Chapter 11

Comparative Aspects of Vertebrate Reproduction

Survival of all vertebrate species depends on successful reproduction and production of successful new generations of offspring. It should not be surprising that reproduction involves an interaction of environmental and endogenous factors to coordinate events within the **hypothalamus−pituitary−gonad (HPG) axis** as well as the regulation of complicated behavioral events involving individuals and often entire populations. Nutritional state is closely tied with reproduction, and chemical signals from adipose tissue appear to be important signals that modulate hypothalamic functions. Reproduction also requires close cooperation of the adrenal **(HPA)** and thyroid **(HPT**) axes, as well as many other hormones that influence reproduction and metabolism in a myriad of ways.

An understanding of reproductive patterns and their hormonal control in vertebrates is central to our concerns about environmental quality and the future of aquatic and terrestrial ecosystems that are affected adversely by human activities. Levels of environmental contamination previously considered "safe" because they were not immediately toxic are now being seen to influence reproductive efforts through more subtle mechanisms than the dramatic thinning of bird eggshells by the pesticide DDT described some decades ago. Furthermore, documented changes in human populations dealing with sex ratios, timing of puberty, declines in reproductive potentials in human males, and dramatic increases in reproductive cancers provide even more incentive to understand endocrine-related reproductive mechanisms. It is imperative that biologists learn more about the endocrine-regulated reproductive mechanisms that are most prone to disturbance, how these disturbances occur, and what remedies might be applied. In this respect, we need more information about the roles of natural environmental influences and nutrition on reproduction as well as the influences of environmental contaminants on reproduction and metabolism.

Because of the great diversity among vertebrates and the important role of natural selection on reproductive phenomena, it is even more difficult to generalize about reproductive patterns in non-mammalian vertebrates than it was for mammals in Chapter 10. Considerable diversity may be observed even within a relatively small taxonomic group. The descriptions of reproductive anatomy and physiology in this chapter are based largely on the mammalian terminology provided in Chapter 10. Those terms defined previously in Chapter 10 will not be redefined here. Similarly, the details of the organization and operation of the HPG axes of vertebrates are provided in Chapters 4 and 5.

I. SOME GENERAL FEATURES OF VERTEBRATE REPRODUCTION

The HPG axis regulates the reproductive success of all vertebrates. It is influenced by a variety of internal factors (i.e., hormones and other bioregulators) and external cues such as temperature, photoperiod, and pheromones. Attainment of sexual maturity (puberty) occurs at a time characteristic for each species and is followed by a series of reproductive cycles closely attuned to certain environmental factors. Bony fishes represent the largest, most diverse, and one of the oldest groups of living vertebrates. Among these fishes, the teleosts illustrate the full range of evolutionary reproductive strategies known for vertebrates, and numerous excellent models for the study of natural reproduction and its disruption by human activities can be found among them. For example, sexual maturity may be achieved during the first year of life (many teleosts), after more than 15 years of juvenile existence (e.g., Atlantic eel, sturgeon) or at some intermediate period. Some animals are **semelparous** and breed only once after attaining sexual maturity and die soon afterward (e.g., Pacific salmon, *Oncorhynchus* spp.), whereas most species are **iteroparous** and exhibit two or more reproductive cycles. Some of these may produce successive broods in a given year or season or may exhibit only one or two cycles per year. A few species may breed as one sex, then change to the opposite sex (termed a *sex reversal*), and breed again. Males may exhibit an **associated reproductive pattern** (also called **prenuptial**) in which gonadal steroids are highest during mating or a **dissociated reproductive pattern** (also called **postnuptial**) where mating occurs when androgens are reduced (Figure 11-1). Natural environmental factors, such as temperature and photoperiod and the presence of suitable breeding or nesting sites, influence the central nervous system and the HPG axis and regulate gonadal maturation and secretion of sex hormones. Steroid hormones, pituitary hormones, or both determine the development of

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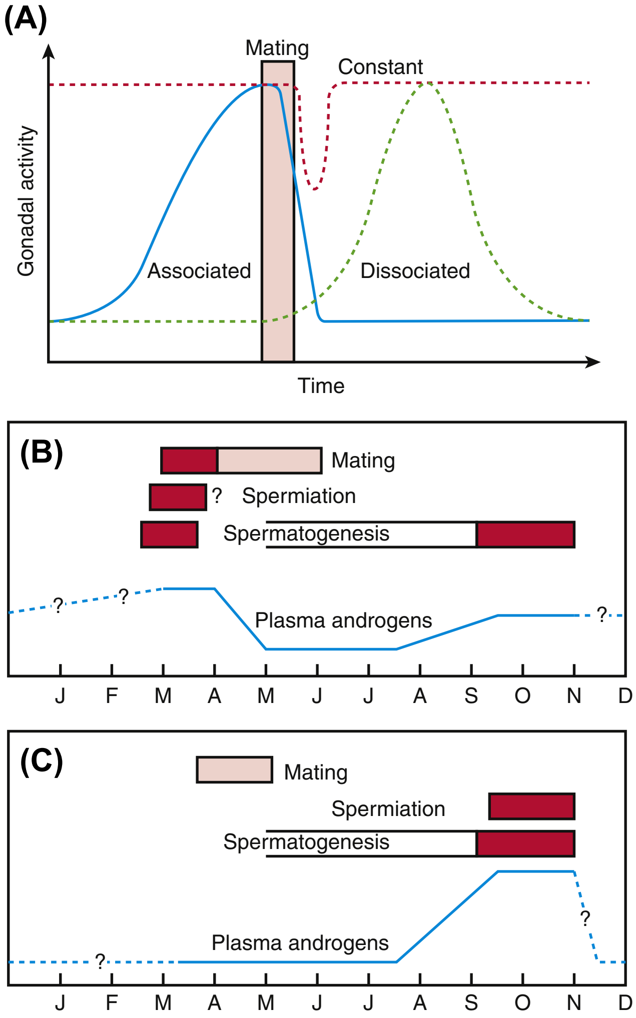


FIGURE 11-1 Associated and dissociated reproductive patterns. Mating occurs at the peak of gonadal activity in species exhibiting the associated pattern, whereas mating occurs when gonadal activity is low in the dissociated pattern. *(Part A is adapted with permission from Whittier, J.M. and Crews, D., in "Hormones and Reproduction in Fishes, Amphibians, and Reptiles" (D.O. Norris and R.E. Jones, Eds.), Plenum Press, New York, 1987, pp. 385–410. Parts B and C are adapted with permission from Houck, L.D. and Woodley, S.K., in "Amphibian Biology. Vol. 2. Social Behaviour" (H. Heatwole, Ed.), Surrey Beatty & Sons, Chipping Norton, New South Wales, Australia, 1994, pp. 677–703.)*

various sex-dependent characters and influence courtship, breeding, and parental behaviors. Pheromones may play critical roles in coordination of male and female physiology and behavior leading to successful reproduction. Many species are known to migrate between feeding and breeding sites.

TABLE 11-1 Patterns of Reproduction Described as Ovoviviparity

Pattern	Description
1	Internal fertilization; partial development of eggs within female reproductive tract; eggs at oviposition contain visible embryos
2	Restricted to anamniotes; site of metamorphosis is central to recognization; young at birth are premetamorphic larvae (amphibians)
3	Nutrients all supplied by yolk and not by placenta; oviductal secretions, or sibling embryos (including yolk)
4	A trace of egg shell appears whereas in viviparous species, no egg shell is shown
5	Includes anurans that brood eggs in vocal sacs, stomachs, dorsal skin pouches, etc.

Adapted with permission from Blackburn, D.G., *Herpetological Journal*, **4**, 65–72, 1994.

Like mammals, chondrichthyean and bony fishes, amphibians, and reptiles may be either **viviparous** or **oviparous** whereas cyclostomes and birds are all oviparous. Females of oviparous species lay eggs but viviparous species give birth to live young. Oviparous species all lay eggs with protective coverings from which a larval or juvenile form later will hatch regardless of the state of development at oviposition. The term **ovoviviparity** has been applied somewhat inconsistently in vertebrates but will not be used here as suggested by Blackburn (1994) (see Table 11-1). For simplicity, use of the term "viviparous" here will indicate live-bearing species regardless of whether there is a placental relationship or not; hence, "viviparous" will include retention of eggs in the body of the parent prior to hatching so that free-living young are released into the environment.

Fertilization of eggs after they leave the female's body (**external fertilization**) is a common practice among fishes and amphibians. However, a prerequisite for vivipartiy requires a technique for transferring sperm from the male to the female prior to release of eggs (**internal fertilization**). Some viviparous anurans (for example, *Nectophrynoides*) transfer sperm through cloacal apposition (as do the oviparous birds) or what has been termed the "cloacal kiss." Aquatic fishes and urodele amphibians, which practice internal fertilization, rely on **spermatophores** for the transfer of sperm. The spermatophore consists of a bundle of sperm that are aggregated and enclosed in a gelatinous substance that will not rapidly dissolve in water. The spermatophore allows the male to directly or indirectly transfer sperm to the female without excessive dilution of the semen. Spermatophore transfer often is facilitated by a sex accessory structure such as a modified fin in fishes or by a copulatory organ. Elasmobranchs, viviparous teleosts, gymnophionid (= caecilians, apodans) amphibians, two anuran species (*Ascaphus*), and many reptiles possess **intromittent organs** that allow direct transfer of sperm or spermatophores from male to female. In birds, sperm transfer is accomplished by direct apposition of the male's cloaca with that of the female. In urodele amphibians, a terrestrial or aquatic male typically deposits his spermatophores on the ground or on the bottom of a pond, respectively, and through a complicated behavioral ritual induces the female to pick one up with her cloaca, thus accomplishing indirect transfer of sperm to the female. Frequently, the female receiving a spermatophore has a special storage site, the **spermatotheca**, which is capable in some species of storing viable sperm for months. The spermatotheca has special mechanisms to disperse and nourish sperm so that they can perform their destined functions at a later time. Eggs can be fertilized and laid at a later time even when males are absent.

A. Gonad Features in Non-Mammals

There is a major difference in the structure of testes in anamniotes and amniote vertebrates. Whereas testes of mammals, birds, and reptiles (amniotes) exhibit a tubular pattern of seminiferous elements with interspersed clumps of interstitial cells, the testes of anamniotes (fishes and amphibians) exhibit a cystic organization. In cyclostomes and elasmobranchs, the testes consist of isolated cellular **cysts** in which spermatogenesis occurs, whereas in bony fishes and amphibians the testes consist of lobes or **lobules**, each of which is composed of large cellular cysts in which spermatogenesis is synchronous. Each testicular cyst is derived from a single **germ cell** that is surrounded by a **Sertoli (sustentacular) cell** (Figure 11-2). Mitotic proliferation of the germ cell occurs, and all of the cells within a cyst (and usually all the cysts within a lobule) will be in the same stage of spermatogenesis. The more posterior lobules may be in a more advanced stage of spermatogenesis in repeating breeders than are the more anterior lobules. Spermiation in anamniotes is usually followed by complete evacuation of sperm from each mature cyst and degeneration of the associated Sertoli cell. In iteroparous species, differentiation of lobules containing new cell nests occurs anteriorly from connective tissue elements and residual germ cells in the connective tissue covering of the testis, the **tunica albuginea**. In some fishes, however, all lobules develop and discharge sperm more or less simultaneously, and if breeding recurs there must be extensive regeneration of new cysts and spermatogonial nests prior to the next breeding season. In urodele amphibians, different lobules mature each breeding season and spent lobules do not regenerate. Although the gonads of anurans have been described by some as consisting of seminiferous tubules, they actually exhibit a pattern of cystic spermatogenesis as seen in other anamniotes.

Cystic spermatogenesis, at least in fishes, is considered to be more efficient than tubular spermatogenesis as exemplified by mammals. Generally, there is less apoptosis of germ cells (about 30 to 40% in fishes), whereas 60 to 80% of all germ cells undergo apoptosis in rats and mice. Furthermore, more sperm are produced in fish per Sertoli cell (e.g., about 100 in guppy, tilapia, or zebrafish) but only 8 to 10 per Sertoli cell in rats and mice. It is hypothesized that a second type of stem cell gives rise to new Sertoli cells throughout the reproductive life of fishes. Sertoli cells in fishes are also extremely efficient at phagocytosis of apoptotic germ cells and at destroying unused sperm remaining in the testes after spawning.

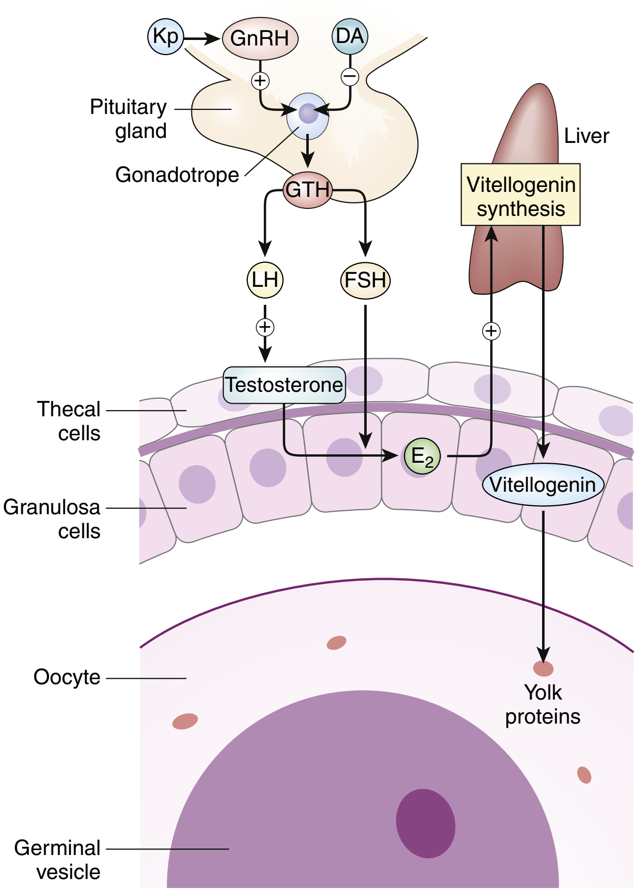


FIGURE 11-2 **Vitellogenin (Vtg) synthesis and incorporation into oocytes in teleosts.** Gonadotropin (GTH) secretion is stimulated by gonadotropin-releasing hormone (GnRH) produced in the hypothalamus of the brain and inhibited by dopamine. Luteinizing hormone (LH) stimulates production of testosterone by thecal cells. Follicle-stimulating hormone (FSH) stimulates conversion of testosterone in granulosa cells to estradiol (E_2) that is secreted into the blood. Estradiol travels to the liver, where it stimulates synthesis of the phosprotein Vtg, which returns via the blood to the ovary. Vtg in turn is incorporated into oocytes and converted under the influence of FSH into yolk proteins. The germinal vesicle is the nucleus of the oocyte. This process occurs in all vertebrates that produce yolky eggs. Kp, kisspeptin. *(Adapted with permission from Connaughton, M.A. and Aida, K., in "Encyclopedia of Reproduction, Vol. 2" (E. Knobil and J.D. Neill, Eds.), Elsevier, Amsterdam, 1999, pp. 193–204.)*

It was formerly believed on the basis of light microscope observations that interstitial tissue (i.e., **Leydig cells**) was lacking in many anamniotes, and the synthesis of androgenic hormones was thought to occur in **lobule boundary cells** associated with the testicular lobule walls. However, the cells formerly identified as lobule boundary cells are actually Sertoli cells. In most cases, Leydig cells are present between cysts in anamniotes, in the periphery of the testes, or in a few cases adjacent to the testes. In the amniote testis, Sertoli cells are associated with spermatogonia in seminiferous tubules and the Leydig cells develop between the tubules in the interstitial regions. Furthermore, the amniote Sertoli cell is involved with the entire range of spermatogenetic stages in levels or layers from the spermatogonia at the outside of the tubule to spermatids and sperm bordering the tubule's lumen (see Chapter 10, Figure 10-9). Furthermore, the amniote Sertoli cell does not degenerate after releasing sperm as occurs in anamniotes. However, in birds and reptiles as well as in some mammals, the Sertoli cell regresses considerably after the breeding season when GTH levels are low.

Ovarian structures and events occurring in the gonads of non-mammalian vertebrates are similar to those described for mammals. Ovarian follicles consist of a surrounding layer of **granulosa cells** that in turn are surrounded by **thecal cells**. Oocyte development is regulated by pituitary **gonadotropins (GTHs)**. The process of yolk protein formation is called **vitellogenesis** (Figure 11-3). Synthesis of lipoprotein yolk precursors or **vitellogenins** by the liver is stimulated by estrogens. When released into the blood, these vitellogenins bind calcium ions and result in an elevation of total blood calcium in females undergoing vitellogenesis. Thus, marked increases in blood calcium can be used as an indicator of vitellogenesis and are a reflection of circulating estrogen levels. Furthermore, vitellogenins are phosphoproteins, and an increase in plasma phosphoproteins can be monitored to provide information on reproductive status. However, sensitive immunoassays (e.g., ELISA, see Chapter 2) are available to measure plasma vitellogenin levels, and measurement of plasma calcium levels or phosphoprotein levels to approximate vitellogenin levels are no longer necessary. Incorporation of vitellogenins by growing oocytes and their conversion to yolk proteins are controlled by estrogens synthesized in the ovary.

An additional steroidogenic tissue, the **interstitial gland**, may develop in the ovaries of non-mammalian gnathostomes similar to that described for mammals in Chapter 10. Interstitial glands develop mainly from cells derived from atretic previtellogenic follicles. It has been suggested that much of the androgen and estrogen synthesized in females during reproductive cycles is from the interstitial gland of the ovary and not from the ovarian follicles.

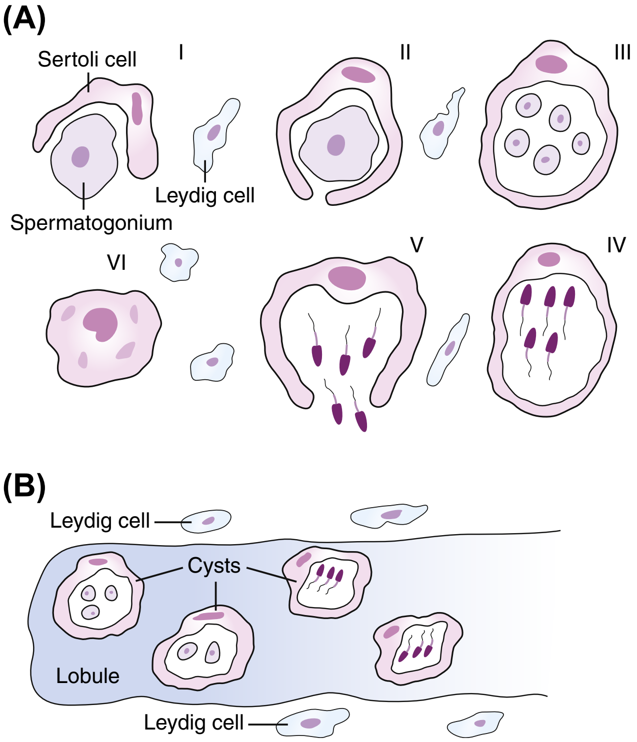


FIGURE 11-3 Phylogenetic organization of the testis. (A) Development of cysts in agnathans and elasmobranches where Sertoli cells envelop a spermatogonium cell to form a cyst. Leydig cells occur in the connective tissue surrounding the cycts. (B) In teleosts and amphibians, the cysts develop in lobules and Leydig cells are located between lobules. *(Adapted with permission from Pudney, J., in "Encyclopedia of Reproduction, Vol. 2" (E. Knobil and J.D. Neill, Eds.), Elsevier, Amsterdam, 1999, pp. 1008–1020. © Elsevier Science, Inc.)*

B. Reproductive Ducts in Non-Mammals

In mammals (Chapter 10), sperm are conducted from the epididymis associated with each testis via a **vas deferens** that is derived from the primitive pronephric (archinephric duct) and embryonically is referred to as a **wolffian duct**. The paired **müllerian ducts** develop adjacent to or possibly from the wolffian ducts and give rise to the oviducts and uterus of females when present. The upper end of the müllerian duct, and hence the entire oviduct, is open to the peritoneal cavity.

You will recall that the müllerian ducts degenerate in male mammals due to production of **anti-müllerian hormone (AMH)**, but in females but they may persist in some non-mammals (e.g., male amphibians). In elasmobranch fishes, the müllerian ducts definitely develop from the pronephric duct. The müllerian ducts give rise to oviducts in primitive bony fishes but they do not develop in teleosts. Instead, some teleosts have a short duct of uncertain homologies that transports ova, but most teleosts have an oviduct associated with each ovary that develops from a fold of peritoneum. In still others, a temporary opening develops in the body wall to allow the extrusions of gametes.

The sperm ducts of teleost fishes like the oviducts are derived from the coelomic walls and are not homologous to the vasa deferentia of other vertebrates that develop from the wolffian ducts. Amphibians, reptiles, and birds all retain the müllerian duct in females, and some male amphibians retain a rudimentary müllerian duct. The wolffian ducts function both as urinary ducts and sperm ducts in male fishes and amphibians. They are also retained in all fish and amphibian females as a urinary duct to drain the mesonephric kidney. In amniotes, the wolffian ducts degenerate in all females since new ducts, the ureters, develop to drain the metanephric kidney.

C. Endocrine Features in Non-Mammals

The endocrine factors in non-mammalian vertebrates are similar to and in many cases identical to those already described for mammals. The reader is reminded, however, that relatively few vertebrates have been examined with respect to endocrine factors and their involvement in reproduction; for example, about 50 of the 4600 mammals and even fewer of the more than 27,000 teleosts have been studied thoroughly. The same is true of amphibians, reptiles, and birds. So, there is much to be learned about the diversity of reproductive patterns in vertebrates.

It is clear in every case that the control of reproduction resides in the hypothalamus that controls pituitary and ultimately gonadal functions. One or more **gonadotropin-releasing hormones (GnRHs)** have been identified in all vertebrate groups (see Chapter 5) and GnRH-1s are responsible for release of GTHs: **follicle-stimulating hormone (FSH)** and **luteinizing hormone (LH)**.

There appear to be two distinct GTHs in most non-mammals that are FSH- and LH-like in their actions. Their release generally is under stimulatory hypothalamic control. Follicular development in females and spermatogonial mitoses in males are stimulated by FSH, with meiotic events in males being influenced locally by androgens. Spermiation and ovulation are generally controlled by LH-like GTHs. Teleost fishes have two distinct GTHs now termed FSH and LH (formerly GTH-I and GTH-II, respectively; see Chapter 5). Amphibians, birds, and most reptiles have separate FSHs and LHs. In contrast to fishes and the other tetrapod vertebrates, reproduction in squamate reptiles requires only an FSH-like GTH, and mammalian LH is ineffective in these reptiles (see Chapter 5).

The major circulating estrogen in non-mammals is **estradiol**, and **testosterone** or a structurally similar androgen such as **5α-dihydrotestosterone (DHT)** or **11-ketotestosterone (11-KT)** is characteristic for males. **Progesterone** or a closely related steroid is commonly produced by non-mammalian ovaries as well as by testes. In males, androgens are typically synthesized by Leydig cells, although Sertoli cells may be important sources in some cases. Ovarian production of androgens is typically carried out by thecal cells, with conversion to estrogens in the granulosa cells as described for mammals in Chapter 10.

Reproductive steroid hormone concentrations in blood plasma generally are greater than in mammals possibly due to high circulating levels of **steroid hormone-binding globulin (SHBG)**. Furthermore, relative levels of androgens and estrogens are not always correlated with a particular sex. For example, female fishes may exhibit levels of androgens at certain times that exceed estrogen levels. Similarly, males may secrete significant amounts of estrogens at particular times. The actions of gonadal steroids, including negative feedback effects on the HPG axis, are similar in non-mammals to those described for mammals. In addition to their feedback effects, gonadal steroids regulate gonaduct differentiation and function, differentiation and maintenance of sex accessory structures, and induction of certain behaviors.

As in mammals, **prolactin (PRL)** exhibits some specialized functions in certain species that are closely linked to reproductive events. The specific involvements of PRL will be discussed in some of the accounts that follow.

The relationships between thyroid hormones and reproductive events in non-mammals were discussed in Chapter 7, and actions of corticosteroids on reproduction were described in Chapter 9. Briefly, thyroid hormones appear to enhance the onset of gametogenesis, especially in males. It is only in amphibians and certain avian species that a negative correlation has been reported between thyroid activity and the onset of sexual maturation. Generally, corticosteroids enhance reproduction and typically are elevated during breeding. Stressful stimuli, however, can activate the hypothalamus–pituitary–adrenal (HPA) axis to a level that results in a reduction in or complete cessation of reproductive activities.

D. Sex Determination in Vertebrates

There are several mechanisms of sex determination in vertebrates (see Figure 11-4). The determination of sex in many vertebrates is associated with distinct differences in one pair of chromosomes known as **sex chromosomes**. The remaining pairs are referred to as *autosomes*. In the **homogametic sex**, both sex chromosomes are identical; hence, all gametes produced by members of that sex are the same with respect to chromosome morphology (i.e., they are homogametic). The opposite sex has unlike sex chromosomes that separate during meiosis, producing two kinds of gametes with respect to the sex chromosomes, hence the

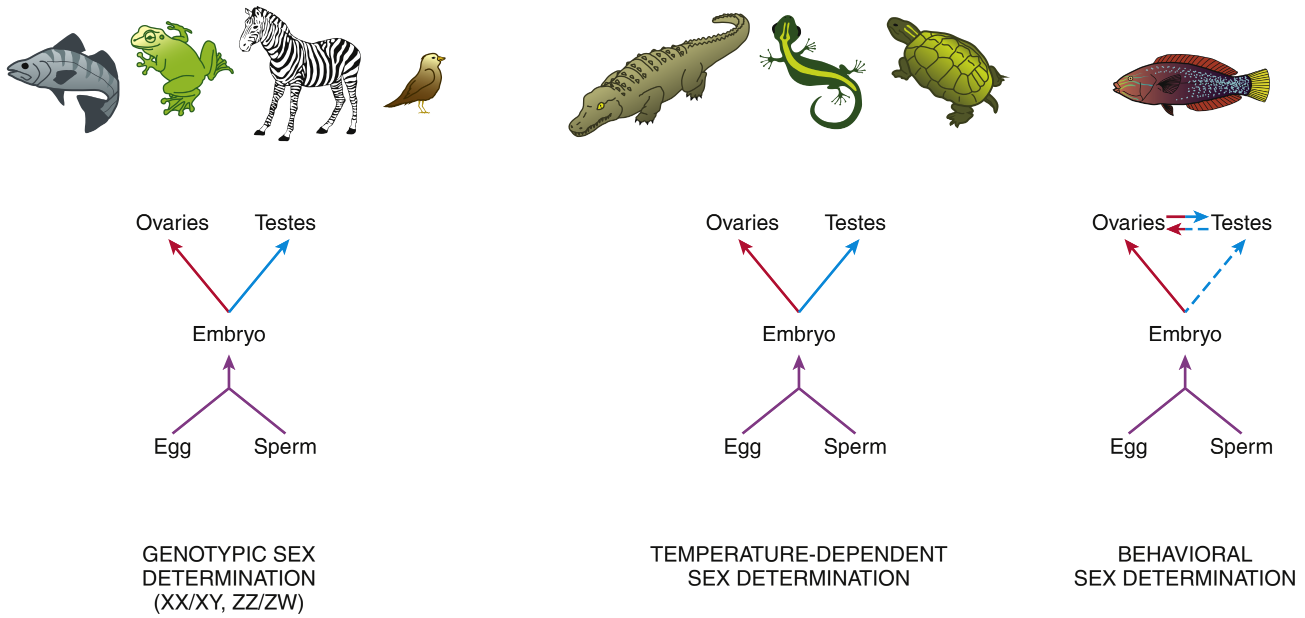


FIGURE 11-4 **Mechanisms of sex determination.** Genotypic sex determination is found among most fishes, amphibians, mammals, and birds. Temperature-dependent sex determination occurs in crocodilians and turtles and some squamate reptiles and amphibians; however, most squamates appear to have genotypic sex determination. Behavioral sex determination involves visual perceptions driving sex changes in adult individuals such as in certain wrasse species that exhibit transformation of mature females into mature males. See text for discussion.

heterogametic sex. When the female is homogametic, as in mammals, most anuran amphibians, some reptiles, and some teleosts, the common sex chromosome type is termed an X chromosome and her diploid sexual genotype is XX. The male is heterogametic, with one X chromosome and one Y chromosome (genotype = XY). When the male is the homogametic sex, as in birds, some reptiles, most urodele amphibians, and numerous teleosts, the male is designated as ZZ and the heterogametic female is ZW. Typically, the homogametic sex does not require gonadal steroids for early differentiation (i.e., it is the default sex), but gonadal steroids must be present for the heterogametic sex to overcome the default sex.

The *sry* gene on the Y chromosome of mammals and *sry*-like genes in some other vertebrates are responsible for initiating heterogametic sexual differentiation (Box 11A). When sex is determined by unique genes located on one chromosome, it is termed **genotypic sex determination (GSD)**, regardless of whether or not there are distinct sex chromosomes. Sex determination in most non-mammals appears to involve GSD even where chromosomal dimorphism is absent. However, specific sex-determining genes have not been identified in non-mammals with the exception of two teleost species identified by Yoshi Nagahama and his colleagues in Japan, although differential sequences of gene activation have been described for males and females of many non-mammalian groups (see Box 11A). Although in numerous instances, genes unique to a given sex have been identified in some non-mammals, they are downstream of unidentified events that determine if and when they become active.

Many vertebrates do not have distinct sex chromosomes and some of these exhibit environmental mechanisms of sex determination. Although the lack of a morphologically distinct pair of sex chromosomes does not mean there is no genetic basis to sex determination, at least two mechanisms depend on environmental temperature or behavior of conspecifics. **Temperature-dependent sex determination (TSD)** has been demonstrated in all crocodilians, many turtles, some lizards and snakes, some amphibians, and some fishes. TSD is correlated with early nest or water temperatures (Table 11-2). Incubation at a high temperature produces all one sex whereas at a lower temperature all the offspring are the other sex. These

TABLE 11-2 Temperature-Dependent Sex Determination in Vertebrates

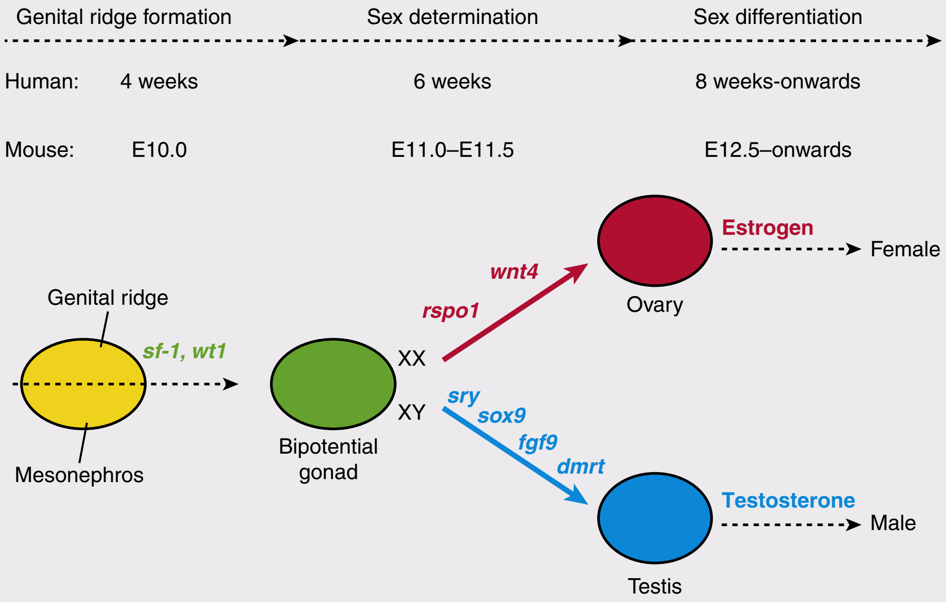
Group	Male-producing temperatures (°C)	Female-producing temperatures (°C)
Crocodilians	> 34	< 30
Turtles	23–27	30–33
Lizards	29–33	24–29
Teleosts	17–25	11–19

BOX 11A Genes Involved in Sex Determination of Non-Mammalian Vertebrates

The pathway of gene activation responsible for sexual differentiation in mammals is provided in Box Figure 11A-1. The *sry* (sex-determining region Y) gene located on the Y-chromosome in mammals is required for testis formation, but the *sry* gene has not been found outside of the placental mammals. Interestingly, the downstream genes or their homologues are widely distributed among vertebrates. Steroidogenic factor-1 (SF-1), an orphan receptor of the steroid receptor superfamily, is a transcription factor, and the *sf-1* gene is thought to be an important activator of *sry* in mammals.

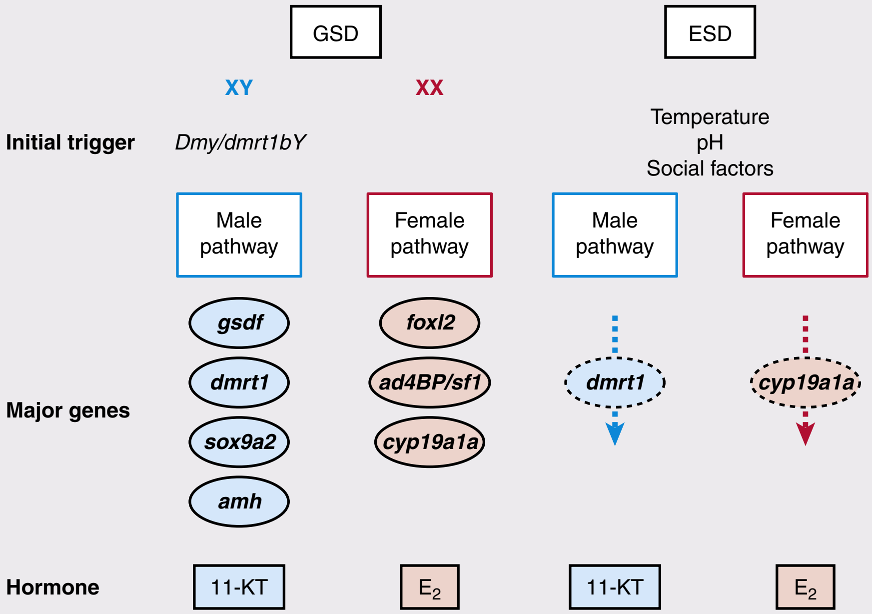
Among teleosts with XX/XY sex determination, a unique male sex-determining gene was discovered in the medaka (*Orizias latipes*), the *dmy/drmt1bY* gene (DM domain of the Y chromosome/doublesex- and mab-3-related transcription factor 1). This medaka gene has not been found in any other teleost except for another species of *Orizias*. However, another gene called the gonadal-soma-derived factor (*gsdf*) is activated in other XX/XY teleosts early in the sex-determining process. This gene appears to be unique to fishes. In males, expression of *gsdf* increases in what will become Sertoli cells. The gene *gsdf* activates the *drmt1* gene coincident with testicular differentiation, starting a cascade of gene activation, including *sox9a2* (Box Figure 11A-2). In mammals, the *sox9* (SRY-related high-mobility group box 9) gene regulates differentiation of Sertoli cells during development of a testis. A different cascade has been identified in females involving expression of *foxl2* (forkhead box L2) and *cyp19a1a* that encodes P450$_{\text{aro}}$ (Box Figure 11A-2). In teleosts that exhibit TSD, the expression of *cyp19a1a* is diminished in males. Conversely, *cyp19a1a* expression is increased in females (Box Figure 11A-2).

Non-mammalian tetrapods employ genes for sex determination that are similar to those found in both fishes and mammals. The gene *dax1* (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) is expressed early in the gonads of both sexes of amphibians and reptiles. In male amphibians, *dmrt1* and *sox9* are expressed later in interstitial cells and Sertoli cells within the testes. In female amphibians, *foxl2* is responsible for activation of the aromatase gene. Among reptiles exhibiting TSD, administration of testosterone and 5α-reductase inhibitors to turtles results in a large proportion of females at male-producing temperatures whereas inhibition of aromatase at female-producing temperatures yields mostly males. Although there are few gene studies done in reptiles, the *sox9* gene appears to be activated after the *amh* gene (anti-müllerian hormone), whereas in mammals the sequence is reversed. In ZZ/ZW birds, a Z-linked *dmrt1* gene activates the *sox9* gene for testis development. Both *foxl2* and the aromatase gene are involved with ovarian differentiation in birds, but the activating gene has not been identified.



BOX FIGURE 11A-1 **Mammalian genes and gonadal development and differentiation.** In XY male mice, activation of some genes inhibits activity of genes in the XX pathway. Similarly, in a female mouse, activation of certain genes prevents activation of male genes downstream of *sry*. E10.0, etc. represent day of embryonic development in the mouse. Developmental times for humans are shown for comparison. The pathway for humans and other mammals is thought to be essentially like the mouse. Genes are indicated in italics. *(Adapted with permission from Sim, H. et al.,* Trends in Endocrinology & Metabolism, ***19****, 213–222.)*

BOX 11A Genes Involved in Sex Determination of Non-Mammalian Vertebrates—cont'd



BOX FIGURE 11A-2 Gonadal differentiation pathway in the medaka, a teleost fish. GSD, genetic sex determination; ESD, environmental sex determination. See text for gene explanations. *(Adapted with permission from Paul-Prasanth, B. et al., in "Hormones and Reproduction of Vertebrates. Vol. 1. Fishes" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 1–14.)*

temperature effects at the extremes are "all-or-none" but intermediate temperatures produce equal ratios of males and females (50:50) rather than intersexes. Hormones do not play an initiating role in TSD, although the levels of certain enzymes, such as **aromatase (P450$_{aro}$)** and **Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD)** are greatest in adrenocortical tissue and mesonephric kidney during sexual differentiation. Brain P450$_{aro}$ may also be involved in brain changes associated with sexual differentiation.

Another type of environmental sex determination is based on social situations (i.e., behavior) that can initiate changes in gonadal sex. In sequential hermaphrodites, sex change may be induced by behavioral events, and such species are said to exhibit **behavioral sex determination (BSD).** Most examples of sex change (often termed *sex reversal*) occur in adult coral reef fishes. **Protogyny** (female to male sex change) is the more common and appears to be triggered by environmental cues. Male to female sex change is called **protandry**. Some sex-changing species are termed **diandric** in that two types of males are found. One male is a genetically determined or **primary phase male**, and the **secondary** or **terminal phase male** results from sex change of an adult female. In many cases of protogyny, sex change is initiated by loss or removal of a dominant male in the social grouping, and the largest ranking female takes his place as the dominant fish. Often there are immediate behavioral changes (e.g., increased aggression) in the dominant female that prevent sex reversal in subordinate females. Changes in skin coloration and transformation of the ovaries to testes usually follow the behavioral changes.

The bluehead wrasse (*Thalassoma bifasciatum*) is a diandric, protogynous sex-changing teleost living in the Caribbean. Spontaneous reversal is accompanied by a twofold increase in the number of GnRH-immunoreactive cells in the preoptic area of the brain. A similar increase in GnRH cells can be induced with implants of the androgen 11-KT into females. Treatment of females with **human chorionic gonadotropin (hCG)** induces gonadal sex change within 1 to 6 weeks. How GnRH and GTHs produce a sex change in females previously employing these same hormones for female reproduction is unclear. Possibly the answer lies in changes in brain function that are initiated by environmental cues.

A similar pattern of sex change occurs in a closely related diandric species, the saddleback wrasse (*Thalassoma duperrey*) living on coral reefs in Hawaii. A very different mechanism for triggering sex change operates in *T. duperrey*, a promiscuously mating fish that does not live in male-dominated groups like the bluehead wrasse. Long-term studies of these fish in underwater cages have determined that the cue for sex change is visual rather than chemical or tactile. The largest female somehow perceives a change in the ratio of larger fish (usually males) to smaller fish (usually females) and undergoes sex reversal when sufficient males are not present. Experimental studies suggest a role for biogenic amines in the events that influence GnRH and GTH release. Isolation of a female *T. duperrey* from the population results in immediate changes in catecholaminergic neuronal activity leading to sex change that can be duplicated with appropriate pharmacological treatments.

1. Steroid Hormones and Sex Determination

Regardless of the mode of sexual differentiation (GSD, TSD, or BSD), gonadal steroids and neurosteroids appear to have important roles. Androgens and estrogens from the gonads traveling in the blood or locally produced in the brain often determine the pattern of HPG function early in development that persists in adults. Furthermore, exposure to exogenous steroids can interfere with this process and cause reversal of one sex to the other. Typically, there is a window of sensitivity in development for this process (Figure 11-5). When during development this window occurs and its width (how long the window is open) vary with the species. Treatment of larval fish, for example, with reproductive steroids during the sensitive window can produce populations of all one sex that may be advantageous in fish farming where one sex may grow more rapidly than the other or where sterile fish spend energy producing muscle and not gametes. Estrogen treatment can reverse the effects of a male-producing temperature in turtles and augment the actions of a female-producing temperature, but androgens usually cannot override effects of a female-producing temperature. In turtle eggs incubated at a neutral temperature (one that should produce a 50:50 ratio), the sex ratio is sensitive to either androgens or estrogens. However, addition of both androgens and estrogens simultaneously at the neutral temperature will produce intersexes.

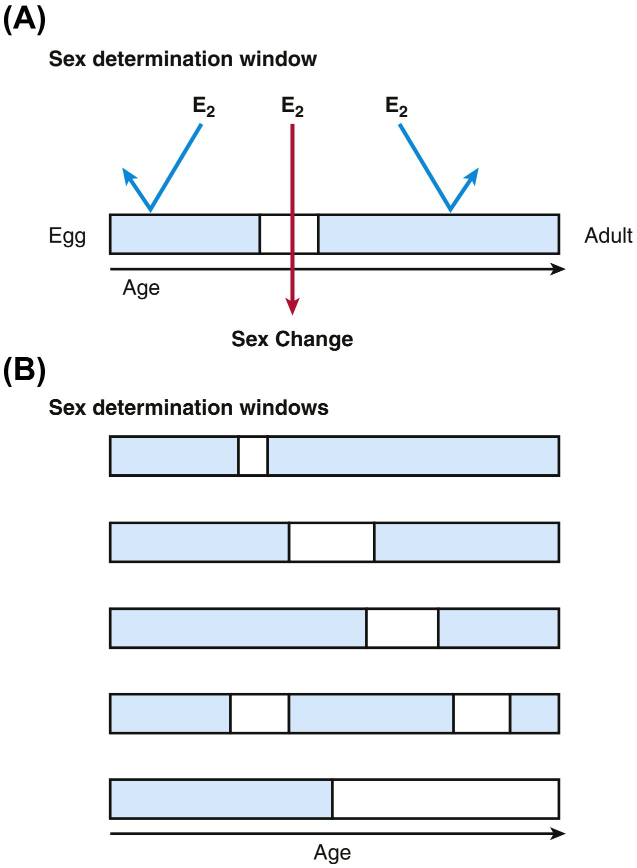


FIGURE 11-5 **Sex determination windows in non-mammals.** (A) Estrogen (E_2) can alter the genetic sex only if it appears during the short time or window (clear area) when sex determination is normally occurring in the embryo or larva. Phenotypic sex is an example of a trait that is determined by an organizational action of steroids. Exogenous estrogen often alters the genetic sex from male to female if it appears at this time. (B) Illustration that such windows (clear areas) can exist at different times in the life history of animals and can be variable in their duration. Thus, adults of some species may undergo sex reversal even after functioning as a male or as a female.

One of the major concerns of exposure of developing animals in nature to environmental estrogens relates to the appearance of an **endocrine-disrupting chemical (EDC)** during this window of sensitivity. Depending on dose and time of exposure, we may see complete or partial (intersexes) sex changes.

Paradoxical effects of steroid exposure are frequently observed, especially in fishes and amphibians. For example, exposure of a juvenile female catfish to testosterone causes precocial but otherwise normal sexual development, whereas exposure to a large dose of testosterone may feminize larval male fish.

This early sexual differentiation based on gonadal steroids or temperature resulting in a permanent designation of sex or of a sexual characteristic is known as an **organizational effect**. Sexual differentiation of the HPG axis, for example, is an example of an early organizational event in most vertebrates. In the situation described above for the coral reef fishes, sex and related structures and behaviors are not organized at an early age but show that sex determination and differentiation of the gonads are due to an **activational effect** that occurs later in life.

II. REPRODUCTION IN AGNATHAN FISHES: CYCLOSTOMES

It appears that the cyclostome gonad arises entirely from the embryonic cortex, whether it is destined to be a testis or an ovary. This singular embryonic origin may account for the common observations of what are believed to be hermaphroditic gonads among the hagfishes. In contrast, the single ovary of hagfishes is due to the failure of one primordium to develop. Some data on circulating levels of steroids for selected species of cyclostomes are provided in Table 11-3. Gonaducts that are characteristic of most vertebrates are absent in cyclostomes, and the gametes are

TABLE 11-3 Circulating Steroid Levels in Fishes (ng/mL)

Class and species	Testosterone[a]	Estradiol	Progestogen
Agnatha			
Petromyzon marinus (sea lamprey)			
Male, prespermiating	275	0.4–1.4	
with mature sperm	216	0.6–1.0	
Female, preovulatory	156		
Female, ovulating		10–13	
	0.02		
Chondrichthyes			
Torpedo marmorata (male)	15.6–35		
Raja radiata			
Male	28–102		
Female	0.2–6		
Raja eglanteria			
Male	42.7	0.022	0.150
Female	14	2.48	0.042
Scyliorhinus canicula (male)	2-6		
Osteichthyes			
Oncorhynchus nerka			
Male	17		
Female	78		
Salmo trutta			
Male	2–33		
Female	20–77		
Oncorhynchus mykiss			
Prespawning female	52–235	24–48	8–15[b]
Spawning female	65–84	2–3	354–416[b]
Spent female	2–5	1–2	8–19[b]

[a] *15α-hydroxylated testosterone in cyclostomes.*
[b] *$17,20\beta$-dihydroxy-4-pregnen-3-one.*

shed into the coelom from which they exit via abdominal pores.

A. Lampreys

Lampreys (Petromyzontidae) are characterized by having no breeding cycle, since all individuals die after a single spawning (i.e., semelparous). The indifferent gonad develops very slowly and appears female-like in both sexes. In males, the posterior portion develops into testicular tissue and oocytes may persist for a time but eventually undergo a form of degeneration called **atresia**. In females, the anterior gonad becomes the functional ovary and the posterior region degenerates. Sex accessory structures include enlarged fins, fusion of the two dorsal fins, and cloacal swelling. Steroidogenesis is unique in lampreys, with the dominant steroids being 15α-hydroxylated compounds. The physiological roles of these compounds is not clear as specific receptors have not been identified for either 1**5α-hydroxyprogesterone** or **15α-hydroxytestosterone**. Testosterone appears to be a precursor for both 15α-hydroxytestosterone and estradiol.

1. Male Lampreys

Male lampreys exhibit a single median lobular testis with a cystic pattern of spermatogenesis. Testicular development is slow, beginning at or after metamorphosis of the ammocoete to the adult form. The testes may contain only primary spermatocytes at the time of migration to the breeding grounds. These spermatocytes are transformed rapidly near the time of spawning into sperm masses. When the cysts have completed formation of sperm, they simultaneously rupture and release sperm into the body cavity.

Typical interstitial cell masses can be identified cytologically between the lobules in testes of migrating lampreys. These cells accumulate cholesterol-positive lipids and have become densely lipoidal by spawning time. Cytologically, interstitial cells appear to be steroidogenic and exhibit maximal 3β-HSD activity in February and March prior to the time of spawning. Plasma testosterone levels are extremely low and do not change with administration of lamprey GnRH nor does gonadal tissue respond *in vitro* with testosterone secretion. However, 15α-testosterone levels in male sea lampreys exhibit a dose—response relationship to administered lamprey GnRH.

2. Female Lampreys

Because of fusion of the paired primordia early in development, the adult female lamprey has a single ovary. Oogenesis has been carefully examined in the parasitic sea lamprey *Petromyzon marinus* and in the river lamprey *Lamperta fluviatilis*. In *P. marinus*, oogonia proliferate mitotically in the larvae to form primary oocytes, but by the time of metamorphosis of the larva to the juvenile, there are no oogonia remaining in the ovary. The primary ovarian follicles become more vascularized at this time. During the prolonged parasitic phase of body growth (about 10 to 20 months), the oocytes continue to enlarge slowly. The single follicular cell layer becomes thinner and less vascularized as spawning approaches, and the oocyte enters a period of rapid enlargement to reach the preovulatory condition. The mature follicles rupture immediately before spawning, and the eggs enter the coelom. Follicular atresia occurs throughout the history of ovarian development, and many oocytes undergo atresia, establishing this basic pattern early in the phylogeny of vertebrates. Phagocytes derived from the follicular cells ingest the yolk, and the follicle layers and surrounding stroma collapse into the area formerly occupied by the oocyte.

Oocyte growth in *L. fluviatilis* accelerates markedly just prior to spawning. The granulosa contacts the oocyte only at the vegetal pole and reaches maximal development about one month prior to spawning. The thecal cells are greatly reduced and cover the granulosa layer and the animal pole. The theca interna consists of a single layer of cells in which there is a marked increase in smooth endoplasmic reticulum and mitochondrial differentiation during vitellogenesis. These cells show maximal cytological activity prior to the time of most intensive vitellogenesis, following which they undergo progressive regression until the time of ovulation. The theca externa consists of fibroblasts, collagen fibers, and capillaries. 3β-HSD activity is apparently confined to the thecal cells, where peak activity is observed about one month prior to the appearance of secondary sex characters and the acceleration of follicular development.

Vitellogenesis appears to be an estrogen-dependent event in lampreys involving cooperative action of the liver, which produces proteins that are secreted into the blood and are sequestered by the ovary to be incorporated in the developing oocyte. Estrogens stimulate liver hypertrophy and elevate plasma protein-bound calcium, suggesting the presence of a mechanism such as the one that has been documented so carefully in other non-mammals (see ahead).

In the free-living lampreys that do not feed after metamorphosis, the ovarian events occur over a much shorter time and are consequently more dramatic. In brook lampreys, the immediate post-metamorphic period is marked by the onset of both vitellogenesis and massive atresia. As many as 70% of the follicles present at metamorphosis may become atretic, and phagocytosis of the yolk may provide an essential nutritional source for growth of the remaining oocytes to maturity.

3. Endocrine Function in Lampreys

The importance of gonadal steroids to development of sex accessory structures has been demonstrated through classical experiments involving hypophysectomy, gonadectomy, and appropriate hormone therapy to either hypophysectomized or castrate animals. However, lampreys secrete primarily 15α-hydroxylated steroids that are not secreted by any other vertebrates. Lamprey GnRH or pituitary GTHs from various vertebrates stimulate gonadal hormone secretions, which in turn stimulate formation of secondary sex characters. Spermiation also can be induced with lamprey GnRH. The gonads of hypophysectomized *L. fluviatilis* are less developed than those of sham-operated controls, indicating a reliance on the HPG axis.

B. Hagfishes

There is a single gonad in adult hagfishes similar to that described in lampreys. In *Myxine glutinosa*, about 41% of the gonads examined are hermaphroditic, about 58% are all female, and less than 1% are male; however, there is to date no evidence of whether hagfishes are functional hermaphrodites. All gonads appear female after hatching, and sexual differentiation of the gonads does not occur until the hagfishes reach about 20 cm in length. Both "preovulatory corpora lutea," referred to here as **corpora atretica** or **atretic follicles**, and "postovulatory corpora lutea," referred to here as **corpora lutea**, have been described in mature females, and in *M. glutinosa* they can convert pregnenolone to progesterone. Although male hagfishes appear to lack identifiable interstitial cells, they do secrete testosterone.

Hagfishes (Myxinoidea) apparently breed more than once, and several species exhibit seasonal migrations and reproduction. The reproductive biology of deepwater myxinoids is poorly known. They typically live on muddy ocean bottoms at depths between 100 and 300 meters in northern or arctic waters. Several hagfishes are seasonal breeders and some exhibit continuous reproduction. *Eptatretus* species, which migrate periodically into the shallow coastal waters of Japan, show a definite seasonal cycle of gonadal activity. The brain content of GnRH of the Atlantic hagfish (*M. glutinosa*) exhibits a seasonal cycle, and peaks of GnRH activity in females are followed by peaks in plasma estradiol and progesterone.

Hypophysectomy of *Eptatretus stouti* results in testicular degeneration in males, indicating evidence of gonadotropic control. However, hypophysectomy of mature females has no effect on either ovarian structure or circulating steroid hormone levels. Vitellogenesis is stimulated by estradiol treatment of *E. stouti*.

III. REPRODUCTION IN CHONDRICHTHYEAN FISHES

Chondrichthyean fishes are oviparous (holocephalans, skates, and some sharks) or viviparous (all rays, 70% of sharks). The prevailing view is that viviparity evolved independently 9 to 10 times, although a more recent suggestion considers the smaller bodied oviparous species as being derived from viviparous species. Viviparity is present in ten families of sharks and four families of rays. Many viviparous sharks and batoid rays are "continuous breeders"; for example, the spotted dogfish (*Scyliorhinus canicula*) is sexually active throughout the year and may have embryos in different stages of development *in utero* at the same time. In contrast, many rays and some viviparous sharks are "seasonal breeders," with entire populations synchronized to environmental factors. Some viviparous and oviparous species are intermittent or "punctuated breeders," meaning that reproductively active periods may be interspersed with one or more non-breeding years.

Fertilization is internal regardless of whether they are viviparous or oviparous, and special intromittent organs called **claspers** are present in mature males. Holocephalans have not been studied extensively, and the reproductive biology of the more common elasmobranchs is better known. The elasmobranch reproductive system is illustrated in Figure 11-6, and plasma levels of gonadal steroids for selected species are provided in Table 11-3.

A. Male Elasmobranchs

Male elasmobranchs have paired testes and paired **epigonal organs**. Efferent ducts connect each testis to a genital duct (ductus deferens) and a sperm storage organ (epididymis). Claspers are associated with ventral (pelvic) fins located near the cloaca and are used for transferring sperm to the female genital tract. In sharks, claspers are not used to grasp the female during mating as the name implies, but rather males grasp the females with their teeth during

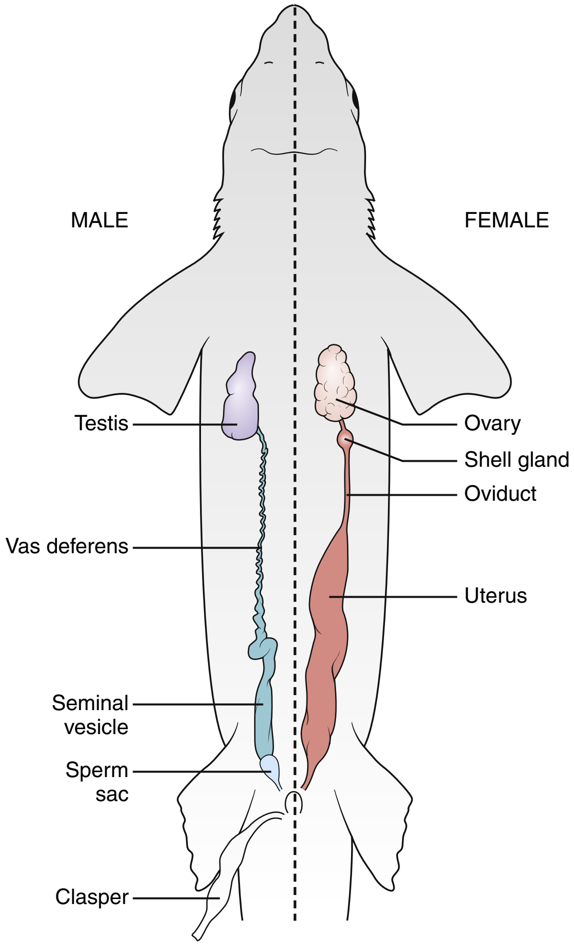


FIGURE 11-6 Reproductive systems in elasmobranchs. *(Adapted with permission from Maruska, K.P. and Gelsleichter, J., in "Hormones and Reproduction of Vertebrates. Vol. 1. Fishes" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 209–237.)*

copulation. Recent bite marks on a female shark are used as evidence of recent copulation.

Spermatogenesis in paired testes is of the cystic type. Sertoli cells are present and possess 3β-HSD and $P450_{aro}$ activity and are considered to be the primary source of androgens and estrogens. Sertoli cells become densely lipoidal and cholesterol positive following spermiation and are eventually resorbed. Sertoli cells for the next cycle differentiate from connective tissue cells (fibroblasts or possible stem cells) in the wall of the testis. Nests of spermatogonia proliferate from germ cells in the same regions, and they are responsible for producing sperm utilized during the next breeding period. In a few species, Leydig cells have been described between cysts although they may not be readily discernible throughout the testicular cycle. They are not considered an important source of androgens, however.

Spermatophores produced by elasmobranchs are the result of secretory activities of male accessory ducts. After spermiation, sperm pass through vasa efferentia and enter the coiled tubules of the **Leydig gland**, which is derived from the anterior portion of the mesonephric kidney. It is not steroidogenic and should not be confused with the Leydig cells. Sperm and secretions of the Leydig gland pass on to an expanded region of the vas deferens known as the **ampulla epididymis**. Here the sperm are consolidated and receive additional secretory material to form complex spermatophores typical for each species.

1. Endocrine Factors in Male Elasmobranchs

The importance of the ventral lobe of the elasmobranch pituitary as the source of GTH controlling spermatogonial proliferation (mitosis) has been demonstrated in the spotted dogfish. Degenerative changes in the testes appear 6 weeks after removal of only the ventral lobe, and 22 months later the testes contain only spermatogonia and mature sperm, indicating that removal of the ventral lobe blocks further differentiation of spermatogonia to spermatocytes, whereas all spermatocytes present at the time of surgery are able to complete meiosis and spermiogenesis. In addition to testosterone, 11-KT and DHT have been reported in many but not all elasmobranchs. Androgen levels are elevated when mature spermatocysts are present in the testes, during development of sex accessory structures, and during semen transport. Circulating estradiol and progesterone also have been reported in males. Estradiol levels are lower in male *Raja eglanteria* than in females, but progesterone levels are greater in males. Corticosterone levels in seasonally breeding elasmobranchs are elevated during testis growth, spermatogenesis, and mating.

A **relaxin** molecule, structurally more similar to mammalian insulins than to mammalian relaxin, is produced by the testis of the shark *Sypyrna tiburo* and is elevated in the circulation during late spermatogenesis and at copulation. The level of relaxin in semen is 1000× greater than that found in the blood. The precise role of relaxin in males needs to be determined.

B. Female Elasmobranchs

The elasmobranch ovary is covered by germinal epithelium and may contain a cavity derived from large lymph spaces within the stroma. Elasmobranch follicles are similar to those of mammals in possessing several distinct layers of cells. The connective tissue near a nest of oogonia will differentiate into the theca. As each follicle begins to develop, some epithelial cells from the germinal epithelium undergo hypertrophy and hyperplasia to become the granulosa. In some species, the granulosa may consist of only a single layer of cells. These cells are responsible for transfer of vitellogenin during follicle growth as well as for yolk resorption should a given follicle become atretic. Granulosa cells also are thought to be the source of testosterone and estrogens since they exhibit more 3β-HSD activity than do thecal cells. Most estrogen synthesis occurs in mature follicles that have a well-developed granulosa. During follicular development, a theca interna and theca externa can be discerned; however, both layers largely consist of connective tissue elements, and only a small amount of 3β-HSD activity has been observed in the theca interna cells. The granulosa cells are the major source of steroidogenic cells in both atretic follicles and corpora lutea, although thecal cells may contribute as well. The connective tissue layers surrounding these structures are derived from the theca.

Elasmobranch females have well-developed müllerian ducts that give rise to the oviducts as well as to the uterus of viviparous species. Oviducts have been examined in oviparous species that secrete horny shells to protect the eggs laid in the ocean as well as in viviparous species, and they possess a number of specialized features. **Oviductal** or **nidamental glands** occur in the anterior oviduct and secrete albumen and mucus. The oviductal glands of oviparous species are often differentiated into an anterior albumin-secreting area and a posterior shell-secreting region. An intermediate mucus-secreting zone may be found in some species. The "shells" secreted by the oviduct are rigid in oviparous species but are pliable in viviparous species. In some species, the anterior oviduct may function as a spermatotheca.

The lower portion of the oviduct is expanded into a uterus where eggs are held prior to spawning or in which young develop in viviparous species. Villus-like structures may develop in the uterine portion of the oviducts of certain viviparous females, and they provide nourishment for their young. Even in aplacental viviparous species, the uterus often supplies nutrients and oxygen and removes metabolic wastes during gestation. In *Squalus acanthias*, contraction of the myometrium flushes fresh seawater into the uterus, brings in fresh electrolytes, and removes wastes. Such mechanisms may operate in other species as well, although this has not been documented. In some aplacental species, unfertilized eggs may be consumed later by developing young. In still others, the first hatching young may cannibalize less developed uterine inhabitants.

1. Endocrine Factors in Female Elasmobranchs

Removal of the ventral lobe of the adenohypophysis blocks oviposition in female *S. canicula*, and all follicles containing oocytes larger than 4 mm in diameter undergo atresia, demonstrating dependence on GTHs. Progesterone can inhibit vitellogenesis in the spiny dogfish *S. acanthias* and prevents reinitiation of oocyte growth during early gestation. Estradiol stimulates vitellogenin synthesis in the liver and growth of the oviduct.

In the oviparous skate, *Raja erinacea*, estradiol and testosterone levels increase during follicular growth but progesterone shows a peak just prior to ovulation. Testosterone rises in females of some species at ovulation or during oviposition by oviparous species. Elevated testosterone has been reported in *S. tiburo* females at mating and during retention of sperm.

After ovulation, there is a decline in steroid secretion until oviposition (Figure 11-7). However, estradiol peaks prior to ovulation in the egg-retaining viviparous *S. acanthias* and progesterone remains elevated during the early part of gestation (Figure 11-8). Corpora lutea of several species possess 3β-HSD activity, and corpora lutea persist during gestation in *S. acanthias*. The corpora lutea from pregnant *S. acanthias* produce twice as much progesterone *in vitro* as do those from nonpregnant females, which possess only atretic follicles and no corpora lutea, supporting an endocrine role for atretic follicles in this species. Viviparous rays, *S. tiburo* and *Dasyatis sabina*, exhibit patterns of estradiol and progesterone similar to those of *S. acanthias* (Figure 11-9).

The ovaries of viviparous sharks produce relaxin with similar biological actions when tested for its uterine-relaxing activity in the guinea pig. A similar molecule called **raylaxin** has been isolated from rays and skates. Relaxin prevents premature uterine contractions in sharks and enlargement of the cervical region prior to pupping or egg laying. Relaxin appears early in vertebrate evolution and performs a similar function in sharks as in mammals.

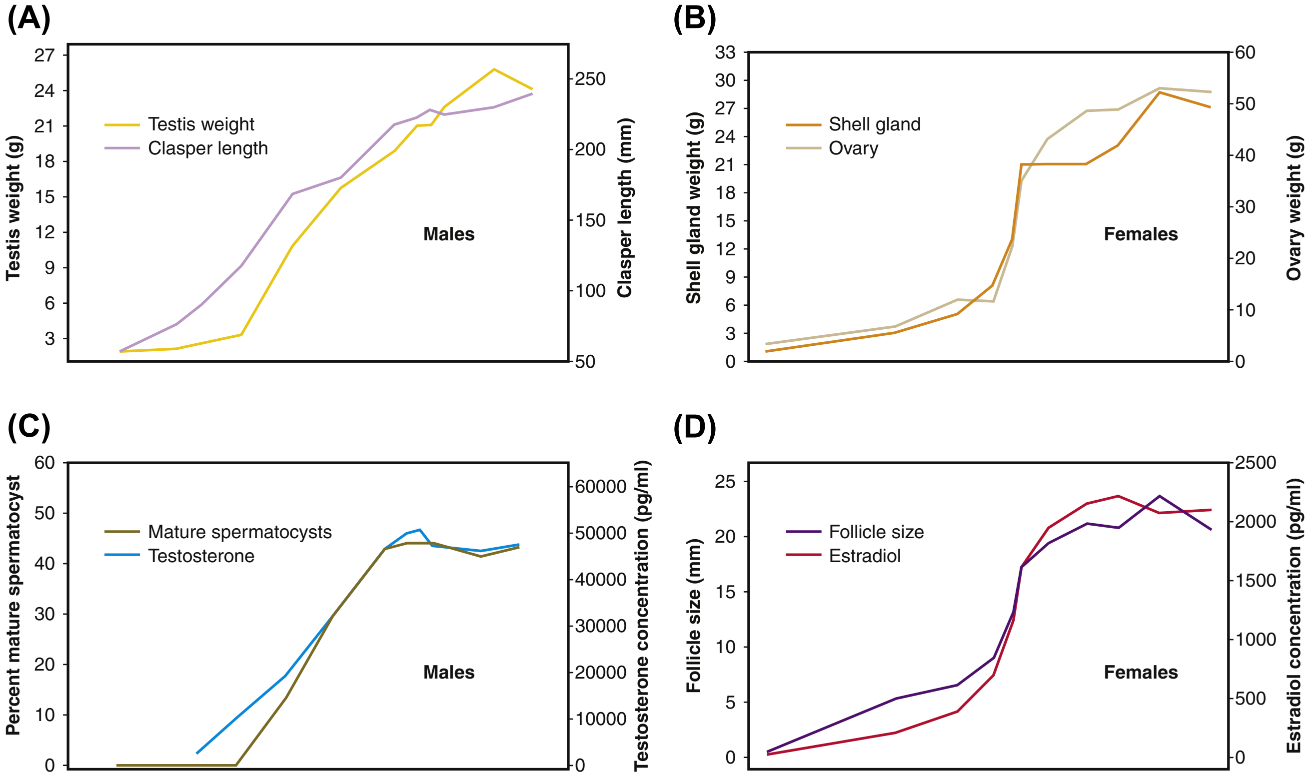


FIGURE 11-7 **Steroid hormone levels and reproductive structures during sexual maturation of male and female winter skates (*Leucoraja ocellata*).** *(Adapted with permission from Maruska, K.P. and Gelsleichter, J., in "Hormones and Reproduction of Vertebrates. Vol. 1. Fishes" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 209–237. Figure originally appeared in Sulikowski, J.A. et al.,* Environmental Biology of Fishes*, **72**, 429–441, 2005.)*

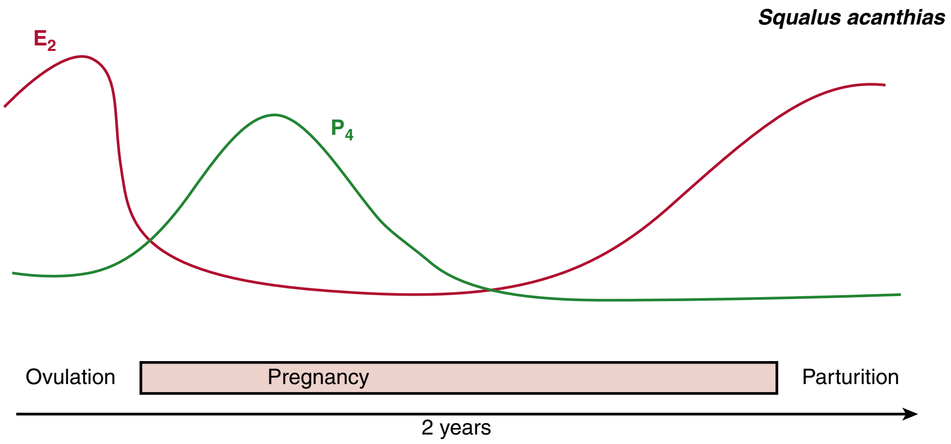


FIGURE 11-8 **Reproductive cycle of the viviparous shark *Squalus acanthias*.** E_2, estradiol; P_4, progesterone. *(Adapted with permission from Koob, T.J. and Callard, I.P.,* Journal of Experimental Zoology, ***284****, 557–574, 1999.)*

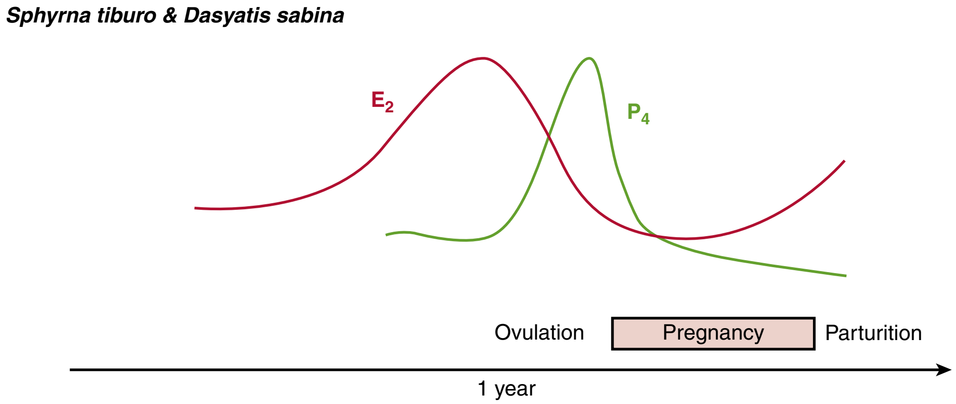


FIGURE 11-9 **Reproductive cycles of viviparous skates, *Sphyrna tiburo, Dasyatis sabina*.** E_2, estradiol; P_4, progesterone. *(Adapted with permission from Koob, T.J. and Callard, I.P.,* Journal of Experimental Zoology, ***284****, 557–574, 1999.)*

However, this is the only group of non-mammalian vertebrates so far shown to produce a relaxin and to respond to mammalian relaxin.

IV. REPRODUCTION IN BONY FISHES

Among the bony fishes, the 27,000 species of teleosts exhibit almost every reproductive pattern and strategy known for vertebrates, including some that are unique to these fishes. Most of the account here is based on teleosts, but there are many similarities between teleosts and the other groups of bony fishes. Some features of these other bony fishes will be illustrated, also, but first some generalizations need to be made.

Like that of cyclostomes, the teleost gonad develops only from a cortical primordium. Bony fishes may be **gonochoristic** (separate sexes), **unisexual** (all female populations), or **ambisexual** (simultaneous or sequential hermaphrodites). **Hermaphroditism** implies that both functional sexes appear in the same body as opposed to **intersex**, which refers to the presence of features normally characteristic of the opposite sex (i.e., presence of a female character in a genetic male). The intersex condition is considered to be an anomaly. A few hermaphroditic species are simultaneous hermaphrodites but most are sequential hermaphrodites. Among the sequential hermaphrodites, there are numerous examples of protandry (function first as males and later transform to females) and protogyny (*gyno*, female). Most bony fishes are oviparous but viviparity has arisen in many forms as well.

Fertilization may be external or internal as in the viviparous teleosts and in the viviparous coelacanths, *Latimeria* spp. In some viviparous teleosts, the fertilized egg is known to develop within the ovary. Elaborate patterns of courtship, nest building, parental care, and other specific reproductive behaviors have been reported among diverse groups.

Breeding typically is cyclic with the exception of semelparous species. Some species are **synchronous spawners** such as in the semelparous Pacific salmon. Others are called **group-synchronous spawners** with most commonly two spawning episodes per season (e.g., Atlantic salmon, rainbow trout). Still other species are **asynchronous spawners** and spawn repeatedly all year (e.g., zebrafish, medaka, killifishes). Each species has a well-defined spawning period regulated by one or more environmental factors (seasonal changes in photoperiod, lunar cycles, temperature, etc.). Many iteroparous species spawn several times during a single breeding season (e.g., *Hyporhamphus melanochir*, an Australian garfish of the halfbeak family). In some viviparous species, such as the guppy *Poecilia reticulata*, a new batch of oocytes is released soon after birth of the young and another brood is raised. Other viviparous species require a longer "interbrood period" for oocyte maturation and vitellogenesis (*Mollienesia* and *Gambusia*). However, in *Quintana atrizoma*, oocyte development occurs during gestation so that a new batch of eggs can be fertilized as soon as the young are born.

The endogenous nature of seasonal or annual rhythms has been shown only in a few species; for example, the killifish (*Fundulus grandis and F. heteroclitus*) and the golden rabbit fish (*Siganus guttatus*), which spawn according to lunar cycles, exhibit free-running lunar-related reproductive cycles in the laboratory (see Box 11B). Seasonal reproductive cycles clearly are evident even in tropical species. The effects of artificial lengthening and decreasing of the photophase may accelerate spawning in spring and fall spawners, respectively. A classical demonstration of environmental phasing of reproduction has been demonstrated by transporting a poecilid, *Jenynsia lineata*, from South America to the Northern Hemisphere. In South America, this species normally spawns in January and February (summer). At the new pond location where photoperiod and related seasons were reversed, the fish switched to spawning in July and August. However, the possible importance of the temperature regimen, which was also switched, should not be overlooked. In some species, temperature is the critical factor in controlling recrudescence regardless of the light regimen imposed on the fish.

A. Male Bony Fishes

The major circulating androgens in teleosts are 11-KT and testosterone. Levels of androgens are correlated with testis size, which increases annually in breeding adults (Figures 11-10 and 11-11). Many species also show marked seasonal development in gonaducts, accessory glands, and secondary sexual characters that are presumed to be under androgenic control. For example, testosterone induces formation of **nuptial tubercles** on the heads of males in some species (Figure 11-12).

Spermatogenesis in the bony fishes is of the cystic type (Figures 11-3 and 11-13). The testes of more ancient groups (i.e., coelacanths, chondrosteans, salmonids, cyprinids) consist of anastomosing tubules, whereas the neoteleosts (e.g., killifishes, bass, cichlids, sunfishes, labrids) exhibit a lobular organization. Nests of spermatogonia proliferate from germ cells along the tubules or at the ends of the lobules located near the testicular surface. Gonadotropins (FSH and LH) stimulate production of a number of paracrine regulators that control various steps in the formation of sperm (Figure 11-14). Production of new spermatogonia for each breeding season is stimulated by FSH and estradiol acting through the Sertoli cell. Production of androgens by Leydig cells is stimulated by LH, and these androgens cause secretion of paracrine regulators by the Sertoli cells that regulate spermatogonial proliferation. Induction of meiosis and spermiogenesis is regulated by **17,20β-dihydroxy-4-pregnen-3-one (17,20β-DHP)** secreted from the Sertoli cell.

Fertilization in most bony fishes is external and gonadal development is synchronized in males and females to spawning. Fertilized eggs may be released directly into the water, attached to vegetation, or reared in nests. Some species are mouth-brooders, which retain the eggs during development that takes place in the parent's mouth. Viviparous teleosts, like their distant elasmobranch relatives, produce spermatophores, employing secretions by the male gonaducts. These males often have an androgen-dependent modified anal fin, called a **gonopodium**, to faciliate sperm transfer to the female.

B. Female Bony Fishes

The teleost ovary (Figure 11-15) has been studied in considerable detail with respect to gonadal differentiation, oogenesis, vitellogenesis, and ovulation, both in oviparous and viviparous species. The ovary of most teleosts is hollow, whereas solid ovaries have been reported in most lungfishes and chondrostean fishes. A few teleosts also have solid ovaries. Unlike the hollow ovary of elasmobranchs and amphibians, the teleost ovarian cavity is lined with germinal epithelium. Each hollow ovary is continuous, with an oviduct that is not homologous to the müllerian duct-derived oviducts of other vertebrates. Eggs are discharged from the ovary directly into the oviduct. In species with solid ovaries, the eggs are discharged into the body cavity from which they pass to the exterior via oviducts or directly through temporary openings in the body wall. Teleost GnRH-1 or LH induces ovulation in teleosts. Viviparous species acquire sperm from males and retain their eggs that are fertilized within the hollow ovary, where they develop prior to birth.

Basically, the teleost ovary consists of masses of follicles embedded in a rather sparse stroma. Development of mature eggs follows a set sequence of stages (Figure 11-16). Each follicle begins as a single-layered epithelium derived from the germinal epithelium and surrounding an oocyte. As the

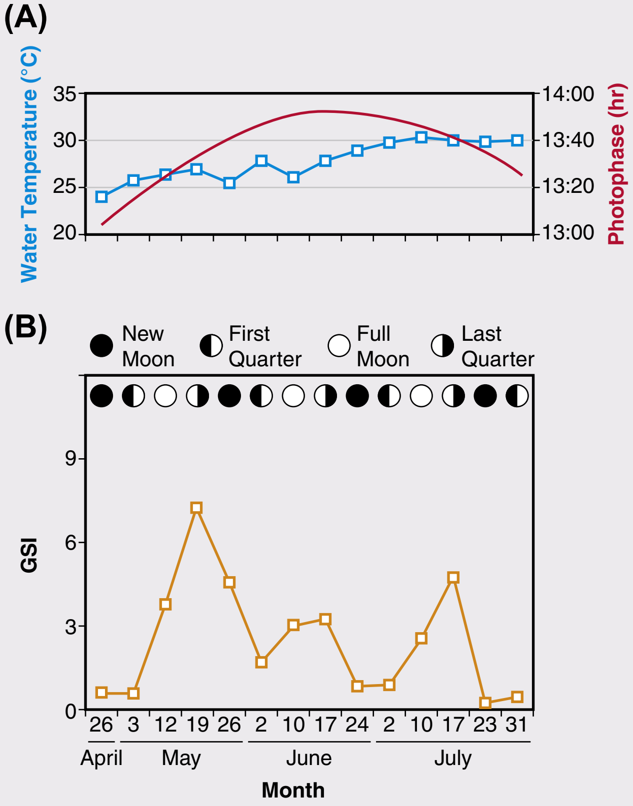
BOX 11B Lunar Cycles and Teleost Reproduction

Rabbitfishes are widespread tropical reef fishes found in the Indo-Pacific, the Red Sea, and the eastern Mediterranean. Photoperiod and temperature provide relatively little variation as environmental clues, and many species of fishes spawn in synchrony with the lunar cycle. Reproduction of many estuarine fishes, such as members of the genus *Fundulus* are linked to lunar cycles. The rotation of the moon around the Earth requires approximately 28 days (called the *sidereal lunar month*, which is 27.32 days in length). Another lunar cycle of about 14 days refers to the interval between the moon's crossing of the equator. The semilunar or tidal cycle is also approximately two weeks long and is a consequence of the relationship of the gravitational influences of the moon, the Earth, and the sun. This cycle is accompanied by continual changes in water level and currents as well as by changes in weak electromagnetic fields.

The foxtail rabbitfish (*Siganus argenteus*), near Japan, shows peak gonadal development from May through July with spawning occurring during the full moon (Box Figure 11B-1). Variations in plasma hormone levels are provided in Box Figure 11B-2. Other rabbitfish species (e.g., the golden rabbitfish, *Siganus guttatus*) in the same area show similar peak cycles but the months of the peaks may vary in different latitudes. For example, *S. argenteus* in the Philippines spawns from February to September. The observation that the golden rabbitfish will spawn all year long in captivity if fed an adequate diet suggests that variations in food availability may influence the reproductive period of these fishes in nature.

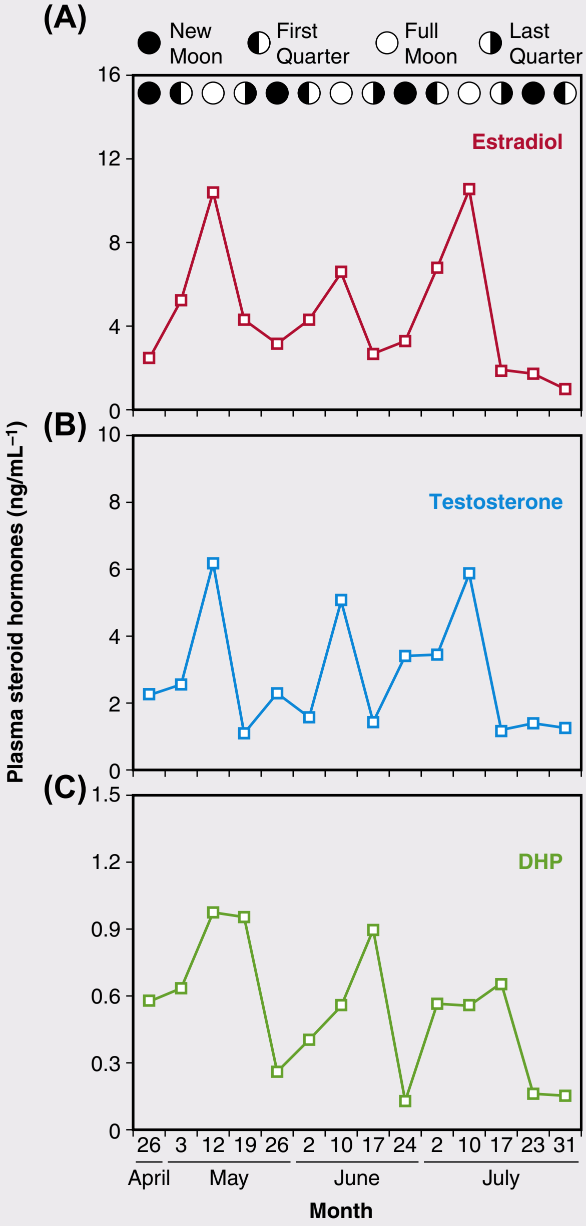
Some tropical species in lakes (e.g., Lake Tanganyika in Africa) that show no tidal influences also spawn during a full moon. Captive *Fundulus* spp. and rabbitfishes also exhibit lunar periodicity in the absence of any tidal influence. These observations suggest that detection of variations in the weak electromagnetic field correlate to tides, as shown for beach crabs transported inland hundreds of miles from the seashore. After a period of adjustment, these crabs showed tidal-related behaviors as if the ocean had moved inland to Indiana.

In some freshwater and brackish water fishes, the reproductive cycles are tuned to the wet and dry seasons. Periodic flooding and drying cause marked changes in water availability and also influence salinity and chemical composition of the aquatic environment. During the dry season, the entire habitat of the South American annual killifish (*Austrofundulus limnaeus*) dries up and all adults die. However, the last eggs produced by these fish enter a state of diapause and survive in a desiccated state until the next rainy season. When the temporary ponds fill with water, the eggs hatch and the young grow rapidly, mature, reproduce repeatedly, and die when the ponds dry again.



BOX FIGURE 11B-1 **Photoperiod, water temperature, and lunar phase related to gonad development in the foxtail rabbitfish (*Siganus argenteus*).** Note that the GSI (gonadosomatic index) decreases dramatically due to spawning under the full moon. *(Adapted with permission from Takemura, A. et al.,* Fish and Fisheries, *5, 317–328, 2004.)*

BOX 11B Lunar Cycles and Teleost Reproduction—cont'd



BOX FIGURE 11B-2 **Variations in plasma steroid levels in female foxtail rabbitfish (*Siganus argenteus*) with phases of the moon.** Note that all three steroids peak with the full moon. *(Adapted with permission from Takemura, A. et al.,* Fish and Fisheries, ***5****, 317–328, 2004.)*

follicle grows, these epithelial cells undergo hyperplasia and hypertrophy to form the granulosa. Connective elements in the stroma near the follicular nest will differentiate into a theca, which may further differentiate into a theca externa and a theca interna. The thecal and granulosa cells cooperate in the synthesis of androgens and estrogens, respectively, as described for mammals (Figure 11-17). The granulosa cells also are responsible for yolk deposition in the oocyte during follicular growth and resorption of yolk during atresia. In salmonids, receptors that will bind FSH are found on both thecal and granulosa cells, and FSH is responsible for synthesis of estrogens during follicular growth. The LH receptors occur only on granulosa cells, where they are associated with synthesis of 17,20β-DHP (sometimes called the **maturation promoting factor, MPF**) that is responsible for final oocyte maturation and ovulation in many species

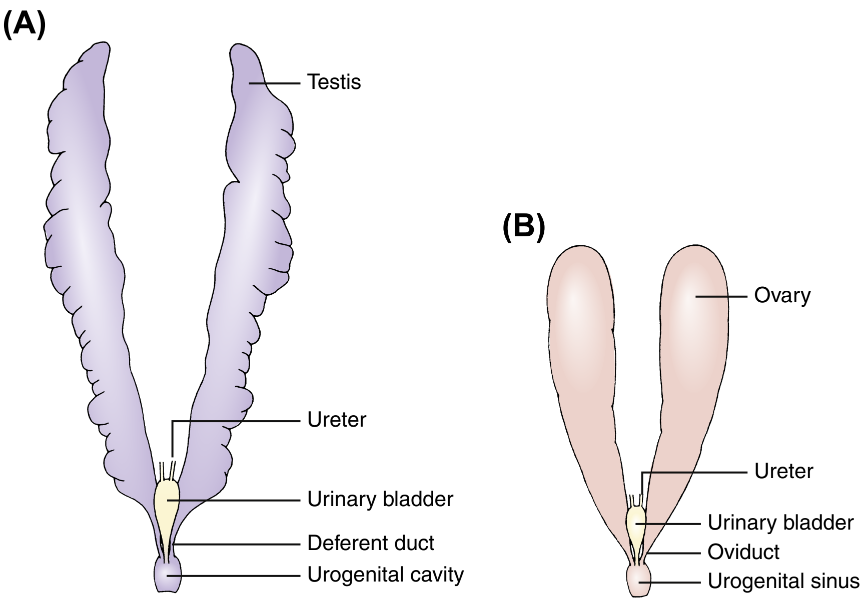


FIGURE 11-10 Reproductive system of teleost fishes as exemplified by the carp *Cyprinus carpio*. (A) Male. (B) Female. Note the absence of the elaborate system of ducts seen in chondrichthyeans. *(Adapted with permission from Matsumoto, A. and Ishii, S., "Atlas of Endocrine Organs: Vertebrates and Invertebrates," Springer-Verlag, Berlin, 1992.)*

(Figure 11-18). A related derivative of progesterone, **17,20β,21-trihydroxy-4-pregnen-3-one (17,20β,21-THP)**, is responsible for these events in some species. A corticosteroid, **deoxycorticosterone (DOC)**, is responsible for ovulation and final oocyte maturation in an Indian catfish, *Heteropneustes fossilis*.

Three developmental patterns for ovaries can be identified in teleosts. In the **synchronous ovary**, all oocytes are in the same stage of development. Species with a synchronous ovary are semelparous (for example, *Anguilla* spp. and most members of the genus *Oncorhynchus*). Species such as rainbow trout, white sucker, and flounder have a **group-synchronous ovary** with at least two populations of oocytes. These iteroparous species generally spawn once each year during a short breeding season. The last type is the **asynchronous ovary**, which has oocytes in all stages of development at all times during the breeding season. These species spawn frequently each year during a prolonged breeding season. Tropical species may be continuous breeders or may be tuned to wet and dry cycles.

Teleost ovarian tissue synthesizes testosterone, estrogens and DOC. These steroids have been identified in the peripheral plasma of females from several teleost species (Figures 11-19 and 11-20). The levels of testosterone often are greater in prespawning females than they are in males, suggesting a behavioral role for androgens in females as reported for some female mammals (e.g., hyenas, European moles of the genus *Talpa*).

All groups of bony fishes develop preovulatory, secretory corpora atretica as a result of atresia of developing follicles and develop short-lived corpora lutea following ovulation. However, a convincing endocrine function for corpora lutea is not yet established.

Vitellogenesis by the liver is stimulated by estradiol, and consequently total plasma calcium and phosphoprotein levels usually are elevated during oogenesis (Table 11-4). Calcium and vitellogenins are sequestered by the granulosa cells and are transferred to growing oocytes where they are transformed into yolk proteins (Figure 11-3).

C. Reproductive Behavior in Bony Fishes

Many aspects of reproductive behavior have been studied in teleosts, including migration to breeding sites, courtship, nest building, spawning, copulation, and parental care. Most of this work has concentrated on roles of testis, testosterone, and synthetic androgens in males. Male behaviors are regulated by 11-KT and hence do not appear in females that rarely produce 11-KT; however, elevated testosterone is correlated with aggressive behavior in the females of some species. Castration of males blocks breeding behavior and causes reversal to the non-breeding condition of androgen-dependent characters.

1. Breeding Behaviors in Teleosts

Male fishes often defend territory or females against other males or females through aggressive **agonistic (combative) behavior**. Androgens are responsible for agonistic behavior in some species. Additionally, dominant fish that hold territory tend to have higher androgen levels and lower cortisol levels than subordinate fish. Castrating male three-spined sticklebacks (*Gasterosteus aculeatus*) eliminates nest building one week later. Agonistic behavior remains high for three or four weeks if castration is performed less than one week within nest building. In some

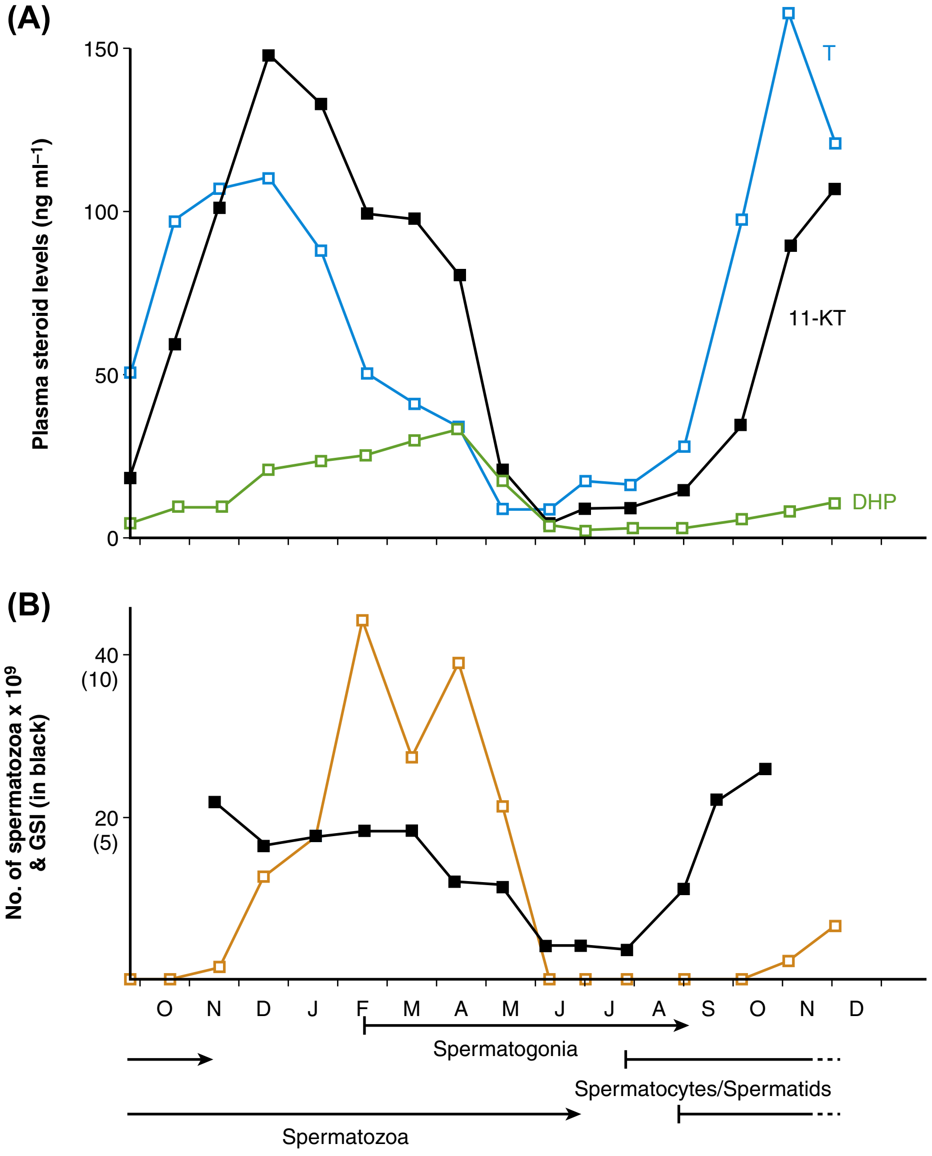


FIGURE 11-11 **Annual reproductive cycle of male rainbow trout, *Oncorhynchus mykiss*.** The top panel depicts plasma levels of testosterone, T; 11-ketotestosterone, 11-KT; and 17,20β-dihydroxy-4-pregnen-3-one, DHP. The lower panel shows the volume of sperm produced (yellow line) and the gonadosomatic index (GSI, gonad weight related to body weight; black line). *(Adapted with permission from Scott, A.P., in "Fundamentals of Comparative Endocrinology" (I. Chester-Jones, P.M. Ingelton, and J.G. Phillips, Eds.), Plenum Press, New York, 1987, pp. 223–256.)*

species, castration does not result in a decrease in agonistic behavior, and androgens may not be required to maintain the behavior once it has been induced in these fishes.

Males sometimes exhibit alternative reproductive tactics, and a population may consist of more than one male phenotype. For example, in some species there are "sneaker" males who live at the edges of a dominant male's territory awaiting a chance to dart in and fertilize some eggs of attendant females. Sneakers are often younger and smaller as compared to the territorial male that controls the nesting site and attracts the females. In some cases, the sneaker male may closely resemble a female and even exhibit female-like behaviors to fool the territorial male. Although androgen levels generally are greater in dominant male teleosts versus subordinate males, levels of testosterone, 11-KT and 17,20β-DHP in smaller sneaker coho salmon "jacks" did not differ from those of larger, hook-nosed males that defended the spawning nest.

The roles of estrogens and androgens in relation to the breeding behaviors of females have been studied less than the roles of androgens in males. Castration of females may result in complete abolition of all reproductive behaviors, loss of only some, or only a decrease in intensity. In one case, ovariectomized *G. aculeatus*, form *leiurus*, show more aggressive behavior than intact females, implying that ovarian steroids normally repress aggressive behavior in females. Estrogens have proven ineffective for inducing female behavior in females. Possible roles for androgens have not been studied, but the relatively high level of plasma androgens in prespawning females is suggestive of a behavioral role.

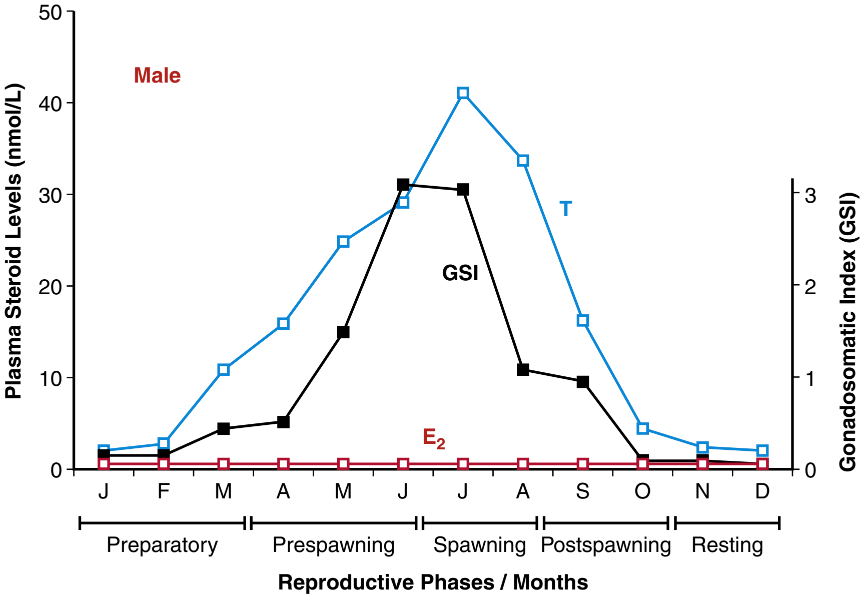


FIGURE 11-12 **Seasonal reproductive cycle in Indian major carp (*Labeo rohita*).** Testosterone (T) levels are highly correlated with growth of the testes (gonadosomatic index, GSI). Estradiol (E_2) levels are minimal in this species. *(Adapted with permission from Suresh, D.V.N.S. et al.,* General and Comparative Endocrinology*, **159**, 143–149, 2008.)*

FIGURE 11-13 **Nuptial tubercles on the snout of a minnow.** These androgen-dependent secondary sexual characters are found on a number of cyprinid teleosts.

Recognition of conspecific (belonging to the same species) genders and/or breeding conditions may occur by behavioral displays, coloration patterns, special adornments, electrical signals, or chemical signals (pheromones). All of these may have endocrine mechanisms of production and/or determination by the receiver.

Spawning behavior in females appears to be under the control of GnRH and GTHs, although in *F. heteroclitus* neurohypophysial preparations or synthetic oxytocin induce reflexive spawning movements in hypophysectomized or castrated females (see Chapter 5). This spawning reflex is a behavior not dependent upon shedding of ova. Similar observations have been reported for a few additional species. Spawning behavior in female goldfish is stimulated by ovarian prostaglandins.

2. Parental Behaviors

Many teleosts exhibit extended care of eggs in a nest prior to hatching and may even protect young hatchlings from predators. Mouth-brooding species retain the fertilized eggs in their oral cavities, and young hatchlings may continue to seek refuge there when threatened. Relatively little is known about the endocrine regulation of these behaviors. Mammalian PRL influences certain aspects of parental behavior in fishes. Fanning behavior associated with aeration of the eggs can be stimulated in *Symphysodon aequifasciata* and *Pterophyllum scalars* by PRL treatment; however, similar treatment inhibits fanning behavior in sticklebacks. PRL stimulates secretion of mucus that is fed to young *S. aequifasciata*, but it is not clear if the behavior of feeding the young also is PRL dependent in these parent fish.

3. Pheromones and Behavior in Teleosts

Communication by pheromones is important in the reproduction of teleosts. Prostaglandins (e.g., $PGF_{2\alpha}$), and a variety of free and conjugated steroids function as pheromones. Following ovulation and prior to oviposition, females of numerous oviparous species emit pheromones that attract and arouse sexual activity in males. For example, female *Bathygobius soporator* secrete a pheromone from the ovary that elicits courtship behavior by

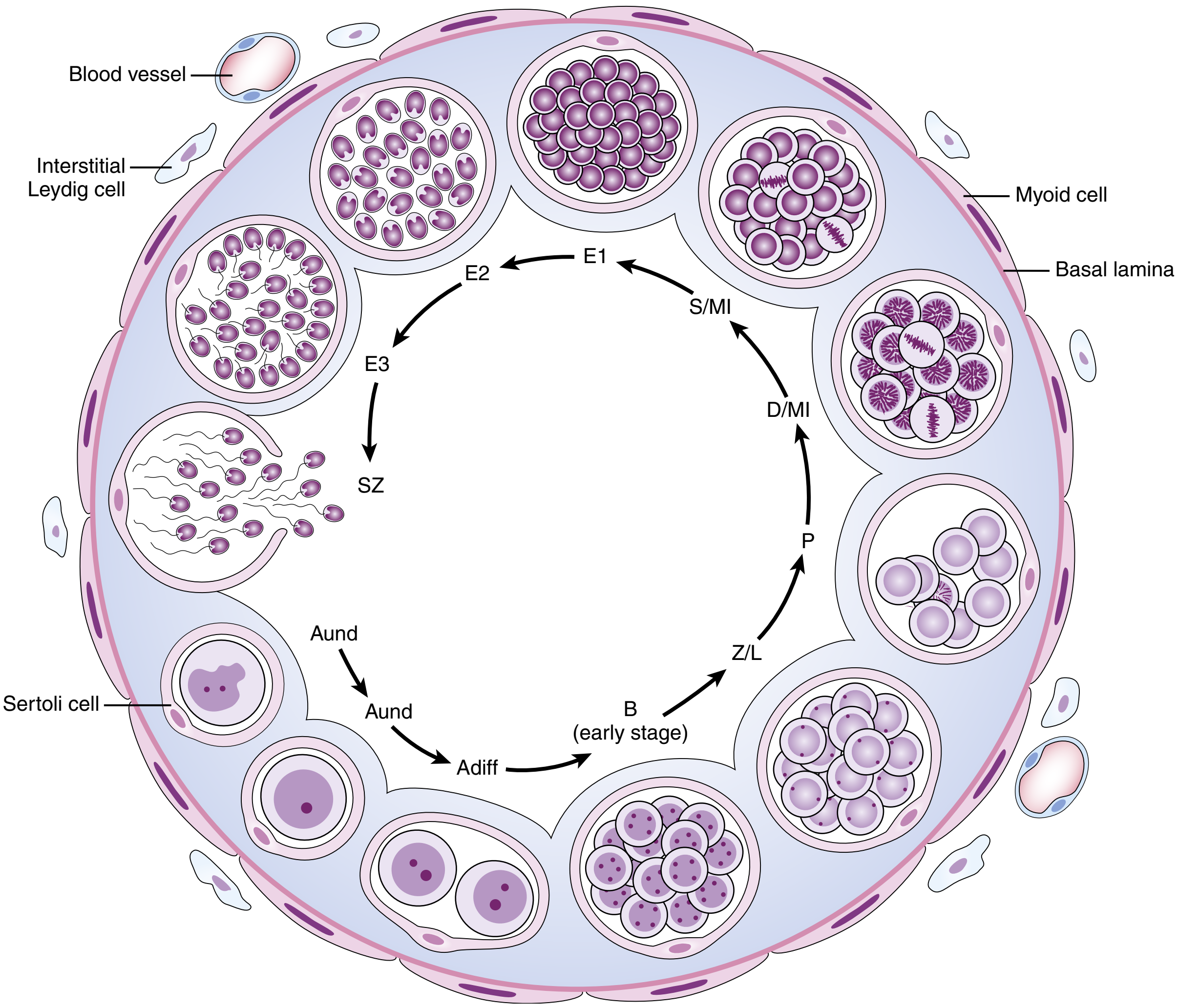


FIGURE 11-14 **Stages in teleost spermatogenesis.** Schematic depiction of spermatogenesis in a seminiferous tubule from zebrafish (*Danio rerio*, Cyprinidae, Cypriniformes) testes. Progression of spermatogenesis is depicted flowing from the lower left corner around to the upper left corner. Abbreviations: Adiff, type A differentiated spermatogonia; Aund, type A undifferentiated spermatogonia (potentially a stem cell); B (early−late), type B spermatogonia; D/MI, diplotene spermatocytes/metaphase I; E1, early spermatids; E2, intermediate spermatids; E3, final spermatids; S/MI, secondary spermatocytes/metaphase I; SZ, sperm; Z/L, leptotene/zygotene primary spermatocytes. *(Adapted with permission from Schulz, R.W. et al.,* General and Comparative Endocrinology, ***165****, 390−411, 2010.)*

intact males but not by anosmic males (treated to prevent olfactory detection). A priming pheromone released in the urine of female salmonids increases production of 17,20β-DHP in recipient males that in turn causes spermiation. The chemical nature of this pheromone is not known. Parental behavior is stimulated in *Heterochromis bimaculatus* by chemicals secreted by the young. Several species recognize their own young by using olfactory cues, and the offspring of some species use chemical recognition to identify their parents.

The goldfish (Figure 11-21) has become a model system for investigations of pheromonal communication in teleost reproduction primarily due to the pioneering discoveries by Norman Stacey at the University of Alberta and his collaborators. During the final stages of oocyte development, the ovaries synthesize a mixture of C_{21}-steroids including 17,20β-DHP and 17,20β,21-THP. These steroids not only induce final oocyte maturation but also act as pheromones in males to induce LH secretion, sperm release, and competence for spawning behavior. In addition, sulfated forms of these steroids are produced by goldfish ovaries and these too are potent stimulators of males. Recent evidence indicates that different regions of the male olfactory epithelium can detect 17,20β-DHP and distinguish selectively between 17,20β-DHP and its sulfated form, 17,20β-DHP-S.

In addition to releasing free and conjugated progestogens, goldfish ovaries also release considerable quantities of the androgen androstenedione that also plays a pheromonal role. Androstenedione release precedes release of

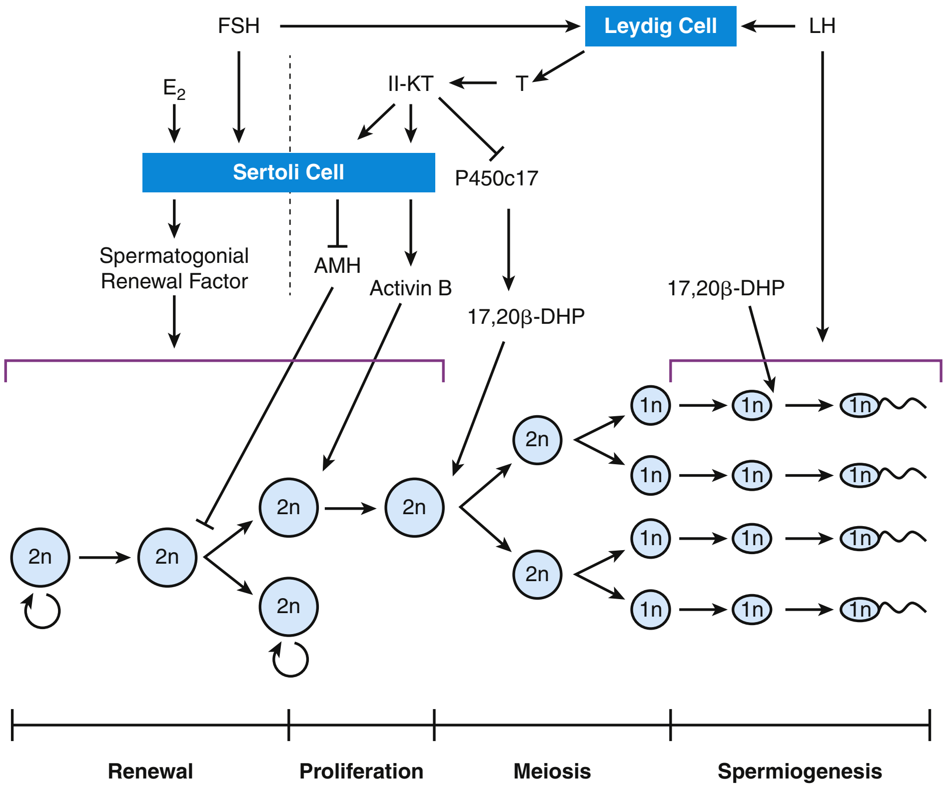


FIGURE 11-15 Gonadotropin control of paracrine production in the teleost testis. Note that some factors are inhibitory. Abbreviations: 2*n*, diploid number of chromosome; 1*n*, haploid number of chromosomes. See Appendix A for explanation of abbreviations. *(Adapted with permission from Knapp, R. and Carlisle, S.L., in "Hormones and Reproduction of Vertebrates. Vol. 1. Fishes" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 43–63.)*

17,20β-DHP and inhibits the responsiveness of males to 17,20β-DHP. This mechanism may prevent premature gamete release in males.

4. Migratory Behavior and Reproduction in Teleosts

The implication of hormones in migratory behavior is largely circumstantial. The gonads and their secretions probably do not play a causative role, since gonadal maturation often occurs during migration. Thyroid hormones in salmon and other species have been claimed to be causative factors of reproductive migratory behavior, and increased thyroid activity coincides with migration as does increased corticosteroid secretion as seen also in seaward migration of juvenile salmon (see Chapter 7, Figure 7-13). It is possible that the increased activity of the thyroid and adrenal glands is correlated to "permissive" effects of these hormones related to metabolism and osmoregulation. Levels of mRNAs for GH, PRL, and **somatolactin (SL)** are elevated during the marine phase of the spawning migration. Levels of GH mRNA decrease when the fish enter the estuaries (brackish water), and both PRL and SL decrease after the fish enter freshwater. These changes also may be related to osmoregulation. These hormones may only enhance the physiological states favorable to migration, whereas the behavioral changes may be controlled through actions of environmental factors such as photoperiod and temperature operating on the nervous system or possibly by endogenous rhythmic neural cycles that are regulated by these environmental factors.

V. REPRODUCTION IN AMPHIBIANS

Many amphibians are characterized as terrestrial or semiterrestrial adults with an aquatic larval form. Typically, eggs are laid in water where they hatch into a larval form. The importance of the aquatic intermediate larval stage is that it allows for an aquatic feeding stage where growth can be optimized without using energy stores or food resources of the terrestrial parents. During metamorphosis of these fish-like amphibian larvae to terrestrial-type tetrapods, the same problems are encountered and solved that confronted the evolution of terrestrial vertebrates from aquatic animals.

Although only a few of the almost 6000 known species of amphibians have been studied thoroughly, it is clear that there is a great diversity of reproductive patterns within this group of animals. Within the modern Amphibia, several trends in reproductive patterns are evident (Figure 11-22).

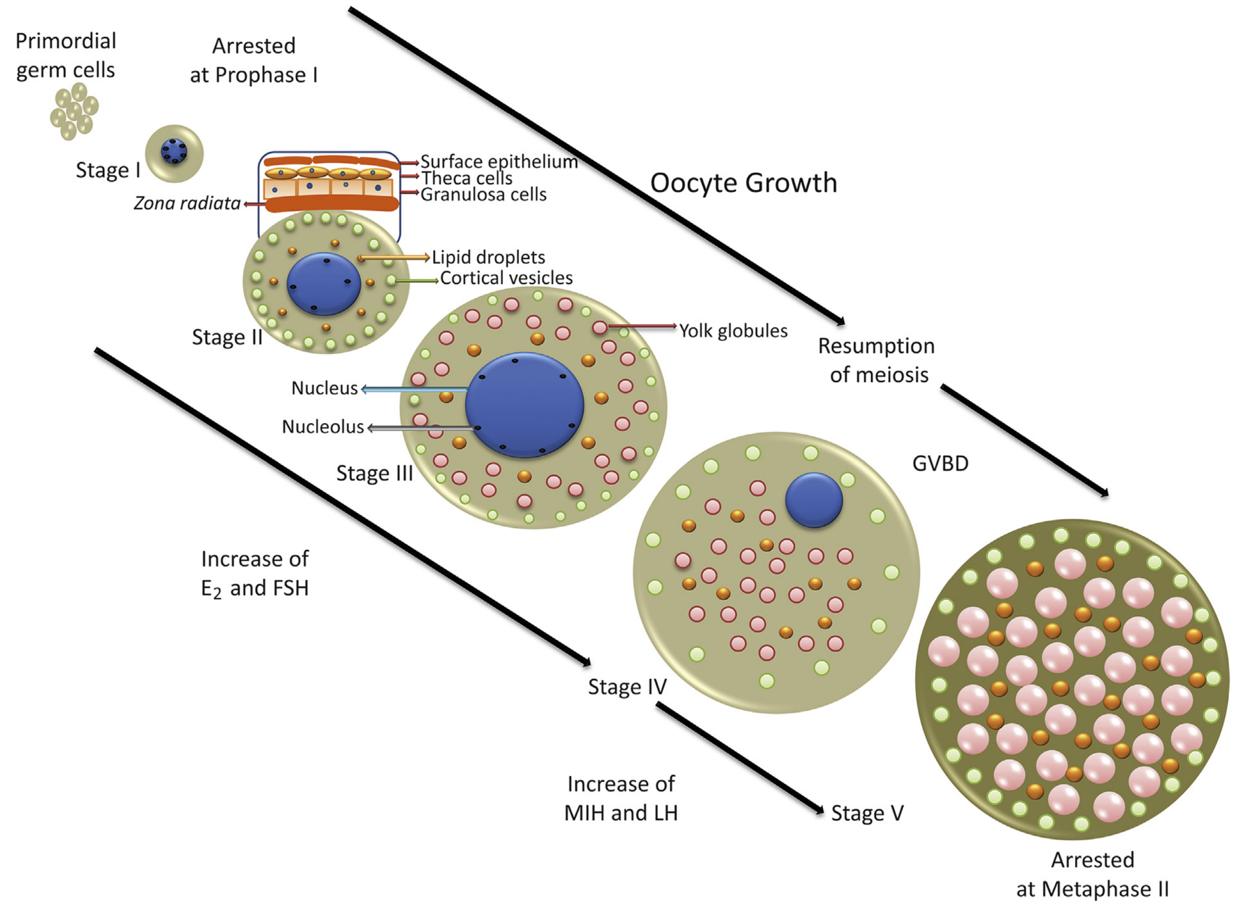


FIGURE 11-16 **Stages of follicle development in teleost oocytes.** The sequence of oocyte stages is as follows: stage I, primary growth; stage II, cortical alveoli growth period; stage III, early vitellogenic oocytes; stage IV, late vitellogenic phase; and stage V, mature/ovulated oocyte, full of yolk (with lipid and protein globules). Oocyte growth is controlled by 17β-estradiol (E_2) and follicle-stimulating hormone (FSH), whereas the resumption of meiosis is regulated by luteinizing hormone (LH) and maturation-inducing hormone (MIH). GVBD, germinal vesicle breakdown. *(Reprinted with permission from Urbatzka, R. et al., in "Hormones and Reproduction of Vertebrates. Vol. 1. Fishes" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 65–82.)*

All three extant orders (anurans, urodeles, and gymnophionids) show a reduction in the use of the aquatic habitat with a tendency toward terrestrial development. This trend is accompanied by greater reliance on internal fertilization, a reduction in clutch size (number of eggs produced per breeding), and development of simple parental care of eggs and young. Mate selection and courtship patterns have become very elaborate in some species, often related to these complex patterns. Finally, oviparity apparently has given rise to viviparity independently on several occasions in each group of anurans, urodeles, and gymnophionids.

Sexes are separate and natural hermaphroditism is rare, but not undocumented, in adult amphibians. Some amphibians may exhibit varying degrees of juvenile hermaphroditisim during development as the gonads mature. A period of juvenile hermaphroditisim has been reported in *Bufo americanus*, *Hyla versicolor*, and *Rana sphenocephala* raised under laboratory conditions. Amphibians exhibiting a **semi-differentiated** type of gonadal development (*Rana curtipes*, for example) may develop ovaries first regardless of their genetic sex followed by further differentiation into testes in genetic males. Amphibians exhibit both XX/XY and ZZ/ZW forms of genetic sex determination, although the sex chromosomes are not morphologically distinct. The gonads of most species develop through an indifferent or bipotential stage and differentiate in the male or female direction early in development (Figure 11-23).

Conspicuous masses of adipose tissue called **fat bodies** are located adjacent to the gonads of amphibians (see ahead). These are structures unique to amphibians. In female anurans and urodeles, the size of the fat body is correlated inversely with gonadal weight, and it has been proposed that the lipoidal substances stored in the fat bodies are utilized for oocyte growth. However, the size of the fat bodies also may be correlated with food availability. Fat bodies of both male and female European newts (*Triturus cristatus*) can synthesize steroids and therefore may influence gonadal function, accessory sex structures, or

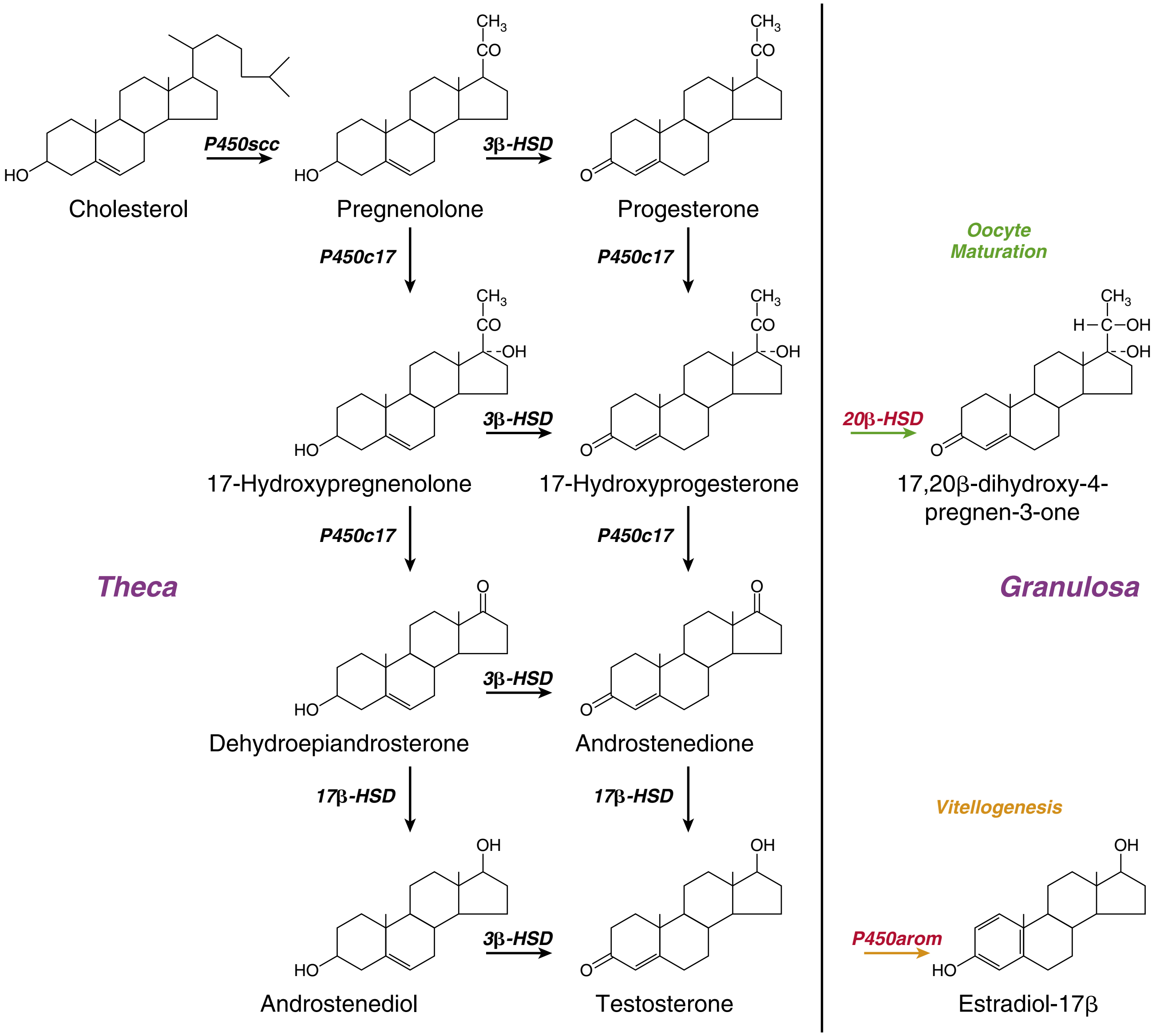


FIGURE 11-17 **Ovarian steroidogenesis in thecal and granulosa cells as occurs in salmonid fishes.** *(Adapted with permission from Lubzens, E. et al.,* General and Comparative Endocrinology, ***165****, 367–389, 2010.)*

both. In the European frog *Rana esculenta*, the steroidogenic function of fat bodies appears to be regulated by pituitary GTHs.

The male gonaducts function as both urinary ducts and as sperm ducts or vasa deferentia. Enlargement and differentiation of the wolffian ducts to the functional male condition is caused by either testosterone or DHT but is antagonized by estrogens. In marked contrast, although normal ovarian differentiation of müllerian ducts can be stimulated by estrogens, both DHT and testosterone are effective at stimulating müllerian duct enlargement in tiger salamanders (Figure 11-24) although only testosterone is effective in frogs.

Reproduction in amphibians is controlled by the hypothalamus and pituitary, acting in response to environmental cues. Administration of GnRH along with a dopamine antagonist induces spawning in a variety of frogs, suggesting an inhibitory role for dopamine as seen in teleost fishes. Treatment with GTHs alone is also effective at inducing spawning and other reproductive events by acting directly on the gonads.

A. Oviparity in Amphibians

Most anuran amphibians studied are oviparous with external fertilization, although internal fertilization occurs in several species. Breeding in oviparous species is tied closely to a seasonal cycle involving photoperiod, temperature, availability of moisture, or a combination of

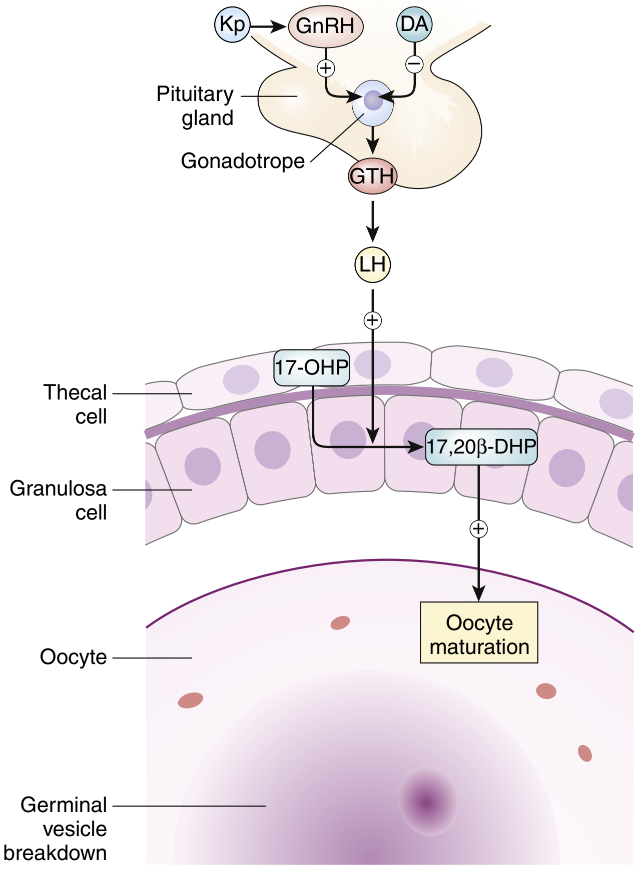


FIGURE 11-18 **Final oocyte maturation in teleosts.** LH stimulates production of 17α,20β-dihydroxyprogesterone (or a related progestogen) by follicle cells. This progestogen is also known as the maturation promoting factor (MPF). MPF causes a breakdown of the oocyte nucleus, the germinal vesicle. This process is called germinal vesicle breakdown (GVBD) and immediately precedes expulsion of the oocyte from the follicle. See text or Appendix A for explanation of abbreviations. *(Adapted with permission from Connaughton, M.A. and Aida, K., in "Encyclopedia of Reproduction, Vol. 2" (E. Knobil and J.D. Neill, Eds.), Elsevier, Amsterdam, 1999, pp. 193–204.)*

these, although a few species are continuous breeders (e.g., the Indian frogs *Rana tigrina* and *R. erytrea* and the South American toads *Bufo arenarum* and *B. paracmenis*). One predominant reproductive pattern is found in temperate oviparous anurans. Spermatogenesis and ovarian follicular development are completed in the fall, and the animals simply "hibernate" until suitable breeding conditions occur in the spring. Many oviparous species lay their eggs in temporary or permanent ponds with the eggs developing into free-swimming larvae. Tadpole larvae are the characteristic limbless, fish-like larval forms of anurans and differ markedly from the larvae of urodeles that possess external gills and four limbs at hatching. Anurans have internal gills like fishes and obtain their limbs later during metamorphosis. One anuran (*Ascaphus*) is known to lay its eggs in streams, and the tadpole larvae that result have special modifications to keep from being swept downstream. Some anuran and urodele species lay their eggs on land, usually in moist places such as under logs, in the axil of tree branches, or in temporary ponds held within the leaves of certain tropical plants. Terrestrial eggs require considerable parent care (see ahead). Terrestrial eggs that are heavily yolked often develop directly into miniature adults, and no aquatic larval stage exists except as a transitional state within the egg.

Oviparous urodeles exhibit several reproductive patterns. In some species (e.g., *T. cristatus*, *Notophthalmus viridescens*), the pattern is similar to that of oviparous anurans. In the hellbender *Cryptobranchus alleganiensis*, spermatogenesis occurs in July shortly before breeding in August and September. Other species such as the mudpuppy (*Necturus* spp.) transfer sperm to the females in the fall, and oviposition and fertilization occur the next spring when males are not present. A number of oviparous gymnophionids have been described, all of which lay terrestrial eggs. In *Ichthyophis*, the eggs are laid in a burrow near a stream, and each newly hatched larva must emerge from the burrow and find its way to the stream. Gymnophionids generally produce larger eggs than do the other amphibian groups, and clutch sizes are proportionally small.

B. Viviparity in Amphibians

Two European land salamanders, *Salamandra salamandra* and *S. atra*, give birth to live offspring that develop in the posterior portion of the oviducts. In *S. atra*, one young develops and undergoes metamorphosis in each oviduct during a 4-year gestation period. Gestation is shorter in *S. salamandra*, which gives birth to larval salamanders. The large size of these offspring indicates that considerable nutrient contributions are made by the mother during these prolonged gestation periods. Details of these contributions and the mechanisms for their transfer have not been reported.

Viviparity in anurans typically involves modifications that allow the eggs to develop into tadpoles on the body of the maternal animal. A fold of skin may develop that completely covers the eggs in a pouch such as that found in the so-called marsupial frogs, *Gastrotheca* spp., of South America. In others, such as the African frog *Pipa pipa*, each egg develops in its own dermal chamber that forms on the back of the parent. Oviductal incubation of eggs occurs in *Nectophrynoides* and *Eleutherodactylus*. One anuran incubates its young in its vocal sacs and at least one Australian species broods its young in its gut. Although this latter species was discovered very recently, it is now believed to be extinct. The South American tree frogs carry their eggs in a single mass on their back.

It is estimated that viviparity occurs in the majority of gymnophionid species. The contribution of maternal

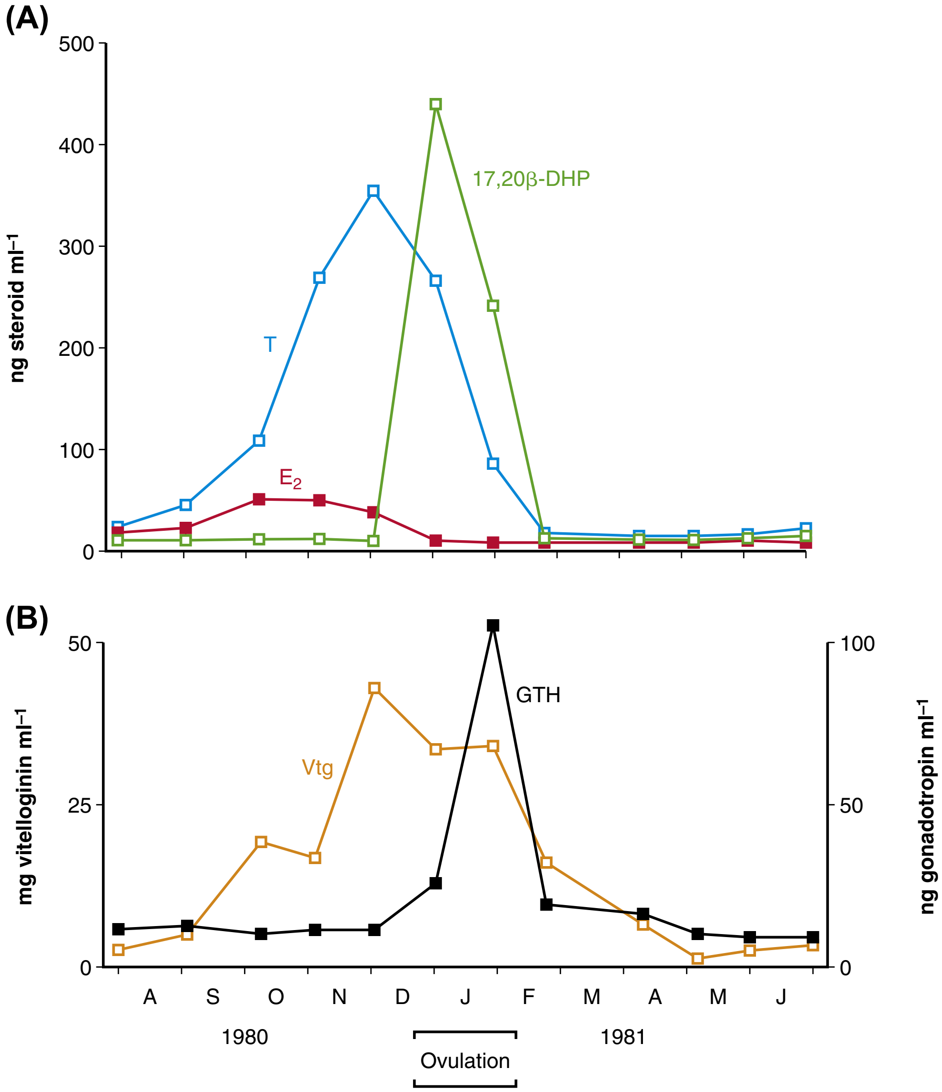


FIGURE 11-19 **Annual reproductive cycle of female rainbow trout (*Oncorhynchus mykiss*).** The top panel shows plasma levels of testosterone, T; estradiol, E_2; and 17,20β-dihydroxy-4-pregnen-3-one, DHP. Plasma levels of vitellogenin, Vtg and gonadotropin, GTH appear in the lower panel. *(Adapted with permission from Scott, A.P. and Sumpter, J.P.,* General and Comparative Endocrinology*, **52**, 79–85, 1983.)*

energy through oviductal secretion to support the developing young also is considerable. In *Typhlonectes*, one female may give birth to as many as nine larvae, each of which weighs about 40% of the mother's body weight at birth.

C. Reproduction in Male Amphibians

Male anurans and urodeles secrete both testosterone and **dihydrotestosterone (DHT)** in response to LH. Additionally, urodeles may produce some 11-KT. Circulating androgens, FSH, and LH vary seasonally, but estradiol levels are very low. Highest values for reproductive hormones occur during mating in some species (e.g., *Rana catesbeiana*) but not in others (*Rana esculenta*, *Ambystoma tigrinum*) where gametogenesis is dissociated from time of spawning and development of accessory structures is dependent on steroids (See Figure 11-1).

1. Male Urodeles

The reproductive system of a male urodele is provided in Figure 11-25. Spermatogenesis is of the cystic type in urodeles, and testicular structure and function are very similar to those of fishes. The urodele testis consists of one or more lobes, each containing several ampullae, which in turn are comprised of several germinal cysts (Figure 11-25). Germ cells associated with a germinal cyst divide mitotically to produce a cluster of secondary spermatogonia. These cells undergo synchronous differentiation to primary spermatocytes and enter meiosis. All

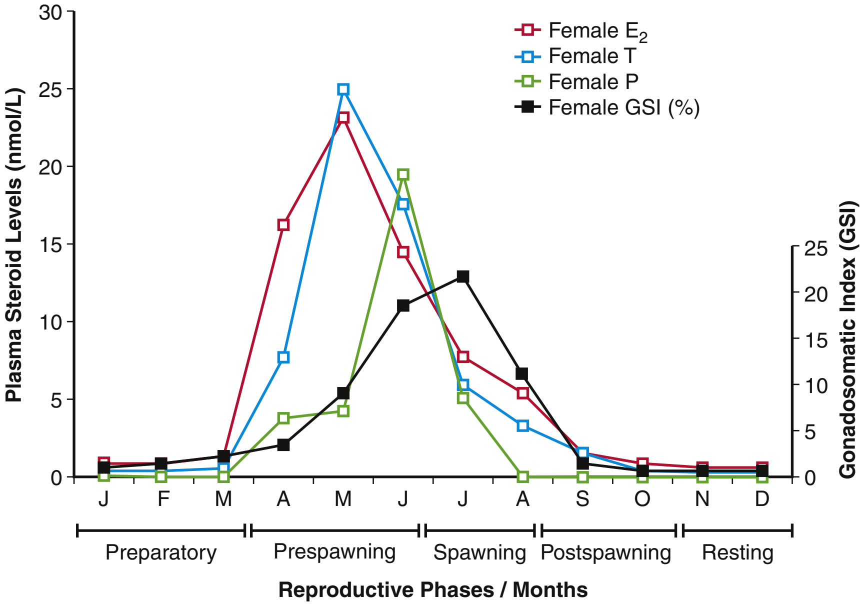


FIGURE 11-20 **Plasma steroid levels and gonadosomatic index (GSI) of female Indian major carp (*Labeo rohita*) during the five phases of the reproductive cycle.** E_2, estradiol; T, testosterone; P, progesterone. *(Adapted with permission from Suresh, D.V.N.S. et al.,* General and Comparative Endocrinology*, **159**, 143–149, 2008.)*

TABLE 11-4 Reproductive State and Serum Calcium Levels during the Spring in Steelhead Trout (*Oncorhynchus mykiss*) from a Natural Population

Sexual state	*N*	Mean body weight (g)	Mean serum calcium (mg/dL ± SE)
Immature males and females	12	38.4	11.6 ± 0.43
Sexually mature males prior to spawning	11	213.6	11.5 ± 0.74
Sexually mature females after ovulation but prior to spawning	9	204.9	15.5 ± 1.44[a]

[a] *Sexually mature female trout differ significantly ($p < 0.01$) from sexuantly mature males and immature trout.*

of the cysts within an ampulla develop synchronously, although it is typically only the more posterior ampullae that exhibit spermatogenesis prior to a given breeding season. The other ampullae represent the source of sperm for future breeding seasons. Sertoli cells develop from fibroblasts in the cyst walls while the spermatogonial divisions are taking place. As the ampullae mature, the posterior portion of the testis becomes swollen with sperm, whereas ampullae of the most anterior portion consist primarily of spermatogonia. The posterior portion of the testis becomes dense and whitish because of masses of sperm. After spermiation occurs the collapsed ampullae that have discharged their sperm into the male ducts are resorbed, and after breeding, spermatogenesis is initiated in the anterior portion of the testis. If spermiation occurs in the fall, spermatogenesis will not be resumed until the next summer. New ampullae differentiate from connective tissue elements and germ cells in the tunica albuginea.

Androgen levels exhibit distinct seasonal cycles in urodeles (Figure 11-26, Table 11-5). Seasonal patterns of androgen secretion have been reported for *Taricha granulosa*, *Cynops pyrrhogaster*, and *A. tigrinum*. Androgen levels appear to be low during breeding, and high levels are more closely associated with development and maintenance of the vas deferentia that store sperm until breeding. Androgens also stimulate development of cloacal glands in males that are associated with spermatophore production and possibly with production of pheromones used in breeding. Contraction of the vasa deferentia and discharge of sperm during mating and spermatophore production are caused by AVT.

Androgens also stimulate development of sex accessory structures called **nuptial pads** in the newt *Notophthalmus viridescens*, but maximal development is obtained by simultaneous treatment with PRL and androgen. Development of skin glands also is influenced by androgens. In urodeles such as *N. viridescens*, *T. cristatus*, and *A. tigrinum*,

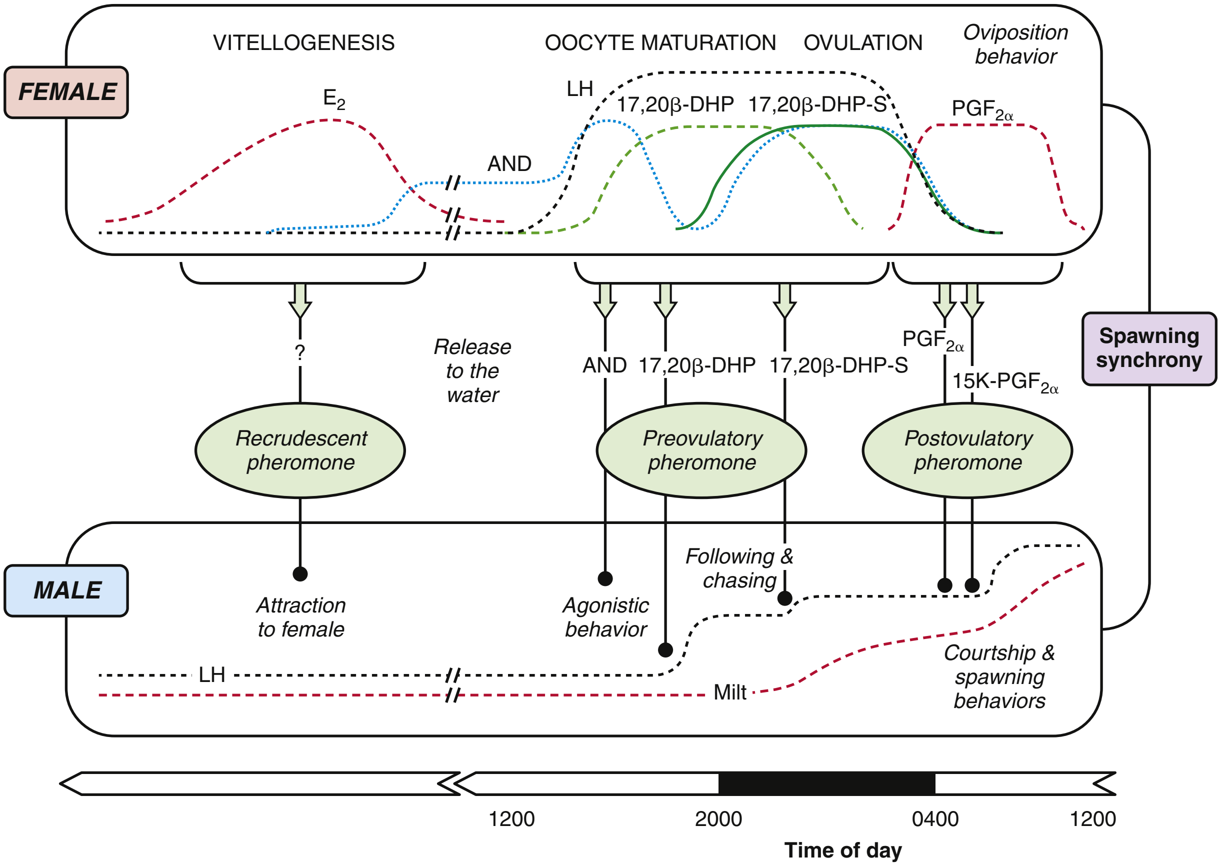


FIGURE 11-21 **Model of action for reproductive pheromones in the goldfish (*Carassius auratus*).** Luteinizing hormone (LH) is involved in production of the female pheromones and influences the responsivity of the male. Abbreviations: AND, androstenedione; PGF$_{2\alpha}$, prostaglandin; 15K-PGF$_{2\alpha}$ 15-keto metabolite of PGF$_{2\alpha}$ 17,20β-P, 4-pregnen-17,20β-dihydroxy-3-one; Milt = sperm. *(Adapted with permission from Stacey, N., in "Hormones and Reproduction of Vertebrates. Vol. 1. Fishes" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 169—192.)*

PRL is known to influence the movement of land-phase animals to water for breeding and also induces heightening of the tail fin, which is a male secondary sex character in some species.

Internal fertilization in both aquatic and terrestrial urodeles occurs through transfer of a spermatophore from the male to the female. The spermatophore is produced through the actions of an array of specialized cloacal glands. The spermatophore consists of a glycoprotein matrix to which a packet of coagulated sperm is attached. The glycoprotein matrix acts as a base upon which the sperm packet rests. Following a complex courtship procedure, the female is induced to pick off the sperm packet using her cloacal lips. The sperm packet may then be stored in a specialized portion of the female's spermatotheca (Figure 11-27) until ovulation and fertilization occur.

2. Male Anurans

The reproductive systems of male frogs and toads are provided in (Figure 11-28). The anuran testis is often described as consisting of seminiferous tubules with a permanent germinal epithelium and conspicuous Leydig cells. However, spermatogenesis clearly is of the cystic pattern as described for all other anamniotes (Figures 11-3 and 11-29). The Leydig cells resemble mammalian Leydig cells ultrastructurally and possess 3β-HSD activity. The lipid cycle within the Leydig cells and the degree of 3β-HSD activity closely parallel the development of androgen-dependent sex accessory structures such as the enlarged thumb pads of ranid frogs. Leydig cells of post-spawning anurans exhibit considerable lipoidal accumulation but very low 3β-HSD activity, and thumb pads regress in male ranids at this time.

During winter months, Sertoli cells in testes of ranid frogs lack lipid, but these cells elongate and exhibit small lipoidal granules as the breeding season approaches. Sertoli cells of breeding animals have a well-developed smooth endoplasmic reticulum, and 3β-HSD activity is detectable. After spermiation the Sertoli cells detach from the tubule wall and degenerate. New cells for the next reproductive period differentiate from fibroblasts in the tubule walls.

Among male bufonid toads are found rudimentary ovaries or **Bidder's organs** (Figure 11-28) that develop

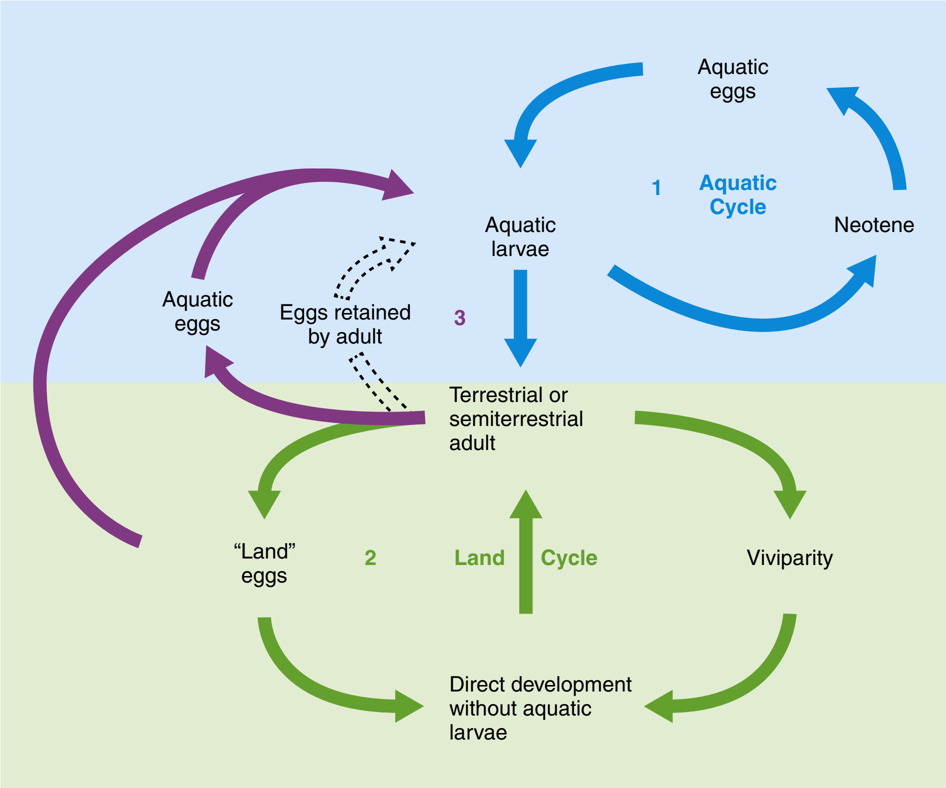


FIGURE 11-22 **Summary of amphibian life history patterns.** Three basic patterns are found in amphibians: (1) a totally aquatic cycle with sexually mature larvae (e.g., neotenes; see text), (2) a totally terrestrial or land cycle, (3) an aquatic—land pattern with terrestrial or semiterrestrial adults and aquatic larval stages. Within these patterns are some distinct variations such as viviparity versus laying an egg on land within the land cycle.

from cortical remnants of the embryonic genital ridge prior to normal gonadal differentiation. Histologically, the Bidder's organ consists of a compact mass of small oocytes. In males, the bidderian oocytes undergo a limited seasonal growth and degeneration cycle correlated with the testicular cycle (Figure 11-30). These bidderian oocytes never reach the vitellogenic stage in males, however. The presence of 3β-HSD activity suggests they are steroidogenic during this time. After castration of male bufonids, the Bidder's organ hypertrophies, presumably under the influence of increased GTHs, and forms a functional ovary. In castrated males, the rudimentary müllerian ducts may develop into functional oviducts, and such sex-reversed animals may breed as females. The presence of isolated oocytes in the testes of bufonids is a common condition in at least one species, *Bufo woodhousei*. Female bufonids also exhibit a Bidder's organ but it is assimilated into the ovary and bidderian oocytes are not distinguishable from ovarian oocytes.

3. Male Gymnophionids (Caecilians)

Male caecilians differ from urodeles and almost all anurans by possessing an elaborate intromittent organ, the **phallodeum**, associated with the posterior part of the cloaca. The pallodeum is employed for transferring sperm to the female reproductive tract; consequently, fertilization is internal in all caecilians. Another unique feature is the retention of only the most posterior portion of the müllerian ducts in males as the **müllerian glands**. These tubular apocrine glands are believed to produce seminal fluid and hence would be analogous to the prostate of mammals. The structure of the lobed gymnophionid testes is similar to that of urodeles, but the cell nests within an ampulla are not synchronized and do not exhibit the same stage of spermatogenesis.

D. Reproduction in Female Amphibians

1. Female Anurans and Urodeles

The reproductive systems of female urodeles and anurans are depicted in Figures 11-31 and 11-32, respectively. Amphibian ovaries are hollow, sac-like structures derived from the embryonic cortex and covered by germinal epithelium. A derivative of embryonic medullary tissues forms the inner lining of the ovary. Oogonia are present in the germinal epithelium, and they give rise to nests of oocytes. The follicular epithelium is derived from the germinal epithelium and consists of a single layer of granulosa cells throughout the maturation period. A very thin thecal layer does form around the follicle, but it is only obvious when viewed with the aid of the electron microscope (compare Figures 11-33 and 11-34).

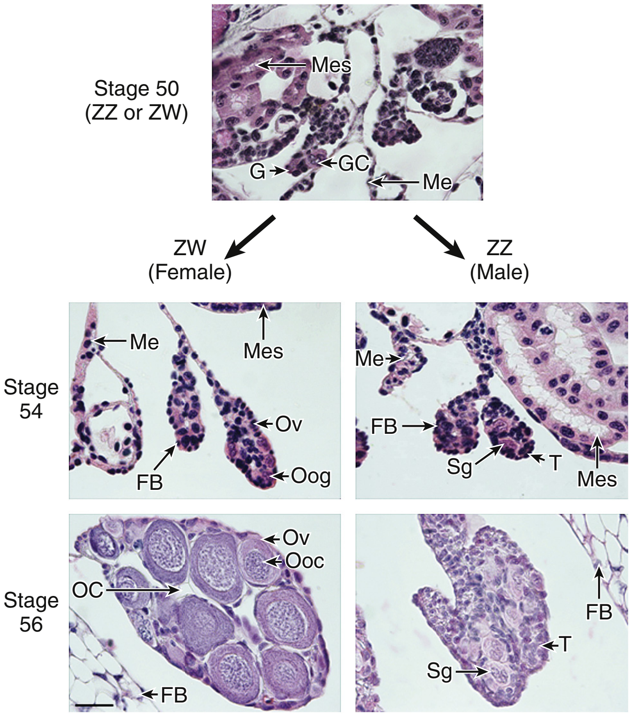


FIGURE 11-23 **Gonadal development in the salamander *Pleurodeles waltl.*** Stage 50 larva has an indifferent or undifferentiated gonad. In ZW females, the beginnings of the ovary and oviduct can be seen. By Stage 56 the ovary is clearly differentiated. In the ZZ male, development is not so obvious as in the female. Fat body, FB; gonad, G; germ cell, GC; gut mesentery, Me; mesonephros, Mes; ovarian cavity, OC; oocytes, Ooc; oogonia, Oog; ovary, Ov; spermatogonia, Sg; testis, T. *(Reprinted with permission from Flament, S. et al., in "Hormones and Reproduction of Vertebrates. Vol. 2. Amphibians" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 1–19.)*

Granulosa cells and thecal cells contain 3β-HSD activity, but there is no steroidogenic activity in the ovarian interstitial cells of the ovary. Both thecal and granulosa cells may be sources of circulating ovarian steroids, but cytological evidence favors the granulosa as the major source. GTHs stimulate these cells to synthesize estrogens.

At the end of a breeding season, the ovary typically contains young follicles that will become the next crop of mature oocytes, numerous cell nests that will become the young follicles of the next vitellogenic period, and primary germ cells that will give rise to new cell nests for future generations of oocytes. Progression from primary germ cells to mature oocytes may require three breeding seasons or more for completion.

Ovarian estrogens control development of sex accessory structures such as the hypertrophy of oviducts prior to sexual maturation and during each season prior to ovulation. The oviducts regress when estrogen synthesis declines after breeding. In the marsupial frog *Gastrotheca riobambae*, the development of the brood pouch that will be used to incubate the eggs is dependent upon estrogens secreted from preovulatory follicles.

Postnuptial ovaries frequently contain postovulatory corpora lutea, which are short-lived in oviparous species. Granulosa cells hypertrophy after ovulation and accumulate cholesterol-positive lipids. The follicle collapses and becomes a central mass of lipoidal cells surrounded by a fibrous capsule derived from the thecal layer. Postovulatory corpora lutea of *T. cristatus* and *R. esculenta* possess 3β-HSD activity and are sources for steroids; however, no functional endocrine role for postovulatory corpora lutea has been demonstrated in these oviparous species.

In viviparous amphibians such as the anuran *Nectophrynoides occidentalis*, postovulatory corpora lutea are capable of converting pregnenolone to progesterone and persist throughout gestation. Corpora lutea are required for about the first 25 to 30 days of the 100- to 125-day gestational period in the pouch of the marsupial frog *G. riobambae*, and ovariectomy after day 40 has no effect on gestation. The granulosa-lutein cells of the postovulatory corpora lutea in viviparous *S. salamandra* possess 3β-HSD activity and appear cytologically to be steroidogenic. Thirty or more corpora lutea persist in each ovary during the first 2 years of gestation and gradually decrease in both size and number over the next 2 years until the young are born. Development of new oocytes may be arrested by the presence of corpora lutea. In *N. occidentalis* as well as in oviparous *Taricha torosa*, the succeeding crop of follicles begins development only after degeneration of the postovulatory corpora lutea. These latter data suggest an inhibitory action of progesterone produced in the postovulatory corpora lutea on release of GTHs from the adenohypophysis.

Atresia occurs frequently during follicular development in amphibians. Granulosa cells are responsible for phagocytosis of yolk and formation of the corpora atretica as they are in fishes. However, these corpora atretica exhibit no 3β-HSD activity and hence are probably not steroidogenic.

The process of vitellogenesis in the liver and yolk deposition in oocytes of oviparous amphibians has been examined extensively. Estrogen levels are sufficient in females to stimulate vitellogenesis and elevate plasma vitellogenin (Figure 11-35). In *Xenopus laevis*, GTH stimulates micropinocytosis of vitellogenin by oocytes. Micropinocytotic vesicles of vitellogenin are hydrolyzed enzymatically in the yolk platelets to produce the yolk proteins **phosvitin** and **lipovitellin**. The yolk platelets containing these yolk proteins are utilized as an energy source during early embryogenesis. Estrogens will induce vitellogenin synthesis in both female and male livers when administered *in vivo*. Circulating vitellogenin binds free calcium ions, resulting in elevated total plasma calcium levels through release of calcium from storage sites.

Hypophysectomy results in atresia of all vitellogenic follicles in excess of about 0.4 mm in diameter, indicating the

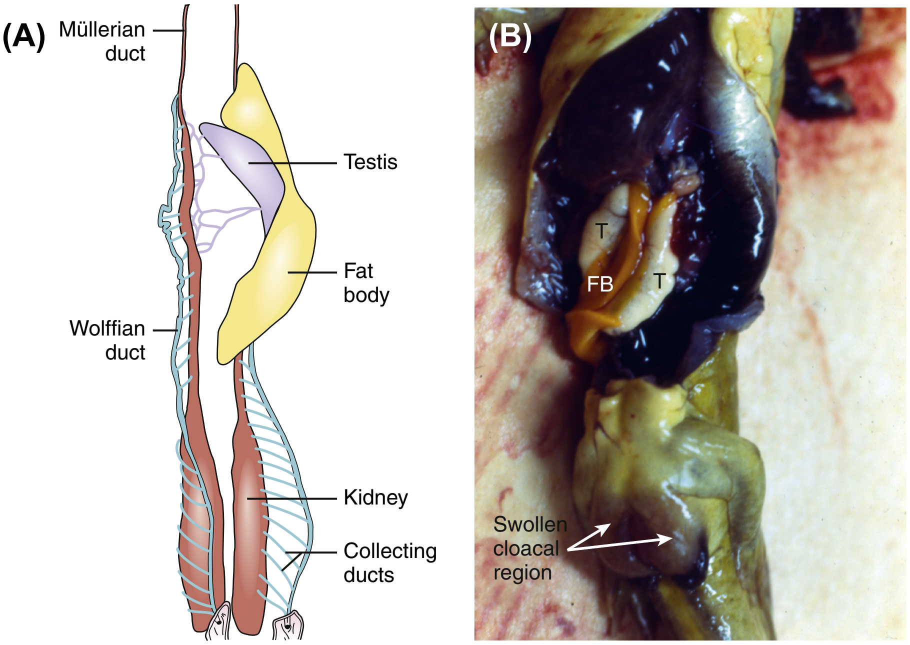


FIGURE 11-24 **Urodele reproductive system in the tiger salamander (*Ambystoma tigrinum*).** (A) Schematic diagram of a sexually mature metamorphosed male. The wolffian duct doubles as both vas deferens and ureter. Many urinary collecting ducts connect the lumbar portion of the kidney to the wolffian duct. One testis (T) and its corresponding fat body (FB) have been removed. Even in the adult, a remnant of the müllerian duct persists. (B) Dissected sexually mature male larvae (neotene) showing white testes swollen with sperm. Note the pair of prominent fat bodies. The swollen cloacal region is caused by development of multiple glands associated with production of the spermatophore and is both an indicator of sexual maturity and sex as the cloacal region of a mature female is only slightly swollen and lacks the darkly pigmented tubercles on the psoterior margin of the swellings. *(Part A adapted with permission from Rodgers, L.T. and Risley, P.L.,* Journal of Morphology, ***63****, 119–139, 1938. © John Wiley & Sons. Part B photograph provided by D.O. Norris.)*

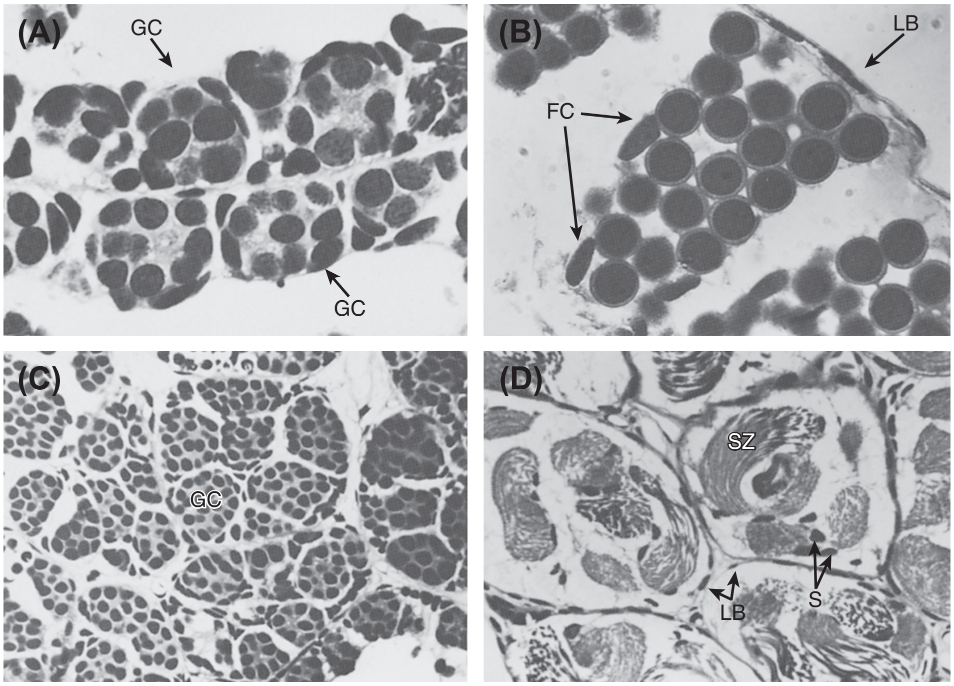


FIGURE 11-25 **Testis of the newt *Taricha granulosa*.** (A) Early germinal cysts (GC). (B) Older cyst containing secondary spermatogonia. Follicle cells (FC) have flattened nuclei. LB, lobule boundary. (C) Lower magnification showing several ampullae each containing six to eight cysts. (D) Enlargement of cysts from another region of the testis containing mature sperm (SZ) and prominent Sertoli cells (S) derived from follicle cells. *(Courtesy of Frank L. Moore, Oregon State University, Corvallis.)*

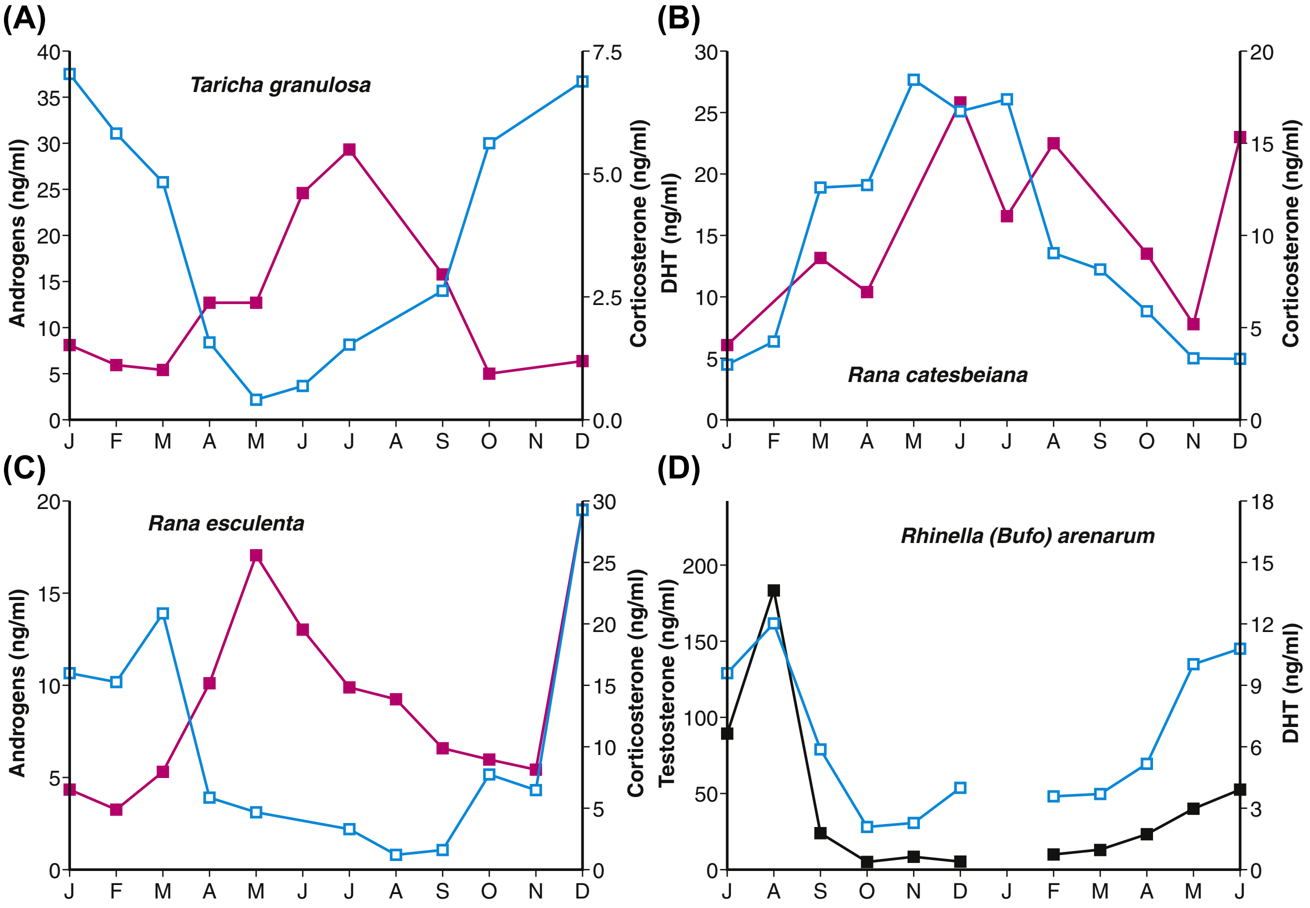


FIGURE 11-26 Comparision of androgen and corticosterone levels during the reproductive cycles of selected amphibians. Androgen levels appear to be negatively correlated to corticosterone levels in the newt *Taricha granulosa* (A) and the frog *Rana esculenta* (C) but dihydrotestosterone (DHT) is positively correlated with corticosterone in bullfrogs (*Rana catesbeiana*) (B). The toad *Rhinella* (*Bufo*) *arenarum* (D) shows a pattern of testosterone (blue) and dihydrotestosterone (DHT; black) secretion similar to androgen secretion in *T. granulosa* and of *R. esculenta*. *(Parts A to C adapted with permission from Moore, F.L. and Deviche, P., in "Processing of Environmental Information in Vertebrates" (M. Stetson, Ed.), Springer-Verlag, Berlin, 1988, pp. 19—45. Part D adapted with permission from Medina, M.F. et al.,* General and Comparative Endocrinology, ***136****, 143—151, 2004.)*

importance of endogenous pituitary GTHs in the process of vitellogenesis and maintenance of follicular growth. Mammalian FSH will augment the growth of vitellogenic follicles and prevent atresia following hypophysectomy. The failure of GTHs to stimulate formation and growth of previtellogenic follicles (i.e., follicles less than 0.4 in mm diameter) coupled with their apparent insensitivity to hypophysectomy has led to the suggestion that these processes are completely independent of pituitary control. However, experimental studies have not ruled out completely a role for GTHs in the development of previtellogenic follicles, and it is possible that low endogenous levels of amphibian GTHs are necessary for even the earliest events in gametogenesis.

Amphibian ovaries *in vitro* produce progesterone, estradiol, estrone, testosterone, DOC (like teleosts), androstenedione, and DHT. The high levels of circulating testosterone reported for some female amphibians may be related to a precursor role for peripheral aromatization to estrogens or to a behavioral role (Figure 11-36).

Ovulation is under control of an LH-like GTH and progesterone. *In vitro* studies of the ovary, initiated by Paul Wright about 60 years ago and subsequently elaborated by others, indicate that LH as well as a wide variety of steroids can induce oocyte maturation (completion of meiosis and **germinal vesicle breakdown, GVBD**) and ovulation *in vitro*. Progesterone is the most potent maturational steroid, and it is reasonable to presume that progesterone or a closely related steroid plays a key role in normal ovulatory events. The synthesis of progesterone is under the control of LH, and the action of progesterone is believed to be indirect, operating through stimulation of a "maturation-promoting factor". Recent studies with *Xenopus laevis* have shown that it may be the enzymatic conversion of progesterone into testosterone in the follicle that actually triggers final oocyte maturation and

TABLE 11-5 Seasonal Extremes in Androgen Levels (ng/mL) in Selected Male Amphibians

	Testosterone		5α-Dihydrotestosterone		Total androgens	
Species	High	Low	High	Low	High	Low
Urodeles						
Ambystoma tigrinum	32	0.4	7	0.3	39	0.7
Taricha granulosa					37	3
Triturus carnifex					28	2
Anurans						
Bufo japonicus	203	47	115	27	318	74
Bufo mauritanicus	183		52			
Rana blythi	4		1			
Rana catesbeiana					5	
Xenopus laevis	39	19			35	3

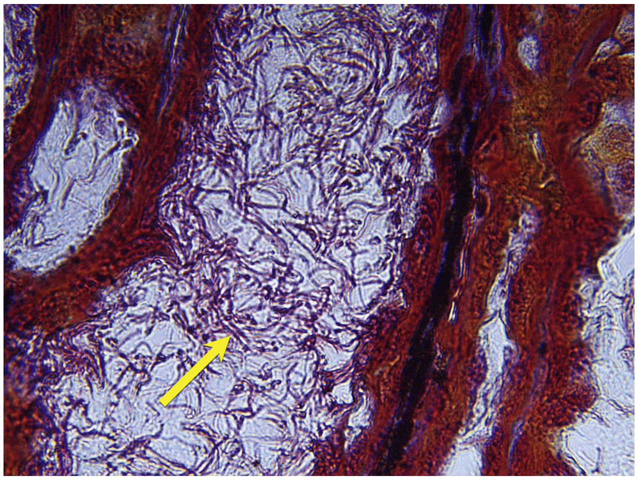


FIGURE 11-27 **Sperm (arrow) in spermatotheca of neotenic female tiger salamander.** *(Reprinted with permission from Norris, D.O., in "Hormones and Reproduction of Vertebrates. Vol. 2. Amphibians" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 187–202.)*

ovulation. PRL enhances the sensitivity of oocytes to GTH or progesterone (or testosterone) both *in vivo* and *in vitro*. This enhancement can be blocked by simultaneous *in vivo* treatment with thyroxine (T_4).

Ovarian maturation typically is completed in autumn, and ovulation is delayed over winter until favorable conditions occur in the spring. The endocrine basis for this diapause is not clear but may involve direct inhibition of ovulation by one or more pituitary hormones. Hypophysectomy of gravid anurans and at least one urodele results in ovulation and oviposition. Furthermore, hypophysectomy of gravid neotenic tiger salamanders increases their sensitivity to induced ovulation in response to a single injection of hCG. This "reflexive" ovulation could be due to inadvertent release of GTHs by the operation itself or to removal of an active inhibitory substance of pituitary origin or to both.

Growth of amphibian oviducts normally is stimulated by estrogens, and the size of the ovaries and the oviducts varies with season (Figure 11-37). Oviducts of some species may respond to treatment with either estrogens or androgens (Figure 11-38). The feminizing action of androgens is another example of a paradoxical effect. In mature females, contraction of oviducts is caused by AVT, which presumably is the hormonal stimulus in oviposition. Oviducts of breeding animals are more sensitive to AVT than are those of non-breeding adults. Progesterone induces responsiveness to AVT in immature oviducts of salamanders, but estrogens are not effective. Possibly the pre- or postovulatory follicle releases sufficient progesterone in response to LH to alter receptor levels for AVT in the muscles of the oviducts. Androgens and estrogens do not affect the sensitivity of oviducts to AVT although they stimulate growth of the oviducts.

2. Female Gymnophionids (Caecilians)

Fertilization in caecilians normally occurs in the upper portion of the oviduct following intromission by the male, and the fertilized eggs are either laid in burrows or are retained in the oviducts until the developing larvae

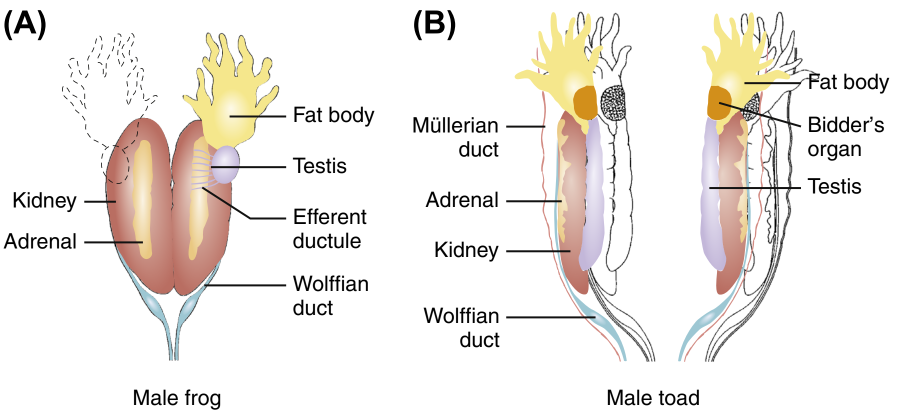


FIGURE 11-28 **Reproductive systems of male anurans.** (A) Bullfrog (*Rana catesbiana*). (B) Toad (*Bufo bufo*) with Bidder's organ, an ovarian remnant commonly found among species of *Bufo*. *(Adapted with permission from Matsumoto, A. and Ishii, S., "Atlas of Endocrine Organs: Vertebrates and Invertebrates," Springer-Verlag, Berlin, 1992.)*

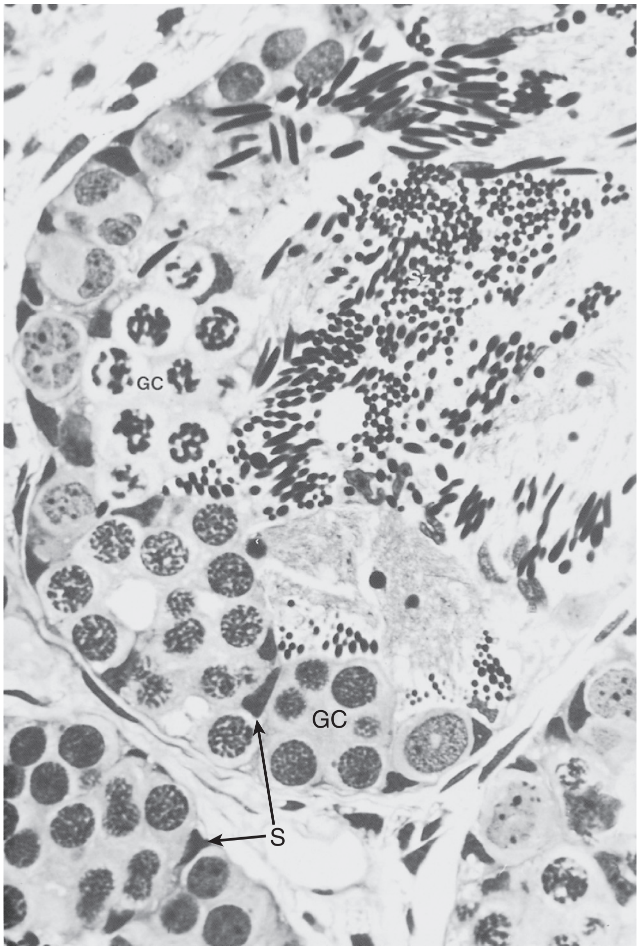


FIGURE 11-29 **Section through testis of the bullfrog (*Rana catesbeiana*).** Note how all cells in a germinal cyst (GC) are in the same stage of development. S, Sertoli cell nucleus. Compare to urodele testis in Figure 11-10. *(Courtesy of Charles H. Muller, University of Washington, Seattle.)*

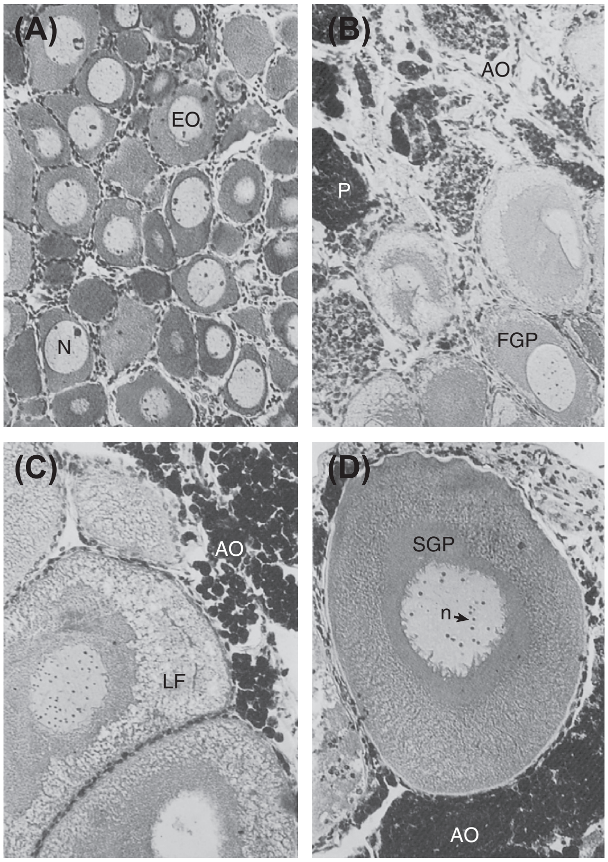


FIGURE 11-30 **Sections through Bidder's organ from male *Bufo woodhousii*.** These figures at the same magnification show the effects of castration and gonadotropin treatment on oocytes of Bidder's organ. (A) Sham-operated toad treated with saline injections for 26 days shows unstimulated follicles. EO, early previtellogenic follicle; N, nucleus. (B) Sham-operated toad treated with mammalian gonadotropins for 26 days shows moderately enlarged follicles. AO, atretic follicle; FGP, first growth phase follicle (previtellogenic); P, pigment granules. (C) Castrated toad treated with saline for 26 days shows oocyte and follicle growth into the late previtellogenic stage. Late first growth phase oocyte, LF. (D) Castrated toad treated with gonadotropins shows vitellogenic or second growth phase oocyte (SGP). *(Reprinted with permission from Pancak-Roessler, M.K. and Norris, D.O.,* Journal of Experimental Zoology, ***260****, 323–336, 1991. © John Wiley & Sons, Inc.)*

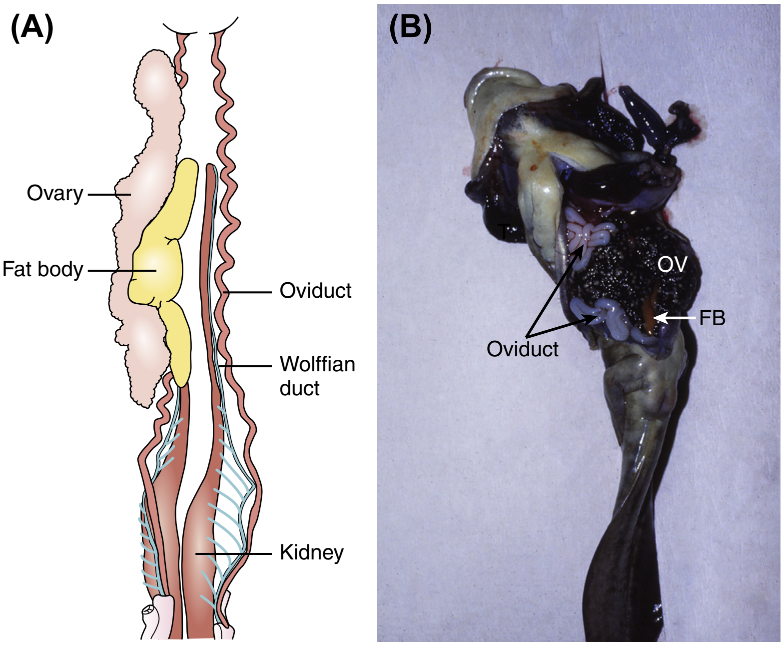


FIGURE 11-31 **Reproductive system of female urodele, the tiger salamander (*Ambystoma tigrinum*).** (A) Schematic diagram of a sexually mature metamorphosed female. (B) Dissection of an adult sexually mature female larva (neotene). Abbreviations: FB, fat body; OV, ovary. *(Part A adapted with permission from Rodgers, L.T. and Risley, P.L.,* Journal of Morphology, ***63****, 119–139, 1938. © John Wiley & Sons. Part B photograph provided by D.O. Norris.)*

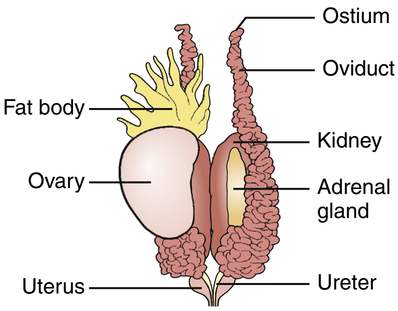


FIGURE 11-32 **Reproductive system of female anuran, the bullfrog (*Rana catesbeiana*).** Ovary has been removed from the right side to expose the kidney, adrenal, and the convoluted oviduct. *(Adapted with permission from Matsumoto, A. and Ishii, S., "Atlas of Endocrine Organs: Vertebrates and Invertebrates," Springer-Verlag, Berlin, 1992.)*

have completed metamorphosis. Ovarian development is very similar to that described for anurans and urodeles, but in caecilians the eggs tend to be larger and fewer. Postovulatory corpora lutea develop in the ovaries, and they appear to be important for maintaining oviductal secretion (even in oviparous species) and pregnancy. Oviductal secretions in viviparous species provide nutrition for the developing young, and these secretions may be controlled by hormones released from the corpora lutea. Relatively few caecilians have been examined, and it is not possible to describe the seasonality of breeding and ovarian cycles in caecilians. Consequently, the endocrine factors involved in reproductive events mainly must be inferred from studies on anurans and urodeles.

E. Reproductive Behavior in Amphibians

Numerous aspects of reproductive behavior have been described for amphibians, but little is known about the mechanisms of endocrine control for most species. Reproductive behavior includes migration, calling, courtship, clasping, spawning, and various kinds of parental care. Studies involving castration, hypophysectomy, and/or injections of pituitary hormones support the conclusion that testicular hormones are involved in calling, courtship, and clasping. In neotenic tiger salamanders, *A. tigrinum*, a species with dissociated mating, androgens are not high at the time of mating (see Figure 11-1). Although most species examined exhibit associated mating, attempts to stimulate reproductive behavior with androgens alone often have not been successful.

Even knowing plasma androgen levels may not tell the entire story. In the European crested newt (*T. carnifex*), inactive males have higher testosterone levels than courting males. Clasping of the female does not occur in these newts and courtship involves progressive stages: approaching, fanning, tail lashing, and spermatophore deposition. $P450_{aro}$ activity increases in the brain and gonad during courtship, and courting males have significantly higher levels of estrogen in brain and plasma during the approach stage of courtship.

Neuropeptides (AVT, GnRH, ACTH) trigger mating behavior in androgen-primed animals. For example, when a female frog that is not ready to spawn is clasped by a courting male, she croaks to signal her non-receptivity. A receptive female will not emit this release call. This

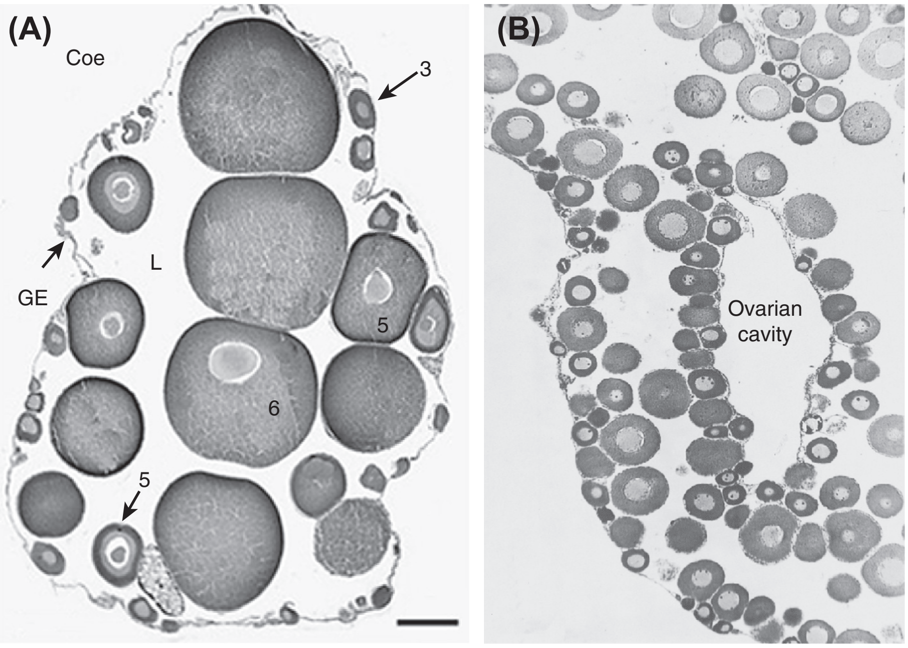


FIGURE 11-33 Hollow amphibian ovaries. (A) Ovary of *Ambystoma dumerlii* showing several stages of amphibian follicle growth according to Uribe (2011). Abbreviations: GE, germinal epithelium; L, ovarian lumen. (B) This ovary from the cane toad (*Bufo marinus*) is typical of the hollow amphibian ovary with follicles attached to the germinal epithelium. *(Part A reprinted with permission from Uribe, M.C.A., in "Hormones and Reproduction of Vertebrates. Vol. 2. Amphibians" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 55–81. Part B courtesy of Charles H. Muller, University of Washington, Seattle.)*

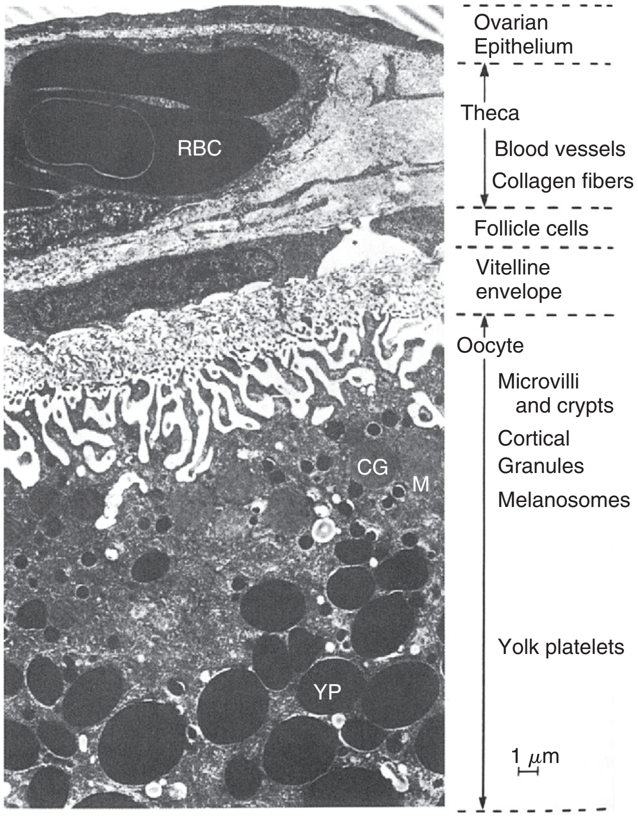


FIGURE 11-34 Vitellogenic follicle of *Xenopus laevis*. This transmission electron micrograph shows the vascular theca and its relationship to microvillous processes projecting from the surface of the developing oocyte. YP, yolk platelets. *(Reprinted with permission from Dumont, J.N. and Brummett, A.R.,* Journal of Morphology, ***155****, 73–97, 1978.)*

receptive female has accumulated water that will be used in ovulation and oviposition. Water retention is caused by AVT (see Chapter 5), and administration of AVT inhibits the release call, possibly through effects on the brain.

According to extensive studies by Frank Moore and associates at Oregon State University, reproductive behavior by male rough-skinned newts (*Taricha granulosa*) also is stimulated by AVT, although androgens and other factors are involved (Figure 11-39). During courtship, males in the breeding pond will attempt to clasp a female along the back. Attempts by several males to clasp the same female result in the formation of mating balls that persist for a time, but the unsuccessful males soon drop off leaving only one clasping the female. A series of behavioral and chemical interactions between the clasping pair eventually will lead to spermatophore transfer. Androgens play a priming role that enhances the sensitivity of the newts to AVT. Stressors or injection of corticosterone can rapidly repress clasping behavior within minutes. The finding that corticosterone rapidly inhibits reproductive behavior in newts has led to the hypothesis that corticosterone may interact with a membrane receptor, rather than a nuclear receptor, to modulate reproductive behavior. Clasping can be activated by cloacal stimulation and is controlled by neurons located in the rostral portion of the medulla. Furthermore, activity of these neurons is increased by AVT treatment but decreased by administration of corticosterone.

1. Pheromones in Amphibian Reproduction

Pheromones are used in courtship by many urodeles that possess **hedonic glands** or **cloacal glands**. These glands are

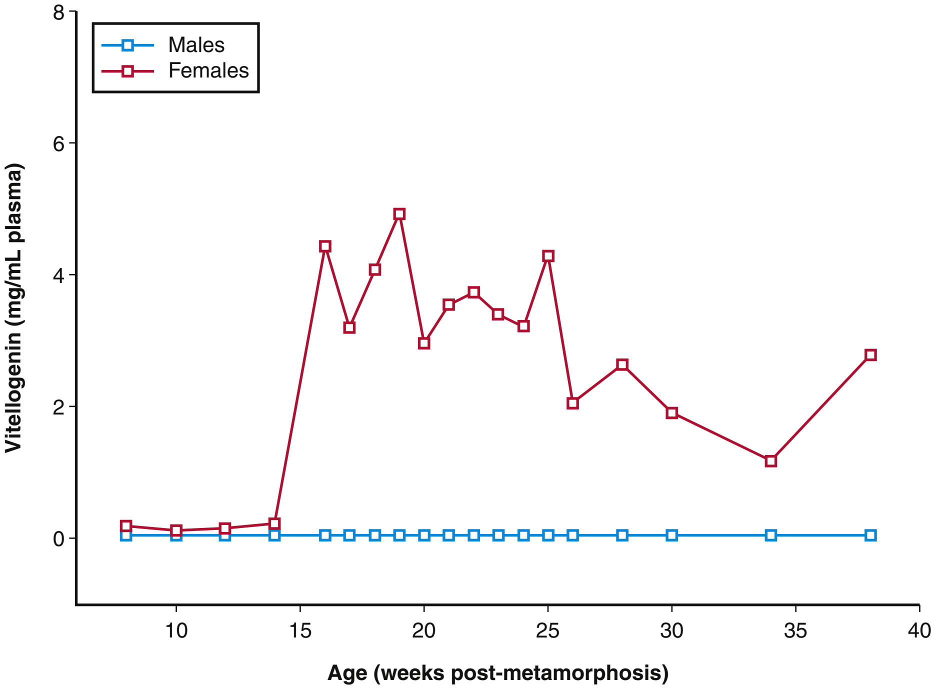


FIGURE 11-35 Estrogen secretion elevates plasma levels of vitellogenin in post-metamorphic *Xenopus tropicalis females.* Animals begin to secrete estrogens about 16 weeks after metamorphosis followed by rapid elevation of vitellogenin production, whereas vitellogenin production is minimal in males. *(Adapted with permission from Olmstead, A.W. et al.,* General and Comparative Endocrinology, ***160**, 117–123, 2009.)*

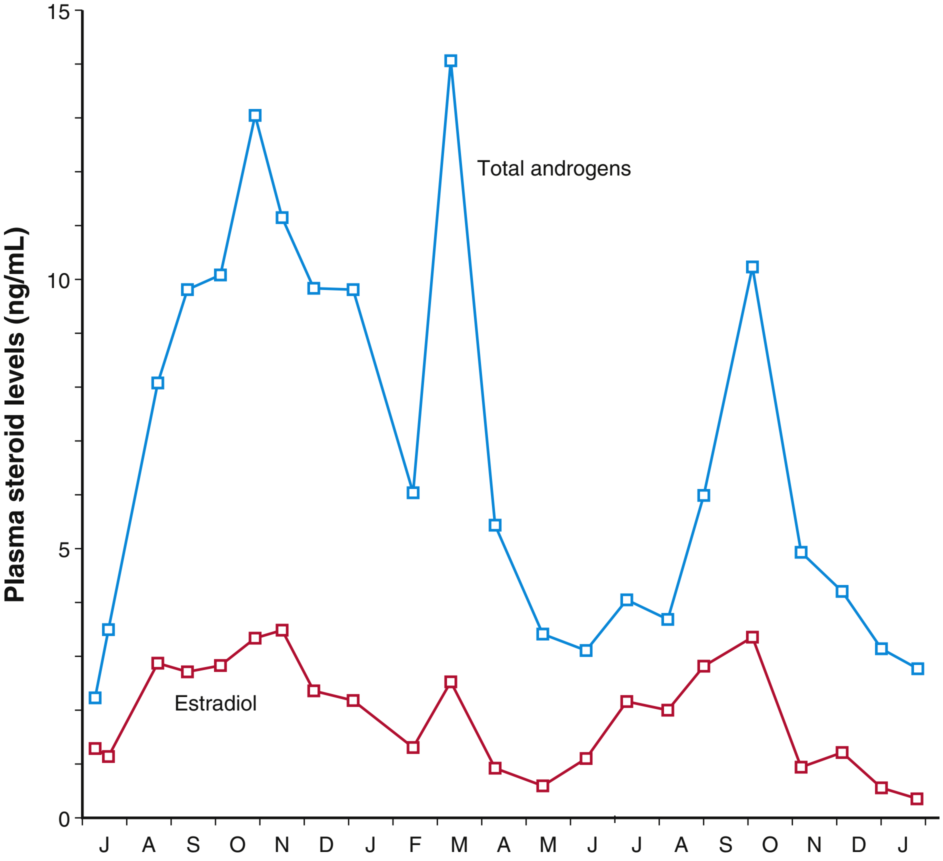


FIGURE 11-36 Seasonal variations in steroid levels in female *Pleurodeles waltl.* *(Adapted with permission from Garnier, D.H.,* General and Comparative Endocrinology, ***60**, 414–418, 1985.)*

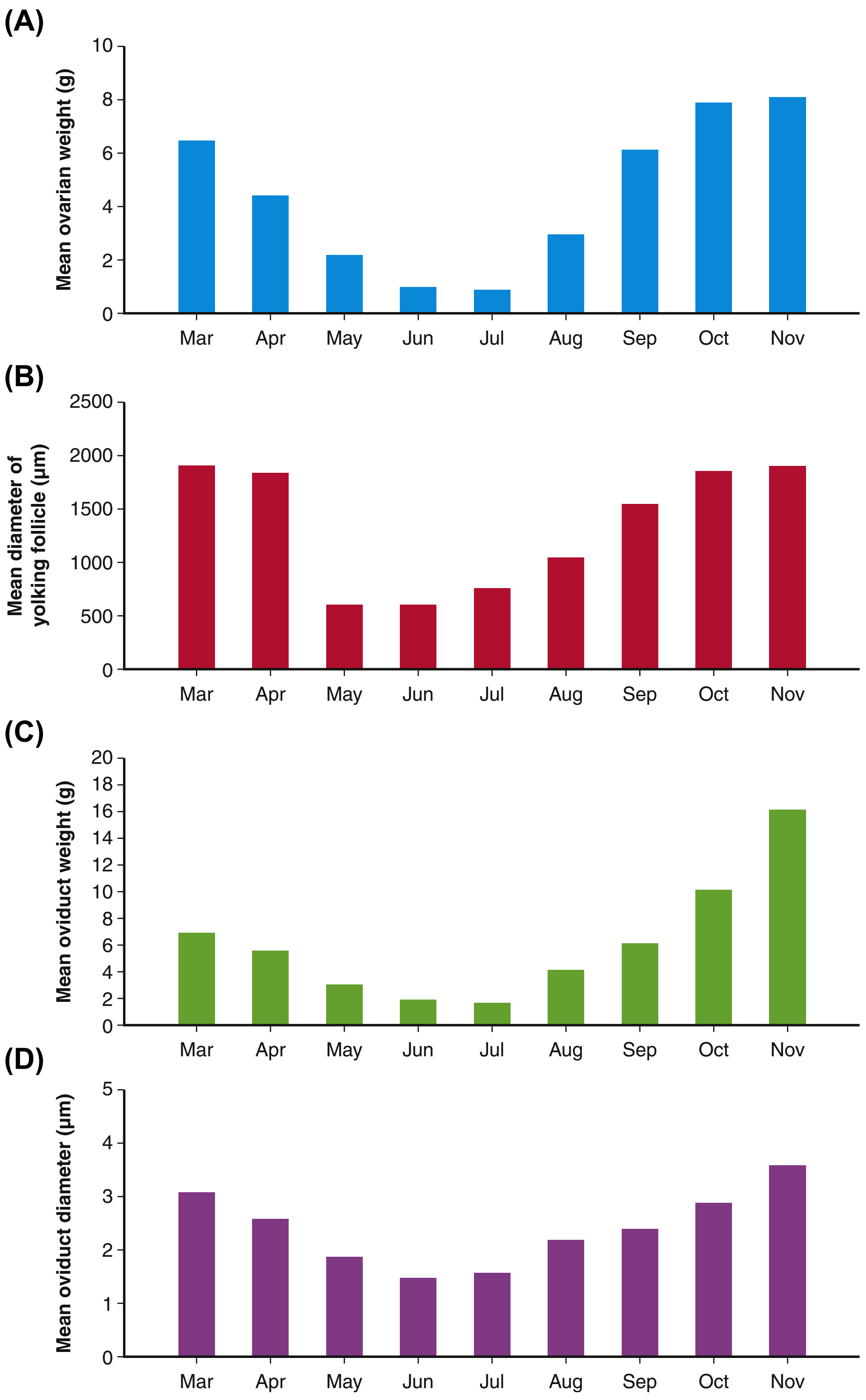


FIGURE 11-37 **Correlative changes in reproductive parameters of sexually mature female tiger salamander larvae (neotenes).** Oviposition occurs in March and April. Pond was frozen over from December through February. Mean ovarian weight, oviduct weight, follicle diameter, and oviduct diameter show identical patterns throughout the year. Numerous studies of estrogen levels in other species allow prediction of a similar pattern for estrogen levels in this species correlated to any one of these parameters. *(Adapted with permission from Norris, D.O., in "Hormones and Reproduction of Vertebrates. Vol. 2. Amphibians" (D.O. Norris, and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 187–202.)*

known to be sources of potent pheromones. Male plethodontid and desmognathid salamanders employ a tubular **mental (chin) gland** for stimulating courtship behavior in females. Ambystomatids and salamandrid salamanders rely on pheromonal secretions from one of their cloacal glands, the **abdominal gland**, to stimulate females. Female attractants released into a stream readily attract reproductively active male *T. granulosa*. Furthermore, males of this species

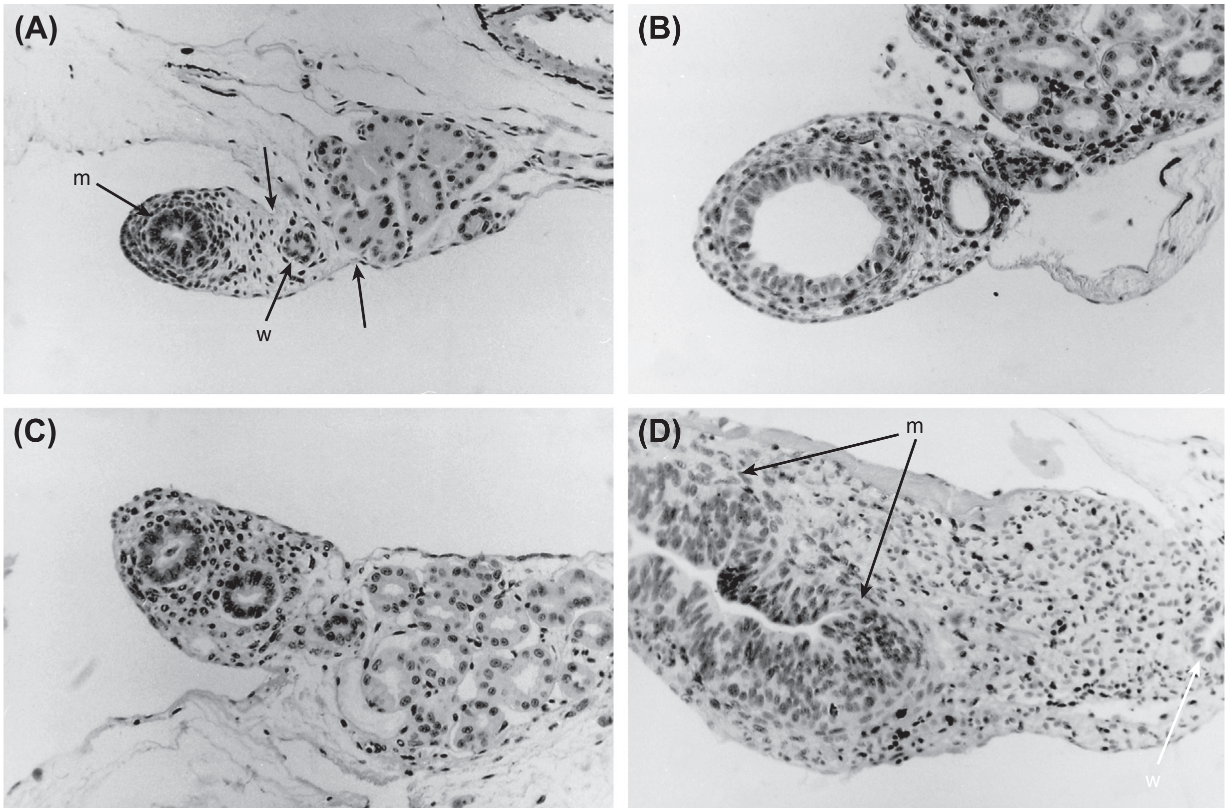


FIGURE 11-38 **Effects of steroid treatment on müllerian ducts of larval tiger salamanders.** (A) Saline-treated control. (B) Effect of 12.5 μg estradiol (E_2). (C) Effect of 12.5 μg dihydrotestosterone (DHT). (D) Synergistic effect of E_2 and DHT. Abbreviations: m, müllerian duct; w, wolffian duct. All photomicrographs were prepared at the same magnification. *(Reprinted with permission from Norris, D.O. et al.,* General and Comparative Endocrinology*, **106**, 348–355, 1997.)*

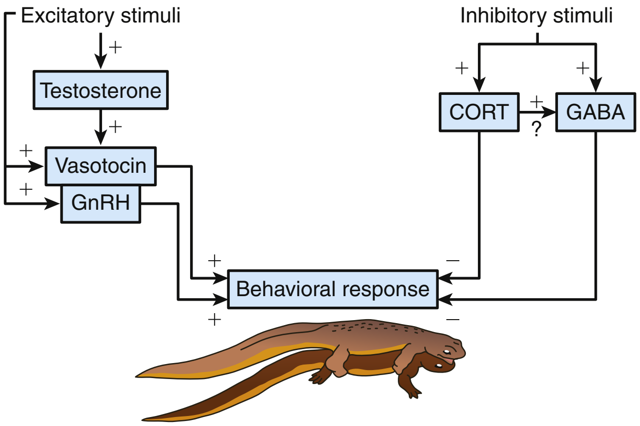


FIGURE 11-39 **Neuroendocrine control of clasping behavior in the rough-skinned newt.** Stress activates inhibitory pathways (CORT, corticosterone; GABA, γ-aminobutyric acid) that antagonize the actions of GnRH and arginine vasotocin activated by external sexual parameters. *(Adapted with permission from Moore, F.L. and Orchinik, M.,* Seminars in Neuroscience*, **3**, 489–496, 1991.)*

are attracted to females by airborne cues as well (see Table 11-6). Two peptide pheromones have been isolated from the abdominal cloacal glands of newts (*Cynops pyrrhogaster, C. ensicauda*) and a 22-kD protein was found in the mental gland of the salamander *Plethodon jordani*. These secretions are used to attract the female to a spermatophore. The pheromone from male *C. pyrrhogaster*, **sodefrin**, is released by the action of AVT on the abdominal gland, and it binds to receptors in the female's vomeronasal organ. The number of receptor cells for sodefrin in the female increases during the breeding season. The responsiveness of the vomeronasal epithelium to sodefrin is enhanced by exposure of the animals to PRL and estrogens.

In contrast, there is little evidence for chemical communication among anuran species, and mating is accomplished presumably by using auditory, tactile, and visual cues. However, another peptide with female attractant properties has been isolated from the skin glands of a male anuran, *Litoria splendida*.

2. Parental Care in Amphibians

Some parental care has evolved in certain amphibian species as a means of ensuring greater survival of young, mostly among the anurans. These parental behaviors are largely an alternative strategy for viviparity and help ensure survival to hatching or in some cases through metamorphosis. Female marsupial frogs (e.g., *G. riobambae*,

TABLE 11-6 Sex-Dependant Conspecific Odor Preferences in *Taricha granulosa*: Directed Locomotor Response of Newts to Newt and Non-Newt Odors

No. of tests	Sex of test newt	Sex of stimulus newt	Ratio of S/Ns[a]	Probability that choices were random
10	Male	Male	7/3	0.0570
10	Male	Male	10/0	0.0003
20	Male	Male/female	17/3	0.0004
10	Female	Male	4/6	0.625
10	Female	Female	6/4	0.625
20	Female	Male/female	10/10	0.987

[a] *Subject chose air from stimulus animal/air from non-stimulus source. Unpublished data of M. Schwartz, D. Duvall, and D.O. Norris.*

described earlier) develop an estrogen-dependent pouch on their backs into which eggs are stuffed and where they develop to hatching. Males of the midwife toad (*Alytes obstetricans*) wrap the strings of jelly containing the fertilized eggs and periodically take them to water to moisten them and finally to allow them to hatch as tadpoles. In terrestrial glass frogs of Central America (e.g., *Hyalinobatrachium fleischmanni*), eggs are attached under leaves; the male keeps them hydrated and defends them from predatory spiders (Figure 11-40A). In the gastric-brooding frog of Australia (*Rheobatrachus silus*), the female swallows the fertilized eggs and they remain in her stomach undigested until they hatch as tadpoles, which are then forcefully regurgitated alive. Unfortunately, this rare frog with its unusual brooding habit is now considered to be extinct.

Nest-building is a complex form of parental care that is exemplified by the tungara frog, which forms foam nests on the surface of ponds (Figure 11-40B), and the terrestrial foam-nest frog *Chiromantis xerampelina*. As many as 40 females of *C. xerampelina* may contribute to production of a huge, communal foam nest in which fertilized eggs are deposited. The females produce the foam by beating water with their hind limbs. Males are much smaller than females and they attract females by making soft clicking sounds. When a female arrives at a calling site, males drop from above onto her back. One male occupies the central position while up to seven peripheral males hang on. This central position is considered to be the most advantageous position for fertilizing eggs emanating from the female's cloaca. In experiments where the cloacae of males were sheathed to prevent sperm release, no eggs were fertilized. When only the central male was sheathed, about half of the eggs were still fertilized, but they were fertilized by the unsheathed peripheral males. In another study, 10 of 15 naturally breeding females were shown to produce clutches having 2 or more fathers. Thus, cooperation occurs not only in nest-building behaviors in this species but also in mating, which ensures a good mixing of genetic material in the following generation.

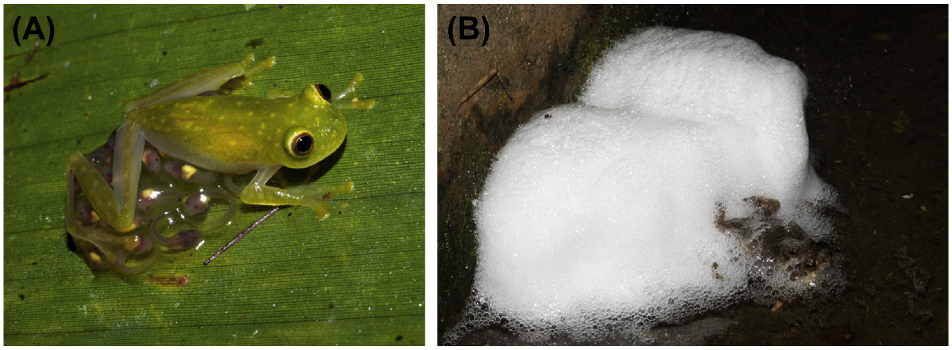


FIGURE 11-40 **Parental behaviors protecting young amphibians.** (A) A male glass frog, *Hyalinobatrachium fleischmanni*, moistens the developing eggs and protects them from predation by spiders (B) A foam nest produced on the pond surface by a breeding pair of Túngara frogs, *Engystomops* (*Physalaemus*) *pustulosus*, in which the fertilized eggs will develop protected from sunlight, dehydrations, predators, etc. *(Photographs courtesy of Jesse Delia.)*

F. Occurrence of Unisexual Species in Amphibia

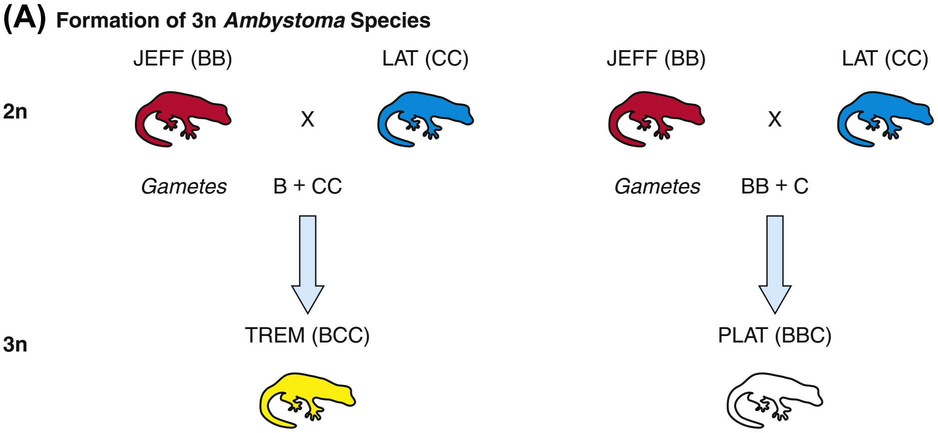
The *Ambystoma jeffersonianum* complex consists of four salamander species found in the Great Lakes region of North America. Two of these species are normal bisexual, diploid ($n = 14$, $2n = 28$) species—*Ambystoma jeffersonianum* ("JEFF") and *Ambystoma laterale* ("LAT")—but two of these are unisexual, consisting of all triploid ($n = 14$, $3n = 42$) females: *Ambystoma platinium* ("PLAT") and *Ambystoma tremblayi* ("TREM"). The two $3n$ species are believed to have resulted from hybridization of the two $2n$ species where one of them produced diploid gametes by mitosis instead of meiosis (Figure 11-41). The $3n$ species are all female and produce $3n$ gametes via mitosis; however, these eggs will not develop unless penetrated by a sperm provided by one of the $2n$ species. At breeding time, the $3n$ females compete with the $2n$ females to get a male to provide sperm to activate their eggs, which then develop parthenogenetically into $3n$ females (the male gametes contribute no genetic material to the embryo). Interestingly, the LAT male prefers to mate with LAT females but his second preference is to mate with a TREM female who has two sets of LAT chromosomes and only one set of JEFF chromosomes. The LAT male's third choice is a PLAT $3n$ female with only one set of LAT chromosomes and two sets of JEFF chromosomes. Least favorite mating choice is a JEFF female. Similarly, The JEFF male shows the same hierarchy: JEFF female, then PLAT $3n$, then TREM $3n$, and finally LAT female.

VI. REPRODUCTION IN REPTILES

Living reptiles are members of diverse orders, and it is not surprising that considerable differences occur, making it difficult to generalize about reptilian reproduction. The squamate reptiles (lizards and snakes) possess features unique to their order, whereas the other orders may be more typical of primitive reptiles that gave rise to four extant reptilian groups (chelonians, crocodilians, squamates, and the tuatara) and also gave rise independently to birds and mammals. Hence, the study of reptilian reproduction is of special evolutionary interest. The reproductive system of a typical lizard is provided in Figure 11-42.

Most reptilian species are oviparous and exhibit well-defined annual reproductive cycles and breeding seasons. In addition, many examples of viviparity are known among snakes and lizards, and it is hypothesized that viviparity evolved 100 times in these groups. Only a few, heavily yolked eggs are produced by most reptilian species although clutch sizes may be relatively large in some turtles, crocodilians, and snakes. Fertilization is internal in all reptiles, and males have intromittent organs (often two hemipeni) for placing sperm into the cloaca of a female. Mating frequently follows complicated behaviors, including male–male territorial and aggressive interactions. Following mating, females of many species can store sperm in spermatothecae for months.

Crocodilians and turtles produce two distinct GTHs, but squamate reptiles rely on a single FSH-like hormone. The reptilian hypothalamus produces GnRH-1 that regulates



(B) Mating Preference by 2n Males

	First Choice	Second Choice	Third Choice	Fourth Choice
LAT Male (CC)	2n LAT female (CC)	3n TREM female (CCB)	3n PLAT female (CBB)	2n JEFF female (BB)
JEFF Male (BB)	2n JEFF female (BB)	3n PLAT female (BBC)	3n TREM female (BCC)	2n LAT female (CC)

FIGURE 11-41 The *Ambystoma jeffersonianum* complex. (A) Formation of the triploid involves hybridization between two diploid species where nondisjunction has occurred in either *A. laterale* (LAT) or *A. jeffersonian* (JEFF). The former results in formation of the $3n$ species *A. tremblayi* and the latter results in the $3n$ species *A. platinium.* (B) Mating preference for a diploid species is for females that have the closest genetic composition. Note that the chromosome sequences are changed in the triploid females to emphasize closeness to the male genotype.

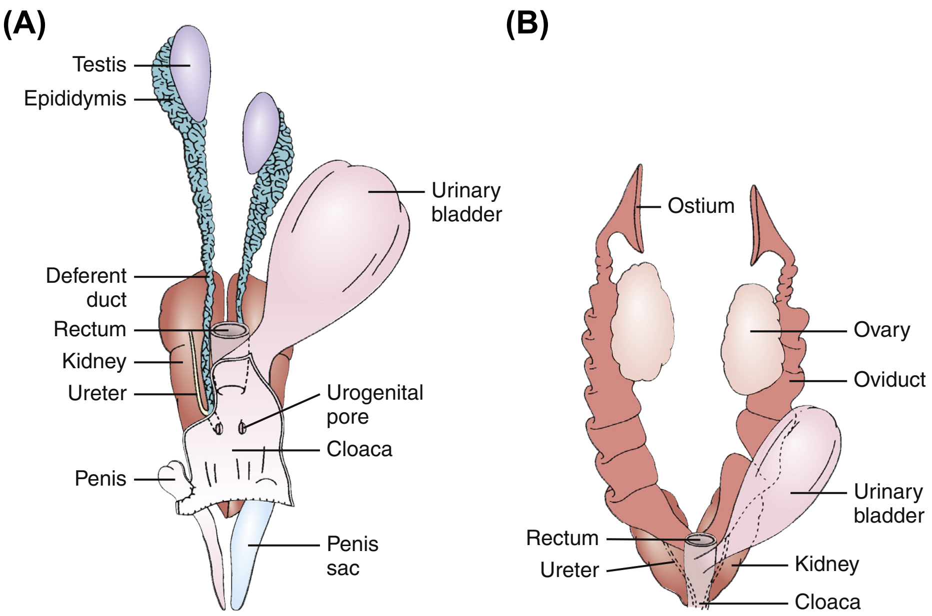


FIGURE 11-42 Reproductive system of a lizard. (A) Male. The top wall of the cloaca has been removed to show the duct openings and the two hemipeni. (B) Female. *(Adapted with permission from Matsumoto, A. and Ishii, S., "Atlas of Endocrine Organs: Vertebrates and Invertebrates," Springer-Verlag, Berlin, 1992.)*

GTH release in response to environmental stimuli such as photoperiod and temperature (see Chapter 5).

A. Sex Determination and Gonadal Development

Temperature-dependent sex determination (TSD) had been observed in all reptilian groups as well as in both XX/XY and ZZ/ZW types of sex determination. The same genes are involved in forming a testis in reptiles with TSD as were described for other vertebrates (see above) although there is some disagreement as to the exact activation sequence. Whether a higher temperature produces males or females varies considerably among different species (Table 11-20). Gonads typically go through an indifferent or bipotential stage prior to differentiation, and hormonal factors can influence which direction differentiation will follow. The development of sex accessory ducts (Figure 11-43) follows the same patterns as in other tetrapods. In males, the vas deferens develops from the wolffian ducts as described for amphibians. The vas deferens conducts no urine in reptiles, however, as the mesonephric kidney is completely replaced in reptiles by the evolution of the metanephric kidney with its own ureter connecting to the urinary bladder. As demonstrated in the American alligator, müllerian ducts degenerate in males prior to hatching as a consequence of AMH secreted by the testes.

B. Male Reptiles

The male reproductive system (Figure 11-42) is typical of amniotes, and the testes consist of convoluted seminiferous tubules (Figure 11-44A), each surrounded by a connective tissue sheath, the tunica propria. The entire testis is enclosed by a tunica albuginea. Spermatogenesis recurs soon after the breeding season and is completed in most species prior to the onset of winter. Pituitary GTHs stimulate spermatogenesis, and sperm may be stored in the vas deferens for up to several months prior to mating. Leydig cells (Figure 11-44A) and Sertoli cells are common, and they are both steroidogenic. After spermiation and testicular collapse, the Sertoli cells fill with cholesterol-positive lipid that is depleted under the influence of FSH at the time mitosis resumes in the spermatogonia. Leydig cells undergo cyclical changes associated with androgen secretion and sexual changes in androgen-dependent sex accessory structures. Representative plasma androgen levels are provided in Table 11-7. In species with associated reproductive patterns (typically crocodilians, squamates, and some turtles), there is typically a single peak of testosterone that coincides with spermatogenesis and development of the vasa deferentia. However, some species, such as the Northern Pacific rattlesnake *Crotalus oreganus*, may show two androgen peaks and breed in both the spring and fall of the year (Figure 11-45). In contrast, species exhibiting dissociated reproductive patterns (mostly turtles) also have two peaks of androgen secretion but neither is associated directly with spermatogenesis.

In sexually active squamates, a portion of the kidney tubules and possibly other portions of the excretory system develop into the **renal sexual segment** of the kidney (Figure 11-44B). Some suggest it may be homologous to the seminal vesicles of male mammals. The sexual segment undergoes hypertrophy under the influence of androgens

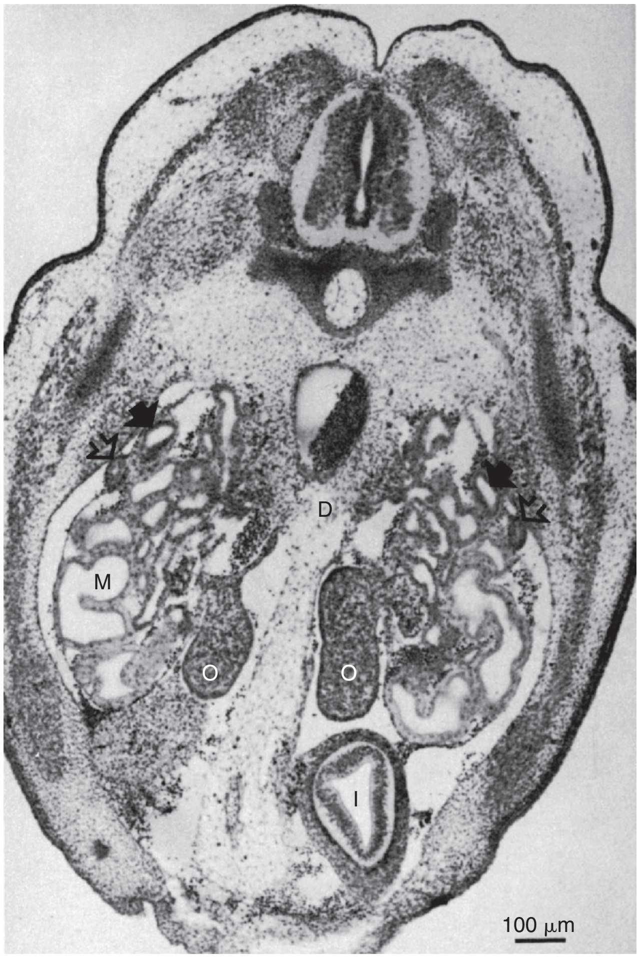


FIGURE 11-43 Cross-section through an early embryo of the lizard *Sceloporus undulatus* prior to sexual differentiation. Developing gonads (O), mesonephric kidney (M), müllerian ducts (open arrows), wolffian ducts (solid arrows), intestine (I), and dorsal mesentery (D). *(Reprinted with permission from Austin, H.,* General and Comparative Endocrinology, ***72****, 351–363, 1988.)*

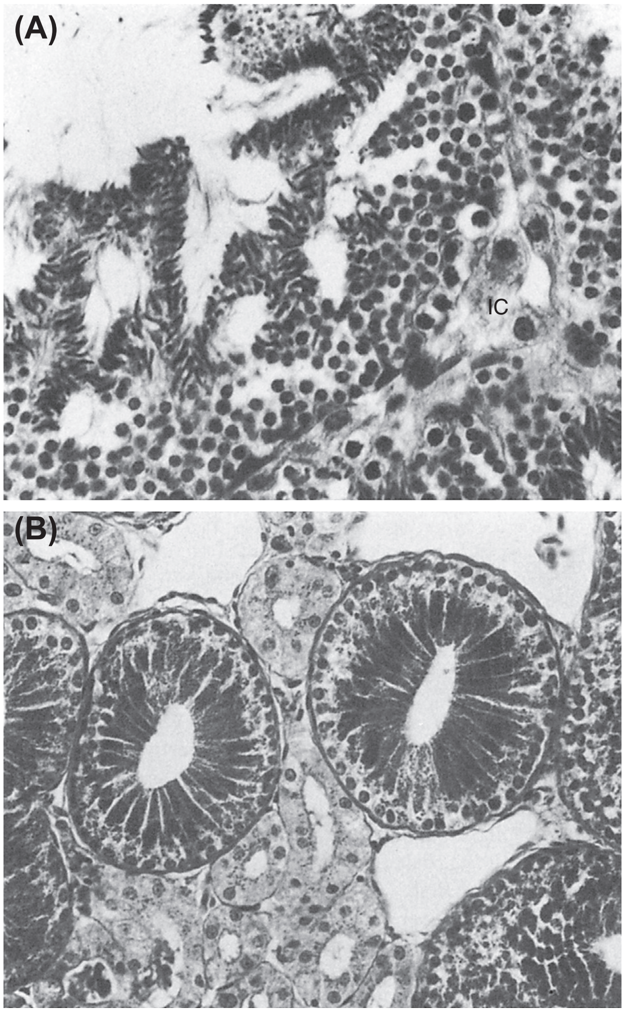


FIGURE 11-44 Spermatogenesis and the sexual segment in sceloporine lizards . (A) Spermatogenesis in the testis of *Scerloporus jarrovi*. Portions of three seminiferous tubules are separated by large Leydig cells (IC). (B) Section through kidney of *Sceloporus undulatus* showing small, lightly stained renal tubules and the large renal sexual segments modified for sperm storage. *(Photo courtesy of Dr. John Matter, Juanita College.)*

and appears to secrete materials that help maintain sperm stored in the vas deferens prior to ejaculation. The renal sexual segment is largest at the time of spermiation. Apparently, in some species it may contribute secretions to the formation of the **copulatory plug** produced to block the female cloaca after copulation and prevent entry of sperm from another male.

C. Female Reptiles

Reptiles have paired hollow ovaries with little stromal tissue. The reproductive system of a female lizard is provided in Figure 11-42. Ovarian cycles as exemplified by steroid hormone secretion for the American alligator and the North Pacific rattlesnake are provided in Figures 11-46 and 11-47, respectively. Oogonia are present in the mature ovary as described for anamniotes and give rise to primary oocytes and follicle growth throughout reproductive life. The developing oocyte becomes invested with granulosa cells derived from the germinal epithelium. The granulosa cells are separated from the surrounding thecal cells by a connective tissue layer, the **membrana propria**. In reptiles as in mammals, the theca is differentiated into an inner, glandular **theca interna** surrounded by a fibrous **theca externa**. The cells of the granulosa in most species are considered the primary source of follicular estrogen during ovarian recrudescence, although histochemical evidence in skinks (genus *Lamproholis*) suggests that only the thecal cells are steroidogenic in those lizards. Follicle cells exhibit changes in cholesterol-positive lipid inclusions and 3β-HSD activity paralleling estrogen-dependent oviductal growth and changes in other sex accessory structures. Changes in

TABLE 11-7 Plasma Steroid Levels (ng/mL) in Reptiles

Species	Testosterone	Estradiol	Progesterone
Turtles			
Chrysemys picta			
Male	15–40		
Preovulatory female	3.2–5.7	0.79–1.37	1.2–1.5
Preovulatory female	0.2	UND[a]	0.3–0.5
Stenotherus ordonuttus			
Female	0.025–1.5	0.5–5	0.7–4
Male	10–75		
Lizards			
Uromastix hardwicki			
Preovulatory female	0.37	0.18	1.6
Gravid female	1.57	0.46	13.41
Iguana iguana			
Male	0.1	0.079	
Female	0.003	0.27	
Snakes			
Natrix fasciata (female)	0.05–1.065	0.010–0.54	0.090–1.445
Naja naja			
Male	0.6–2.3		
Female	0.03–0.7	10–310	1.4–25
Nerodia sipedon (male)	2–21		

[a] *UND, undetectable.*

the gonadotropes of the adenohypophysis parallel follicular changes indicating an important role for gonadotropin. As the oocyte enlarges, it begins to project into the ovarian cavity and out from the surface of the ovary.

The squamate granulosa contains a unique flask-shaped cell type, the **pyriform cell** (Figure 11-48) that is in direct contact with the developing oocyte. These cells apparently are involved with early steps in oocyte development as they either degenerate or transform into typical granulosa cells soon after the onset of vitellogenesis. Thus, the multilayered granulosa becomes a single layer of cells in mature follicles.

As ovulation approaches, the granulosa cells as well as some thecal cells accumulate cholesterol-positive lipids and, following ovulation, proliferate and luteinize to form corpora lutea. These corpora lutea are well vascularized, exhibit 3β-HSD activity, and synthesize progesterone. They persist throughout egg laying in oviparous species or during gestation in most viviparous forms. Corpora lutea of viviparous species synthesize greater amounts of progesterone than do those of oviparous species and persist much longer. Plasma progesterone levels are greatest following ovulation and are maintained at elevated levels throughout gestation in most viviparous lizards and snakes. Preovulatory peaks of progesterone are found in oviparous turtles, crocodilians, and lizards (Table 11-8).

Only a few of the follicles that begin development reach maturity at a given time; the majority undergo atresia. Follicular atresia and formation of corpora atretica is a common occurrence in reptilian ovaries as in other vertebrates. The importance of corpora atretica is unknown, but the absence of 3β-HSD activity in corpora atretica makes it unlikely they would have an endocrine function. However, steroidogenic cells from atretic follicles may give rise to an “interstitial gland” that is believed to be a major source of ovarian estrogens.

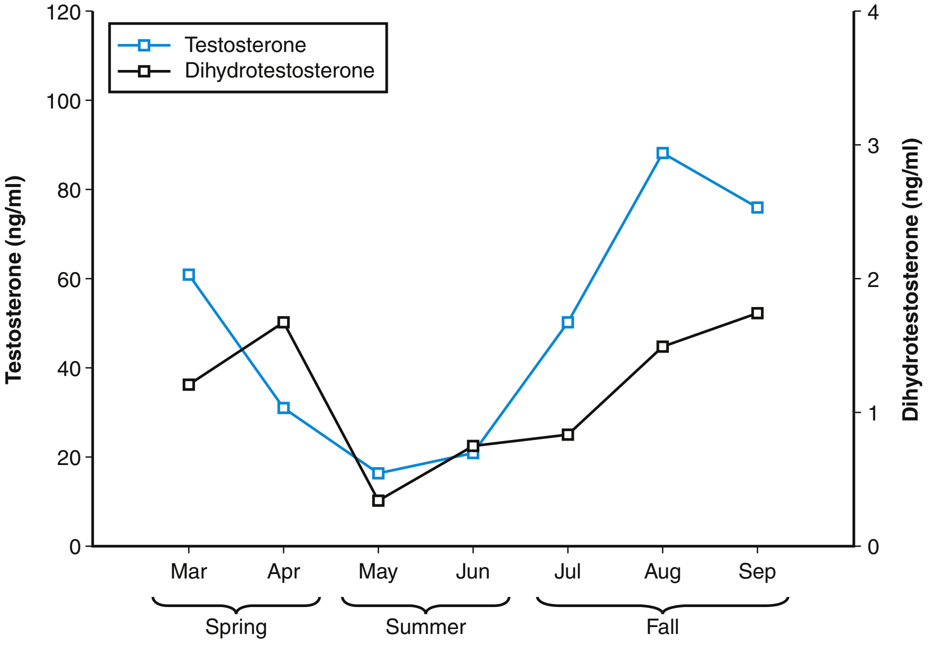


FIGURE 11-45 Seasonal androgen levels in male northern Pacific rattlesnakes (*Crotalus oreganus*). Testosterone is the predominant androgen. Males may mate with females either in the spring or fall when androgen levels are highest. *(Adapted with permission from Lind, C.M. et al.,* General and Comparative Endocrinology, ***166****, 590–599, 2010.)*

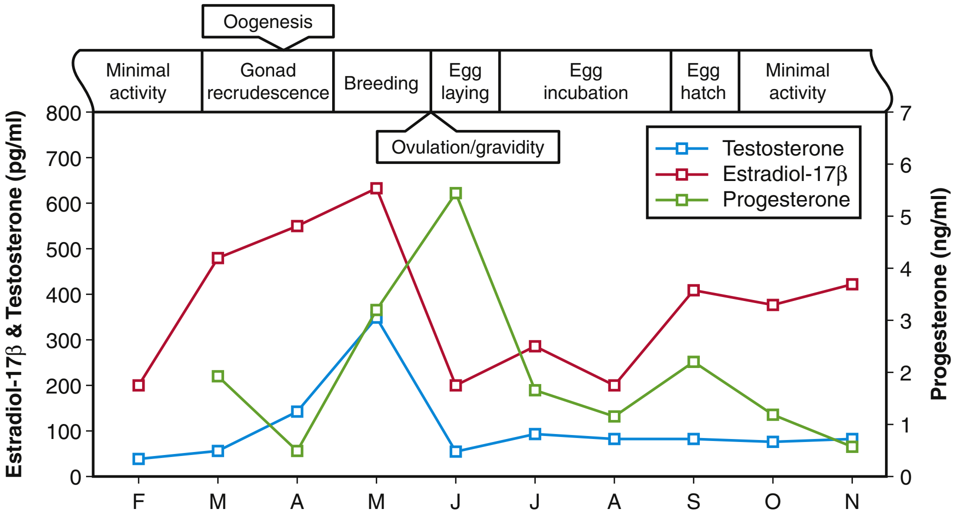


FIGURE 11-46 Seasonal reproductive cycle for female *Alligator mississippiensis*. *(Adapted with permission from Milnes, M.R., in "Hormones and Reproduction of Vertebrates. Vol. 3. Reptiles" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 305–319.)*

Ovaries of reptiles show different patterns of follicular maturation and ovulation. Some produce several eggs simultaneously from each ovary (most reptiles). Others may alternate production of a single egg from each ovary (anoline lizards). Differences in follicular atresia rather than in the number of oocytes beginning development may be responsible for these patterns. Still others (e.g., certain turtles) may produce most of their eggs in one ovary during one season and most from the other ovary the next season.

Exogenous GnRH produces direct actions on ovarian follicles that may reflect a paracrine role for ovarian GnRH. Treatment of lizards (*Podacris sicula sicula*) with salmon GnRH (sGnRH) increases secretion of prostaglandin ($PGF_{2\alpha}$) from mid- to late follicles and early corpora lutea and increases secretion of progesterone from follicles. Antagonists of GnRH produce opposite results. The physiological relevance of these observations is open to interpretation, but it does suggest that paracrine effects of GnRH are not limited to mammals.

Alternation of ovulation in the ovaries of the anoline lizard *Anolis carolinensis* has been investigated extensively. There is a definite alteration in catecholamine activity in the hypothalamus that mirrors ovarian alternation. These

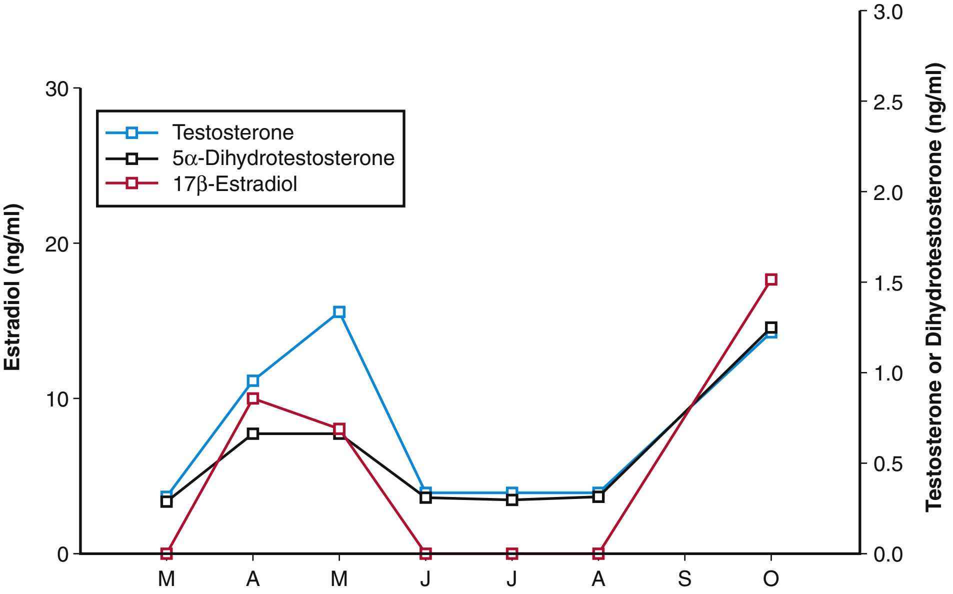


FIGURE 11-47 **Seasonal steroid levels in female northern Pacific rattlesnakes (*Crotalus oreganus*).** *(Adapted with permission from Lind, C.M. et al.,* General and Comparative Endocrinology, ***166****, 590–599, 2010.)*

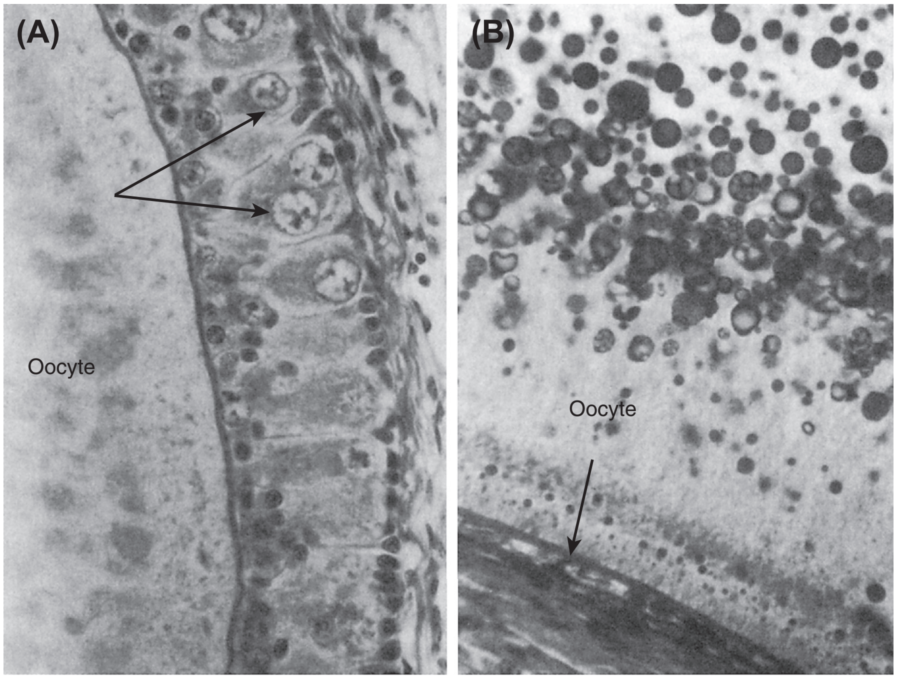


FIGURE 11-48 **Ovary of the iguanid lizard *Ctenosaura pectinata*.** (A) Section through a perivitellogenic follicle with large pyriform cells (arrows) in the granulosa layer. (B) Lower magnification of a vitellogenic follicle. Note the flattened granulosa (arrow). *(Courtesy of Dr. Mari Carmen Uribe, Facultad de Ciencias, UNAM, Mexico.)*

observations may be explained by sensory neural connections between the ovaries and brainstem areas projecting to the hypothalamus that are transmitting information responsible for regulating the alternating pattern of ovulation.

Reptilian oviducts are differentiated into several regions. Squamate oviducts have only three distinct regions: a thin-walled region, the **infundibulum**; a muscular **uterus** with many glands; and a **vagina** connecting the uterus to the cloaca. The uterine glands are responsible for first secreting a fibrous layer around the egg and a surrounding shell that is calcified in oviparous species. The oviducts of crocodilians and turtles, species that generally make much larger eggs than squamates, have two additional regions between the infundibulum and the uterus. The major region is the glandular **uterine tube** that secretes the egg white and may

TABLE 11-8 Circulating Progesterone Levels (ng/mL) during Reproductive Cycles of Turtles, Lizards, and Snakes

Species	Period of early follicle growth	Preovulatory stage	Early postovulatory	Mid-pregnancy	Late pregnancy
Turtles					
Chrysemys picta (oviparous)	0.2 ± 0.06	5.0 ± 1.02	0.5 ± 0.01	—	—
Chelonia mydas (oviparous)	0.2 ± 0.08	1.8 ± 0.13	0.7 ± 0.88	—	—
Lizards					
Sceloporus cyanogenys	0.7 ± 0.15	0.9 ± 0.38	3.3 ± 0.48	—	3.5 ± 0.34
Chamaelo pumillis	0.9	1.0 ± 0.71	5.0 ± 3.90	2.3 ± 0.34	—
Snakes					
Natrix taxispilota	0.4 ± 0.04	0.9 ± 0.08	1.9 ± 0.24	—	—
Nerodia sipedon	1.3 ± 0.19	3.9 ± 0.83	5.0 ± 1.41	6.9 ± 0.78	2.8 ± 0.44
Thamnophis elegans	—	—	1.7 ± 0.30	6.2 ± 1.00	—

Adapted with permission from Lance, V. and Callard, I.P., *General and Comparative Endocrinology*, **35**, 295–301, 1978.

constitute 40% of the oviduct in these oviparous species. A short isthmus connects the uterine tube to the uterus where the shell is secreted. This arrangement is very similar to the oviducts of birds.

Oviductal development apparently is under the influence of ovarian estrogens, and progesterone is without effect. In oviparous species, estrogens probably influence the secretion around the egg of albumin and shell from the anterior end of each oviduct. Estrogens also stimulate synthesis of vitellogenic proteins by the liver necessary for oocyte growth and cause increases in serum calcium of oviparous snakes, lizards, and turtles that contributes to shell formation (Table 11-9). Crocodilians produce yolk proteins that are biochemically similar to those of birds.

Oviposition or birth of live young is controlled by AVT, prostaglandins, and β-adrenergic innervation in turtles, lizards, and snakes. In the American chameleon (*A. carolinensis*), the sensitivity of the uterus to AVT is determined by the presence or absence of a corpus luteum in the adjacent ovary.

TABLE 11-9 Effect of Estradiol on Serum Calcium Levels of Ovariectomized Female Lizards (*Anolis carolinesis*)[a]

Treatment	*N*	Serum calcium (mg/dL ± SE)
Ovariectomized saline-injected females	5	14.8 ± 1.05
Reproductively active, sham-operated females	4	21.2 ± 3.47
Ovariectomized females injected with 1.0 μg of estradiol per day for 7 days	5	213.2 ± 9.88
Ovariectomized females injected with 10 μg of estradiol per day for 7 days	7	256.0 ± 22.13

[a] *Unpublished data of K. Faber and D. Norris.*

D. Environment, Behavior, and Reproduction in Reptiles

The role that physical and biological components of the environment play in sexual behavior and reproduction has been extensively studied in reptiles. Species living in temperate climates exhibit distinct seasonal patterns of hormonal secretion and reproductive events. Reproduction in tropical reptilian species varies from cyclic patterns to continuous breeding (see Figure 11-49). There is a strong tendency for an observed increase in the incidence of viviparity among species inhabiting colder climates (altitude or latitude), but it is not clear which is cause or consequence.

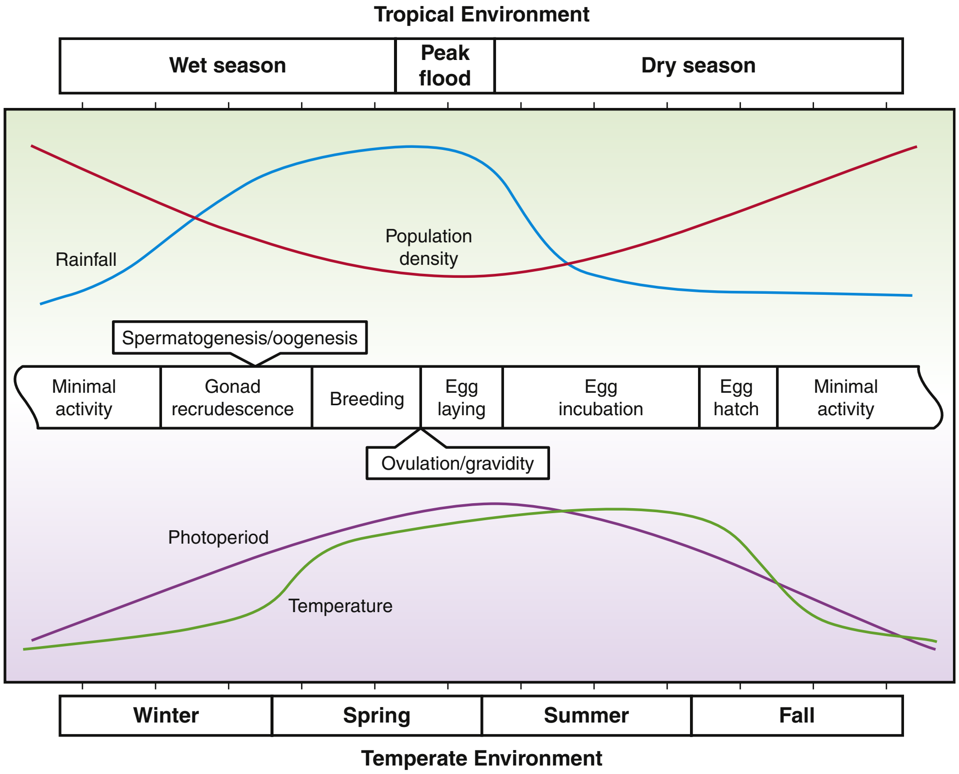


FIGURE 11-49 Comparison of reproductive cycles of crocodilians living in tropical and temperate environments. In tropical situations, population density increase during the dry season inhibits reproduction. Gonadal recrudescence occurs during the wet season. Temperate species are controlled by temperature and photoperiod. *(Adapted with permission from Milnes, M.R., in "Hormones and Reproduction of Vertebrates. Vol. 3. Reptiles" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 305—319.)*

Among temperate lizards, temperature is the dominant environmental factor influencing reproduction. Photoperiod, humidity, and nutritional status play decisive roles in some species. Other groups of reptiles have not been studied as extensively as lizards.

Although visual cues are the primary mechanism employed in reptilian courtship, evidence for pheromonal communication can be inferred from some experimental studies in all major reptilian groups. Male lizards of several families (Scincidae, Lacertidae, Teidae, Gekkonidae) have androgen-dependent **femoral glands** located on the inner thighs as well as special cloacal glands that seem to play important roles in courtship and territorial behavior in association with breeding. Female garter snakes, *Thamnophis sirtalis parietalis*, produce pheromones that are exuded from the skin and elicit courtship by males. Cloacal secretions by brown tree snake (*Boiga irregularis*) females apparently prevent courtship by unwanted suitors. Inguinal and axillary glands of chelonians have been implicated in reproductive behavior, too. Crocodilians appear to use chemical communication in courtship, but few details are available.

When a female red-sided garter snake (*Thamnophis sirtalis*) emerges from its winter hibernaculum, she is immediately courted by a large number of males who had emerged previously. This behavior produces a mating ball of males, all attempting to copulate with a single female. The skin of the reproductive female produces methyl ketone that apparently in the presence of estrogens attracts the males and signals that she is ready to mate. As soon as one male successfully copulates with the female, she secretes another semiochemical that immediately turns off male mating behavior. Although injection of estrogens into adult males does not make them attractive to other males, some males with high testosterone and aromatase levels also are attractive to normal males, presumably because of the conversion of testosterone to estrogens. These "she-males" are more successful in achieving copulation with females than are normal males, presumably because the "she-males" confuse normal males who attempt to mate with them rather than with the true female, thus reducing their competition. Interestingly, males that are courting have lower corticosterone levels than males that are dispersing from the breeding area. Furthermore, the mating males show no stress response.

One model for studying reptilian behavior is exemplified by the work of David Crews and his collaborators at the University of Texas. In general, estrogen and progesterone are responsible in reptiles as in most vertebrates for stimulating female receptive and mating behaviors, whereas androgens, typically testosterone, control male behaviors. As previously mentioned, in a number of cases, androgens may be converted to estrogens by aromatase in order to produce behavioral effects. Among the species of whiptail lizards in the genus *Cnemidophorus*, about one-third are unisexual. These species consist only of females that are further unusual in that these females are all triploid (3*n*). Studies by Crews have employed one 3*n* species, the desert-grasslands whiptail (*C. uniparens*), which apparently evolved from hybridization of two 2*n* species, the rusty rump whiptail (*C. burti*) and the little striped whiptail (*C. inornatus*). Because *C. uniparens* has two sets of chromosomes derived from *C. inornatus* and only one from *C. burti*, Crews focused his behavioral studies on *C. uniparens* and *C. inornatus* (which exhibits normal sexual reproduction). In *C. uniparens*, mating does not occur with males of diploid species, but rather the 3*n* females alternate between expressing female (receptive) and male (mounting) mating behaviors (Figure 11-50). Although estradiol levels in the 3*n* females is five times less than in 2*n* females, this results in higher hypothalamic levels of estrogen receptor mRNA in the 3*n* brains and hence greater sensitivity to low plasma levels of estradiol. The postovulatory decrease in estrogen allows for the appearance of male-like mounting behavior or **pseudocopulation** although the exact chemical stimulus is not clear.

E. Parental Behavior in Reptiles

Reptiles classically have been characterized by a lack of parental behavior and, in some cases, even lack of recognition of their offspring. In recent years, however, studies have demonstrated that there is considerable investment in parental care even among oviparous species. Although members of the oldest extant group, the chelonians, typically abandon their nests once the eggs are laid, many squamates exhibit parental behavior. Crocodilian parents participate in the hatching process and in protecting the young. Evidence of nest building and parental care has been unearthed for some extinct dinosaurs, as well. Thus, complex parental care did not appear *de novo* in birds and mammals but already had evolved in early reptiles.

VII. REPRODUCTION IN BIRDS

Knowledge of the reproductive endocrinology of birds is based largely on the intense investigations of only a few domestic (e.g., chicken, Japanese quail, emu, turkey, ostrich) and wild migratory (e.g., white-crowned sparrow) species with limited information from numerous other species. The HPG axis of birds appears to be controlled by both the production of GnRH-1 in the PVN and by a GRIH neuropeptide from the same nucleus in the hypothalamus

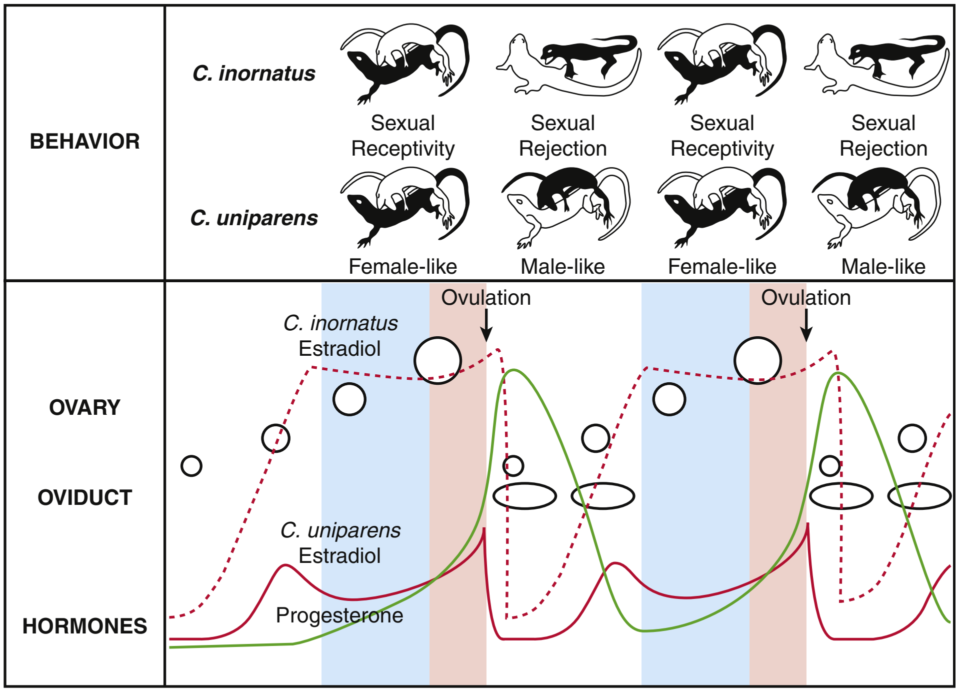


FIGURE 11-50 Comparison of mating behavior in unisexual and bisexual lizards. Female-like and male-like behavior in the all female desert-grasslands whiptail lizard (*Cnemidophorus uniparens*) is compared with female receptive behavior in a bisexual lizard, the little striped whiptail (*C. inornatus*). The differences in estradiol levels for the two species are illustrated (red lines). Female-like behavior in *C. uniparens* is elicited by a lower estrogen level and is followed by male-like copulatory behavior. The circles represent size of the ovarian follicles and the ovals indicate presence of eggs in the oviduct. *(Adapted with permission of the publisher from Young, L.J. and Crews, D.,* Trends in Endocrinology & Metabolism, ***6****, 317–323, 1995.)*

controlling secretion of LH and FSH from the pituitary (see Chapter 5). Secretion of GRIH appears to be regulated by melatonin from the pineal gland. These dual factors regulating reproduction in migratory birds are controlled by photoperiodic information.

Avian reproductive organs (Figure 11-51) reflect the anatomical adaptations to flight that characterize most bird systems. In females of most species, only the left ovary and its attendant oviduct develop, whereas the right-hand components remain in a rudimentary state. Should the left ovary be removed surgically or destroyed by disease, the right rudiment may develop, but it will usually form an ovotestis or a testis rather than another ovary. This left—right asymmetry is reflected in the male where the left testis usually is larger than the right although both are functional.

Avian gonads develop from a pair of undifferentiated primordia associated with the embryonic nephrotome. These primordia are invaded by primordial germ cells that migrate through blood vessels to reach the developing gonads, where they develop into the germinal epithelium. As in other vertebrates, the embryonic gonad goes through a bipotential state in which both cortical and medullary components are present. Differentiation of cortical tissue is necessary for ovarian development, and the medullary portion is suppressed. The reverse condition prevails in male birds. In contrast to mammals it is the male bird that is the homogametic sex (ZZ), and it is the female that has unlike sex chromosomes (ZW). Developing a female phenotype requires estrogens, and castration of a young female may result in development of male plumage.

Development or suppression of the müllerian and wolffian ducts eventually depends on the direction of gonadal development as it does in amphibians and reptiles. In females in which only the left half of the reproductive system usually develops, the left ovary receives the larger proportion of germ cells that migrate to the gonads. The mechanism behind this disproportionate distribution of germ cells is not known. Degeneration of the müllerian duct occurs only on the side of the smaller ovary and is induced by AMH produced in ovarian cells. Local secretion of estrogens by the larger ovary is believed responsible for preventing oviduct degeneration on that side.

All birds are oviparous but display significantly more parental behavior than any non-mammalian group of vertebrates. No placenta has evolved in birds although they are common in reptiles; however, studies of the **chorioallantoic membrane (CAM)** of birds indicate this membrane has the capacity to synthesize progesterone *in vitro* if given the appropriate precursor. This membrane gives rise to placentas in many viviparous reptiles and in mammals. Birds have evolved other mechanisms to provide extended care to their progeny (see ahead).

Birds are endothermic like mammals and use body heat to support development of the embryo within the egg much as the mammal does *in utero*; consequently, birds can breed successfully under conditions that are too cold for their reptilian relatives. The adaptation of long-distance flight coupled with high body temperatures allows utilization of polar and subpolar regions where winter conditions are too severe for survival but where the summer months provide adequate warmth and food to breed and rear young birds to a size sufficient for successful fall migration to warmer latitudes for winter.

Reproduction is decisively cyclic in adult birds and is closely attuned to environmental factors, especially temperature and photoperiod (Figure 11-52). Olfactory systems are minimally developed in most birds, and

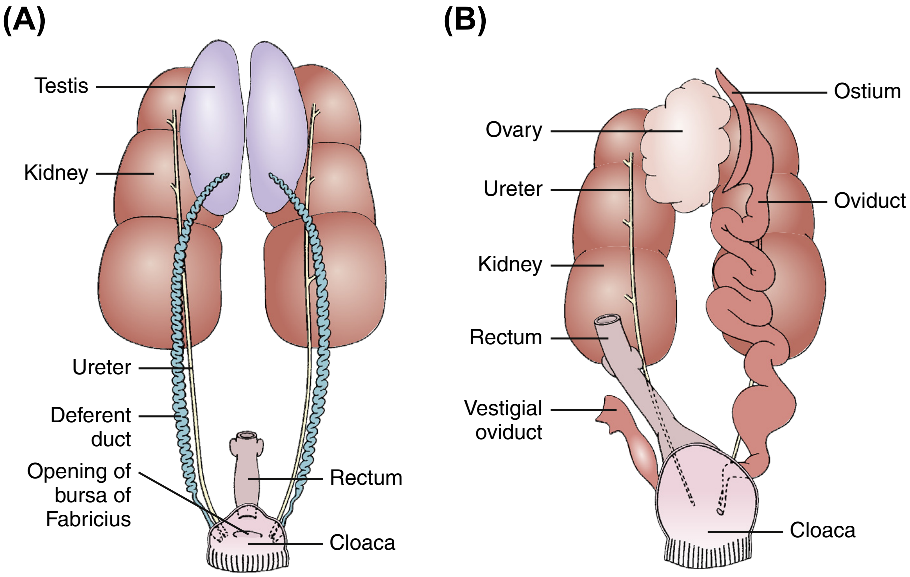


FIGURE 11-51 Reproductive organs of the pigeon. The top of the cloaca has been removed in both sexes to illustrate connection of the gonaducts. (A) Male. (B) Female. Note that the right ovary is absent in most birds (the left in others) as well as the corresponding oviduct. This regression is due to production of AMH in the embryo by the remaining ovary which secretes estradiol locally and protects the müllerian duct on that side from AMH. *(Adapted with permission from Matsumoto, A. and Ishii, S., "Atlas of Endocrine Organs: Vertebrates and Invertebrates," Springer-Verlag, Berlin, 1992.)*

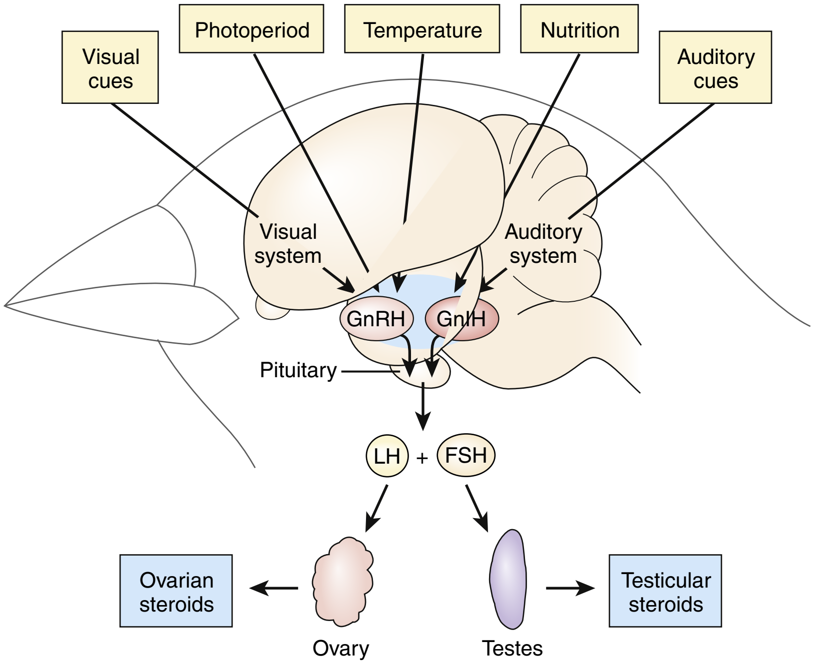


FIGURE 11-52 Environmental and endocrine factors controlling reproduction in birds. Environmental factors influence secretion of GnRH or GnIH from the hypothalamus that control release of gonadotropins (LH and FSH) from the adenohypophysis (Pit). The gonadotropins stimulate secretion of estrogens and other hormones by the ovary (OV) and androgens and other hormones by the testes (T). *(Adapted with permission from Ramenofsky, M., in "Hormones and Reproduction of Vertebrates. Vol. 4. Birds" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 205–237.)*

pheromones do not appear to be important in regulating reproduction as compared to all other terrestrial vertebrates. Both migratory and non-migratory species exhibit seasonal cycles, with breeding occurring in the spring and sometimes continuing through much of the summer (Figure 11-53). On the other hand, species occupying arid regions may show irregular cycles cued to the availability of water, which may not occur with any seasonal regularity.

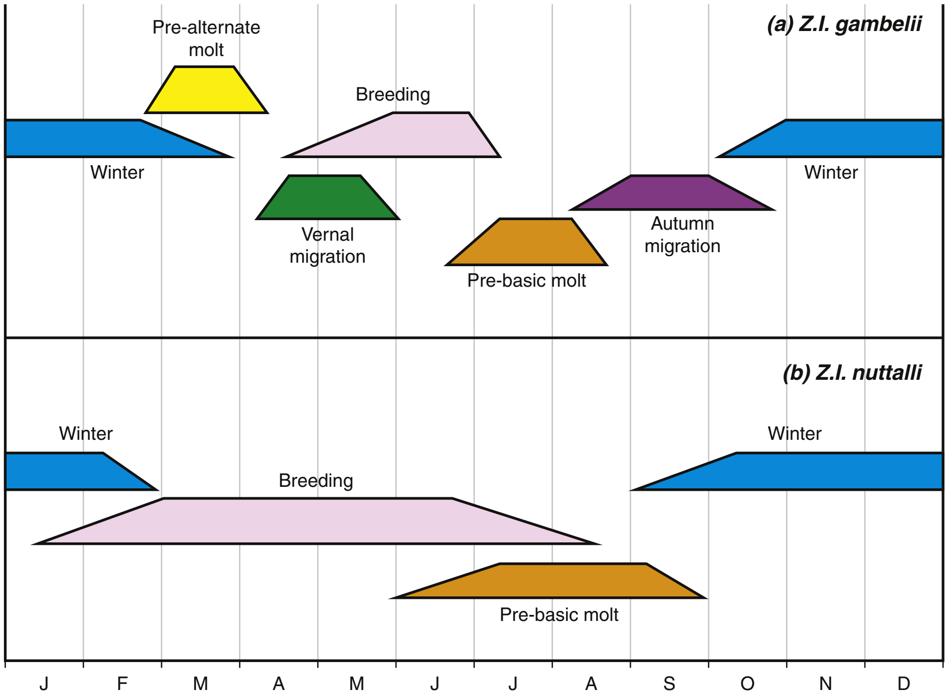


FIGURE 11-53 Reproductive patterns for two subspecies of white-crown sparrows (*Zonotrichia leucophrys*). The *Z. l. gambelii* subspecies migrates great distances to breed in northern habitats where winter survival is not a possibility. The sedentary subspecies *Z. l. nutalli* exhibits the pattern typical of temperate non-migratory species. Thickness of the lines reflects intensity of activity. *(Adapted with permission from Ramenofsky, M., in "Hormones and Reproduction of Vertebrates. Vol. 4. Birds" (D.O. Norris and K.H. Lopez, Eds.), Academic Press, San Diego, CA, 2011, pp. 205–237.)*

One American species, the rufous-collared sparrow (*Zonotrichia capensis*), has been reported to breed every 6 months regardless of rainfall as long as food is available.

Generally, ovaries and testes remain small in non-breeding birds and undergo tremendous hypertrophy in a very short time at the onset of the breeding season. This is especially advantageous for migratory species or species relying on particular stimuli for breeding where gonadal recrudescence can await arrival on the breeding grounds or appearance of suitable conditions such as abundant food.

A. Male Birds

Each testis consists of a mass of convoluted seminiferous tubules lined with a germinal epithelium and surrounded by connective tissue. Both developing germ cells and steroidogenic Sertoli cells as well as numerous fibroblasts can be seen in the germinal epithelium. As in other vertebrates the cytoplasm of the Sertoli cells completely envelops the germ cells. Typical steroidogenic interstitial cells occur between the seminiferous tubules as seen in reptiles and mammals.

In non-breeding birds, testes are very small, and histologically these quiescent testes appear to be composed largely of interstitial cells due to a marked post-breeding regression of spermatogenetic tissue. The onset of spermatogenesis (recrudescence) results in a rapid and marked increase in testicular size. Such rapid and extreme growth (to as much as 500 times the resting gonad weight in some species) results in considerable strain and damage to the tunica albuginea surrounding the testis, and it must be replaced each year during the postnuptial phase of the testicular cycle. Replacement is accomplished through differentiation of fibroblasts and formation of a new tunica directly beneath the damaged one. It is often possible to distinguish histologically between juvenile birds and postnuptial birds by the presence of two connective tissue capsules around the testis in the latter.

Testicular recrudescence may involve a single synchronous spermatogenetic event or separate spermatogenetic waves, depending on whether a given species produces successive clutches during a particular breeding season. In either event, following spermiation, sperm migrate to expanded distal ends of the vasa deferentia known as **seminal sacs** from which sperm will be ejaculated forcefully during mating.

1. The Avian Testicular Cycle

The annual testicular cycle of temperate birds has three more or less distinct phases: (1) regeneration or **preparatory phase**, (2) acceleration or **progressive phase**, and (3) **culmination phase**. Similar phases can be identified in all birds regardless of the seasonal nature of their reproductive cycles or what environmental factors control testicular events. The most common environmental factor influencing development of the avian testis is **photoperiod**. Placing quiescent, temperate birds, such as the ostrich (*Struthio camelus*) on long day photoperiods will typically stimulate testicular recrudescence, whereas maintenance of these birds on short-day photoperiods even into the normal breeding season represses anticipated testicular events. In contrast, some birds, such as the emu (*Dromaius novaehollandiae*), are short-day breeders with peak testicular function occurring during the winter months and quiescence being maintained under long-day photoperiods.

The preparatory phase of testicular development begins immediately after the reproductive period and is characterized by marked collapse of the testis. Animals in the preparatory phase are insensitive to effects of long photoperiod and are termed **photorefractory**. The end of the preparatory phase is heralded by restoration of **photosensitivity** that characterizes the following two phases. The endocrine basis for the photorefractory period in birds is not clear and more than one mechanism may be involved in different species. Some studies suggest that feedback of testosterone on the hypothalamus is responsible for induction of the photorefractory period and for lower levels of LH during the photorefractory period. Other investigations point to changes in hypothalamic sensitivity and/or steroid metabolism and not testosterone feedback. An intriguing hypothesis proposed by Peter Sharp involves the inhibitory role of PRL that determines GnRH secretion in both long-day and short-day breeders.

During the progressive phase, there is an increase in GTH secretion brought about by actions of lengthening photoperiod on the HPG axis. Increased circulating GTHs stimulate both spermatogenesis and androgen secretion by the interstitial cells. An increasingly intensive period of sexual activity (often including singing) occurs, and males of some species may begin exhibiting territorial behavior and mate selection. This effect of long photoperiod can be blocked by low temperatures.

The culmination phase coincides with the time of ovulation in females and includes the time of insemination. The male typically is ready for breeding before the female, and his testes will be bulging with sperm. Successful breeding involves a complex, hormonally dependent series of events involving precise male-female behavioral interactions.

2. Leydig Cells in Birds

A characteristic lipid cycle occurs in avian Leydig cells similar to that described for other vertebrates. There is accumulation of lipid in young birds followed by rapid depletion coincident with onset of the first breeding season and spermatogenesis. The Leydig cells of adult birds are small and sparsely lipoidal in winter, although they occupy a large proportion of the testis because of the regressed nature of the seminiferous tubules. There is a gradual accumulation of lipids, including cholesterol, throughout the progressive phase as well as an increase in 3β-HSD activity. At the time of maximal sexual display, there is rapid depletion of Leydig cell lipid. Cholesterol disappears completely, but 3β-HSD activity remains strong, indicating that lipid depletion is a consequence of rapid synthesis and secretion of androgens. The activity of 17α-hydroxylase is also high at this time (see Chapter 3 for its specific role in androgen synthesis). A massive disintegration of interstitial cells occurs during the preparatory phase, and new Leydig cells differentiate from fibroblasts.

3. Sertoli Cells in Birds

Cyclical changes in lipid content are characteristic of avian Sertoli cells that ultrastructurally resemble steroidogenic cells. Both 3β-HSD and 17β-HSD activities have been reported for these cells. They become densely lipoidal following the breeding season, and no detectable 3β-HSD activity remains. The stored lipid is depleted with the onset of the next period of spermatogenesis.

4. Endocrine Control of Testicular Function

The hypothalamus controls release of LH and FSH from the adenohypophysis through secretion of GnRH or GnIH (Figure 11-52). LH causes hyperplasia of Leydig cells, which become lipoidal and exhibit increased 3β-HSD activity. Androgens secreted by Leydig cells stimulate sex accessory structures and secondary sexual characters. Mitotic divisions of spermatogonia are stimulated by FSH, and local effects of androgens from Sertoli cells are responsible for stimulating meiosis. Once initiated, androgens can maintain spermatogenesis in hypophysectomized birds. The patterns of LH and androgen secretion are illustrated for ostrich (Figure 11-54) and king penguins (Figure 11-55). Testosterone levels for black kites are provided in Figure 11-56. Prolactin is present in the male pituitary and has been reported to inhibit FSH release and block spermatogenesis in some species. The formation of incubation patches (see ahead) on males of certain species

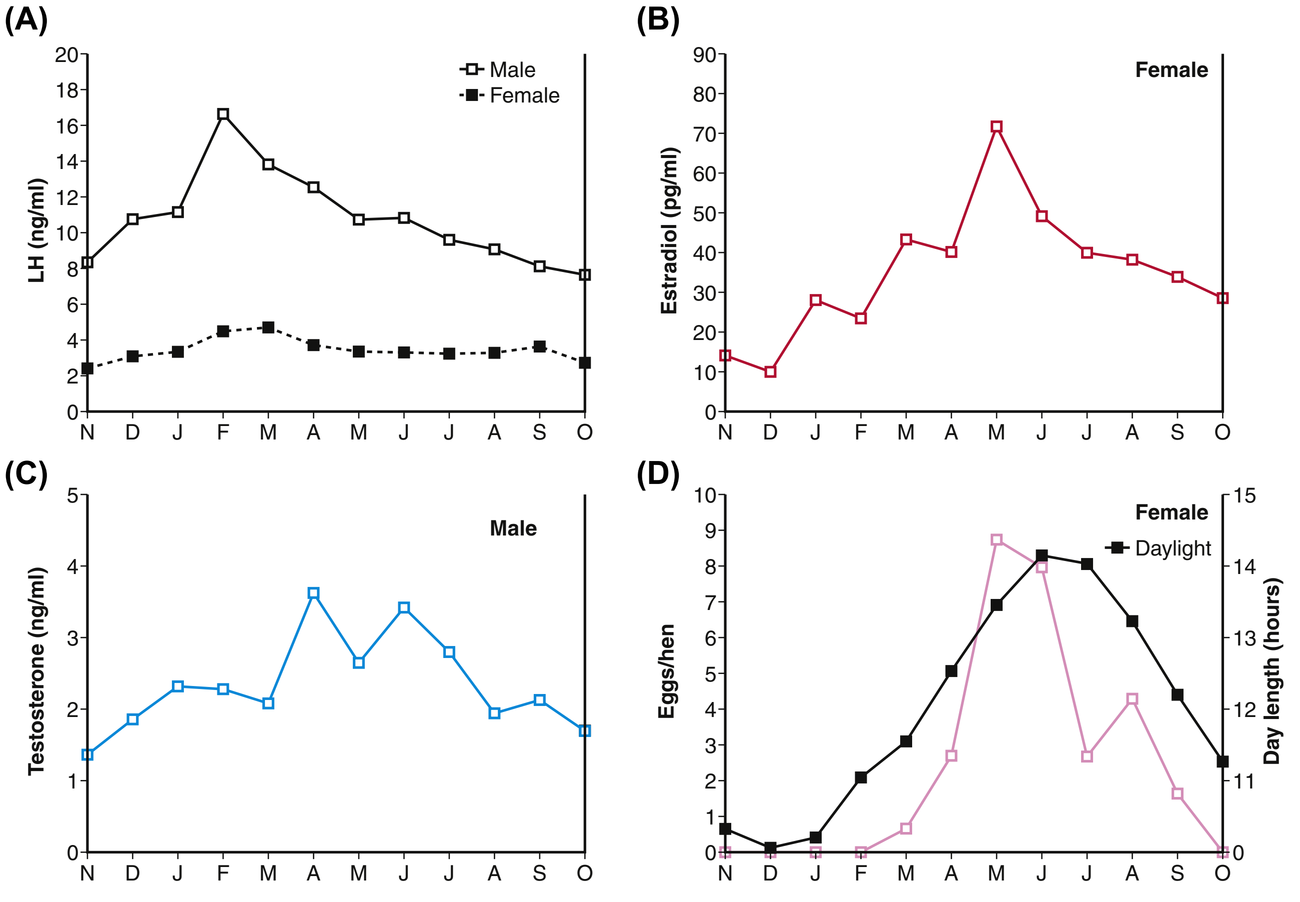


FIGURE 11-54 **Plasma LH, plasma estradiol, and egg production (light bars) in female South African ostriches are affected by photoperiod.** *(Adapted with permission from Degen, A.A. et al.,* General and Comparative Endocrinology, ***93****, 21–27, 1994.)*

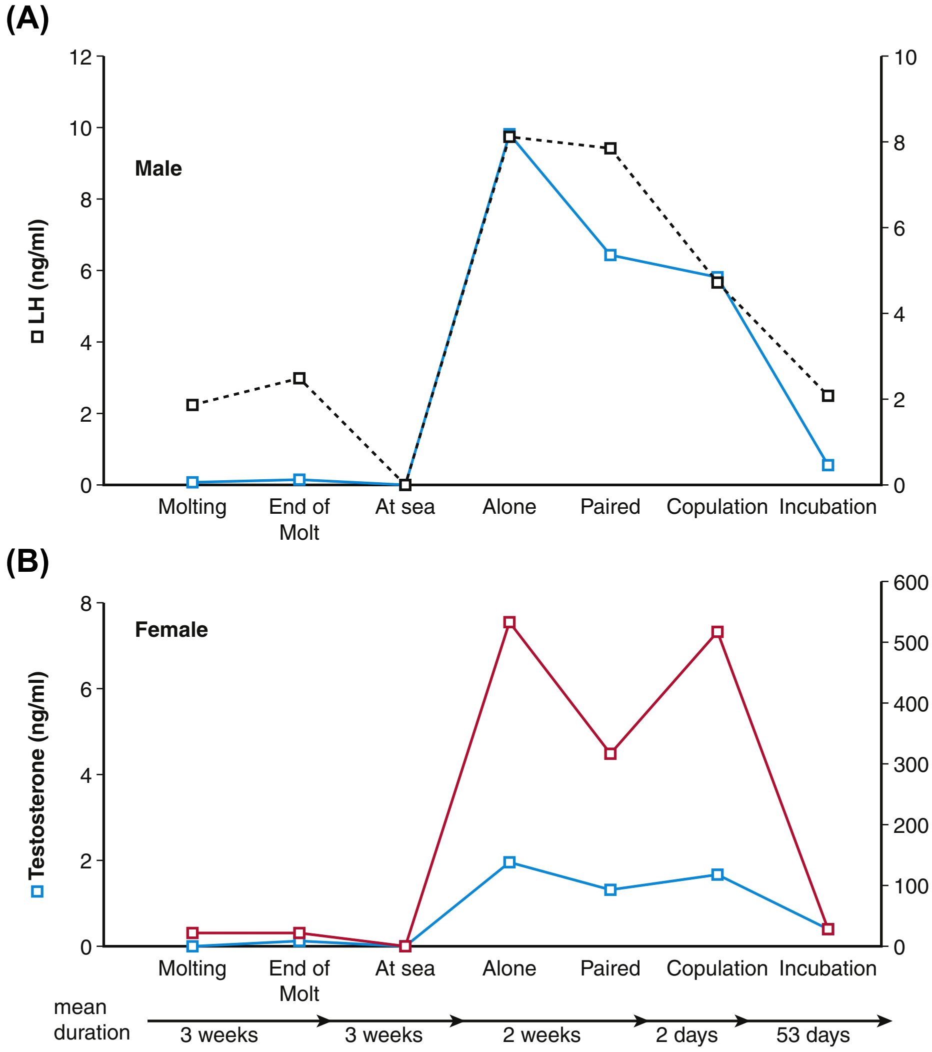


FIGURE 11-55 **Plasma hormone levels in male and female King penguins from molting to the onset of egg incubation.** (A) Plasma LH and testosterone levels in males. (B) Plasma, testosterone and estradiol in females. LH levels (not shown) paralleled that for the males in (A). *(Adapted with permission from Mauget, R. et al.,* General and Comparative Endocrinology, ***93****, 36–43, 1994.)*

is induced in part by PRL working cooperatively with testicular steroids.

5. Sex Accessory Structures in Male Birds

Wolffian ducts give rise to paired vasa deferentia, vasa efferentia, and the epididymides. These structures exhibit hypertrophy with the onset of sexual activity that is dependent on testicular androgens. Each testis is connected to vasa efferentia by small rete tubules in the tunica albuginea that become enlarged during the breeding season. The vasa efferentia show increased secretory activity during the breeding season and coalesce to form a long, coiled tube, the epididymis. Hypertrophy of the epididymis is accompanied by secretion of seminal fluid. Mature sperm leave the epididymis and enter the enlarged vas deferens for storage. The distal end of each vas deferens (seminal sac) fills with sperm. The posterior walls of the seminal sacs protrude into the cloaca as erectile papillae that facilitate transfer of sperm to the female. During copulation, the male's cloaca is everted and these erectile papillae are brought into contact with the vagina of the female. In some species, the cloaca actually is modified into a penis-like intromittent organ.

B. Female Birds

Assessment of ovarian function in domestic species is relatively complete and provides a basis for comparison with wild species. In many respects, ovarian function in birds is like that of their oviparous ancestors, the chelonians and especially the crocodilians. However, females of domestic species differ most importantly from wild birds

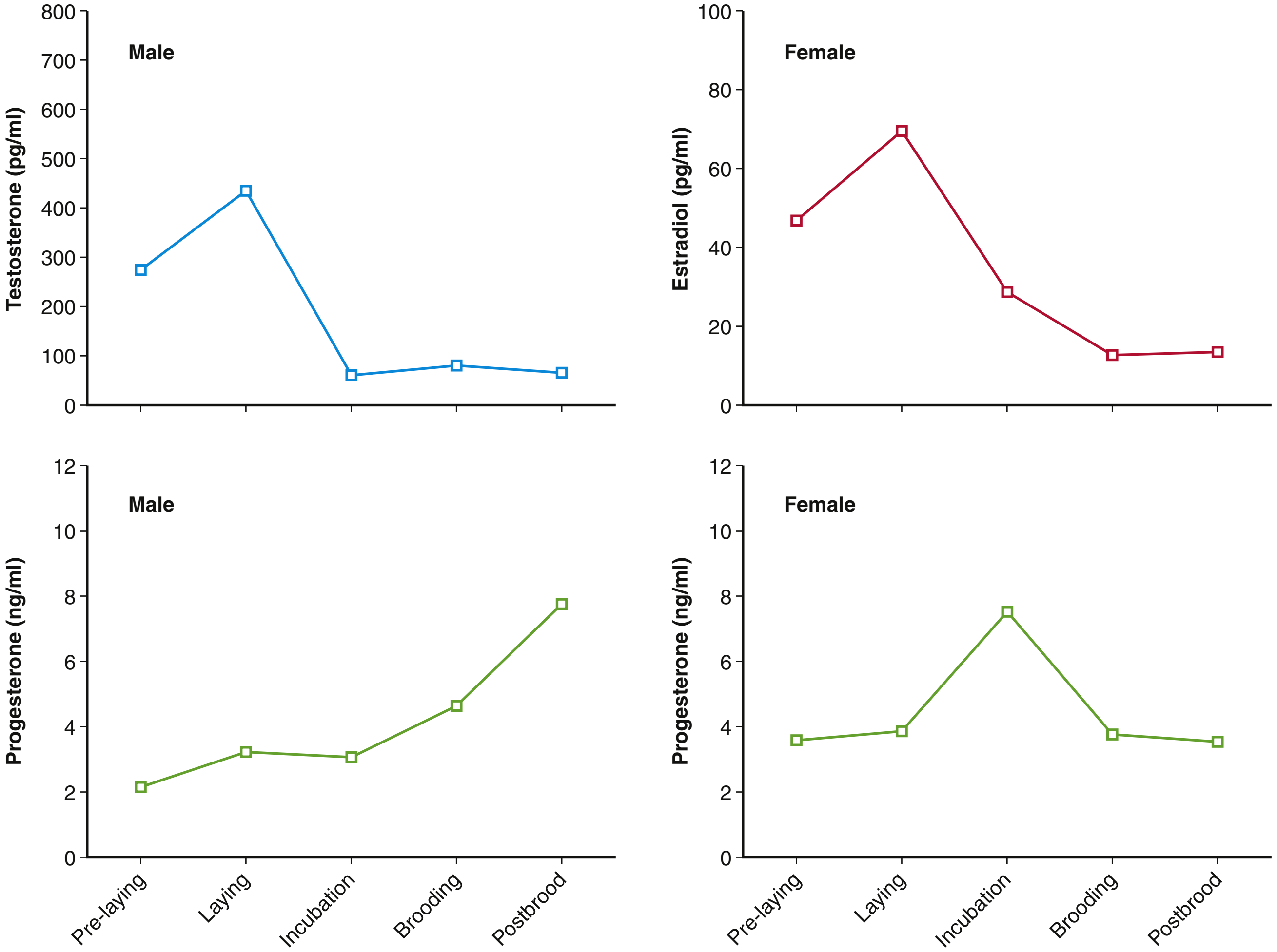


FIGURE 11-56 **Plasma steroid levels in male and female black kites (*Milvus migrans*).** *(Adapted with permission from Blas, J. et al.,* General and Comparative Endocrinology, ***168****, 22–28, 2010.)*

by being selected for continuous breeding. The following account is based largely on studies of the domestic hen.

Prior to hatching there is a proliferation of oogonia to produce thousands of primary oocytes that will serve the hen throughout her long and busy reproductive life. Whether new oocytes will be formed after hatching, as recently described for mammals, remains to be demonstrated. However, most of these oocytes will undergo atresia during early maturational stages.

A primary follicle consists of an oocyte surrounded by a layer of granulosa cells. As the follicle grows, thecal layers are added, and the follicle becomes highly vascularized. Both granulosa and thecal cells are steroidogenic, possess 3β-HSD activity and produce steroids as described for mammals. However, in birds, the entire steroidogenic process may involve three cells rather than two. For example, in the domestic turkey, progesterone is synthesized by the granulosa cell, whereas androgens are made in cells of the theca interna and converted to estrogens in the theca externa. This pattern has yet to be confirmed in wild species.

Estrogens secreted from the follicular cells cause the liver to produce large quantities of calcium-binding vitellogenin and triglycerides for the growing oocytes. This mechanism brings calcium to the oviducts where it is used to construct the shell. Vitellogenin is enzymatically hydrolyzed to produce phosvitin and lipovitellin, which are stored in the yolk of the egg. Incorporation of phosphoproteins and triglycerides into growing oocytes is stimulated by FSH. Avian follicles, like those of other nonmammals, do not form an antrum as described for mammalian follicles (Chapter 4).

Not only does the female place nutrients in the egg to support early development but she also provides a number of hormones that influence the offspring in many ways. For example, differences in androgen levels in eggs have been shown to influence behavior, growth morphology, immune functions, and survival. Studies show that a female can influence the levels of androgens deposited in different eggs or in different clutches of eggs, thus perhaps adjusting development to environmental conditions. Nestlings of the

Florida scrub jay (*Aphelocoma coerulescens*) that exhibit higher corticosterone levels of maternal origin are more fearful as adults. Furthermore, nestlings with lower corticosterone levels breed early than their siblings that have higher corticosterone levels as nestlings. Although the underlying mechanisms of controlling egg hormone levels are not understood, this is an intriguing area that deserves more attention.

The large developing follicles of birds bulge conspicuously from the surface of the ovary, giving it the appearance of a bunch of grapes. The largest follicles are highly vascularized except for a rough, avascular spot, the **stigma**, where the follicle will rupture at ovulation.

Atresia of developing follicles may occur at any time during follicular development. These atretic follicles can be easily recognized histologically by an influx of fibroblasts that phagocytize the yolk materials. Granulosa and thecal cells are lipoidal and contain cholesterol. There are many corpora atretica at all times in the ovary, but their endocrine importance is not known. However, some of the cells of the disintegrating corpora atretica may become stromal interstitial cells and secrete estrogens.

Birds are characterized by the absence of persistent corpora lutea following ovulation. This feature is correlated with the absence of viviparity among all of the more than 8000 known species of birds. Following ovulation, collapsed follicles consist largely of granulosa cells containing progesterone, abundant smooth endoplasmic reticulum, and considerable 3β-HSD activity. The only evidence for a functional role, however, is the observation that surgical removal of these ruptured follicles increases the time that the ovulated egg is retained in the oviduct.

1. Endocrine Control of Ovarian Functions in Birds

There is a close correlation between pituitary GTH content and ovarian function in both domestic and wild birds as evidenced by patterns of hormone secretion (see Figures 11-53, 11-54, and 11-55). Hypophysectomy results in ovarian regression and extensive follicular atresia, which can be prevented by GTH replacement therapy. Follicle development is stimulated by FSH, and FSH will maintain oviducts in hypophysectomized but not in ovariectomized birds. Secretion of estrogens is controlled by both LH and FSH. Although mammalian GTHs are not always as effective as avian GTHs, avian FSHs are very effective at stimulating follicle development in lizards, emphasizing the close similarity between reptilian and avian pituitary hormones.

As is the case for certain reptiles, growth of follicles and ovulation is a continual process throughout the breeding season. Ovarian function is regulated so that typically only one egg is discharged at a time. This condition is reminiscent of the human and the lizard *A. carolinensis*, in which only one ovum is discharged, and the ovaries alternate in providing the ovum. However, recall that birds have only one functional ovary with a hierarchy of graded follicle sizes. The endocrine basis for establishment and maintenance of this hierarchy is not known for reptiles, birds, or mammals, and it represents one of the major unanswered questions in reproductive biology.

Estrogens induce the synthesis of vitellogenin by the avian liver. Total serum calcium concomitantly increases, which is related to the binding of calcium by vitellogenin (Table 11-10). In addition to incorporation of vitellogenic proteins into the oocyte, circulating calcium is sequestered by the shell glands of the "uterus" (an expanded region of the oviduct; see ahead) for construction of the eggshell.

Pituitary LH is responsible for triggering ovulation of the fully mature follicle. Plasma LH peaks about 6 to 8 hours before ovulation in domestic hens as well as in Japanese quail, but the magnitude of the avian LH surge is considerably smaller than that observed in mammals. This lower surge of LH might be an adaptation to ensure only sufficient LH for ovulating the largest follicle.

Calcium availability may be a potent factor regulating reproduction in female birds. Production of shelled eggs in domestic species directs as much as 10% of the body calcium stores per day into eggs. If large amounts of calcium are not available in the diet, the reproductive axis of the egg factory is shut down before damage to the skeleton occurs. When sufficient calcium becomes available, the birds resume laying. Although continuous egg laying does not occur in wild birds as it does in domestic fowl, it is possible that calcium depletion in wild birds contributes to cessation of breeding and induction of the refractory period.

Another pituitary hormone, PRL, plays essential roles in reproduction, including the development of a specialized, de-feathered region in some species known as an **incubation patch**, which aids in incubating eggs (see ahead). Secretion of **crop milk** by the crop sac of some birds for use in feeding young hatchlings is induced by

TABLE 11-10 Effect of Mammalian Parathyroid Extract and the Influence of the Egg-Laying Cycle on Total Serum Calcium of Chicken

Subject	Control (mg/dL ± SE)	Treated with parathyroid extract (mg/dL ± SE)
Roosters	10.1 ± 0.2	19.5 ± 3
Nonlaying hens	13.4 ± 2	19.5 ± 4
Laying hens	29.8 ± 11*	47.7 ± 9[a]

[a] *Significantly different from roosters and non-laying hens.*
**From Assenmacher, I., in "Avian Biology, Vol. 3" (D.S. Farner and J.R. King, Eds.), Academic Press, New York, 1973, pp. 183–286.*

PRL. This feature of PRL has resulted in development of a most useful biological assay for tetrapod vertebrates called the **pigeon crop sac assay** (see Chapters 4 and 5 and Appendix D). Prolactin does not affect steroidogenesis in cultured chick granulosa cells and possibly has no effect on progesterone synthesis.

2. The Avian Oviduct

Estrogens secreted by the growing follicle stimulate hypertrophy of the oviduct and differentiation of secretory regions. Five differentiated regions can be identified in the mature avian oviduct which are like those of crocodilians (see above): **infundibulum**, **magnum**, **isthmus**, **shell gland**, and **vagina**. After ovulation, the ovum moves through the body cavity, enters the open end of the infundibulum, and is fertilized in the upper portion of the oviduct before the egg white protein **albumen** is added. The middle portion of the oviduct or magnum becomes highly glandular under the influence of estrogens, forming tubular glands and goblet cells. Estrogens stimulate synthesis of ovalbumen protein by these tubular glands, whereas progesterone stimulates the goblet cells to secrete the other major egg white protein, **avidin**. After accumulation of several coatings of albumen, the egg passes from the magnum to the muscular isthmus, where two shell membranes are applied. These membranes are composed of fibrous proteins cemented together with albumen. The shell consists largely of calcium salts supported by a fibrous protein matrix deposited on the outermost shell membrane by the shell gland or "uterus" (see Chapter 14 for the role of hormones in making Ca^{2+} available for eggshell formation). After the shell has been applied, contraction of a powerful sphincter muscle causes the egg to rotate in the muscular vagina and enter the cloaca pointed-end first. Movement of the egg into the cloaca as well as its extrusion into the nest (oviposition) is controlled by AVT and prostaglandins. An increase in plasma AVT together with a concomitant decrease in neurohypophysial AVT coincides with oviposition. Treatment with AVT can cause premature oviposition.

3. Incubation Patches

In many avian species, a ventral region of skin called the **apterium** becomes de-feathered, highly vascularized, and edematous just prior to or during egg laying to form the incubation or brood patch. In addition, the epidermis of this region may exhibit hyperplasia. When in contact with the eggs it provides an efficient transfer of warmth from the parent's incubation patch to the eggs. Incubation patches may form in females, males or both, depending upon the species and which sex is responsible for incubating eggs; however, possession of an incubation patch by a male is not requisite for exhibition of incubating behavior. Male house sparrows (*Passer domesticus*) have no incubation patch, yet exhibit incubating behavior. Conversely, male flycatchers (genus *Empidonax*) develop an incubation patch like that of the female but do not show incubating behavior.

Formation of incubation patches involves cooperative actions of both estrogens and PRL (see Figure 11-56). Estrogens stimulate vascularization of the patch region, and PRL stimulates de-feathering and epidermal hyperplasia. Typically, pituitary lactotropes transform at this time to a stimulated morphology known as "broody cells." Both PRL and estrogens are necessary for complete patch development. Furthermore, the response of the ventral skin epidermis to form a patch is both site specific and tissue specific. After transplant to the dorsal surface, ventral skin will still respond to PRL although not to estrogen. Vascularization of the ventral skin occurs only when it is in its normal location. Dorsal skin transplanted to the normal patch site will not respond to either estrogens or PRL.

C. Androgen-Dependent Secondary Sex Characters in Male and Females Birds

Androgens play important roles in both male and female birds. In a number of species, a change in bill color is associated with breeding. Such changes are induced in both sexes by androgens but not by estrogens or progesterone. However, there is at least one case in which bill color change occurs only in the female, and, in that case, the color change is induced by estrogens.

Plumage color changes also may be controlled by androgens. This can occur even in the case of phalarope birds where the females possess the more colorful or **nuptial plumage**. One cannot presume that androgens are responsible unless specific studies have been performed to verify this fact, because androgens are not always responsible for nuptial plumage. For example, development of nuptial plumage in castrated male weaver finches (*Euplectes orix*) has been used as the classical bioassay for LH (see Appendix D). In some instances, estrogens actively inhibit formation of nuptial dress, and castration of females will cause development of male plumage. Assumption of nuptial dress in males also can be blocked with estrogens, verifying that it is the absence of estrogens that allows male plumage to develop in both sexes and not the presence of androgens.

In some strains of domestic chickens, both sexes have female type plumage, and castration causes development of male plumage. Treatment of castrated males of these strains with testosterone causes a return to female-type plumage, but growth of the comb and wattle is stimulated (a normal male trait). If castrated males that developed male plumage are treated with DHT, the growth of the comb and wattle is stimulated but there is no reversion to female plumage. The explanation appears to be that, in these strains of chicken, the skin aromatizes testosterone to estrogens and stimulates female plumage. In the comb and wattle, 5α-reductase converts testosterone to DHT, causing comb and wattle development, whereas the plumage of DHT-treated, castrated males does not revert to the female type.

D. Reproductive Behaviors in Birds

Most wild avian species exhibit a precise sequence of endocrine-dependent behaviors such as migration, acquisition of territory, advertisement by song, attraction of mate, pairing, nest building, egg laying, incubating eggs, and rearing young birds. Some of these behaviors appear in domesticated species as well. The actual sequence of events and their endocrine bases are species specific and cannot easily be generalized. Successful breeding involves a complex interaction of male and female behaviors in precise sequences (that is, if male does A, then female does B, which stimulates male to do C, etc.) as well as presence of suitable environmental cues such as proper nesting material, availability of water, etc. Little experimental work has been done with wild birds, as it is difficult to get them to perform under laboratory conditions although several descriptive studies on hormone levels and behavior are available. Much of our knowledge of wild birds comes from the work of John Wingfield, Peter Sharp, Eberhard Gwinner, Thomas Hahn, Marilyn Rand, and their many associates.

Androgens appear to be responsible for territorial display and aggression in wild birds as it is in domesticated species. Aggressive behavior also can be stimulated by FSH but not by LH in males. Courtship appears to involve negative feedback of testosterone on FSH levels, which results in reduction in circulating androgens and allows for subsequent, less aggressive behaviors. In domestic ring doves, the initial aggressive behavior involves testosterone, and copulatory behavior coincides with reduced androgens and increased $P450_{aro}$ activity. Androgens antagonize incubation patch development, and a reduction in circulating testosterone may be necessary for patch development in males of certain species.

Bowing behavior in feral pigeons coincides with maximal androgen synthesis but decreases prior to egg laying, coincident with an increase in progesterone levels. Progesterone is a well-known stimulus for incubation behavior in laying pigeons, and removal of the post-ovulatory follicle from chickens blocks nesting behavior. In black kites, progesterone is elevated in females while they incubate eggs and are elevated in males as they take care of the hatchlings (Figure 11-55). Progesterone decreases at that time in the females that do not care for the hatchlings in this species.

E. Parental Behavior in Birds

Birds exhibit high levels of parental care of eggs and young to ensure survival of the relatively small number of offspring they produce as compared to fishes, amphibians, and reptiles. Generally, both sexes of birds are involved in parental care. Incubation is regulated largely by PRL levels (Figure 11-57) although PRL often interacts with other hormones such as described earlier for incubation patch development. In altricial birds, there is a long period of total dependence on the adults for both temperature

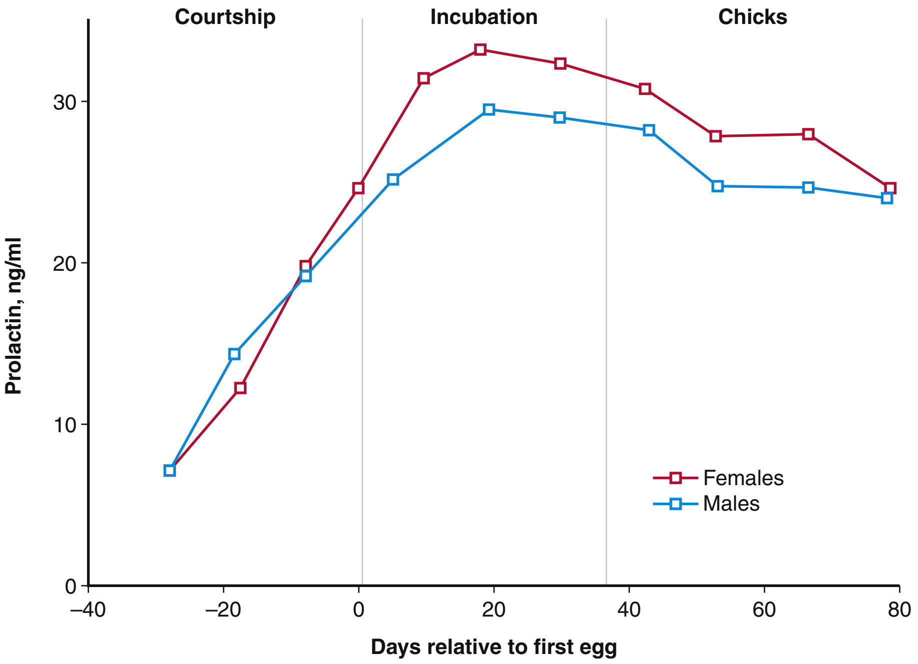


FIGURE 11-57 Plasma prolactin levels during incubation by male and female penguins. *(Adapted with permission from Vleck, C.M. et al., in "Proceedings of the 22nd International Ornithological Congress, Durban" (N.J. Adams and R.H. Slotow, Eds.), BirdLife South Africa, Johannesburg, 1999, pp. 1210–1223.)*

regulation and food requiring considerable effort by the parents to ensure their survival. Elevated androgen levels are not compatible with parental behaviors. Environmental stressors that result in elevation of corticosterone levels inhibit PRL secretion in adults and favor adult survival over that of the offspring, hence the need to reduce human interference in areas where wild birds are breeding.

VIII. ENDOCRINE DISRUPTION OF REPRODUCTION IN NON-MAMMALIAN VERTEBRATES

Endocrine disruption (see Chapter 1) of reproduction has been reported in all vertebrate groups, including mammals (see Chapter 10). The most detailed studies in non-mammals are those on teleost fishes and frogs. Because they are aquatic or semiaquatic they are more susceptible to chemicals that are dumped into the environment that readily find their way into lakes and rivers and eventually reach estuaries and the coastal areas of the continents. Summaries of the rapidly expanding literature on this topic are provided in the suggested readings at the end of this chapter.

In general, the effects observed in these groups are similar to those described in reptiles and birds. Fishes and amphibians tend to be most susceptible to laboratory exposures at environmentally relevant concentrations of natural estrogens, and estrogen mimics, including certain pesticides, **bisphenyl A (BPA)**, nonylphenols, and phytoestrogens such as genistein, as well as anti-androgens, cause induction of intersexes, feminization, and/or demasculinization of male fish, in addition to producing contraceptive-like effects in adult fishes of both sexes. Similar effects are seen in fish populations downstream of wastewater treatment plants (WWTPs), and these effects are directly a result of exposure to the wastewater effluent being added to the streams and rivers. One controlled Canadian study conducted in an experimental lake demonstrated that the contraceptive pharmaceutical ethinylestradiol at a concentration of less than 6 ng/L completely blocked reproduction and virtually eliminated a population of fathead minnows from the lake within 3 years. Intersex has been observed in populations of amphibians in proximity to suburban and agricultural landscapes, but identifying which anthropogenic chemicals may place amphibians most at risk for gonadal abnormalities, aside from cases where clear estrogenic contamination is evident, has been problematic due to the complex soup of chemicals to which animals may be exposed. Male alligators in a Florida lake exposed to metabolites of the pesticide DDT contaminating the lake exhibit feminization (e.g., reduced penis size) due to anti-androgenic actions. Females also showed numerous examples of abnormal follicles in their ovaries. Early exposure of female songbirds, specifically zebra finches (*Taeniopygia guttata*), to estrogens causes masculinization of the brain region necessary for production of song in males. Exposure of adult zebra finches to the insecticide methoxyclor at environmentally relevant doses decreases reproductive performance. In contrast, brains of developing male Japanese quail were feminized by estrogen exposure and they lacked normal male reproductive behavior.

Clearly, the contamination of the environment by chemicals that disrupt reproduction in wildlife are of great concern, as the human population depends on the aquatic and marine environments as major food sources. If concentrations of contraceptive-type chemicals continue to increase, the potential of widespread reproductive decline will markedly change the characteristics of Earth's ecosystem. It is also important to recognize that other classes of endocrine disruptors can alter thyroid and adrenal function as well as metabolism, all of which can influence reproduction. Additionally, neuroactive pharmacological agents (e.g., antidepressants affecting norepinephrine or serotonin activities) may also alter hypothalamic control of pituitary secretion, thus affecting reproduction.

IX. SUMMARY

Reproduction involves a precise integration of environmental factors (e.g., photoperiod, temperature, availability of nesting sites), physiological factors (e.g., nutritional state, general endocrine state with respect to thyroid hormones, adrenocortical functions), and specific endocrine secretions (e.g., FSH, LH, androgens, estrogens, progestogens, PRL). Reproductive patterns are finely tuned to environmental conditions in order to maximize evolutionary success, and this results in frequent observations of greater similarities in reproductive patterns between phylogenetically divergent species facing similar environmental problems than between closely related species living in diverse environments.

Environmental factors operate through the nervous system and specifically the hypothalamus to control the release of GTHs and in certain cases PRL. Prolactin molecules or PRL activity as well as FSH and LH molecules have been identified in all tetrapods. Fishes have a unique PRL-like hormone. In both fishes and tetrapods, FSH initiates spermatogenesis in males and follicular development in females. Local androgens secreted from testicular cells under the influence of FSH appear to be necessary for initiating reductional division (meiosis) of primary spermatocytes. Luteinizing hormone induces androgen synthesis by interstitial (Leydig) or lobule-boundary cells in males and estrogen synthesis and ovulation in females. Androgen synthesis in female mammals also may be stimulated in thecal cells or ovarian interstitial cells by LH. It is thought that thecal androgens are converted to estrogens by granulosa cells.

Follicular atresia associated with formation of corpora atretica is a common occurrence in female vertebrates. Atresia appears to be a mechanism for effectively reducing the biotic potential and placing reliance in production of a smaller number of offspring with better individual survival for evolutionary success. Corpora lutea form in many vertebrates primarily from granulosa cells of ruptured follicles, and corpora lutea usually synthesize progesterone, which is related to gestation or behavior in many viviparous species. Many examples of autocrine and paracrine regulation in the gonads are known.

Courtship, breeding, and parental behaviors appear to be controlled primarily by gonadal steroids although evidence is accumulating for participation of other hormones and pheromones. In addition, estrogens produce dramatic effects on vitellogenesis in non-mammalian liver and bring about a consequent disturbance in calcium metabolism. The basic oviparous mode of reproduction has become modified with respect to the development of viviparity in all non-mammalian classes except Aves and Agnatha.

Endocrine disruption of reproductive biology of non-mammals may contribute to environmental instability. It is imperative that we reduce levels of such chemicals in the environment.

STUDY QUESTIONS

1. Characterize the mechanisms for sex determination for bony fishes, amphibians, reptiles, and birds, indicating those aspects unique to non-mammals.
2. Describe the patterns of reproduction in non-mammalian vertebrates.
3. Compare and contrast hormonal components of the HPG axis of the major vertebrate groups discussed in this chapter with the mammalian HPG axis.
4. Describe the process of sperm formation and the roles hormones play in vertebrates.
5. Describe the process of oogenesis and the roles of hormones in viviparous and oviparous vertebrates.
6. Develop a scenario to explain the evolution of (a) sex-changing fishes, and (b) parthenogenesis.
7. How do environmental factors influence reproduction in teleosts, amphibians, reptiles, and birds?
8. What does the *Ambystoma jeffersonianum* complex in amphibian salamanders suggest to you about the type of sex determination operating in these species?
9. How would you explain the results of transplantation of ventral or dorsal skin described for birds that normally exhibit an incubation patch?
10. From an endocrine and evolutionary point of view, why are there no live-bearing birds?

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