all aspects of reproduction from sexual maturity to mating behavior and seasonal changes in gonadal physiology (Figure 11.1).

1.1. Reproductive Modes

Chondrichthyan fishes can be separated into two main groups, based on reproductive mode: oviparous (egg-laying) and viviparous (live-bearing), the latter of which can be further divided into placental and aplacental forms. Oviparous species retain their eggs for varying amounts of time, enclose them in a protective egg case, and then deposit them on some substrate for development. Oviparity is the reproductive mode found in all of the Holocephali and the skates (Elasmobranchii; Rajiformes), and occurs in several families of sharks (e.g., Orectolobidae, Scyliorhinidae, Heterodontidae) (Wourms, Grove, & Lombardi, 1988). Oviparity is also thought to be the ancestral condition in chondrichthyan fishes, from which viviparity arose independently ~9–10 times (Wourms, 1977; Dulvy & Reynolds, 1997). However, a more recent analysis argues that viviparity is the ancestral state in chondrichthyans, and that oviparity then evolved to increase fecundity in small-bodied species that have limited coelomic space for numerous live offspring (Musick & Ellis, 2005).

Viviparity is characteristic of all rays and occurs in about 70% of all shark species. Viviparous species retain their embryos in the uterus until they are fully developed and then give birth to live young that are miniature versions of the adults. Viviparity can be divided into placental and aplacental varieties, depending on whether a placental connection exists between the mother and embryo. In placental species, the embryo is initially nourished by

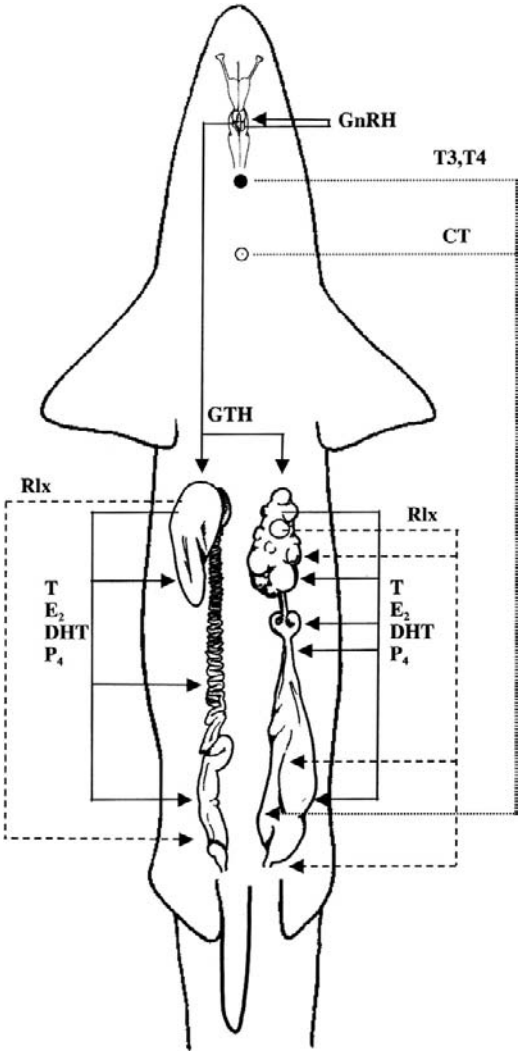

FIGURE 11.1 Summary of the proposed mechanism for hormonal regulation of chondrichthyan reproduction. Male reproductive tract is on the left, and female on the right. Open circle, ultimobranchial gland; closed circle, thyroid gland. CT, calcitonin; DHT, dihydrotestosterone; E_2, 17β-estradiol; GnRH, gonadotropin-releasing hormone; GTH, gonadotropins; P_4, progesterone; Rlx, relaxin; T, testosterone; T_3, triiodothyronine; T_4, thyroxine. See text for additional details. *Reproduced from Gelsleichter (2004), with permission.*

a yolk sac, which, when depleted, elongates and forms a highly vascularized connection with the uterine wall of the mother to form a yolk sac placenta. Nutrients are then delivered directly from the bloodstream of the mother to the developing embryo. Placental elasmobranchs generally have long gestation periods, relatively low numbers of offspring (a few to over a hundred depending on the species), increased maternal protection during embryonic development, and greater survival at birth due to the large size of fully developed young. At birth, the umbilical cord (former yolk stalk) breaks off and leaves a small scar on the body between the pectoral fins of the newborn offspring. The status of this umbilical scar (from fresh/open to fully healed) is often used by researchers to determine the relative age of newborn pups as well as the seasonal timing of parturition (birth).

Aplacental viviparity can be separated into three functional groups based on how the embryo receives its nutrition: (1) yolk dependency: embryos depend solely on the yolk deposited in the egg at ovulation, with no supplemental nourishment from the mother. This type is found in Squaliformes, Hexanchiformes, Squatiniformes, and some Carcharhiniformes, Orectolobiformes, and rays. (2) Intrauterine cannibalism: the most common of these forms, oophagy (egg-eating), occurs when embryos develop initially on yolk reserves and then begin to ingest a supply of unfertilized eggs in the uterus with precocial teeth. This type is found primarily in the lamnoid sharks (e.g., mako, thresher, great white) (Gilmore, Putz, & Dodrill, 2005), but may also exist in Carcharhinidae and Orectolobidae families. In the sandtiger shark *Carcharius taurus*, a more extreme form called embryophagy occurs when the largest and strongest embryo(s) develops quickly and then consumes the other developing young within the uterus. (3) Placental analogs: embryos are nourished by regions of the uterine epithelium called trophonemata that secrete a 'uterine milk' or 'histotroph,' a process known as uterolactation. This type is prevalent in the majority of rays (Myliobatiformes). For a more detailed description of the evolution and diversity of reproductive modes found in chondrichthyan fishes, the reader is directed to the following references: Wourms (1977); Wourms et al. (1988); Wourms and Demski (1993); Carrier, Pratt, and Castro (2004); Gilmore et al. (2005); Hamlett, Kormanik, Storrie, Stevens, and Walker (2005); Musick and Ellis (2005).

1.2. Reproductive Cycles

Annual reproductive cycles in chondrichthyan fishes are complex and diverse, but most species can be grouped into one of three different types based on the ovarian cycle and gestation period in females (Hamlett & Koob, 1999; Koob & Callard, 1999). Continuous breeders (many viviparous sharks, some batoids) are reproductively active year-round with pregnancy lasting almost a full year, and mating, pregnancy, and parturition are generally coupled with environmental factors and synchronized within the population. Seasonal breeders (most ray species, some viviparous sharks) are reproductively active for only a portion of the annual cycle, with pregnancy lasting several months and the remainder of the year spent nonpregnant. Similar to continuous breeders, mating, pregnancy, and parturition are correlated with environmental cues and occur at approximately the same time each year to synchronize the population. Punctuated breeders (some viviparous sharks and

rays, some oviparous species) are often pregnant for about a full year, but spend one or more intervening years in a nonpregnant state. For example, in blue (*Prionace glauca*) and sandbar (*Carcharhinus plumbeus*) sharks, females are pregnant for a full year but only deliver young every two years, spending the intervening year nonpregnant. There are also cases of 2−3.5 year pregnancies in some elasmobranchs (e.g., *Squalus acanthias*, *Chlamydoselachus anguineus*) and new studies continue to describe diverse reproductive cycles in chondrichthyans.

1.3. Mating and Reproductive Behaviors

Direct observations of courtship and mating behaviors in chondrichthyans are rare, especially in the wild, but the available descriptions reveal a complex suite of reproductive behaviors in this group of fishes including dominance hierarchies, pair and group mating behaviors, sexual segregation, and cooperative breeding (Pratt & Carrier, 2001; Carrier et al., 2004; Pratt & Carrier, 2005). Precopulatory behaviors (e.g., following, parallel swimming, biting, female avoidance and acceptance, clasper flexion) may be brief or prolonged, but ultimately culminate in the male grasping the female on the fin or body so that clasper insertion and internal fertilization can occur. This oral grasping often leaves mating scars on the female, which can be used as an indicator of active courtship and/or mating season (Kajiura, Sebastian, & Tricas, 2000). Once the female accepts (often becoming immobile and flaring or cupping her pelvic fins) and the male has an adequate grip, a single clasper is rotated forward, inserted, and often anchored in the female by opening of the terminal cartilages that bear a hook or spur in some species, and semen is then transferred into the female's reproductive tract. Copulation can be brief (seconds to minutes), or may last for several hours, as observed in some benthic skate species maintained in captive settings (Luer & Gilbert, 1985). Females of several elasmobranch species can also store sperm (for weeks to months) in the reproductive tract for later fertilization (Pratt, 1993; Hamlett, Knight, Pereira, Steele, & Sever, 2005). Females may mate with several males, and multiple paternity has been documented in several elasmobranch species, possibly to maintain genetic diversity in animals that produce only a few broods during their lifetime (Chapman, Corcoran, Harvey, Malan, & Shivji, 2003; Carrier et al., 2004; Feldheim, Gruber, & Ashley, 2004; Heist, 2004; Pratt & Carrier, 2005; Daly-Engel, Grubbs, Holland, Toonen, & Bowen, 2006; Chevolot, Ellis, Rijnsdorp, Stam, & Olsen, 2007; Portnoy, Piercy, Musick, Burgess, & Graves, 2007). However, high rates of single male paternity also occur in certain species, such as the bonnethead shark, suggesting that pre- and/or postcopulatory factors that influence the mating systems of these animals may be diverse (Chapman, Prodohl, Gelsleichter, Manire, & Shivji, 2004). In contrast to most other vertebrate taxa, parental care has not yet been described in chondrichthyan fishes.

2. THE HYPOTHALAMIC−PITUITARY−GONADAL (HPG) AXIS

As in all vertebrates, reproduction in chondrichthyan fishes is regulated by the hypothalamic−pituitary−gonadal (HPG) axis. External cues from the environment such as photoperiod and temperature, or internal physiological cues associated with sexual maturation (or puberty), initiate the endocrine cascade that begins with activation of the gonadotropin-releasing hormone (GnRH) neurons in the brain. Gonadotropin-releasing hormone then stimulates production and release of the gonadotropin (GTH) hormones from the pituitary into the bloodstream, where they travel to the gonads (ovary and testis) to promote gametogenesis and steroidogenesis (Figure 11.1). Gonadal steroids are also responsible for further regulating gamete production (via autocrine and paracrine mechanisms in the gonads and feedback mechanisms to the brain and pituitary), and, presumably, secondary sex characteristics, sensory function, and reproductive behavior. While the basic structure and function of the HPG axis is conserved among vertebrates, there are several distinct differences found in the chondrichthyan fishes compared to both higher and lower taxa that are discussed below.

2.1. Gonadotropin-releasing Hormone (GnRH)

Gonadotropin-releasing hormone is represented by a family of decapeptides produced in the brain of all vertebrates and is a critical regulator of reproduction (see Chapter 2, this volume). In chondrichthyan fishes, distinct populations of GnRH neurons are found in the basal forebrain or preoptic area (POA) (GnRH1), the terminal nerve (TN) ganglia, and the midbrain tegmentum (GnRH2) (Figure 11.2) (Sherwood & Lovejoy, 1993; Demski, Beaver, Sudberry, & Custis, 1997). Each of these regions expresses a GnRH variant with a slightly different amino acid sequence. In all chondrichthyan fishes studied to date, multiple forms (i.e., as many as seven) of GnRH are found in the brain of the same species, and in some species multiple forms are localized to the same brain region (Powell, Millar, & King, 1986; Lovejoy et al., 1991; 1992; D'Antonio et al., 1995; Demski et al., 1997; Forlano, Maruska, Sower, King, & Tricas, 2000; Masini, Prato, Vacchi, & Uva, 2008). Despite their important taxonomic position and diversity of reproductive modes, there are no published molecular studies on the sequence or gene structure of chondrichthyan GnRH peptides or GnRH receptors. As a result, recent analyses on

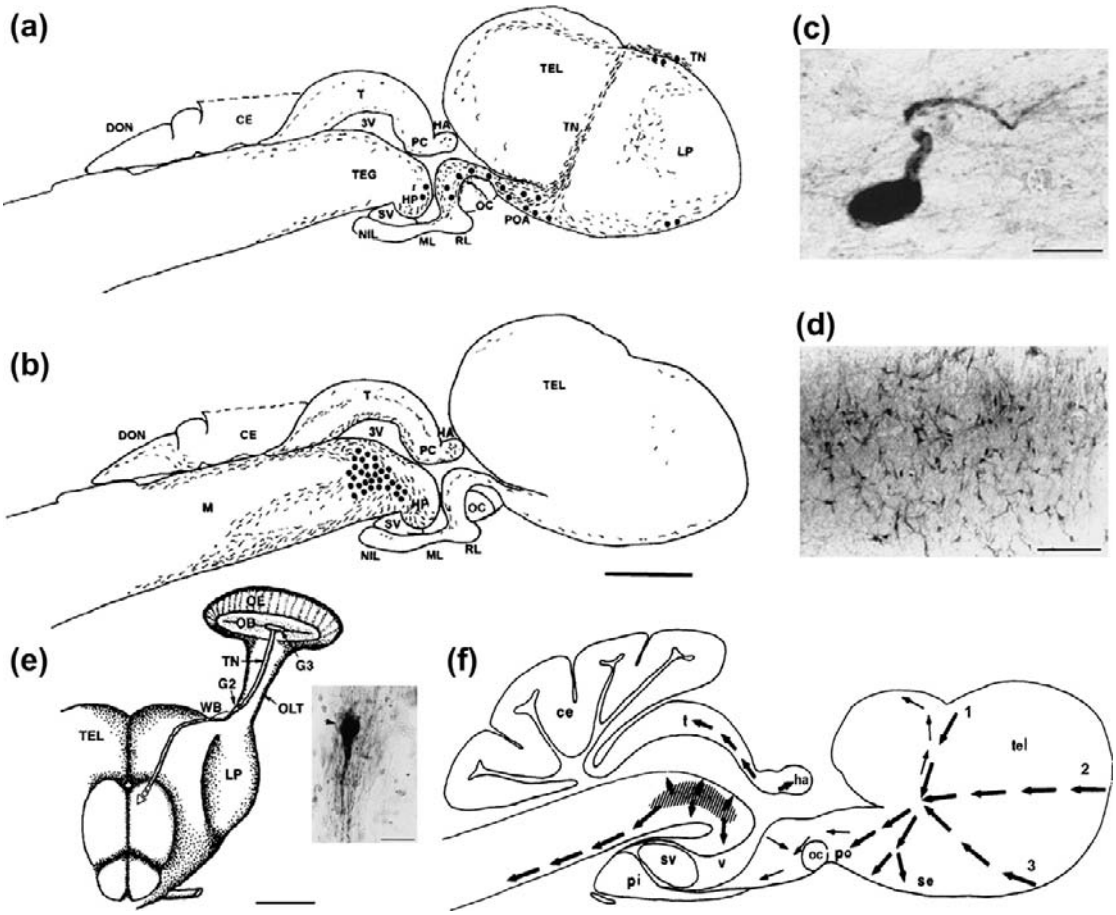

FIGURE 11.2 Distribution of gonadotropin-releasing hormone (GnRH)-immunoreactive (ir) somata and fibers in the elasmobranch brain. Diagrammatic parasagittal representations show the distribution of forebrain (a) and midbrain (b) GnRH-ir somata (dots) and fibers (lines) in the brain of the Atlantic stingray (*Dasyatis sabina*). (c) Representative monopolar GnRH-ir cell in the preoptic area of *D. sabina*. (d) Representative sagittal section showing the large GnRH-ir cell group in the midbrain of *D. sabina*. (e) Schematic drawing of the terminal nerve (TN) and ganglia on the dorsal forebrain of *D. sabina*. Inset shows a monopolar GnRH-ir neuron (arrow) from the white body (WB) of the TN. (f) Summary schematic diagram illustrates the GnRH-ir fiber pathways (arrows) in the elasmobranch brain. Hatched area represents the midbrain GnRH-ir cell group. Numbers 1−3 represent the positions of terminal nerve entry in different species: (1) *Urobatis* (*Urolophus) halleri* and *Platyrhinoidis triseriata*; (2) *Squalus acanthias* and *Triakis semifasciata*; and (3) *Triakis semifasciata*. 3V, third ventricle; CE, corpus cerebellum; DON, dorsal octaval nucleus; G2, ganglion 2 of TN; G3, ganglion 3 of TN; HA, habenula; HP, hypothalamus; LP, lateral pallium; M, medulla; ML, median lobe of pituitary; NIL, neurointermediate lobe of pituitary; OB, olfactory bulb; OC, optic chiasm; OE, olfactory epithelium; OLT, olfactory tract; PC, posterior commissure; pi, pituitary; POA, preoptic area; RL, rostral lobe of pituitary; se, septal area; SV, saccus vasculosus; T, tectum; TEG, tegmentum; TEL, telencephalon; v, ventricle. Scale bars = 0.5 cm (a, b); 20 µm (c, e inset); 200 µm (d); 1 cm (e). (a−e) *Modified from Forlano et al. (2000), with permission*. (f) *Modified from Wright and Demski (1993), with permission*.

the molecular evolution of GnRH and its receptor have not included any chondrichthyan representatives (Guilgur, Moncaut, Canario, & Somoza, 2006; Flanagan et al., 2007; Chen & Fernald, 2008; Kavanaugh, Nozaki, & Sower, 2008; Okubo & Nagahama, 2008). Until the localization of distinct GnRH molecular variants within the TN ganglia, POA, hypothalamus, and midbrain tegmentum are determined in chondrichthyans, it will be difficult to fully appreciate the function and evolutionary relationships of these different cell groups.

Gonadotropin-releasing hormone neurons found in the forebrain region (e.g., ventral telencephalon, POA, hypothalamus) of vertebrates generally project to the pituitary and are responsible for GTH production and release (i.e., the releasing form; GnRH1). Gonadotropin-releasing hormone neurons in the POA of chondrichthyan fishes do project to the rostral, median, and neurointermediate lobes of the pituitary (Demski et al., 1997; Forlano et al., 2000), but do not reach the isolated ventral lobe that contains the most GTH activity (see Section 2.2). In male Atlantic stingrays (*Dasyatis sabina*), the number of forebrain GnRH neurons varies with the seasonal reproductive cycle, consistent with a role in GTH control and gonadal recrudescence (Forlano et al., 2000). However,

chondrichthyans differ from most vertebrates in that GnRH is hypothesized to reach the ventral lobe via the general circulation, and there is some evidence for GnRH release to the bloodstream and cerebrospinal fluid from all three GnRH cell groups (Demski et al., 1997). Consequently, it remains unclear whether reproductive competence is regulated by a single (i.e., GnRH1) or multiple GnRH forms released by one or possibly several GnRH cell groups.

The TN is a ganglionated cranial nerve that is distinct and separate from the olfactory nerve in chondrichthyans, and is another major source of GnRH in these fishes (Demski, Fields, Bullock, Schriebman, & Margolis-Nunno, 1987; Demski, 1993; White & Meredith, 1995; Demski et al., 1997; Forlano et al., 2000). There is some evidence that GnRH from the TN functions both in reproductive competence as a releasing factor (i.e., gonadal recrudescence and steroidogenesis) and in the modulation of sensory-mediated reproductive behaviors. Gonadotropin-releasing hormone-immunoreactive (ir) fibers are closely associated with both cerebral vasculature and ventricles (Demski & Fields, 1988; Demski et al., 1997), which provides a potential route for TN control of reproduction via GnRH release to the systemic circulation. Further, electrical stimulation of the TN in the Atlantic stingray results in increased GnRH levels in the cerebrospinal fluid, which provides support for GnRH access to other brain regions via an intraventricular route (Moeller & Meredith, 1998). Direct projections from the TN GnRH neurons to both the retina (Demski et al., 1997) and olfactory regions (e.g., olfactory bulb, and region between the olfactory bulb and olfactory epithelium) (Demski et al., 1997; Forlano et al., 2000) also suggest a role in the integration of visual and olfactory (i.e., pheromonal) cues with seasonal reproductive behaviors (Demski & Northcutt, 1983; Demski, 1991). As stated above, however, without an understanding of which specific GnRH variant(s) is found in the TN and whether this same form is present in the blood and CSF and has physiological effects on the gonads, the function of TN GnRH in chondrichthyans remains unclear.

The GnRH neurons in the elasmobranch midbrain are an order of magnitude more numerous than those found in bony fishes (hundreds vs. tens), and have been shown to contain the evolutionarily conserved GnRH2 variant by both immunocytochemistry and radioimmunoassay (Figure 11.2) (Forlano et al., 2000). Gonadotropin-releasing hormone-ir axons from this midbrain group project to regions of visual, electrosensory, and mechanosensory processing in both the midbrain and hindbrain, as well as to motor neurons in the spinal cord that may regulate clasper movements (Forlano et al., 2000). Thus, it is hypothesized that midbrain GnRH influences reproductive success by modulating the sensitivity of the visual, electrosensory, somatosensory, and lateral line systems for mate detection and courtship behaviors, as well as regulating motor aspects of copulation including clasper movements. However, this neuromodulatory role for GnRH2 requires experimental confirmation.

2.2. Pituitary Structure and Gonadotropins (GTHs)

As previously mentioned, the pituitary gland in elasmobranchs differs from that of bony fishes and higher vertebrate taxa because there is no direct neural or portal blood connection to deliver GnRH from the brain to the ventral lobe of the pituitary where the GTHs are produced (Figure 11.3) (J. Dodd, M. Dodd, & Duggan, 1983). Holocephalans, on the other hand, lack a ventral lobe and do have neural and vascular connections to the pituitary

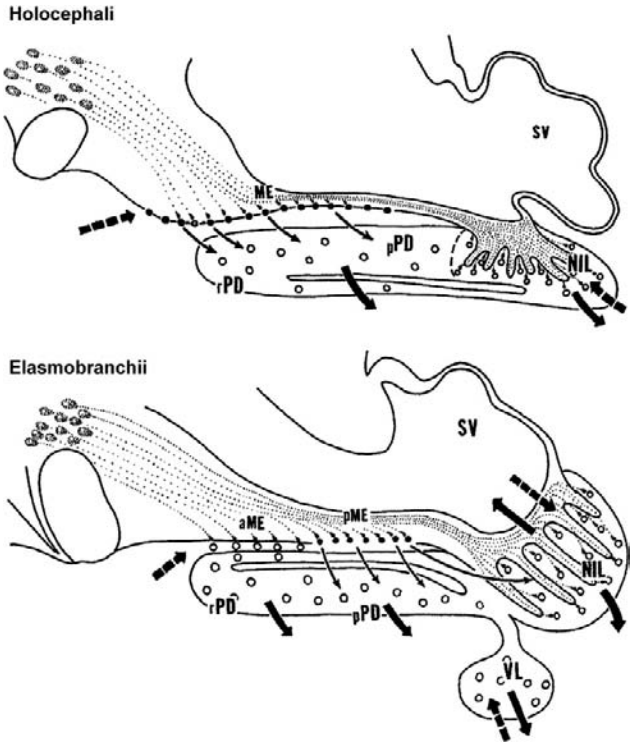

FIGURE 11.3 Diagrammatic sagittal section of the hypothalamic–pituitary gland vascular and neurosecretory connections in Holocephali and Elasmobranchii. In the species examined to date, direct vascular or neurosecretory connections to the ventral pituitary lobe appear to be absent in elasmobranchs. Holocephalans lack the ventral lobe entirely, but possess a buccal lobe (or rachendachhypophyse) (not shown) that is separated from the rest of the pituitary in mature adults. Dotted circles, neurosecretory cells of the nucleus preopticus; dotted lines, neurosecretory axons with axon terminals marked at their endings; filled circles, prehypophyseal plexus; open circles, intrahypophyseal capillaries; large interrupted arrows, arteries; large solid arrows, veins; thin arrows, portal veins. Rostral is to the left. rPD, rostral zone of pars distalis; pPD, proximal zone of pars distalis; NIL, neurointermediate lobe; aME, anterior median eminence; pME, posterior median eminence; SV, saccus vasculosus; VL, ventral lobe. *Modified from Jasinski (1969), with permission.*

(Jasinski, 1969) (Figure 11.3). However, holocephalans also possess a buccal pituitary lobe, or 'rachendachhypophyse,' that in mature adults is completely separated from the rest of the pituitary but contains strong GTH activity (J. Dodd & M. Dodd, 1985). A capillary system does exist in elasmobranchs to join the hypothalamus with the rostral, median, and neurointermediate lobes of the pituitary, and in some species these lobes are also innervated by GnRH axons (Demski et al., 1997; Forlano et al., 2000), but a direct connection to the anatomically separate ventral lobe (elasmobranchs) or buccal lobe (holocephalans) has not been demonstrated (Dodd, 1975; Dodd et al., 1983; J. Dodd & M. Dodd, 1985). Instead, GnRH is hypothesized to reach the elasmobranch ventral lobe via an intraventricular route or the general circulation (King et al., 1992; Sherwood & Lovejoy, 1993; Demski et al., 1997; Moeller & Meredith, 1998). This is supported by the detection of both GnRH and GnRH-binding proteins in the cerebrospinal fluid and peripheral blood of many elasmobranchs (Powell et al., 1986; King et al., 1992; Pierantoni, D'Antonio, & Fasano, 1993; Sherwood & Lovejoy, 1993; D'Antonio et al., 1995; Moeller & Meredith, 1998). The ventral lobe is vascularized from the systemic circulation by the internal carotid artery, which forms a vascular bed (Dodd et al., 1983; Honma, Toda, & Chiba, 1987). This GnRH presence in the bloodstream at levels equivalent to the mammalian hypothalamo−hypophysial portal system (Sherwood & Lovejoy, 1993), the fact that exogenous administration of GnRH analogs also influences gonadal steroidogenesis (Jenkins & Dodd, 1980; Fasano et al., 1989; Callard, Fileti, & Koob, 1993), and the result that ventral lobectomy only partially impairs gametogenesis and steroidogenesis (Dobson & Dodd, 1977a; 1977b; Sumpter, Jenkins, & Dodd, 1978b) also raise the possibility of direct actions of GnRH on the gonads in elasmobranchs. Gonadotropin-releasing hormone receptors have been detected in the gonads of bony fishes and other taxa, and therefore this putative direct hypothalamic−gonadal connection that circumvents the pituitary gland certainly deserves future attention and experimentation.

The presence of GTHs in the ventral lobe of the elasmobranch pituitary has been demonstrated by immunocytochemistry (Mellinger & DuBois, 1973) and radioimmunoassay (Scanes, Dobson, Follett, & Dodd, 1972), and several studies show that extracts of the ventral lobe can stimulate steroidogenesis in testicular cell culture (Lance & Callard, 1978; Sumpter, Jenkins, Duggan, & Dodd, 1980; Sourdaine, Garnier, & Jegou, 1990) as well as *in vivo* in both male and female elasmobranchs (Sumpter et al., 1978b ; Callard & Pasmanik, 1987). Extracts from the ventral lobe showed 10−100 times more GTH activity compared to intermediate and median pituitary lobe extracts, as measured by testosterone (T) production from dispersed reptilian testicular cells (Lance & Callard, 1978). Further, hypophysectomy, or removal of the ventral lobe, alone causes partial regression of the testes and reduced androgen concentrations in some male elasmobranchs (Dodd, Evennett, & Goddard, 1960; Dobson & Dodd, 1977a; Sumpter, Follett, Jenkins, & Dodd, 1978; Fasano et al., 1989) and follicular atresia and impaired oviposition in female *Scyliorhinus canicula* (Norris, 1997). However, these removal experiments did not completely suppress gametogenesis or steroidogenesis and the responses may depend on reproductive stage and/or environmental stimuli (Dobson & Dodd, 1977c), a finding consistent with seasonal variation in pituitary GTHs. Further, it is important to note that the relative role of the ventral lobe in GTH release, and the regulation of gonadal steroidogenesis either via direct GnRH action or the traditional HPG axis, may be very different among diverse chondrichthyan species, especially those with different reproductive modes. For example, the majority of work on ventral lobe function is based on the dogfish *S. canicula* (Dodd, 1975; Dobson & Dodd, 1977a). However, in skates such as *Leucoraja* (*Raja*) *erinacea*, the ventral lobe is less developed and, although GnRH directly stimulates steroid synthesis in follicular cells of females *in vitro*, extracts of the ventral pituitary lobe do not (Callard et al., 1993; Demski et al., 1997). These differences in pituitary structure and function among the limited species examined thus far highlight that future comparative work on pituitary morphology, GTH localization and function, and the role of direct GnRH action in the gonads among sharks, skates, rays, and chimaeras is clearly needed.

The GTHs, follicle stimulating hormone (FSH) and luteinizing hormone (LH), are both heterodimeric glycoproteins with identical α-subunits and distinct β-subunits. In most vertebrates, FSH targets the Sertoli cells of the testis and the follicular and granulosa cells of the ovary to stimulate gametogenesis. On the other hand, LH targets the interstitial Leydig cells of the testes and the granulosa and thecal cells of the ovaries and primarily functions to regulate steroidogenesis and final gamete maturation and release (spermiation and ovulation). One α-subunit and two distinct β-subunits have been cloned and sequenced from the ventral pituitary lobe of the dogfish *S. canicula* and the latter subunits are orthologs of FSH and LH found in bony fishes and tetrapods (Querat, Tonnerre-Doncarli, Genies, & Salmon, 2001). Thus, it seems plausible that the duality of the GTHs occurred prior to the Chondrichthyes. Experimental studies in male dogfish also indicate that GTHs are important for the transition from spermatogonia to spermatocytes, and that GTH levels fluctuate seasonally with the breeding cycle (Dobson & Dodd, 1977a; 1977b; 1977c). However, the differential distribution of FSH and LH receptors in the chondrichthyan gonad is unknown and evidence of discrete functions on steroidogenesis and gametogenesis is lacking.

3. HORMONAL REGULATION IN FEMALES

3.1. Structure and Function of the Female Reproductive Tract

The general structure and morphology of the chondrichthyan ovary and other components of the reproductive tract have been reviewed in detail elsewhere (Hamlett & Koob, 1999; Carrier et al., 2004; Callard, St. George, & Koob, 2005; Hamlett et al., 2005a; Lutton, St. George, Murrin, Fileti, & Callard, 2005) and will only be briefly summarized here. The female chondrichthyan reproductive system consists of paired or single ovaries and the oviducts. Each oviduct is differentiated into an ostium, anterior oviduct, oviducal gland (also known as the shell or nidamental gland), isthmus, uterus, cervix, and the common urogenital sinus. In elasmobranchs, at least two different ovarian types are recognized based on the relationship between the ovary and the epigonal organ, a lymphomyeloid organ associated with the gonad in both sexes (Figure 11.4) (Pratt, 1988). 'Internal' ovaries, found in lamnoid sharks, have a germinal epithelium encapsulated within the epigonal organ and produce numerous small ova. In contrast, 'external' ovaries lie on the distal surface of the epigonal organ, produce fewer but larger eggs, and are found in many different elasmobranch species. Elasmobranch ova are large relative to bony fishes, and yolk accumulation is a slow process taking several months to a year in most species.

Eggs released from an ovary are collected by the ostium (single or paired) and travel through the anterior oviduct to the oviducal gland, where they become fertilized, are surrounded by egg jelly, and are encapsulated by either rigid (in oviparous species) or more pliable (in viviparous species) egg capsules. These oviducal glands are unique to chondrichthyan fishes and all consist of morphologically distinct zones (i.e., the club, papillary, baffle, and terminal zones), which appear to perform different functions in the overall process of egg fertilization and encapsulation (Hamlett et al., 2005a). Oviducal glands vary tremendously among species, but their size and structural complexity correlates with reproductive mode, being larger and more complex in oviparous species that produce hard or leathery egg cases designed for external embryonic development and protection. In addition to egg jelly and tertiary egg envelope formation, oviducal glands in some species also function to store sperm within the female tract for later fertilization (Hamlett et al., 2005a).

Following fertilization and encapsulation, eggs are transported from the oviducal gland to the uterus via a morphologically distinct component of the reproductive tract known as the isthmus. This region undergoes structural modifications associated with the reproductive cycle that first allow it to permit transit of fragile ova into the uterus, and later reduce its compliance to make the uterus an isolated compartment where modification of egg

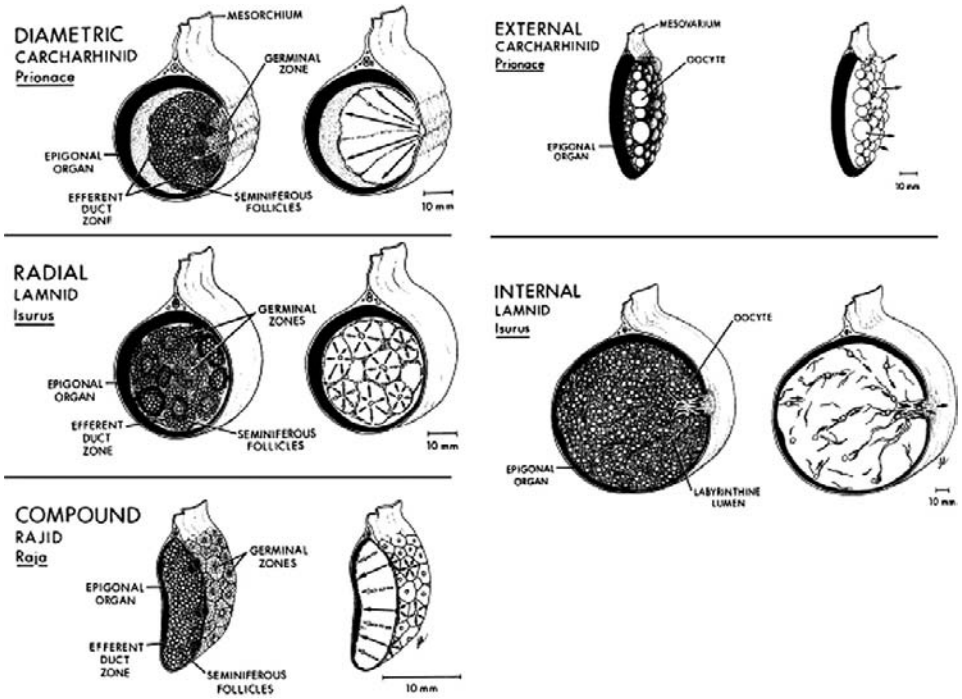

FIGURE 11.4 Schematic cross-section representations of the different forms of elasmobranch testes (left panel: diametric, radial, compound) and ovaries (right panel: 'external,' 'internal'). Left illustrations in each panel are diagrammatic with size of follicles (spermatocysts) exaggerated to show development. Arrows on right illustrations in each panel indicate paths of follicle or spermatocyst development (testis), and oocyte travel and release (ovary). *Modified from Pratt (1988), with permission.*

capsules or embryonic development can take place. In oviparous species, the uterus holds the egg capsule during polymerization of egg capsule materials and tanning; a process collectively known as sclerotization. In viviparous species, the uterus houses the developing embryos until parturition occurs; a period during which it functions to regulate conditions in the intrauterine environment, supply oxygen for respiratory demands, and provide nutrients and waste disposal for growing young (Hamlett & Koob, 1999). This can involve a number of diverse morphological modifications to the uterine structure, such as significant enlargements in size; compartmentalization of the uterus to separate individual embryos; extensive folding and vascularization of the uterine epithelium to oxygenate uterine fluids or, in some species, produce various forms of matrotrophic nourishment (e.g., histotroph); and/or in placental viviparous species form often elaborate placental connections between pregnant females and offspring. Even in species in which embryos derive no additional nourishment beyond that supplied in yolk reserves, proper uterine function is critical to the wellbeing and survival of developing young. An often-cited example of such actions occurs in the aplacental viviparous shark *S. acanthias*, in which contractions of the myometrium serve to periodically flush seawater into the uterus, presumably to maintain electrolyte balance and remove embryonic wastes (Kormanik, 1993).

During oviposition or parturition in oviparous and viviparous species, respectively, eggs or full-term young are released to the external environment through increased compliance of the previously rigid and inextensible cervix. In placental species, distinct scarring of the uterus occurs as a result of uterine compartmentalization and placentation, which then undergoes repair in preparation for the subsequent breeding season (Hamlett, Musick, Hysell, & Sever, 2002).

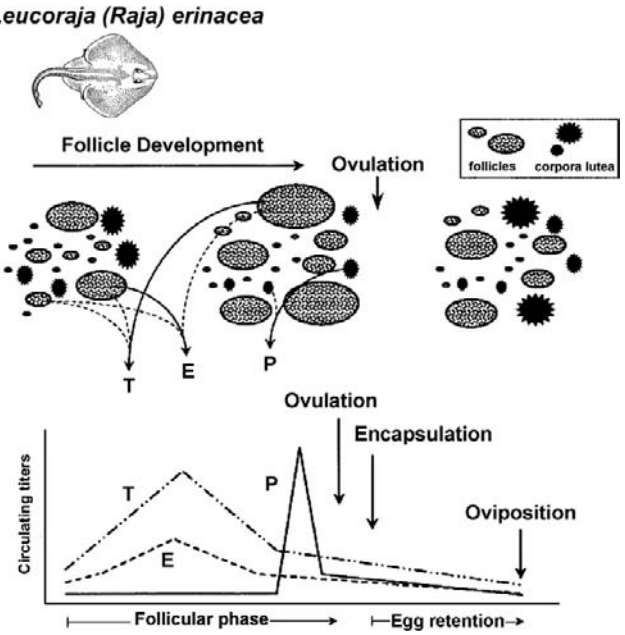

FIGURE 11.5 Tissue source and profiles of circulating 17β-estradiol (E_2), testosterone (T), and progesterone (P_4) titers during the ovulatory cycle of the female oviparous skate *Leucoraja (Raja) erinacea*. The principal source of each steroid is indicated by the solid lines in the upper panel, while additional sources are indicated by dashed lines. 17β-estradiol and T titers are elevated during the follicular phase of each ovulatory cycle, whereas P_4 is elevated for only a brief period during the preovulatory phase and is low during ovulation, egg encapsulation, and oviposition. *Modified from Koob and Callard (1999), with permission.*

3.2. Steroidogenesis, Steroidogenic Enzymes, and Steroid Receptors

As in other vertebrate groups, sex steroids including 17β-estradiol (E_2), T, and progesterone (P_4) are produced by various cell types (i.e., granulosa cells, thecal cells, and corpora luteal tissue) in the ovary of female elasmobranchs (Figure 11.5). As previously discussed, this is likely regulated by pituitary GTHs; a premise supported by the reduction in steroidogenesis observed in certain elasmobranch species (i.e., the skate *L. erinacea*) following ventral lobectomy. Additionally, as illustrated by the stimulatory effects of GnRH on *in-vitro* and *in-vivo* gonadal steroidogenesis in *L. erinacea* and *S. canicula*, respectively, it is possible that the hypothalamus also may play a role in regulating ovarian steroid production in this group (Jenkins & Dodd, 1980; Lutton et al., 2005). Lastly, as implied by its intimate association with the gonads in both sexes, the epigonal organ also appears to contribute to the regulation of gonadal steroidogenesis in elasmobranchs (Lutton & Callard, 2007; 2008a; 2008b). For example, recent studies have demonstrated that epigonal cells and conditioned media can inhibit *in-vitro* production of E_2 and T in the skate ovary (Lutton et al., 2005), and, since blood flow from the gonadal artery first enters the epigonal organ and then perfuses the ovarian follicles, epigonal organ-secreted factors could directly influence follicular steroid production (Lutton & Callard, 2008b). Further, circulating gonadal steroids also may produce either stimulatory or inhibitory changes in leukocyte proliferation within epigonal tissue depending on physiological state and/or environmental cues (Lutton & Callard, 2008a), suggesting a functional interaction between reproductive and immune systems.

As demonstrated for both the skate *L. erinacea* and the shark *S. acanthias*, E_2 and T primarily are produced by both granulosa cells and theca cells of ovarian follicles (Figure 11.5) (Tsang & Callard, 1987b; Fileti & Callard, 1990; Tsang & Callard, 1992; Callard et al., 1993). However, the contribution of granulosa cells to E_2 synthesis

appears to be much greater than that of theca cells in at least some elasmobranchs. This is supported by *in-vitro* studies on steroidogenesis in granulosa cell and theca cell isolates from the skate ovary, which have demonstrated far greater increases in E_2 production in the former in response to hormonal stimulation by GnRH (Lutton et al., 2005). In comparison to that for E_2, T production by these two cell types is believed to be more equivalent. Although the follicles are generally considered to be the primary source of T in females, *in-vitro* studies on the skate ovary have demonstrated that the corpus luteum is also capable of T synthesis and may also contribute to circulating levels of this hormone (Callard et al., 1993).

In contrast to E_2 and T, the primary source of circulating P_4 in female elasmobranchs is corpora luteal tissue, which forms primarily from granulosa cells just prior to or after ovulation (Figure 11.5). The presence of steroidogenic enzymes involved in P_4 synthesis has been detected in luteal tissue of *S. acanthias* and substantial quantities of P_4 are produced by luteal minces from both the shark and skate ovary *in vitro* (Tsang & Callard, 1987a; Fileti & Callard, 1988; Callard et al., 1992). As observed in these studies, production of P_4 by the corpus luteum increases with its development, but declines with its age (Tsang & Callard, 1987a; Fileti & Callard, 1988). Granulosa cells from mid- to large-sized ovarian follicles of the skate *L. erinacea* also are capable of synthesizing P_4, but at lower levels than E_2 or T, and to a far lesser degree than luteal tissue (Lutton et al., 2005).

Very few studies have examined steroid-binding activity and/or the occurrence of steroid receptors in female elasmobranchs. None-the-less, limited data on this topic suggest that both estrogen receptors (ERs) and progesterone receptors (PRs) are present in most of the major organs that contribute to reproduction, such as the liver and various components of the reproductive tract (Reese & Callard, 1991; Paolucci & Callard, 1998; Koob & Callard, 1999). To the best of the authors' knowledge, no published studies to date have explored the distribution of androgen receptors (ARs) in female sharks and rays. A more detailed description of putative target organs for gonadal steroids in female elasmobranchs is provided in the following section, along with possible roles for these hormones in reproduction.

3.3. Gonadal Steroid Cycling and Functions

3.3.1. 17β-estradiol (E_2)

Circulating E_2 concentrations generally peak during the period of follicular development in both oviparous and viviparous elasmobranchs (Figure 11.6), suggesting a role for this hormone in regulating synthesis of the yolk protein precursor, vitellogenin (Vtg). The regulation of Vtg production by E_2 is well-conserved across nonmammalian vertebrate taxa, and generally occurs in the liver via interactions of E_2 with hepatic ERs (Polzonetti-Magni, Mosconi, Soverchia, Kikuyama, & Carnevali, 2004). Following its synthesis, Vtg is transported via the general

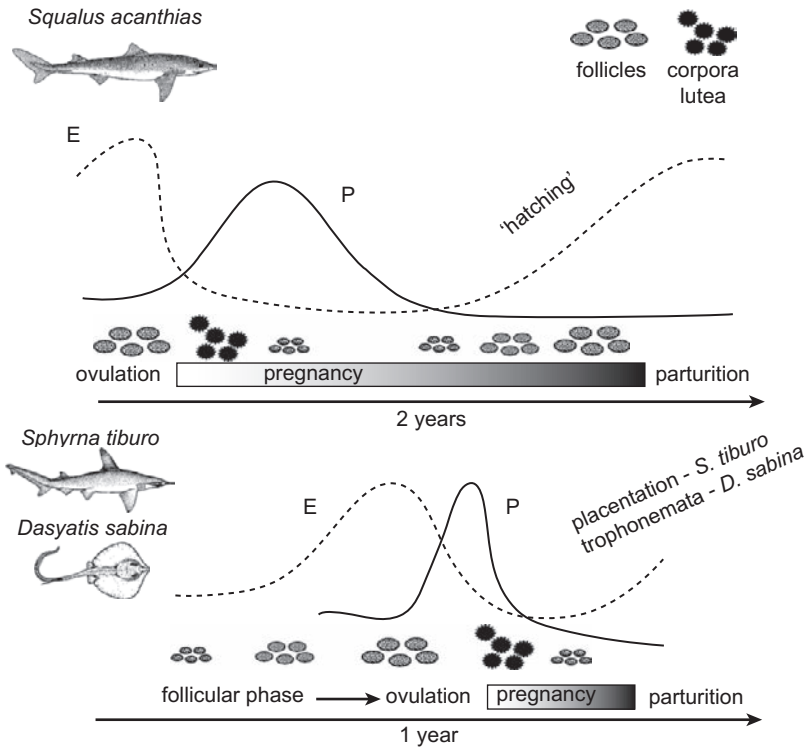

FIGURE 11.6 Patterns of circulating 17β-estradiol (E_2) and progesterone (P_4) levels during the reproductive cycles of females of three different viviparous elasmobranch species. In *Squalus acanthias*, P_4 levels are high during the first half of pregnancy and then decline to low levels for the second half of pregnancy, when E_2 levels are high. In both *Sphyrna tiburo* and *Dasyatis sabina*, P_4 levels rise during the periovulatory period and remain elevated during early pregnancy, but then decline and remain relatively low for the remainder of pregnancy. 17β-estradiol levels rise during late pregnancy in all three species, and, in the placental *S. tiburo* and aplacental *D. sabina*, this correlates with a shift in embryonic nutrient supply, indicating that E_2 may play an important role in regulating nutrients to developing young. *Modified from Koob and Callard (1999), with permission.*

circulation to the ovary, where it is taken up by growing oocytes through the process of receptor-mediated endocytosis. A role for E_2 in regulating hepatic Vtg production in sharks and their relatives is supported by evidence for hepatic ERs in the skate *L. erinacea* (Koob & Callard, 1999), and experimental studies that have demonstrated induction of Vtg production in elasmobranchs in response to E_2 treatment (Figure 11.7) (Craik, 1978; Callard, Etheridge, Giannoukos, Lamb, & Perez, 1991; Perez & Callard, 1992; 1993; Prisco et al., 2008b). In addition, increased levels of circulating Vtg coincide with the preovulatory rise in E_2 observed in both *L. erinacea* and *S. canicula* (Craik, 1978; Craik, 1979; Perez & Callard, 1993). A secondary rise in circulating E_2 concentrations also has been observed in the continuous breeder *S. acanthias* during the latter half of gestation, and appears to regulate hepatic Vtg production and follicular development for the succeeding reproductive cycle. Although recent studies have suggested that the ovarian follicle also may contribute to the production of Vtg in some elasmobranchs (Prisco et al., 2002b), the extent to which this occurs in this group and the importance of E_2 in regulating ovarian vitellogenesis is not known.

Since the rise in circulating E_2 concentrations that occurs during the follicular stage in most female elasmobranchs coincides with increased growth and activity of the oviducal gland, it is possible that E_2 also regulates certain aspects of the development and/or function of this organ (Koob, Tsang, & Callard, 1986; Heupel, Whittier, & Bennett, 1999; Gelsleichter, 2004; Sulikowski, Tsang, & Howell, 2004; 2005). This argument is supported by the presence of ERs in the oviducal gland of some elasmobranchs (Reese & Callard, 1991), as well as observations of increased growth and capsule protein synthesis in the oviducal gland of E_2-treated female *S. canicula* (Dodd & Goddard, 1961). However, since no published studies have explored the distribution of ERs among the diverse cell types that make up the oviducal gland, the specific role of E_2 in this component of the female reproductive tract remains largely speculative.

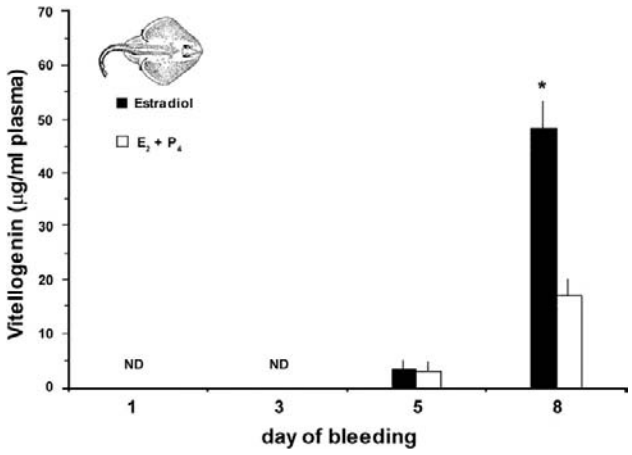

FIGURE 11.7 The effects of 17β-estradiol (E_2) and progesterone (P_4) on plasma vitellogenin in male skates *Leucoraja (Raja) erinacea*. Intact mature male skates were treated with vehicle, E_2, P_4, or a combination of E_2 and P_4, and plasma was assayed for vitellogenin on days 1, 3, 5, and 8. Vitellogenin was detectable in E_2-treated animals by day five and rose sharply by day eight. Progesterone treatment in combination with E_2 attenuated the E_2 response by day eight. Vitellogenin was not detected in control (vehicle) or P_4-treated skates. ND, nondetectable. Asterisk indicates $p < 0.01$ for E_2 vs. $E_2 + P_4$ on day eight. *Modified from Perez and Callard (1993), with permission.*

As for egg fertilization and encapsulation, the passage of fertilized ova from the oviducal gland to the uterus via the isthmus also overlaps with elevations in circulating E_2 concentrations that occur in females of some elasmobranch species just prior to and around the time of ovulation. Since ER expression appears to be high in the isthmus (Callard et al., 2005), it is likely that E_2 alters its structure in a manner that would favorably influence this transport process. This is well supported by experimental studies, which have demonstrated that E_2 can increase the compliance of the isthmus in female *S. acanthias* (Koob, Laffan, Elger, & Callard, 1983), and the circumference of this compartment in female *L. erinacea* (Callard & Koob, 1993). Since the isthmus also appears to possess secretory activity, it is possible that E_2 regulates other aspects of its physiology that contribute to reproduction.

Postovulatory rises in serum E_2 concentrations also have been reported to occur during mid to late pregnancy in females of some seasonally breeding elasmobranch species, such as the bonnethead shark and the Atlantic stingray (Figure 11.6) (Manire, Rasmussen, Hess, & Hueter, 1995; Snelson, Rasmussen, Johnson, & Hess, 1997; Tricas, Maruska, & Rasmussen, 2000). Since follicular development for the subsequent breeding cycle does not begin until after parturition in these species, increased levels of E_2 may reflect a possible role for this hormone in gestation or parturition. In both cases, increased E_2 concentrations coincide with morphological and functional changes in the uterus associated with shifts in embryonic nutrient supply. In the placental viviparous species *Sphyrna tiburo*, the postovulatory rise in E_2 concentrations occurs during formation of placental connections between the gravid female and developing offspring. Similarly, in the aplacental viviparous species *D. sabina*, E_2 levels increase dramatically during a period when the gravid female produces a nutrient-rich uterine histotroph ('uterine milk'), which nourishes embryos from the middle to late stages of pregnancy. Perhaps E_2 plays direct roles in modifying uterine morphology and functions to permit placentation and uterolactation to occur in these species; however, experimental support for this hypothesis is lacking.

Lastly, E_2 is believed to play an accessory role in modulating uterine contractility and cervical compliance in female elasmobranchs in a manner that would initially retain egg capsules or embryos in the reproductive tract during

sclerotization or gestation, and later facilitate oviposition or parturition at the appropriate time. This appears to be related to E_2's ability to potentiate the effects of the ovarian-derived peptide hormone relaxin on the uterus and cervix and is discussed further below (see Section 5).

3.3.2. Progesterone (P_4)

In most female elasmobranchs studied to date, circulating P_4 levels increase at or around the time of ovulation and may play a role in suppressing hepatic production of Vtg (Figure 11.6) (Tsang & Callard, 1987b; Fasano, D'Antonio, Pierantoni, & Chieffi, 1992; Manire et al., 1995; Tricas et al., 2000; Mull, Lowe, & Young, 2010). This is supported by the presence of PRs in the liver of female *L. erinacea,* as well as the ability of P_4 to block E_2-stimulated vitellogenesis and overall follicular development in this species (see Figure 11.7) (Perez & Callard, 1992; 1993; Paolucci & Callard, 1998). Additional support for this hypothesis comes from studies on *S. acanthias*, in which E_2 treatment was incapable of inducing Vtg production in gravid females during the first half of pregnancy, when endogenous P_4 levels remain elevated. This suggests that P_4 may restrict Vtg production in female *S. acanthias* until the second half of pregnancy, when P_4 levels decline and E_2 levels increase and stimulate yolk production for the subsequent breeding period. More recently, support for P_4-regulated inhibition of vitellogenesis also has been observed in the ray *Torpedo marmorata*, in which circulating P_4 concentrations and Vtg expression are inversely correlated (Prisco et al., 2008b).

A potential role for P_4 in regulating some aspects of the egg-laying process in oviparous elasmobranchs also has been proposed due to the changes in circulating P_4 concentrations that occur between ovulation and oviposition in a number of species (Koob et al., 1986; Heupel et al., 1999; Rasmussen, Hess, & Luer, 1999; Sulikowski et al., 2004; Awruch, Pankhurst, Frusher, & Stevens, 2008). This hypothesis is best supported in skates, based on localization of PRs in the reproductive tract of *L. erinacea* and the observation that P_4 treatment can cause early oviposition in this species (Koob & Callard, 1985). Progesterone levels decline in *L. erinacea* following ovulation and remain low until oviposition occurs, suggesting that P_4 may play a role in regulating egg retention (Koob et al., 1986). This is supported by recent studies on the skate *Malacoraja senta*, in which females without egg cases had higher P_4 concentrations than those carrying egg cases of any developmental stage (Kneebone, Ferguson, Sulikowski, & Tsang, 2007). However, in *Raja eglanteria*, a significant rise in serum P_4 concentrations occurs at the time of oviposition, perhaps suggesting an opposing role for P_4 in this species (Rasmussen et al., 1999). Based on these findings, as well as the apparent lack of a relationship between endogenous P_4 concentrations and egg laying or retention in other skate species (i.e., *Amblyraja radiata*), it is still premature to make generalities about the putative functions that P_4 may have in this process.

3.3.3. Androgens

As previously mentioned, no published studies to date have investigated the presence of ARs in the reproductive tract of female elasmobranchs. None-the-less, a number of hypotheses regarding the possible functions of androgens in female chondrichthyans have been proposed, based on alterations in circulating T, 11-ketotestosterone (11-KT), or 5α-dihydrotestosterone (DHT) concentrations that have been observed in relation to the reproductive cycle in both oviparous and viviparous species. For example, a preovulatory rise in endogenous T levels occurs in females of several elasmobranch species and likely serves as a precursor for E_2 synthesis during this period (Koob et al., 1986; Manire et al., 1995; Snelson et al., 1997; Rasmussen et al., 1999; Tricas et al., 2000). In addition, since increased levels of T coincide with the mating period in some sharks and rays, androgens also have been suggested to play a role in modulating copulatory behavior or aggression in these species (Manire et al., 1995; Rasmussen et al., 1999; Tricas et al., 2000; Mull et al., 2010). In female *S. tiburo*, the rise in androgen levels that occurs during the mating period persists during the six-month sperm storage period, suggesting that it may regulate certain aspects of this process (Manire et al., 1995). Lastly, based on the increase in serum androgen concentrations in the oviparous skate *R. eglanteria*, draughtboard shark *Cephaloscyllium laticeps*, and ratfish *Hydrolagus colliei* during the egg-laying period, a role for androgens in oviposition and/or encapsulation also has been proposed (Rasmussen et al., 1999; Awruch et al., 2008b; Barnett, Earley, Ebert, & Cailliet, 2009). Although unconfirmed, all of these hypotheses are intriguing and warrant further attention.

4. HORMONAL REGULATION IN MALES

4.1. Testicular Structure and Spermatogenesis

The general anatomy of the chondrichthyan male reproductive system has been reviewed in detail by others (Pratt, 1988; Carrier et al., 2004; Engel & Callard, 2005; Jones et al., 2005), and only that information needed to understand the hormonal regulation discussed below will be briefly presented here. The elasmobranch testes are paired structures located anterior to the coelom and suspended from the dorsal body wall by mesorchia. The morphology and functional structure of the testes varies tremendously between species, but can be generally categorized into three different types according to the spatial organization of

spermatogenesis: (1) diametric or linear testes, found in squalomorph, galeomorph, sphyrnid, and carcharhinid sharks; (2) radial testes, found in lamnoid sharks; and (3) compound testes, found in batoids (Figure 11.4) (Pratt, 1988). In addition to testes, the male reproductive system consists of the associated paired epigonal organs, paired genital ducts (each consisting of efferent ducts, epididymis, and the sperm storage organ—the ampulla epididymis or ductus deferens), the urogenital papilla, the siphon sacs (sharks), the alkaline and clasper glands (batoids), and the intromittent organs, the claspers.

Each testis contains two main cell types that differ in embryonic origin and function: germ cells and somatic cells (Leydig and Sertoli cells). The existence of true Leydig cells in chondrichthyan fishes remains controversial (see Section 4.2), but, in those species where they are described, they primarily function in steroid production. Chondrichthyan Sertoli cells are closely associated with the germ cells, and, similar to mammals, likely function to physically support germinal elements, control the microenvironment through secreted products, and serve both as a target and a source of molecules involved in regulating spermatogenesis (Engel & Callard, 2005). However, elasmobranch Sertoli cells differ from those of mammals because they (1) undergo cycles of proliferation, differentiation, and degeneration not seen in mature mammals; (2) are developmentally synchronized with germ cell clones at the same spermatogenetic stage, rather than multiple stages as in mammals; and (3) are primary steroidogenic cells in the testis (Engel & Callard, 2005).

Spermatogenesis in chondrichthyan fishes occurs within a functional testicular unit called a 'spermatocyst' (or sometimes described as a 'follicle,' 'ampulla,' or 'lobule'). The spermatocyst is an anatomically discrete sphere bounded by an acellular basal lamina that consists of a developing syncytial germ cell clone and its associated Sertoli cells. Spermatocysts are formed when a single spermatogonium in the germinal zone of the testis associates with a pre-Sertoli cell. The germ cell−Sertoli cell spermatocyst unit then undergoes a sequence of complex cellular events (i.e., mitosis, meiosis, apoptosis, maturation) that is very similar to the spermatogenetic process in all vertebrates, which ultimately results in formation of mature sperm that are released into the efferent ductules when the spermatocyst bursts.

4.2. Steroidogenesis, Steroidogenic Enzymes, and Steroid Receptors

The exact location of testicular steroid synthesis in chondrichthyans is still debated. In most male vertebrates, gonadal steroids are produced by cells that lie outside of the germ cell or testicular spermatocyst compartments (i.e., the interstitial or Leydig cells). Early studies described Leydig-like cells in the interstitial tissue of the dogfish shark, but they were portrayed as undifferentiated and did not undergo structural changes associated with spermatogenesis (Pudney & Callard, 1984b). More recent studies have demonstrated that true Leydig cells do exist in elasmobranchs, but that Sertoli cells are likely the more ancestral producers of steroid hormones (Pudney & Callard, 1984b; Callard, Mak, DuBois, & Cuevas, 1989; Prisco et al., 2008a). Elasmobranch Sertoli cells contain both cytological and enzymatic characteristics of steroid-producing cells and are thought to be the major source of androgens in these fishes (Simpson & Wardle, 1967; Pudney & Callard, 1984a; Dubois & Callard, 1989; Engel & Callard, 2005). This is supported by studies in the dogfish that show increased activity of key enzymes that control androgen biosynthesis in Sertoli cells as spermatogenesis proceeds (Callard, Pudney, Mak, & Canick, 1985). However, in both the dogfish (McClusky, 2005) and the ray *T. marmorata* (Prisco et al., 2002a; 2003; 2008a), androgens are now believed to be produced by several different cell types: Leydig cells, Sertoli cells, and possibly germ cells (i.e., spermatogonia), and the relative role(s) of Leydig and Sertoli cells in steroid production appears to change during spermatogenesis. Immunocytochemical examination of the *Torpedo* ray testis has shown that key enzymes involved in the T biosynthetic pathway, such as 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), are expressed in Sertoli cells before meiosis and after spermiation, and in Leydig cells only during meiosis (Prisco et al., 2008a).

Activity of the enzyme aromatase ($P450_{aro}$ = CYP19) involved in converting T to E_2 is higher in elasmobranch testis regions that are undergoing meiosis compared with both less mature and more mature areas (Callard et al., 1985). Although $P450_{aro}$ has been cloned in several elasmobranch species (Ijiri, Berard, & Trant, 2000; Engel & Callard, 2005), its role during spermatogenesis and other aspects of male reproduction remains unknown. In contrast to $P450_{aro}$, the enzyme that converts T to DHT (i.e., 5α-reductase) is higher in regions with premeiotic spermatocysts compared with both postmeiotic and meiotic stages (Cuevas, Collins, & Callard, 1993), which is also consistent with the presence of DHT in premeiotic areas following *in-situ* perfusion of the dogfish testis with [^{3}H]-T (Callard et al., 1989; Cuevas & Callard, 1992).

There are relatively few published studies on testicular steroid receptors in chondrichthyan fishes, and virtually all have been performed in the dogfish *Squalus* (Callard & Mak, 1985; Callard et al., 1985; Cuevas & Callard, 1992). Androgen-binding activity in *Squalus* testis was highest in regions with premeiotic-stage spermatocysts (Cuevas & Callard, 1992), and immunocytochemistry showed ARs localized to somatic cells (i.e., Sertoli cells) but not germ

cells (Engel & Callard, 2005). This stage-related pattern of AR expression in premeiotic regions of the dogfish testis was later confirmed by measurement of AR mRNA levels (Engel & Callard, 2007), and implies that androgens serve an important function during early spermatogenesis. Androgen receptor has also been recently cloned from the testis of the brown-banded bambooshark *Chiloscyllium punctatum*, and, similar to other vertebrates, classical androgens (T, 11-KT, DHT) binding to this shark AR have been shown to activate target genes via an androgen response element (Ogino, Katoh, Kuraku, & Yamada, 2009).

Estrogen receptors in the dogfish testis are also highest in regions with stem cells, spermatogonia, and premeiotic stages, but virtually absent in areas of mature germ cells (Callard et al., 1985; Ruh, Singh, Mak, & Callard, 1986; Engel & Callard, 2005; 2007). In contrast, $P450_{aro}$ was localized to more mature meiotic regions, and, because the blood flow in the dogfish testis is directed from more advanced to less advanced spermatocyst stages (Cuevas, Miller, & Callard, 1992), E_2 may play a paracrine role to signal the advance of spermatogenetic development (Engel & Callard, 2007). The prevalence of both AR and ER in early premeiotic stages indicates that androgens and estrogens may cooperate to regulate gene expression prior to meiosis in the shark testis (Callard, 1991).

Progesterone receptors also have been described in dogfish testes (Cuevas & Callard, 1992), but, in contrast to AR and ER, their distribution is associated with regions of mature or late-stage spermatocysts and thus P_4 may function in spermiogenesis and sperm release. Progesterone receptor mRNA expression later confirmed these stage-dependent binding studies, but also revealed high expression of PRs in premeiotic stages (Engel & Callard, 2007).

In addition to the steroid receptors mentioned above, a cytosolic nonreceptor-binding protein has been characterized from subfractions of dogfish testis (Mak & Callard, 1987). Based on its high affinity, broad specificity, molecular weight, isoelectric point, and dimeric structure, it was thought to be related to androgen-binding protein (ABP), found in mammalian testis. The concentration of this putative ABP increases as spermatogenesis proceeds, which is coincident with Sertoli cell development and androgen biosynthesis, and thus ABP may serve as a testicular steroid reservoir in sharks.

4.3. Gonadal Steroid Cycling and Functions

Numerous gonadal steroids have been described in male elasmobranch fishes (Callard, 1988; Garnier, Sourdaine, & Jegou, 1999; Manire, Rasmussen, & Gross, 1999), but only a few have been investigated in detail in relation to the reproductive cycle (T, DHT, 11-KT, E_2, and P_4) and will be discussed below. Further, little is known about circulating gonadal steroid levels in male holocephalans (but see Barnett et al., 2009) and therefore all of the information discussed below refers primarily to elasmobranchs.

4.3.1. Androgens

Testis development and seasonal spermatogenesis both appear to be regulated by androgens. Serum T levels (and/or other androgens such as DHT) are often elevated during the middle to late stages of spermatogenesis, which is coincident with the presence of mature spermatocysts in the testes (Manire & Rasmussen, 1997; Snelson et al., 1997; Heupel et al., 1999; Tricas et al., 2000; Sulikowski et al., 2004; Awruch et al., 2008b). Serum T levels also increase with sexual maturation and clasper length, and are elevated during the mating season in some elasmobranch species. Therefore, androgens may have effects on the development of both secondary sex characteristics and copulatory and aggressive behaviors, as seen in other vertebrates. Serum androgen concentrations also are elevated during periods of increased semen transport in sharks and batoids (Manire & Rasmussen, 1997; Garnier et al., 1999; Heupel et al., 1999; Tricas et al., 2000), and thus may influence development and function of the gonoducts and/or maturation of sperm. The seminal fluid of some sharks also contains high concentrations of steroids and steroidogenic enzymes (Simpson, Wright, & Gottfried, 1963; Simpson, Wright, & Hunt, 1964) that may play an important yet undescribed role in regulation of the male reproductive tract, semen transport and storage, and/or copulation and fertilization.

In seasonally breeding elasmobranchs, T is often correlated with gonadal recrudescence, final sperm maturation, and the onset of copulatory activity. However, studies that have examined both circulating steroids and histological stage of the testes indicate that circulating T levels more closely reflect spermatogenetic stage than overall testis mass or gonadosomatic index (GSI) (Figure 11.8) (Heupel et al., 1999; Tricas et al., 2000; Sulikowski et al., 2004; Mull, Lowe, & Young, 2008). This is also confirmed by the presence of stage-specific steroidogenic enzymes in the testis, and high levels of T produced by cultures of more mature spermatogenetic stages (Sourdaine et al., 1990; Sourdaine & Garnier, 1993; Engel & Callard, 2005). In species that are capable of reproducing year-round, however, T levels remain relatively constant throughout the year and there is only a weak (or absent) correlation between circulating T concentration and the number of late-stage spermatocysts (Kneebone et al., 2007). Future studies that account for the different reproductive modes and breeding cycles found in chondrichthyans are needed to fully interpret the relationship between T, spermatogenesis, and male reproductive competence.

11-ketotestosterone is thought to be the main androgen in teleost fishes, but its function in male elasmobranchs is

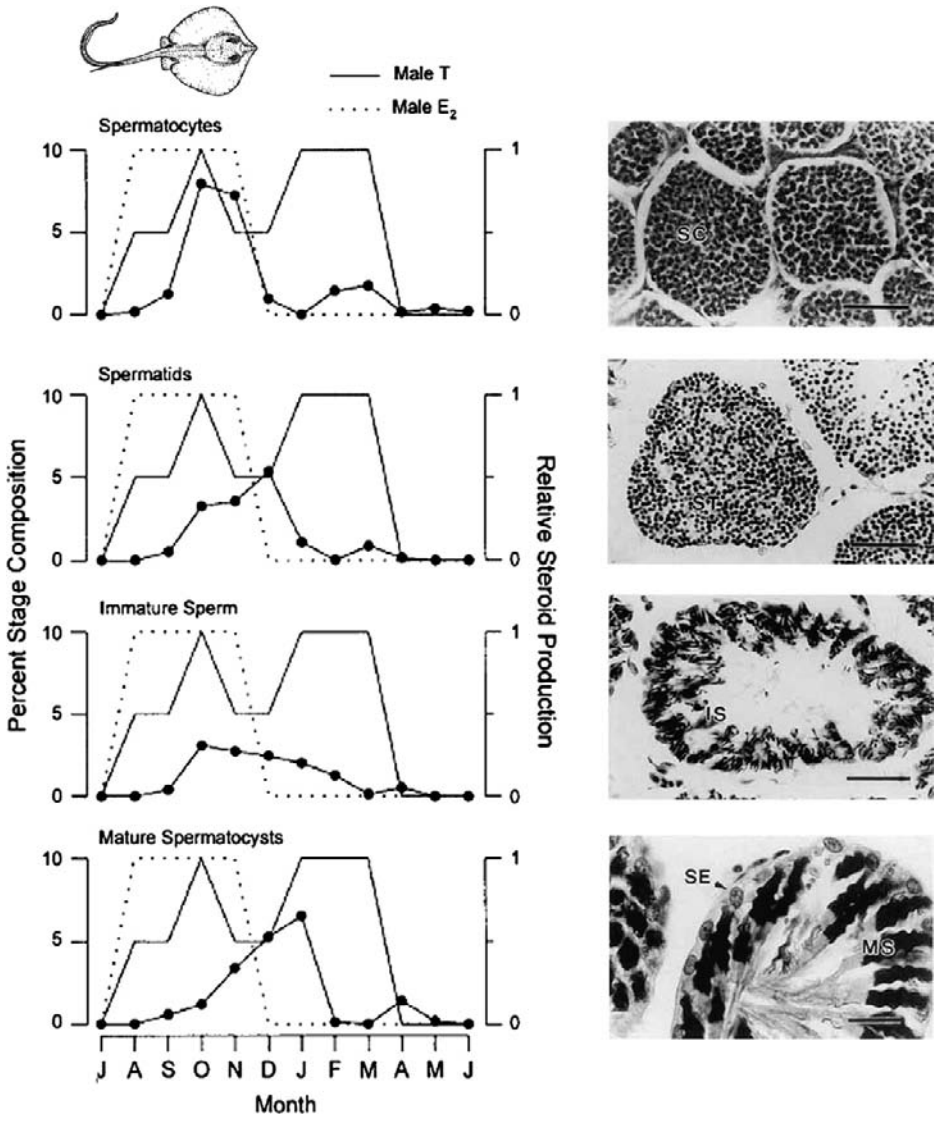

FIGURE 11.8 Stages of testes development and changes in circulating steroid levels in male Atlantic stingrays (*Dasyatis sabina*). Left side shows four histological stages of spermatogenesis expressed as a percentage of total gonad weight for each monthly sample (closed circles), plotted with steroid concentrations for testosterone (T) and 17β-estradiol (E_2) expressed in relative values. Right side shows representative histological sections of the testis from each stage. The first peak in circulating T levels is coincident with spermatocyte presence, while the second peak is coincident with a relatively brief activity of spermatocytes and the peak of sperm maturation. Peak circulating E_2 levels are associated only with spermatocyte production. IS, immature sperm; MS, mature sperm; SC, spermatocytes; SE, Sertoli cells; ST, spermatids. Scale bars = 50 μm. (left panel) *Modified from Tricas et al. (2000).* (right panel) *Modified from Maruska, Cowie, and Tricas (1996), with permission.*

less clear. Circulating 11-KT has been detected in the viviparous bonnethead shark *S. tiburo* (Manire et al., 1999) and stingray *U. halleri* (Mull et al., 2008), and the oviparous dogfish *S. canicula* (Garnier et al., 1999), where it may contribute to testicular development. However, 11-KT was undetectable in another oviparous shark, *C. laticeps* (Awruch et al., 2008b). In studies where both T and 11-KT were examined, serum T levels were higher but the patterns of both androgens were very similar. Serum levels of the androgen DHT also parallel those of T in most species, and DHT is thought to be an important reproductive hormone in male elasmobranchs. Plasma levels of T, DHT, and 11-KT within a single species are only known for the placental viviparous bonnethead (Manire et al., 1999) and oviparous dogfish sharks (Garnier et al., 1999), and future studies are needed to determine the relative roles of these different androgens in male chondrichthyan reproduction.

4.3.2. Estrogens

The role of E_2 in male elasmobranch reproduction is still enigmatic, partially because it is often not measured in males. Many elasmobranch species show distinct seasonal patterns in circulating E_2 concentrations that are related to early and middle stages of spermatogenesis (Manire & Rasmussen, 1997; Snelson et al., 1997; Tricas et al., 2000; Sulikowski et al., 2004). However, other studies show either little or irregular seasonal variation in circulating E_2 levels in males (Garnier et al., 1999; Awruch et al., 2008b), which makes any generalizations on function premature until more species with different testicular organization and breeding cycles are examined. In male Atlantic stingrays, plasma E_2 levels follow testis growth and primary androgen increases during peak spermatocyte activity, but E_2 levels are not elevated during a secondary androgen increase that

occurs in this species (see Figure 11.8) (Tricas et al., 2000). Aromatase enzyme activity has been shown to be highest in primary and secondary spermatocytes of the dogfish (Callard et al., 1985), and thus may contribute to the elevated E_2 levels during times of high circulating T in the stingray and other male elasmobranchs. Estrogen receptors are also localized to testicular regions that contain premeiotic spermatocysts (Callard et al., 1985; Callard, 1992), and E_2 may have paracrine effects on germ cells in earlier stages of spermatogenesis. Treatment of these premeiotic germ cells with E_2 results in a dose-dependent reduction in both cell proliferation and programmed cell death, which indicates it may regulate spermatogenesis via a negative feedback system on developmental arrest (Betka & Callard, 1998). Elevated E_2 concentrations also coincide with increased cell proliferation and growth in the epididymis and seminal vesicle of male Atlantic stingrays (Gelsleichter, 2004), which indicates it may play a role in genital tract function similar to that described for mammals (Hess, 2003; Shayu, Hardy, & Rao, 2007).

4.3.3. Progesterone (P_4)

In male elasmobranchs, P_4 is thought to be a substrate for androgen synthesis during later stages of spermatogenesis, or it may play a role in the regulation of spermiogenesis and/or spermiation (Gelsleichter, 2004). The androgen precursor role for P_4 stems from the fact that circulating P_4 levels coincide with testicular development and mirror the concentrations of T and DHT in some male elasmobranchs (Rasmussen & Gruber, 1990; 1993; Manire & Rasmussen, 1997; Tricas et al., 2000). However, other studies show no correlation between P_4 and androgen concentrations (Snelson et al., 1997; Garnier et al., 1999; Awruch et al., 2008b). The increase in P_4 levels during later stages of spermatogenesis in some species (Gelsleichter et al., 2002), and the observation that PRs show greater expression in late-stage (postmeiotic) compared with early-stage spermatocysts (Cuevas & Callard, 1992), support the hypothesized role of P_4 in spermiogenesis and/or spermiation. In addition, the higher P_4 concentrations in males compared with females of some elasmobranch species (Manire & Rasmussen, 1997; Rasmussen et al., 1999) indicate some important role in male reproductive physiology that warrants future study.

5. OTHER HORMONES INVOLVED IN REPRODUCTION IN MALES AND FEMALES

5.1. Corticosterone (CORT)

Serum concentrations of the steroid hormone corticosterone (CORT) show sex-specific and seasonal variations associated with reproduction in several elasmobranchs (Snelson et al., 1997; Manire, Rasmussen, Maruska, & Tricas, 2007). In bonnethead sharks, serum CORT levels covary with gonadal steroids in both sexes; are correlated with testicular growth, spermatogenesis, and mating in males; and are correlated with vitellogenesis, sperm storage, migration, mating, and early pregnancy in females. In the stingray *D. sabina*, male CORT levels were similarly elevated during testis development and mating, but, in contrast to the bonnethead, female CORT levels were correlated with late pregnancy, parturition, and postpartum stages (Figure 11.9) (Manire et al., 2007). These studies indicate that CORT plays some important role in seasonally breeding elasmobranchs possibly related to the stress axis, but its function may differ between the sexes and among species with different reproductive modes.

5.2. Relaxin

The peptide hormone relaxin is in the insulin superfamily, and is best known for its role in preparing the female reproductive tract for parturition. Relaxin has been detected in the ovaries of both sharks and batoids (Gowan et al., 1981; Reinig et al., 1981; Bullesbach et al., 1986; Bullesbach, Schwabe, & Callard, 1987; Steinetz, Schwabe, Callard, & Goldsmith, 1998), and may play a role during pupping (birth) and/or egg-laying in elasmobranchs. For example, relaxin can increase cervical cross-sectional area in late-stage pregnant *S. acanthias* (Koob, Laffan, & Callard, 1984), and it increases compliance of the female reproductive tract including the cervix in the skate *L. erinacea* (Callard et al., 1993). These effects are similar to the ability of relaxin to increase the circumference of the mammalian birth canal (Steinetz, O'Byrne, Butler, & Hickman, 1983). Relaxin also inhibits uterine contractions in the dogfish, which would protect the encapsulated embryos during early pregnancy and prevent early parturition (Figure 11.10) (Sorbera & Callard, 1995), and, as previously mentioned, these effects are potentiated by E_2.

Relaxin is also produced by male reproductive structures in several vertebrates; is found in the testis, blood, and semen of some elasmobranchs (Steinetz et al., 1998; Gelsleichter, Steinetz, Manire, & Ange, 2003); and is hypothesized to play a role in regulating male fertility (Weiss, 1989). Serum relaxin concentrations are elevated specifically during late spermatogenesis and the copulatory period in male *S. tiburo* (Figure 11.10) (Gelsleichter et al., 2003), and relaxin levels in the semen are 1000 times higher than in the circulation (Gelsleichter, 2004). These data, along with the presence of a relaxin-like compound (raylaxin) produced by the alkaline gland of skates and rays (Bullesbach, Schwabe, & Lacy, 1997), indicate relaxin-related compounds may regulate semen quality or sperm motility, or perhaps facilitate insemination by controlling uterine contractility in postmated females.

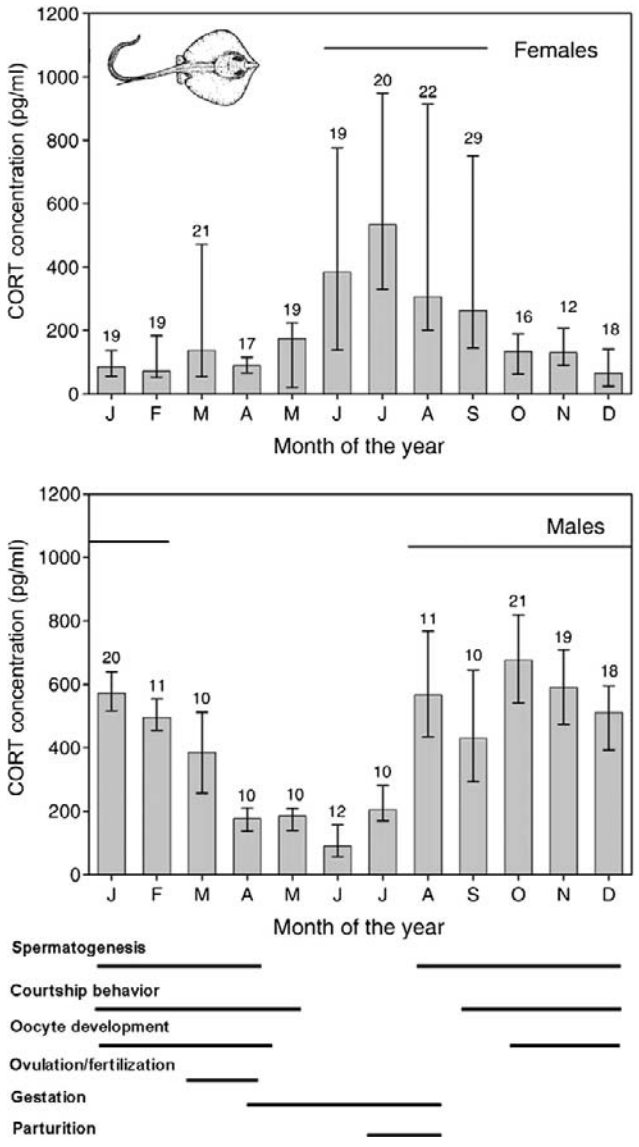

FIGURE 11.9 Monthly plasma corticosterone (CORT) concentrations in male and female Atlantic stingrays (*Dasyatis sabina*) in relation to reproductive events. Corticosterone levels in females were elevated during late pregnancy, parturition, and postpartum stages, while male CORT levels were elevated during spermatogenesis and the protracted mating season. Bars show medians and 25th–75th quartiles (error bars), and sample sizes are indicated above each bar. Lines above bars indicate groups that were significantly different from remaining groups (KW ANOVA, $p < 0.05$). *Modified from Manire et al. (2007), with permission.*

5.3. Thyroid Hormones

The thyroid hormones triiodothyronine (T_3) and thyroxine (T_4) are thought to interact with the HPG axis to regulate vertebrate reproduction, and serum levels are correlated with reproductive and developmental events in several elasmobranch species (Crow, Ron, Atkinson, & Rasmussen, 1999; Gash, 2000; McComb et al., 2005; Volkoff et al., 1999). Elevated circulating thyroid hormones during the period of ovulation, gestation, and maternal–fetal nourishment in both the stingray *D. sabina* and bonnethead shark suggest increased production of these hormones may relate to greater metabolic demand during the energetically costly processes used to provide nutrients to developing embryos (Volkoff, Wourms, Amesbury, & Snelson, 1999; McComb, Gelsleichter, Manire, Brinn, & Brown, 2005). Maternal serum and yolk levels of T_3 and T_4 in the bonnethead shark increased from the preovulatory to postovulatory periods, and peaked during pregnancy (Figure 11.11) (McComb et al., 2005). Further, higher yolk TH levels were found in a bonnethead population in Tampa Bay that shows faster rates of embryonic development compared with a Florida Bay population with slower development, indicating that thyroid hormones may play some role in controlling the rate of embryonic development in these animals (Figure 11.11) (McComb et al., 2005).

5.4. Calcitonin (CT)

The polypeptide hormone calcitonin (CT) is thought to regulate certain aspects of reproduction in many vertebrates, including pregnancy, follicular development, and embryonic development (Zaidi, Inzerillo, Moonga, Bevis, & Huang, 2002). Calcitonin is produced by the ultimobranchial gland in elasmobranchs, a paired organ embedded in the musculature between the pharynx and pericardial cavity. In the stingray *Dasyatis akajei*, ERs were localized to the ultimobranchial organ and E_2 increased CT production (Takagi, Suzuki, Sasayama, & Kambegawa, 1995; Yamamoto, Suzuki, Takahashi, Sasayama, & Kikuyama, 1996). In female bonnethead sharks, serum CT concentrations showed a temporal pattern where peak levels occurred during the yolk-dependent stage of pregnancy, and CT-ir was localized to the duodenum and pancreas of developing embryos during this same stage, suggesting it may be involved in digestion of yolk and fetal nutrition in this placental species (Figure 11.12) (Nichols, Gelsleichter, Manire, & Cailliet, 2003).

5.5. Serotonin (5-HT)

High concentrations of serotonin (5-HT) have been found in siphon sac secretions of mature male spiny dogfish *S. acanthias*, but 5-HT was either absent or found only in trace amounts in the semen of immature males (Mann, 1960). Siphon sacs in mature males had 200 times more 5-HT than immature males, and siphon sac secretions stimulated uterine contractions *in vitro* in *S. acanthias* (Mann, 1960; Mann & Prosser, 1963). These data indicate that, during copulation, 5-HT from the male may cause

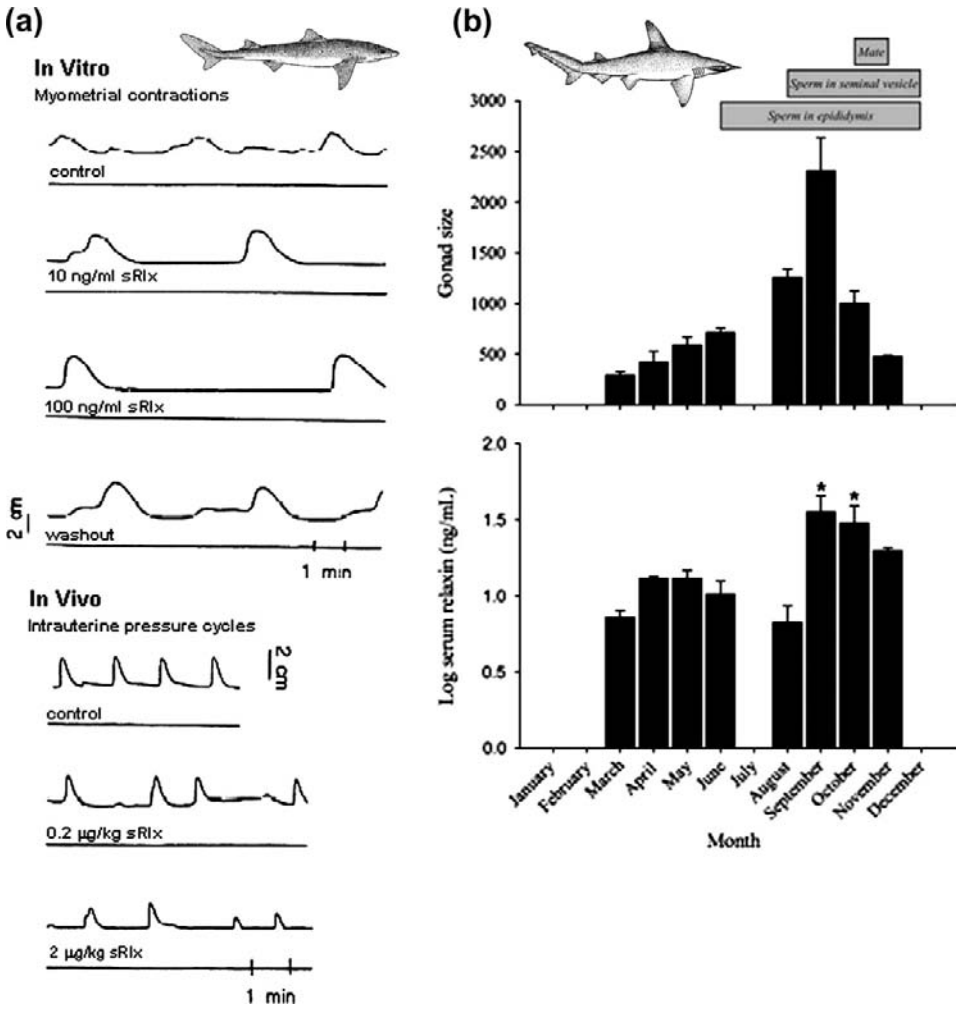

FIGURE 11.10 Relaxin function in female and male elasmobranch reproduction. (a) Effect of homologous *Squalus* relaxin (sRlx) on spontaneous myometrial contractions and intrauterine pressure cycles in the female dogfish *Squalus acanthias*. *In-vitro* myometrial contraction recordings from a uterine tissue strip of a stage C dogfish (ovary contains large 30−34 mm diameter follicles and 12−20 cm embryos free in uterine lumen) before (control) and after intravenous injection of sRlx. *Squalus* relaxin significantly decreased the frequency of contractions in a dose-dependent manner by eliminating smaller low-intensity contractile activity interspersed between the larger contractions. Contraction patterns then returned to control patterns after washout. Intrauterine pressure cycles recorded from a stage C dogfish *in vivo* showed that Rlx caused a dose-dependent decrease in mean frequency of contractions. Thus, sRlx slows the frequency of uterine contractions in third-trimester sharks, a time when progesterone (P_4) is reduced and 17β-estradiol (E_2) levels are rising. (b) Mean serum relaxin concentrations in relation to gonad size (testes width multiplied by length) and reproductive events in mature male bonnethead sharks (*Sphyrna tiburo*). Serum Rlx concentrations were significantly increased during late spermatogenesis and mating (i.e., September and October levels were greater than all other months except November). (a) *Reproduced from Sorbera and Callard (1995).* (b) *Reproduced from Gelsleichter et al. (2003), with permission.*

uterine contractions in the female that aid in sperm transport and fertilization (Mann & Prosser, 1963).

Serotonin is also a ubiquitous neurotransmitter in the brain that plays a role in sexual behaviors and aggression in many vertebrates. The embryonic and ontogenetic development of the primary 5-HT system in the brain of *S. canicula* was recently described as similar to mammals (Carrera, Molist, Anadon, & Rodriguez-Moldes, 2008), suggesting that conserved functions of 5-HT in reproductive-related behaviors may extend to chondrichthyans as well.

5.6. Neurohypophysial Hormones

Neurohypophysial hormones are a family of structurally and functionally related nonapeptides that include the vasopressin and oxytocin homologs. Chondrichthyans contain the arginine vasopressin homolog, arginine vasotocin (AVT), as well as at least eight types of oxytocin-family peptides identified in different species (i.e., oxytocin, isotocin, glumitocin, valitocin, aspargtocin, asvatocin, phasitocin, phasvatocin) (Gwee, Tay, Brenner, & Venkatesh, 2009). Arginine vasotocin is an important

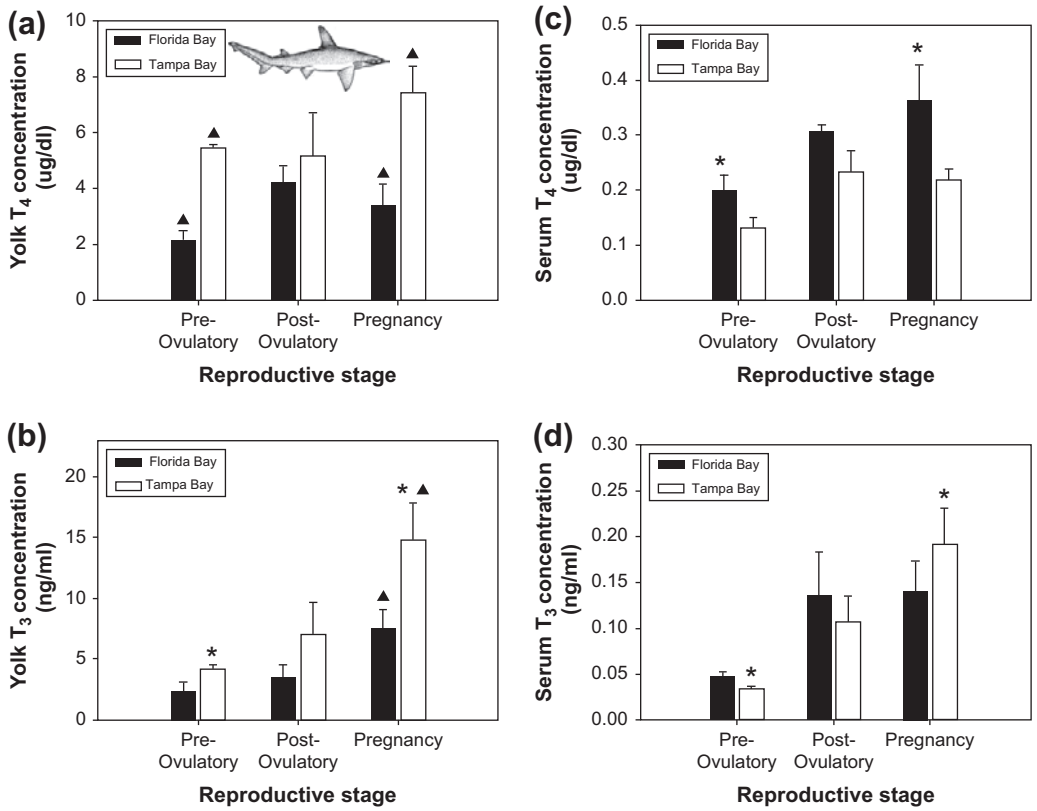

FIGURE 11.11 Yolk (a, b) and maternal serum (c, d) thyroid hormone concentrations during different reproductive stages of female bonnethead sharks (*Sphyrna tiburo*) sampled from two different sites (Florida Bay and Tampa Bay, FL). Asterisks indicate significant differences between reproductive stages within a site, and illustrate increases in thyroid hormone levels from preovulatory to pregnancy stages. Triangles indicate significant differences between sites during a specific reproductive stage, which may be related to reported differences in embryonic development rates between these sites. T_3, triiodothyronine; T_4, thyroxine. *Reproduced from McComb et al. (2005), with permission.*

regulator of reproductive and social behaviors across vertebrate taxa, and its distribution in the central nervous system is relatively well conserved (Goodson & Bass, 2001). The distribution of AVT-ir neurons and fibers in the brain is known for only a single chondrichthyan fish, the dogfish *S. canicula* (Vallarino, Viglietti-Panzica, & Panzica, 1990). While nothing is known of the reproductive physiological function of AVT in chondrichthyans, the peptide distribution in the dogfish brain is similar to that of other fishes and tetrapods and consistent with roles as both a hypophysiotropic molecule influencing the hypothalamic−pituitary−adrenal (HPA) axis and a neuromodulator of reproductive-related functions (Vallarino et al., 1990; Goodson & Bass, 2001). In mammals, oxytocin influences smooth muscle contraction in the ovary and uterus and plays a role in ovulation and parturition. A recent study also detected both AVT and oxytocin in the ovary of the holocephalan elephant shark (*Callorhinchus milii*) during the peak spawning season, suggesting a paracrine role for these hormones in ovulation and parturition (Gwee et al., 2009).

6. HORMONES, SEXUAL DIFFERENTIATION, AND SEXUAL MATURATION

Although studies on sexual differentiation and sex determination in chondrichthyan fishes are limited, the mechanisms involved are different from those of teleosts, but similar to those in amphibians and amniotes (Hayes, 1998). Sex determination is relatively stable in chondrichthyans, as transplantation studies show that gonadal tissue differentiates into ovary or testis according to its genetic sex and is independent of the sex of the transplantation host (Thiebold, 1964). However, as in all vertebrates, development of the reproductive system in chondrichthyan fishes is also influenced by endogenous steroid hormones. Chieffi (1967) observed feminization of embryonic gonads following injections of E_2, P_4, T, and deoxycorticosterone into the external yolk supply of embryonic *Torpedo* rays, and similar effects were seen after T and E_2 injections in the dogfish *S. canicula* (Thiebold, 1953; 1954). During pregnancy, E_2 may

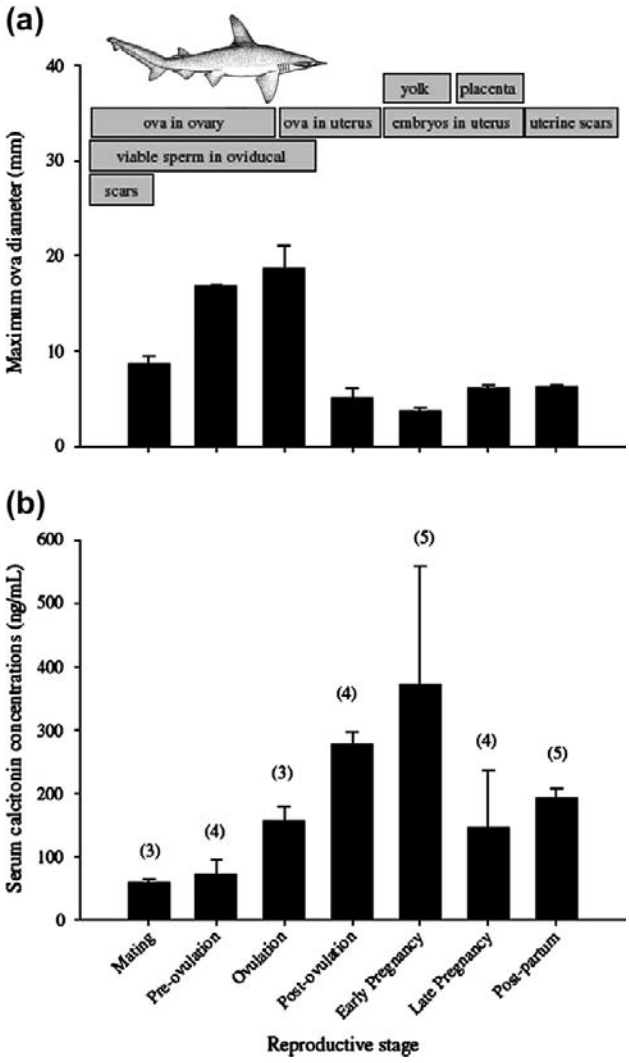

FIGURE 11.12 Maximal ova size (a) and serum calcitonin concentrations (b) in relation to reproductive events in mature female bonnethead sharks (*Sphyrna tiburo*). Serum calcitonin concentrations during early pregnancy are significantly greater than at all other stages except the postovulatory period ($p < 0.05$), suggesting an important role in embryonic development. Values displayed are means + standard error of mean (SEM) for maximal ova size, and medians + semi-interquartile range for serum calcitonin concentrations. Values in parentheses represent sample sizes for each reproductive stage. *Reproduced from Nichols et al. (2003), with permission.*

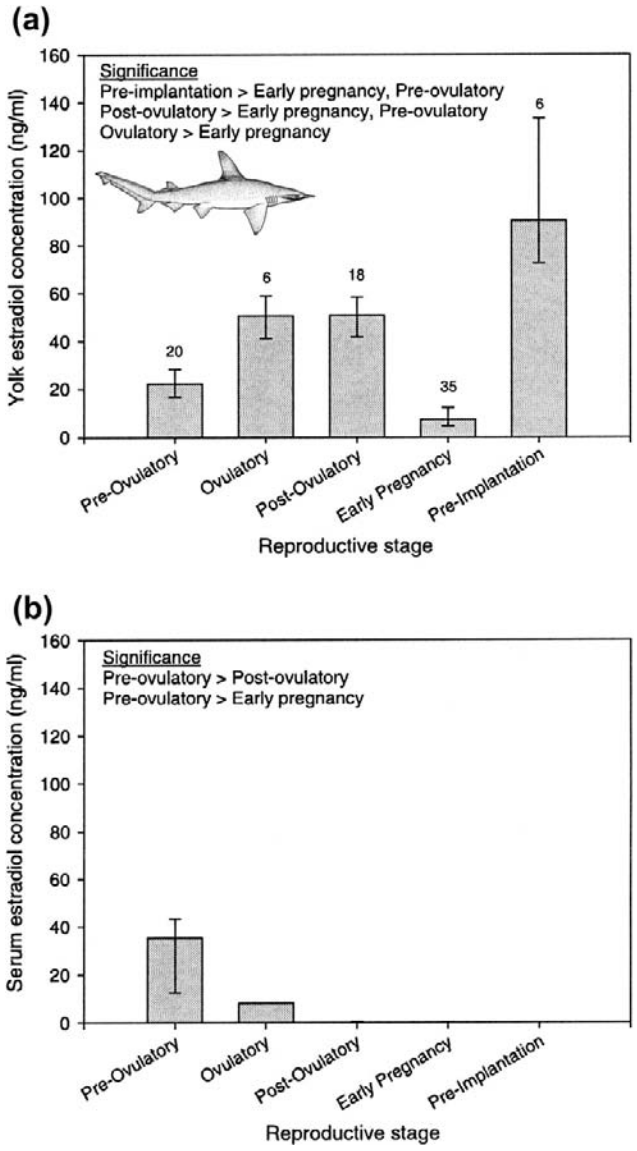

FIGURE 11.13 Yolk (a) and serum (b) 17β-estradiol (E_2) concentrations during reproductive stages from preovulatory to preimplantation (preplacental) stages of the female placental viviparous bonnethead shark (*Sphyrna tiburo*). Yolk E_2 concentrations were greater than serum concentrations in postovulatory and early pregnancy stages. Preovulatory E_2 serum concentrations were also significantly less than yolk E_2 concentrations during ovulatory, postovulatory, and preimplantation stages, and greater than yolk E_2 concentrations during early pregnancy. The decline in yolk E_2 levels between postovulatory and early pregnancy is consistent with E_2 utilization during embryonic development, and the subsequent dramatic increase in the postimplantation stage may be due to hormone production by the embryo; possibly concurrent with or following sex differentiation. Data are plotted as medians with 25th and 75th percentiles (error bars), and sample sizes are indicated above bars. *Reproduced from Manire et al. (2004), with permission.*

regulate structures that provide nutrients to the developing young, such as hepatic yolk synthesis in oviparous species, and placental or trophonemata growth and function in viviparous species (Callard et al., 2005). Steroid hormones (E_2, P_4, T) also are transferred from mother to early-stage embryos via yolk provisions in the bonnethead shark, and E_2 especially may be involved in sexual differentiation and the initiation of embryonic steroidogenesis (Figure 11.13) (Manire, Rasmussen, Gelsleichter, & Hess, 2004).

Sexual maturation (or puberty) is associated with activation of the HPG axis in most vertebrates, and chondrichthyan fishes are likely no exception. Elevated levels of circulating steroid hormones appear to be

essential for development of gonads, reproductive tract, and accessory sex organs (e.g., claspers); mating behavior; and feedback regulation of the brain and pituitary. This is supported by studies that show higher levels of circulating gonadal steroids in mature vs. immature elasmobranchs (Rasmussen & Murru, 1992; Rasmussen & Gruber, 1993; Manire et al., 1999; Gelsleichter et al., 2002). Only a few studies have coupled measurements of circulating gonadal steroid levels with morphological and histological data to more precisely determine the endocrine correlates and age at sexual maturity in sharks (Gelsleichter et al., 2002; Awruch, Frusher, Pankhurst, & Stevens, 2008; Awruch et al., 2008b), skates (Sulikowski et al., 2005; 2006), and holocephalans (Barnett et al., 2009). These studies show concurrent increases in spermatogenesis, clasper length, and plasma T concentrations associated with sexual maturity in males, and increased E_2 levels positively correlated with ovary mass, follicle size, and shell gland mass at sexual maturity in females (Figure 11.14).

Several studies in male elasmobranchs show increased serum androgen concentrations as sexual maturation and clasper length increase (Garnier et al., 1999; Heupel et al., 1999; Awruch et al., 2008a; 2008b). However, androgen sensitivity of the claspers and hormonal regulation of these organs are relatively unstudied. A small increment of clasper growth has been observed following androgen injection or implantation in immature skates and dogfish (Hisaw & Abramowitz, 1938; Dodd, 1960). In contrast, there was no direct relationship between androgen concentration and clasper elongation during puberty in *S. tiburo* (Gelsleichter et al., 2002), and T treatment of clasper cartilage explants from pubertal male bonnethead sharks had no effect on their growth (Gelsleichter, 2004). Hypophysectomy and T treatment also had no effect on *in-vivo* clasper growth in immature male elasmobranchs (Wourms, 1977). These apparently contradictory findings highlight the need for future studies on additional species, as well as examination of the role of aromatization on clasper growth, since E_2 and the growth

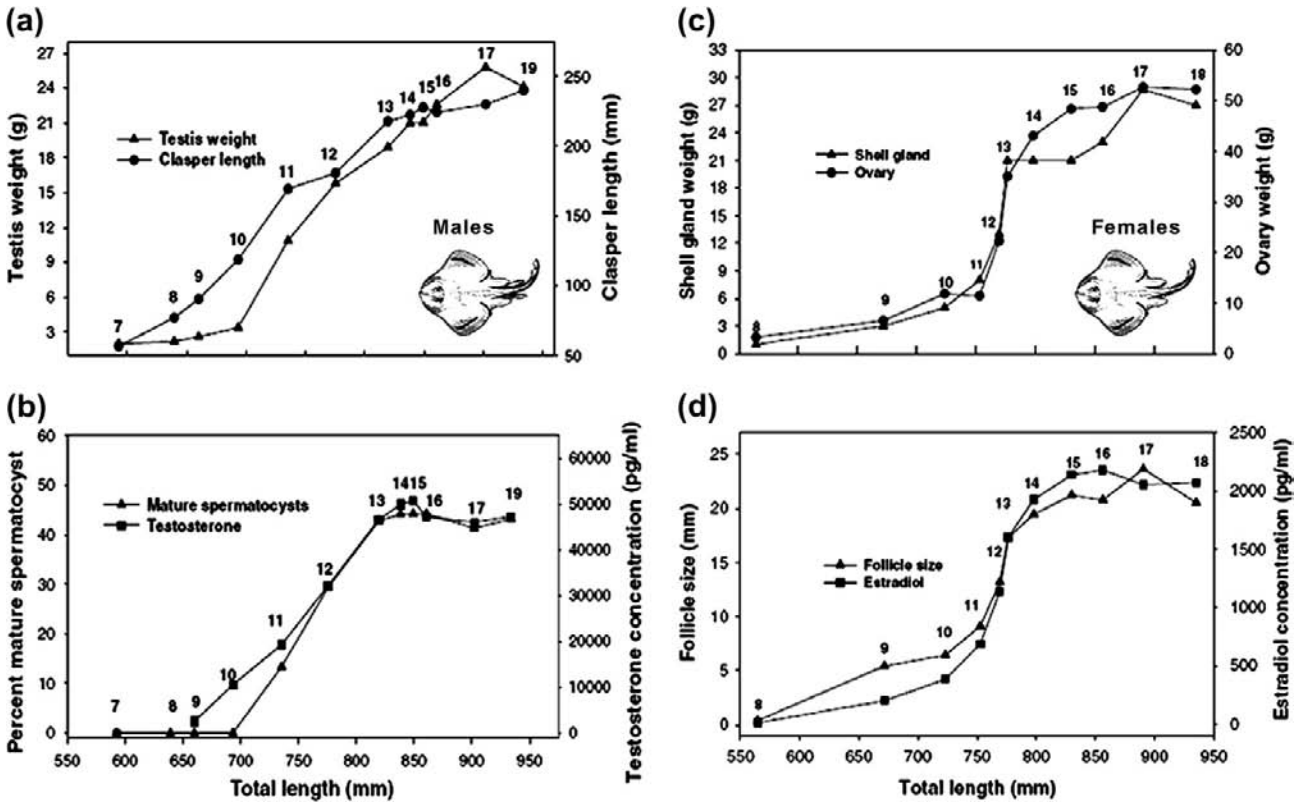

FIGURE 11.14 Relationship between sexual maturity, morphology, and steroid hormone levels in male and female winter skates (*Leucoraja ocellata*). Morphological, histological, and steroid hormone levels were used to assess size and age at sexual maturity: 50% maturity for males was at 730 mm total length and 11 years; 50% maturity for females was at 760 mm total length and 11−12 years. (a) Clasper length and testis mass as males progress through sexual maturity and increase in total length. (b) Proportion of mature spermatocysts and circulating testosterone (T) levels as males progress through sexual maturity. (c) Ovary and shell (nidamental or oviducal) gland mass as females progress through sexual maturity and increase in total length. (d) Follicle diameter and circulating 17β-estradiol (E_2) concentrations as females progress through sexual maturity. Average age is given above each representative size class. Values expressed as mean standard error of mean (SEM). *Modified from Sulikowski et al. (2005), with permission.*

hormone-insulin-like growth factor I axis is thought to mediate the effect of T on pubertal growth of the mammalian skeletal system (Grumbach, 2000), and thus may operate similarly in chondrichthyan species.

7. HORMONES, REPRODUCTIVE BEHAVIORS, AND SENSORY FUNCTION

Reproductive behaviors, including those associated with aggression and territorial defense, courtship and mating, and parental care, are influenced by or correlated with gonadal steroids in many vertebrates. In contrast, there are very few examples of the relationship between reproductive behaviors and gonadal steroids in chondrichthyan fishes. Correlational studies show that circulating androgen levels often are elevated during the mating season in some male elasmobranchs (Heupel et al., 1999; Rasmussen et al., 1999; Tricas et al., 2000), and possibly are associated with aggression and courtship behaviors, but steroid profiles in other species show androgen peaks that occur several months prior to mating (Manire & Rasmussen, 1997; Henningsen, Murru, Rasmussen, Whitaker, & Violetta, 2008). Direct observations of elasmobranch mating events in the wild are rare, and thus there is little information on steroid profiles from actively courting individuals, without which transient increases in circulating steroid levels due to social interactions or the act of copulation would not be detected. However, Rasmussen and Gruber (1990) did find elevated levels of circulating E_2 and T in female lemon sharks during active courtship. Henningsen et al. (2008) showed that some components of sexual conflict behaviors such as increased swimming speed, reduced interest in food, tailing (one male follows another male so closely that the lead shark's tail movement is restricted), and male dominance biting were associated with steroid profiles in male sandtiger sharks (*C. taurus*), whereas other behaviors such as nosing, following, and copulation attempts were not. Androgen levels within individual sharks also were related to their social position in the dominance hierarchy, with lower levels found in subordinate animals, possibly due to androgen-induced reproductive suppression (Henningsen et al., 2008).

In Atlantic stingrays (*D. sabina*), androgens shift the frequency tuning of the electrosensory system, thus allowing males to better detect the bioelectric fields of buried females during the breeding season (Sisneros & Tricas, 2000). This finding is confirmed by both the natural seasonal cycling of androgens in this species (Tricas et al., 2000) and laboratory implants of the nonaromatizable androgen, DHT (Sisneros & Tricas, 2000). Steroid action on the electrosensory system is further supported by preliminary studies that localized ARs to the peripheral electrosensory ampullae of Lorenzini (Sisneros, 1999). As mentioned above, GnRH also may influence the sensitivity of visual, auditory, mechanosensory, electrosensory, and olfactory systems to facilitate mate detection and copulatory behaviors, but this requires further study.

Courtship in chondrichthyan fishes often includes a precopulatory behavior called 'following' (also termed 'close follow,' 'parallel swimming,' or 'chasing'), where the male closely follows the female (within ~one body length) (Carrier et al., 2004; Pratt & Carrier, 2005). This behavior coupled with the existence of a well-developed olfactory system in most elasmobranchs (Schluessel, Bennett, Bleckmann, Blomberg, & Collin, 2008) has led to the hypothesis that pheromones or hormone metabolites are released by the female to trigger or initiate sexual behavior and readiness in males (Johnson & Nelson, 1978; Demski, 1990; Pratt & Carrier, 2001; Hueter, Mann, Maruska, Sisneros, & Demski, 2004). However, there are currently no published experimental studies on pheromones or the use of olfaction during reproductive behaviors in chondrichthyans. Given the importance of olfactory cues during reproduction demonstrated in both higher (e.g., bony fishes, amphibians, reptiles, and mammals) and lower (e.g., lampreys) vertebrate taxa coupled with a legendary olfactory sensing capability, this area of research deserves future attention in chondrichthyans.

8. ENVIRONMENTAL INFLUENCES ON CIRCULATING HORMONE LEVELS AND REPRODUCTION

Water temperature and photoperiod are common environmental cues used by seasonally breeding fishes to synchronize reproductive activities and ensure successful mating. Many elasmobranchs are seasonal breeders, and are hypothesized to use seasonal changes in water temperature and/or day length to coordinate reproductive physiology, gametogenesis, and behavior. There is some correlation between circulating steroid hormones and water temperature and/or day length in both males and females of several elasmobranch species (Garnier et al., 1999; Heupel et al., 1999; Rasmussen et al., 1999; Tricas et al., 2000; Mull et al., 2008; 2010). For example, circulating androgen (T and/or 11-KT) concentrations in males were negatively correlated with ambient water temperature and photoperiod (Garnier et al., 1999; Heupel et al., 1999; Mull et al., 2008), and an experimental increase in water temperature from ambient (18−20 C) up to 25 C caused a 15-fold decrease in plasma T levels in male round stingrays (Figure 11.15) (Mull et al., 2008). Steroid production in testicular tissue was also shown to be temperature- but not photoperiod-dependent in the dogfish *S. canicula* (Dobson & Dodd, 1977c; Kime & Hews, 1982). Although limited, these studies indicate that temperature and/or day length

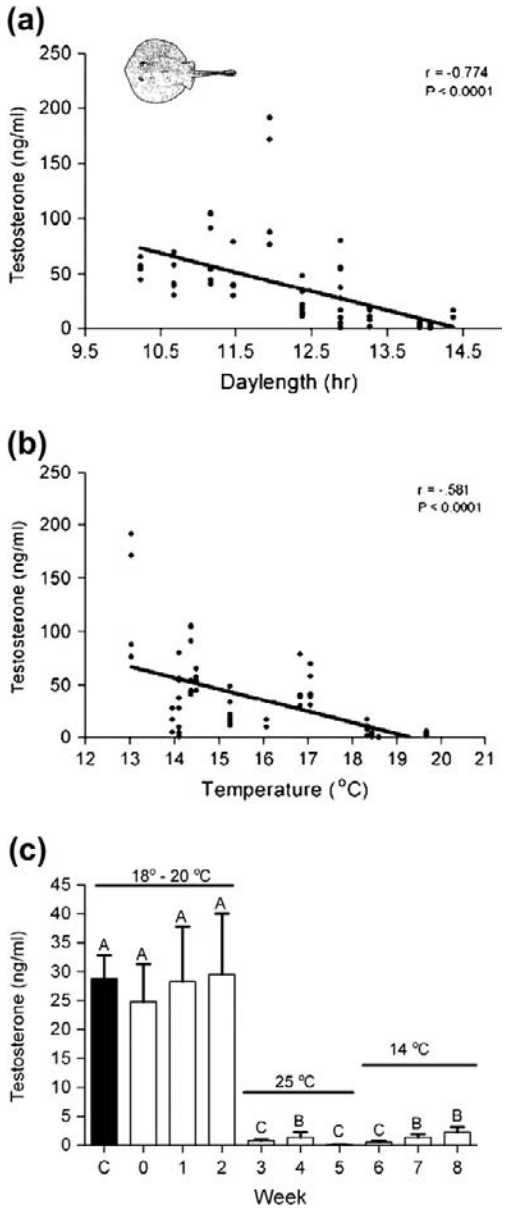

FIGURE 11.15 Relationship between temperature and photoperiod and circulating testosterone (T) concentrations in the round stingray (*Urobatis halleri*). Plasma T levels were negatively correlated with both day length (a) and water temperature (b). (c) Plasma T levels also decreased 15-fold when the water temperature was experimentally raised from 18–20 C to 25 C, and then increased again when it was reduced to 14 C. Bars represent mean + standard error of mean (SEM) and letters indicate significant groupings ($\alpha = 0.05$). Black bar shows the control group, C, which was not subject to water temperature manipulations. *Modified from Mull et al. (2008), with permission.*

probably play important roles in the regulation of circulating steroid levels and reproduction in seasonally breeding chondrichthyan fishes, which is similar to that observed in other vertebrate taxa, but the relative roles of different environmental cues likely depend on species and habitat.

Environmental contaminants (e.g., heavy metals, dioxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons) can also potentially alter endocrine function and possibly reproductive fitness in some chondrichthyan fishes (see Chapter XX, this volume). In freshwater Atlantic stingrays, serum steroid levels were elevated in subpopulations sampled in a high organochlorine pesticide (OC)-contaminated site compared to intermediate and low OC sites, but there was no evidence of reproductive impairment (Gelsleichter, Walsh, Szabo, & Rasmussen, 2006). The heavy metal cadmium (Cd) is a strong spermatotoxicant that increases germ cell apoptosis, causes spermiation failure, and compromises blood–testis barrier function in mammals, but also has been shown to have similar effects and accumulate in a stage-specific manner (premeiotic > meiotic > postmeiotic) in the elasmobranch testis (Betka & Callard, 1999; McClusky, 2006; 2008). Although not yet directly tested in elasmobranchs, Cd is a known endocrine-disrupter in other vertebrates, where it mimics estrogen and has sex steroid receptor-activation and -inhibition effects. These detrimental effects on endocrine and reproductive functions are worrisome given the abundance of elasmobranch species that inhabit often heavily polluted estuarine and inshore waters.

9. CONCLUSIONS AND FUTURE DIRECTIONS

Studies on chondrichthyan reproductive endocrinology have provided important information on how hormones regulate reproduction in this diverse and successful group of fishes, and highlight the fact that many regulatory mechanisms are conserved through vertebrate evolution. However, despite their critical phylogenetic position, there is still a paucity of information on the *function* of certain hormones in regulating various aspects of chondrichthyan reproduction when compared to most other vertebrate groups.

The diversity of breeding strategies and maternal–fetal nutritional modes found in sharks, skates, rays, and chimaeras also makes it difficult to generalize about hormonal regulation of reproduction. The majority of information on reproductive hormone function is centered on only a few species (e.g., *S. canicula*, *S. acanthias*, *L. erinacea*, *D. sabina*, *S. tiburo*). Although these species do represent different reproductive modes, they are only a limited sampling, and future studies should use the comparative approach to include additional species. The role of hormones in holocephalan reproduction is also largely unknown and deserves attention; however, information on this group will certainly be advanced in the near future because of the recent survey-sequencing of the holocephalan elephant shark (*C. milii*) genome. This sequencing project has already provided important

information on evolutionary relationships and tissue distributions of several reproductive-related hormone families (Gwee et al., 2009; Larsson et al., 2009). In addition, there is a need for more experimental and functional studies to define the role of certain hormones during reproductive events, but this first requires fundamental information on the biochemical and physiological mechanisms employed by different species. It also will be important to further our understanding of the distribution of hormone receptors in both male and female chondrichthyans in order to fully appreciate the functions of reproductive hormones in these fishes and the evolution of these systems in all vertebrates.

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ABBREVIATIONS

11-KT	11-ketotestosterone
17β-HSD	17β-hydroxysteroid dehydrogenase
3β-HSD	3β-hydroxysteroid dehydrogenase
5-HT	Serotonin
ABP	Androgen-binding protein
AR	Androgen receptor
AVT	Arginine vasotocin
Cd	Cadmium
CORT	Corticosterone
CT	Calcitonin
CYP19	See $P450_{aro}$
DHT	5α-dihydrotestosterone
E_2	17β-estradiol
ER	Estrogen receptor
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
GSI	Gonadosomatic index
GTH	Gonadotropin
HPA	Hypothalamic−pituitary−adrenal
HPG	Hypothalamic−pituitary−gonadal
ir	Immunoreactive
LH	Luteinizing hormone
OC	Organochlorine pesticide
P_4	Progesterone
$P450_{aro}$	Aromatase enzyme
POA	Preoptic area
PR	Progesterone receptor
T	Testosterone
T_3	Triiodothyronine
T_4	Thyroxine
TN	Terminal nerve
Vtg	Vitellogenin

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