SEX DIFFERENCES IN PHYSIOLOGY

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CHAPTER

1

Introduction for Sex Differences in Physiology

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The study of the human body dates back to ancient times but was not named as the discipline "physiology" until the 16th century by the French physician Jean François Fernel, who introduced the term to describe the study of bodily functions. Since that time, physiologists have contributed fundamental and critical information needed for the evidence-based practice of modern medicine. However, like all scientific disciplines, physiology and physiologists are not immune from political, societal, and cultural trends. In part, because science was historically a male-dominated profession, except for studies related to the physiology of reproduction, most human and animal physiological studies enrolled male volunteers and utilized male animals. Other considerations impacting a male bias in research included concerns about variability in measured parameters resulting from cyclic hormonal variation in females and potential risk for teratogenic effects of interventions and procedures to the fetus in women of child-bearing age. Although the human population can be defined by sex as either male or female, assigned by chromosomal complement and reproductive organs (XX for female and XY for male) [1], sex as a biological variable is rarely considered in the design of basic physiological studies. Thus, physiological principles contained in classical physiological and medical textbooks and graduate and medical curricula have been based on the 70 kg healthy male (usually between 18 and 40 years of age) or on male animals [1].

In 2001, the Institute of Medicine report "Exploring the Biological Contribution of Sex" concluded that sex matters in all aspects of cellular function and physiology from "womb to tomb" [1]. What logically follows, then, is that physiological principles and regulatory mechanisms need to be defined in males and females (animals and humans), so that findings from basic science can be translated to clinical research for the development of evidence-based, individualized medical strategies or practice guidelines.

In the United States, a legislative approach was taken to correct the scientific problem of too few women in clinical trials by the passage of the National Institutes of Health (NIH) Revitalization Act of 1993. This law mandated that women be included in human studies supported by the NIH. Although women have since been included in clinical studies, results of those studies in the clinical setting have rarely reported data separated by sex, thus making it difficult, if not impossible, to understand where the two sexes fell within the distribution of results. In the NIH Revitalization Act, there was no mention of basic human physiological functions or mechanistic studies utilizing isolated cells or tissues. Although the Office of Research on Women's Health of the NIH was founded in 1991, it was not until 2002 that the Office developed and implanted an interdisciplinary targeted funding mechanism (Specialized Centers of Research on Sex Differences) specifically to begin to fill the knowledge gap in information for areas of women's health and sex differences research. Since the inception of the program, 33 awards have been made to 26 academic centers (see http://orwh.od.nih.gov/sexinscience/researchtrainingresources/scor.asp). Advocacy groups such as the Society of Women's Health Research and scientists themselves through original research articles, editorials, and editorial policies of professional societies (eg, Organization for the Study of Sex Differences, that was founded in 2006, the American Physiological Society, and the Endocrine Society) began to draw attention to the lack of experiments and reporting of data on females in basic and translational animal and human studies [2–7] (see http://genderedinnovations.stanford.edu for an up-to-date list of editorial policies for other journals). As a result of these efforts, the

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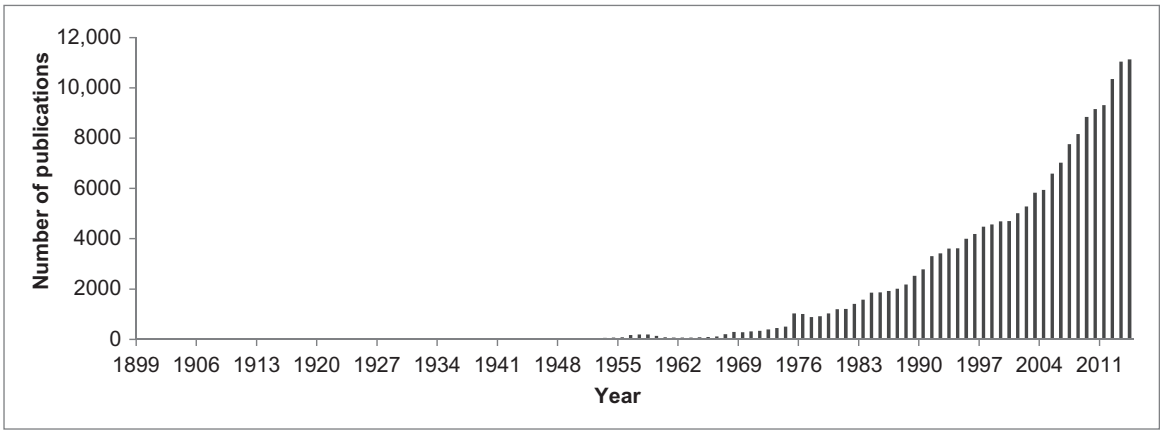

FIGURE 1.1 The graph depicts the number of publications listed in PubMed (www.pubmed.gov), a NIH resource for biomedical literature, that included reference to "sex differences" for each year from 1899–2014.

number of publications addressing biological sex differences has risen dramatically (Fig. 1.1). In addition, problems with reproducibility of basic science experiments, including lack of reporting of the sex of the experimental animals or human participants prompted announcements in 2014 by the NIH that steps would be taken to address these deficiencies [8,9]. These steps were announced in Jun. 2015 (NOT-OD-15-102: Consideration of Sex as a Biological Variable in NIH Funded Research and NOT-OD-15-103: Enhancing Reproducibility through Rigor and Transparency) to be implemented in grant applications funded by the NIH starting in 2016.

So, where do we stand with data upon which to construct a book on "Sex Differences in Physiology"? Epidemiological studies have consistently identified differences in disease incidence, prevalence, morbidity, and mortality between men and women. These statistics reflect differences in the underlying physiological processes that arise from the basic genetic difference between males/females and men/women, coupled with modulation of these processes by hormonal fluctuations and aging. Chromosomal and hormonal differences allow for reproductive competence which brings us full circle: sex differences in reproduction (specific to one sex) drive physiological processes that express as sex differences throughout the lifespan. What is meant by this? Consider the physiological processes that allow successful pregnancy: changes in respiration, metabolism, blood volume, renal function, cardiac output, musculoskeletal locomotion, neurological regulatory processes, and sensory and immune function. These changes are female-specific and suggest differences in underlying mechanisms of adaptability in female physiological processes that may (or may not) be present in males. Although males do not undergo the tremendous physiological changes associated with pregnancy and birth, this does not definitely indicate that there are no hormonally mediated shifts in physiological processes within males. Conversely, if there is an absence of adaptability of physiology within males, could this absence of adaptation render males susceptible to diseases or disorders? Further, although regulatory processes maintain homeostasis in both males and females, for example, blood pressure or glucose or electrolytes, within defined ranges, differing underlying processes may facilitate these regulatory processes and these variations could vary across the lifespan. These processes need to be understood in order to inform adequate diagnosis and treatment of disease and disorders in both males and females.

It is difficult to gather sufficient information about physiological processes in females, as the data may be published either as single sex studies, or as comparative studies labeled as "sex-differences" or "gender differences." Studies utilizing cell cultures rarely are comparative or are based on cell lines of unidentified or single sex. Although gender is related to sex, gender defines behavioral, psychological, and cultural characteristics that are influenced by sociocultural expectations [10]. Curricula in graduate and medical courses do not specifically address differences in physiology between males and females because data and resources are sparse. Both sex and behaviors influenced by gender will affect physiology and pathophysiology. Development of resources and consideration of physiological principles are often segregated into women's specific or men's specific knowledge repositories. Since the first textbooks on *Principles of Sex-Based Differences in Physiology* [11] and *Principles of Gender-Specific Medicine* [12] were published in 2004, updates and additional collective resources are

few [13–15]. Thus, *Sex Differences in Physiology* provides an important update and focus on basic physiological control systems and mechanisms in females and males that contribute to health and disease across the lifespan. The approach is systematic considering the first developmental aspects of sexual differentiation including neuroanatomical and neurophysiological aspects of brain function. Each physiological system is then considered separately, including highlighting body composition and metabolism with influenced risk factors for pathophysiology. In the final section, experts in sex-differences research provide guidelines for strategies to study sex differences.

In order to improve the health of women and men, it is essential for scientists and clinicians to consider sex differences as one of the underlying physiological mechanisms of disease. These chapters will lead the way to new discoveries about basic female physiology across the lifespan and about differences in physiology between females and males, thus providing building blocks for evidence-based, individualized medicine.

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CHAPTER

2

Chromosomal and Endocrinological Origins of Sex

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INTRODUCTION

As described in chapter "Introduction," this book provides a survey of each of the organ systems of the body that is influenced by the biological variable of sex. An obvious omission is the reproductive system, which we exclude because of the well-developed literature on sex differences in the male and female reproductive systems. Before we progress through each of the systems of the body, we first provide a review in this chapter that covers the process of sexual differentiation and the forces that can influence this process. We focus on mammals throughout this book, although the process of sexual differentiation occurs in other classes and under other strictures, and still involves powerful and long-lasting/permanent effects of steroid hormones. We will describe the genetic and endocrinological origins of sex and sexual differentiation throughout different stages of male and female development. For instance, the amount of steroid hormones available pre- versus postpubertal, is significant [1], which, in turn, reshapes the activity of brain circuits during adolescent development and affects numerous other physiological functions. Furthermore, the sensitivity of the system, as reflected by the presence of receptors for the aforementioned hormones, likewise changes. The net result is a system that is responsive to the specific endocrine milieu characteristic of the stage of development, as well as the specific male and female environments [2].

CHROMOSOMAL SEX

The basics of sex determination and sexual differentiation are well understood [3] (see Fig. 2.1). In the human, males and females produce gametes, ova in females and spermatozoa in males, in which the number of chromosomes, compared to the other cells of the body, are halved. Thus, instead of the 23 pairs (46 total), the production of gametes involves a process (meiosis) that results in one member of the 23 pairs of chromosomes, compared to the diploid parent cells (which contain 23 pairs), plus a sex chromosome. The latter in the male's spermatozoa contains a particular genetic trait, the presence of a Y-chromosome or an X-chromosome. When added to the female's egg-bearing X-chromosome, the resulting genotype will be XX and female. If paired with a Y-bearing sperm, the resulting ovum will be XY or male. Thus, in the mammal, for example, the father determines sex by providing an X or a Y chromosome, but in what manner does the XX versus the XY chromosomal pairing initiate its effects? Nature produces in the XX or XY model the potential to grow and elaborate a single underlying substrate into the male or female phenotype. The Y-chromosome contains the *SRY* gene, which encodes the proteins that will facilitate the development of the male phenotype. Regardless of sex/genotype, the embryo has an internal undifferentiated gonad that will develop—or not—depending on the hormonal milieu to which it is exposed. The version that will be present

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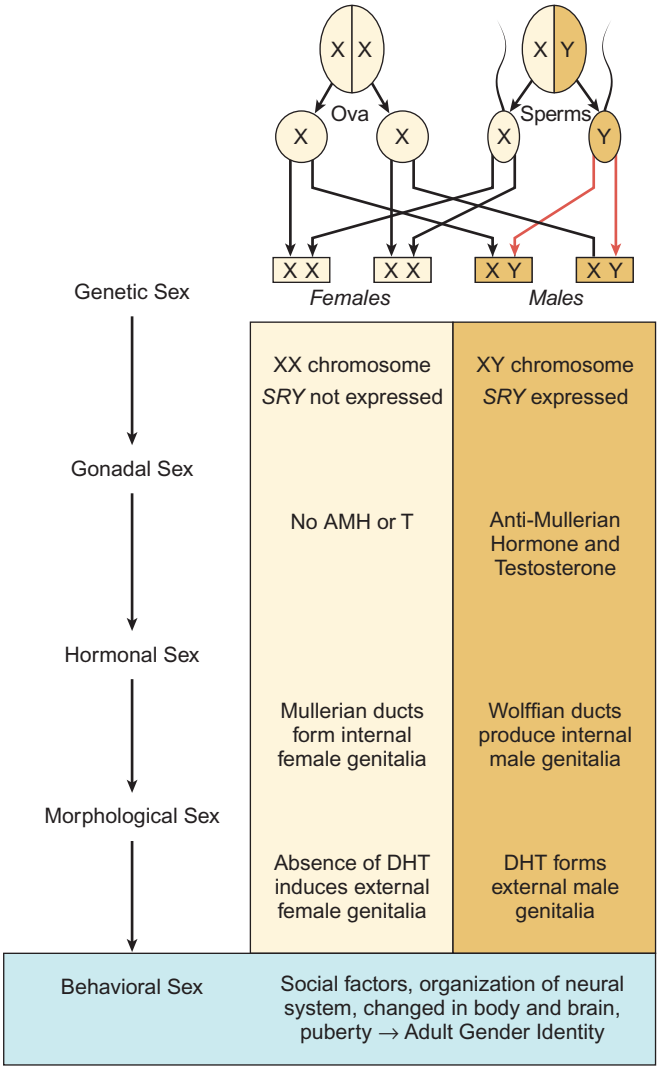


FIGURE 2.1 Process of sexual differentiation in the human. This figure displays and summarizes the process of sexual differentiation in the human. As can be seen, the process represents the interaction among a large number of phenomena and events, any point along which variation can occur subtly shifting the manner of development and its outcomes.

in the average female is the Müllerian system; in the male, the Wolffian. The tendency then is to produce the female version, which develops in the absence of any alternative signal. The Y-chromosome codes for the production of Müllerian-inhibiting factor (MIF), which suppresses the development of the Mullerian system and expresses the development of the Wolffian system, and the male-like structures that follow. The bipotential gonad and its associated structures follow the sex-typical program and either develop or wither away.

In the human, during the first 6 weeks of embryonic development, the gonadal ridge, germ cells, internal ducts, and external genitalia are formed—the basic anatomy of the reproductive tract. Unlike most other developing organs in the embryo that have a specific developmental trajectory, the gonads are bipotential in both genetic configurations (46-XX and 46-XY). At this stage, even after the initial genetic sex determination, the embryonic structures determining the individual's sex can develop either into ovaries or testes (in relation to the tissue of the gonadal ridge), oocytes or spermatocytes (regarding the germ cells), the male's or female's internal organs (the trajectory of the internal ducts), and average external masculine or feminine genitalia. The bipotential gonadal ridge is located medially on the urogenital ridge. Germ cells migrate to the gonadal ridge at approximately 5 weeks of development. Finally, these cells undergo rapid mitotic divisions in both the fetal testis and ovary [4,5].

In mammals, the primordial gonad, for both male and female duct systems, is expressed in the embryonic excretory organ known as the mesonephros. The Wolffian duct system will form male-typical structures such as the epididymis, vas deferens, and seminal vesicles; the Müllerian duct system will form the fallopian tubes, uterus, and posterior part of the vagina. Experimental work in animal models has shown that differentiation and subsequent gonad development is dependent on the inductive interaction between the Wolffian ducts and the intermediate mesoderm [6]. At 5 weeks of embryonic development, tissue destined to form the external genitalia is detectable at the cranial region of the cloacal folds, and it is still bipotential, basically identical in both male and female embryos [7].

GONADAL/HORMONAL SEX

On average, in the human, the bipotential gonads and germ cells begin to form either testis or the ovary around 6 weeks of embryonic development—or around 16% into the embryonic development. In general, in mammals, gonadal sex determination is regulated by a DNA-binding protein expressed in the Y chromosome by the gene known as *SRY* (sex-determining region on the Y chromosome), followed by its downstream mediators, including Sf1 (encoding for the steroidogenic factor 1) and SOX9 (encoding for the transcription factor SOX-9), which, interestingly, induces and maintains neural stem cells—further strengthening the ties between the brain and the reproductive system [8]. The evidence suggests that at this early stage the supporting cell precursors can develop into either Sertoli cells, which promote the development of the testis and their powerful chemical products, or ovary follicle cells [9]. Activation of the encoded proteins cited above can cause cells of the bipotential tissue to develop in the core regions at

the expense of the outer layer. This, then, initiates a process that will lead to the formation of Sertoli cells, which in turn, organize themselves into the seminiferous tubules. Sertoli cells then secrete Müllerian-inhibiting chemicals, including the anti-Müllerian hormone, which causes regression of the Müllerian or female-typical morphological system. The next step is marked by the appearance of Leydig cells, which are capable of producing and secreting testosterone, the powerful, largely male steroid hormone. The final step in male sex determination is constituted by the development of male germ cells, which are influenced by retinoid signals within the mesonephros [10].

In contrast to the continuous proliferation of male germ cells, female germ cell development occurs only during embryogenesis, very early in the female's life. This process is characterized by the proliferation and morphogenesis of the granulosa cells into their cuboidal state, which induces oocytes to increase in size, the production of the zona pellucida, an extracellular glycoprotein matrix deposit between the oocytes and the granulosa cells, and following production of thecal cells [11]. In other words, the embryonic female produces the eggs she will carry with her for the rest of her life.

After differentiation of the gonads, sex steroid hormones are responsible for the next crucial stage of sexual determination: organizational effects of sex steroids. It should be noted that sex hormones must bind to the proper receptors on or within the cell in order to be physiologically active. Many of these hormone receptors are nuclear receptors that function as transcription factors enabling them to exert widespread effects on cell function. Thus, any environmental or cellular phenomenon interfering with the correct binding can prevent fully developed sexual differentiation. For example, as discussed later in the chapter, certain environmental agents, fertilizers, for example, can wreak havoc with the above cellular events. The hormone–receptor complex activates a specific response of the promoter of steroid-responsive genes and interacts with RNA polymerase II to form a large transcriptional activation complex, which in turn is responsible for the appropriate protein synthesis. If any one of the steps involved in the androgen transcription/translation is defective, the result is lack of masculinization of internal sex ducts and external genitalia [11]. It is like disconnecting some links in a chain: what worked before no longer does.

Müllerian-inhibiting substances (MISs) are important for the regression of the Müllerian duct system, which contributes to the successful proliferation of the male, or Wolffian duct system, and its associated testicular development. MISs are also detected in females, but only later on, after the Müllerian ducts have already begun their development.

At this stage of development (8–13 weeks, approx. 22–36% through prenatal development), testosterone is produced by fetal Leydig cells. This hormone is crucial for the development of both the Wolffian duct system and the masculinization of the external genitalia. The biochemical precursor of testosterone is cholesterol, which is produced using a biochemical pathway involving four recognized enzymes. Moreover, cells of the external masculine genitalia contain a 5α-steroid reductase which potentiates masculinization by transforming testosterone to dihydrotestosterone, the most biologically active androgen [12].

In females, the path to feminization is led by a specific cytochrome P450, aromatase, which converts androgens to estrogens [13] and is detectable in fetal tissue. Once again, feminization constitutes the default state, and masculinization can only be started as an active process involving the pathway detailed above.

Hormonal effects on phenotypical sex include discrete anatomical features, internal ducts, and external genitalia. External genitalia in males typically begins forming early in gestation. If androgens are not present until week 12 or later, full masculinization cannot take place. Whereas testosterone is of critical importance for the development of internal ducts, dihydrotestosterone is crucial in the development of external genitalia [13]. Masculinization in this stage includes increasing anogenital distance, fusion of urethral folds, and growth of scrotal swelling. The penis forms from the genital tubercle and continues to grow throughout gestation. In the absence of androgens, the labia majora and labia minora form from the genital swelling and urethral folds. The clitoris, therefore, forms from the genital tubercle.

Other phenotypical sex differences include qualitative differences (males are generally larger and heavier), different trajectory for puberty (females generally reach puberty sooner than males), and the development of secondary sex characteristics (body and facial hair, change in voice, and so forth) [14]. These differences also affect behavioral characteristics and qualitative as well as quantitative differences in behavior between the sexes. The classic dogma concerning phenotypical activation of sex differences links gonadal hormones as the basic factor controlling the sex differentiation of nongonadal tissue, including the brain. Classic studies showed the importance of the influence of sex steroids on brain sexual differentiation [15]. In these studies the removal of the testes early in neonatal life resulted in feminization of brain-regulated functions and behavior in adulthood; whereas, administration of exogenous testosterone to the neonatal female induced masculinization. Several more recent reports, however, have indicated that

sexual differentiation of the embryonic neural tissue occurs before the activation of the gonadal hormones. These studies suggest that early events in sex differentiation, such as cell migration and the activation of sex germ cells, are also dependent on the activation of several genes linked to the Y chromosome [16]. Furthermore, appreciation has grown for direct effects of chromosomal sex on both physiology and behavior, in some cases, separate from the contribution of hormones (see Box 2.1).

Many aspects of the mechanisms of actions of the hormonal sex differentiation of the brain have been demonstrated in the last two decades [14]. In the brain, the involvement of a large variety of intracellular pathways mediated by steroid actions can explain sex behavioral differences. The involvement of neurotransmitters in sexual differentiation of the brain and behavior is now well understood. In most cases, the neurotransmitters act as mediators of steroid action, initiating biological negative feedback loops and acting as modulators of steroid activity, like a volume or gain switch on one's stereo. These are, needless to say, complex, subtle, and multilayered. The influence of sex on brain development is discussed in detail in the chapter "Sex Differences in Neuroanatomy and Neurophysiology: Implications for Brain Function, Behavior, and Neuropsychiatric Disease."

PUBERTY

The influence of sex steroids on development, physiology, and behavior is relatively quiescent from approximately 6 months after birth until the onset of puberty. Puberty specifically consists of the hormonal changes that lead to the sexual maturation of an organism. The increases in sex steroids during puberty also exert activational effects on other organ systems and many of these are discussed in the subsequent chapters of this book. Before we proceed with a discussion of puberty and the governing biological signals, let us first distinguish between puberty and adolescence. Puberty refers specifically to sexual maturation and the related hormonally driven events. Adolescence is a longer period of time and consists of both biological changes and sociocultural influences. We focus here on puberty, but for a discussion of adolescence and related neuronal and behavioral changes, see the work of Blakemore and Robbins [17]. Regarding puberty, this is not a single event but rather a process that occurs over a normal

BOX 2.1

SEPARATING CHROMOSOMAL AND ENDOCRINOLOGICAL SEX: FOUR-CORE GENOTYPES MODEL

Researchers have long sought to understand the physiological effects of sex chromosomes independently from sex hormones and have traditionally manipulated these two systems through gonad removal. This technique results in an immediate cessation of gonadally derived sex hormone production and a precipitous drop in circulating sex steroids. It also allows for the addition of exogenous hormones to determine the impact of sex chromosomes (without hormone replacement) and of varying concentrations of sex hormones (with hormone replacement) in both males and females. Depending on the timing of gonadectomy, partial isolation of organizational and activational effects of hormones is possible. Sex steroids have dramatic organizational influences on the developing fetus, including masculinization or feminization of the genitalia and brain. Recent studies also indicate that sex steroids exert some permanent organizational effects across a broad developmental window from perinatal to the end of puberty. These organizational influences are permanent, remaining into adulthood, and are not affected by gonad removal.

A relatively new animal model, called the Four-Core Genotypes Model, addresses these drawbacks by removing the *Sry* gene from the Y chromosome (Y^-) in mice. Given that the *Sry* gene is necessary for the development of the male phenotype, XY^- are characteristically female. Additionally, the model incorporates mice expressing the *Sry* transgene on an autosome to produce genetically female mice (XX) that express *Sry* and are thus phenotypically male, as well as to produce XY^- mice expressing *Sry* independently of the Y chromosome. This model and others that are targeted at isolation of the origin of observed sex differences are described in greater detail in the chapter "Strategies and Approaches for Studying Sex Differences in Physiology."

range of ages in the human. For girls, puberty is generally initiated between 8 and 12 years of age. For boys, the changes, on average, occur later with a window of 10–14 years of age. When considering common animal models such as rodents, puberty is initiated in the range of 32–38 days postnatally [18].

Major Hormonal Events of Puberty

Gonadotropin-releasing hormone (GnRH) or luteinizing hormone–releasing hormone is well-established as the essential trigger of puberty. GnRH is present during fetal and early postnatal development but then becomes quiescent from about 6 months of age until puberty approaches. As puberty approaches, pulses of GnRH increase in frequency and eventually surges in the hormone trigger puberty. The release of GnRH leads to the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These events are similar in both males and females but the subsequent events diverge between the sexes.

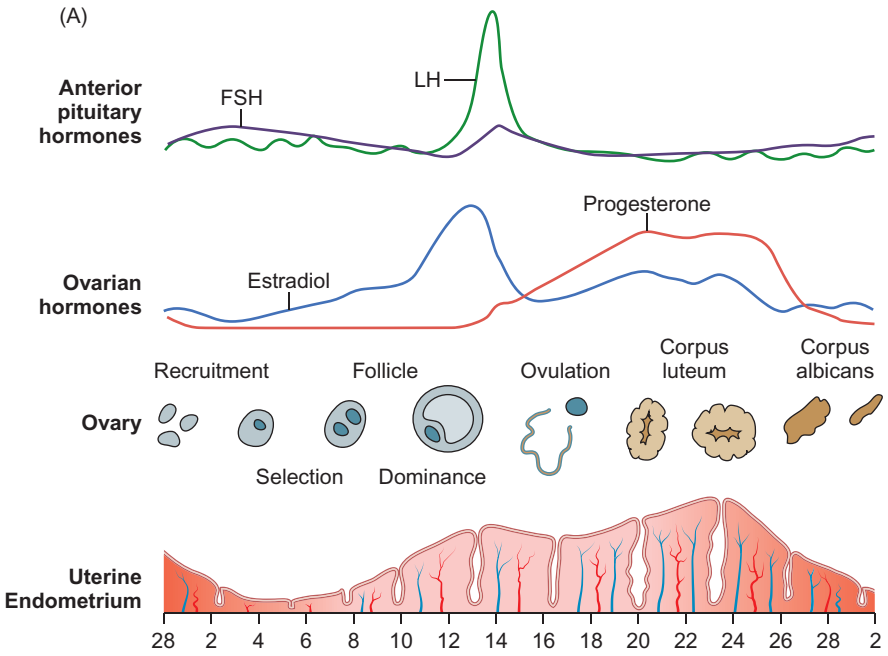
The main site of action for GnRH-induced release of FSH and LH in females is the ovary. Stimulation of the ovaries by LH and FSH leads to secretion of estrogen and progesterone and follicular development. Estrogen mediates the appearance of secondary sex characteristics and the maturation of the genital organs. Following the hormone events of female puberty, the pattern of hormonal secretion transitions to the adult ovarian cycle (Fig. 2.2). In the adult female, there is an alternating cyclic pattern of GnRH release which shifts between tonic and cyclic release, an effect which influences the estrous cycle. The pattern of the cyclic release is essential to appropriate hormone coordination to maintain reproductive viability. Hypothalamic release of GnRH stimulates the anterior pituitary to produce LH and FSH which stimulate estradiol (E2) and progesterone (P4) production in the ovaries. E2 rises during the follicular phase and P4 rises during the luteal phase of the menstrual cycle. This cyclicity in the female will continue until menopause (discussed below).

Puberty is also stimulated by GnRH in the male, but the subsequent events diverge from the female. GnRH-stimulated release of LH leads to the production of testosterone in the Leydig cells and thereby the initiation of spermatogenesis and development of secondary sex characteristics. Testosterone also serves as a modulator of negative feedback on the hypothalamus and anterior pituitary. FSH stimulates the Sertoli cells to release Inhibin which provides negative feedback at the level of the anterior pituitary. The pulsatile release of GnRH and the subsequent negative feedback that is initiated at puberty continues throughout the life of the adult male and no cyclic pattern in activity is present.

Initiation of Puberty

We have established that GnRH is essential to puberty and there is a wealth of literature demonstrating the essential nature of GnRH. So, when one asks "what triggers puberty" what they are really asking is "what triggers the pubertal increase in GnRH?" There are two basic means by which GnRH surges could originate at puberty. One possibility is that the release of GnRH is stimulated de novo at puberty. The second possibility is that tonic inhibition of GnRH is released at puberty. The first of these possibilities is referred to as the Gonadostat Hypothesis and was proposed by Dohrn & Hohlweg in 1931. The Gonandostat Hypothesis proposed that before puberty minute quantities of estrogen can block GnRH function and over time this inhibitory ability decreases releasing GnRH activity. If this were true, high doses of exogenous estrogen should stimulate puberty. This appears to be true for sheep but has not been found to account for puberty in other species. The second hypothesis is termed the GnRH Pulse Hypothesis. The basic principle of this hypothesis is that GnRH is inhibited centrally until puberty, but not by sex steroids. A stimulus triggers an increase in GnRH pulsatility which then leads to puberty. If this hypothesis is true, then a hypothalamic lesion which disinhibits GnRH should lead to puberty. Two lines of evidence for this hypothesis are that GnRH secretion in the absence of gonads remains intact until puberty despite the absence of gonadal steroids and precocious puberty is, in fact, caused by hypothalamic lesions, which is reversible with amelioration of the lesion.

Collectively, it appears that in most mammals GnRH is present but tonically inhibited. The determination of what specifically is inhibiting GnRH until puberty has been an area of intense study [19]. A few important themes from this work are as follows. The control of GnRH pre- and postpubertally is not identical. GABA, leptin, and NPY all contribute to the start of puberty but no one independently accounts for the initiation. Norepinephrine, opioids, and other neurotransmitters and neuropeptides are involved in the coordination of GnRH release—but none of these messengers completely accounts for the initiation of GnRH pulsatile release at puberty. A relatively new neuropeptide first documented in 2000, kisspeptin, appears to be the critical factor in the initiation of pubertal patterns of GnRH release.



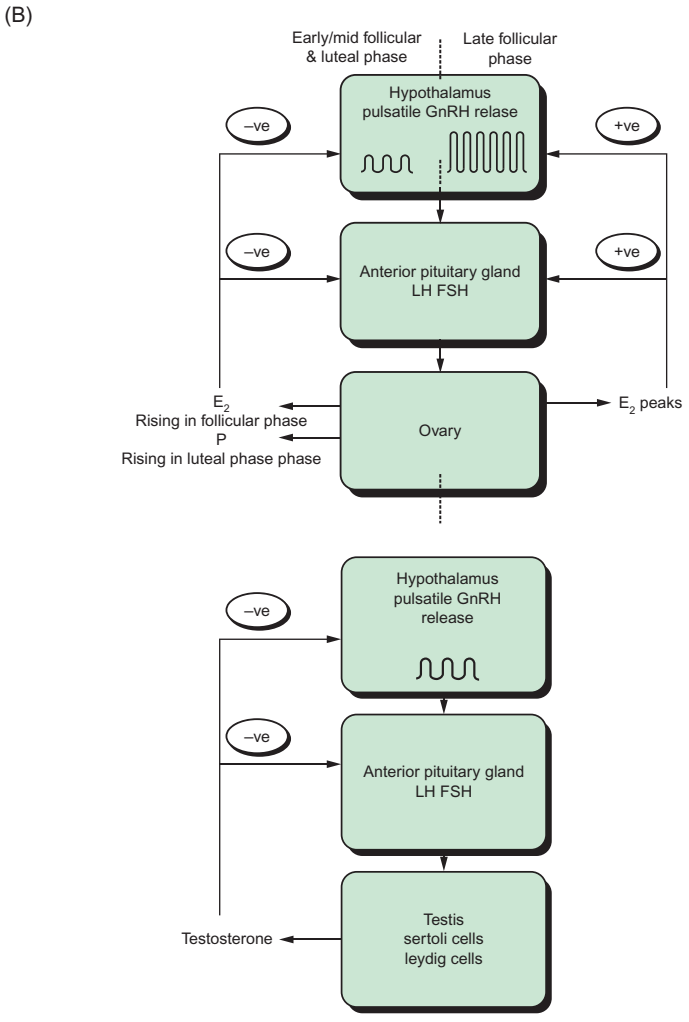


FIGURE 2.2 **Hormonal variations across the menstrual cycle.** (A) The coordinated release of LH and FSH along with changes in estradiol and progesterone coordinate the adult female menstrual cycle. The alternating pattern between tonic pulsatile release of GnRH and coordinated surges is unique to the female once puberty is complete and essential to the continuation of the cycle. (B) In the adult female, there is a cyclic pattern of GnRH release which stimulates the cycle. The pattern of the cyclic release is essential to appropriate hormone coordination to maintain reproductive viability. In the adult male, pulsatile release of GnRH continues, but no surge activity or variable cyclic pattern is present.

The discovery of kisspeptin stems from the documentation of a family with a high incidence of hypogonadotropic hypogonadism. Multiple members of this family had an absence of spontaneous puberty and partial or absent LH pulses. However, the individuals had a normal response to GnRH replacement. This pinpointed the abnormality to GnRH synthesis, secretion, or activity. Genetic analysis of the family determined that there was a leucine to serine substitution at position 148 on the GPR54 gene in the affected individuals [20]. Further study revealed that Gpr54 is the cognate ligand of kisspeptin, GnRH neurons express GPR54, and GPR54 mRNA expression increases in the hypothalamus at puberty. In addition, Gpr54 knockout mice fail to initiate puberty [20]. Adding to the connection between GnRH, kisspeptin, and puberty are demonstrations of increasing kisspeptin neuron apposition to GnRH neurons with progression toward puberty in rodents [21]. Furthermore, kisspeptin antagonists blunt the release of GnRH suggesting a causal relationship [22]. The precise relationship between kisspeptin and GnRH is still being established and the factors that initiate maturation of kisspeptin are still being recognized, but it is clear at this time that kisspeptin is a strong initiating factor in the stimulation of GnRH pulses which initiate puberty. For more detailed information on the initiation of puberty, detailed reviews are available [23–25].

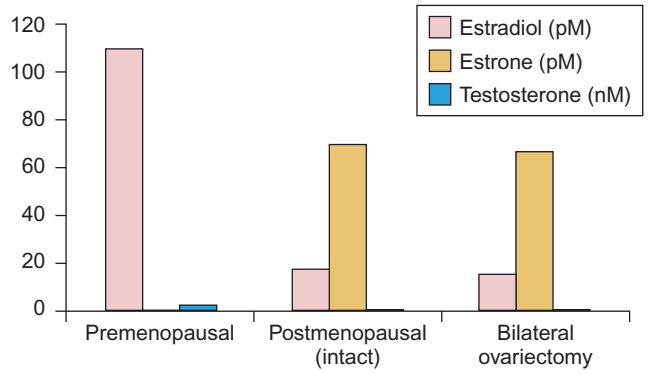


FIGURE 2.3 Changes in circulating levels of sex steroid hormones across life span in women. In premenopausal women, circulating estradiol varies from 18 to 110 pM, depending on the phase of the menstrual cycle. After menopause or bilateral ovariectomy, levels drop to 16–18 pM. Estrone is not produced in premenopausal women and increases to ~70 pM following menopause (intact and ovariectomized). In premenopausal women, testosterone varies from 1.3 to 2.6 nM and drops to 0.29–0.4 nM after menopause (intact and ovariectomized). *Source: Figure adapted from Knochenhauer E, Azziz R. Ovarian hormones and adrenal androgens during a woman's life span.* J Am Acad Dermatol *2001; 45:S105–15.*

ENDOCRINE FUNCTIONS ACROSS LIFE SPAN: AN EMPHASIS ON MAMMALIAN MENOPAUSE

Levels of reproductive hormones vary greatly across the life span. During human fetal development, reproductive hormones are high but then enter a phase of quiescence during childhood before a cascade of endocrine changes commence during puberty [26]. In human adults, reproductive hormones in men are generally consistent across the life span although slow age-related declines in hormone and sexual activity exist [27,28]. Further, whereas sperm production diminishes across the male's life span, fertility may exist throughout the aging process [26]. On the contrary, endocrine hormones fluctuate across the menstrual cycles in women and, as they age, women experience drastic reductions in endocrine function (Fig. 2.3) [29]. In women, menopause refers to the final menstrual cycle associated with the natural cessation of ovulation [30]. Around the time of menopause, physiological changes affecting secondary sex characteristics, vasomotor instability in the form of hot flashes, loss of bone density, and increased cardiovascular vulnerabilities are observed. Further, research suggests a decrease in sexual desire in menopausal women, accompanied by diminished frequency of sexual activity [31], an effect that may be mitigated with hormone replacement therapy [32].

Although many reproductive processes have been conserved through evolution, the phenomenon of menopause is rather unique. Specifically, as human females age, intervals between menstrual cycles increase, accompanied by lower estradiol levels and higher FSH concentrations [33], decreased ovary size, and increased androgen secretion [30,34,35]. Although human females' average life span is 80 years, they experience menopause at approximately 50 years of age, resulting in an extended postmenopausal phase at the end of the life span that is unique among mammals [30]. In fact, using the criterion of the cessation of breeding many years prior to the end of the life span, across all mammals, only pilot and killer whales are thought to have a similar menopause to humans [36]. However, other nonhuman primates are also considered to undergo variations of menopause [30].

Although certain mammals have evolved to live a significant portion of their lives in a postmenopausal state, this final reproductive stage in females presents many questions about the adaptive significance of this phenomenon [36]. There are also many additional effects of drastic reductions in reproductive hormones in women, apart from the physiological effects described above. For example, cognition

and mood have been reported to be adversely affected, resulting in attempts to mitigate these symptoms with interventions such as hormone replacement therapy [37]. With all the uncertainties surrounding these questions about variations in reproductive endocrine levels in aging females, more research is necessary to provide the most informed responses to questions about how women can live the healthiest postmenopausal lives.

INFLUENCES ON SEXUAL DIFFERENTIATION AND DEVELOPMENT

Thus far in this chapter, the trajectory of sexual differentiation has been presented in a typical manner in which developmental events follow in an expected sequence; however, exceptions to these developmental processes exist that can disrupt the organizational impact of sex hormones. Genetic variations, modified environmental and chemical contexts, and altered endocrinological patterns may contribute to various forms of alterations in sexual differentiation.

Environmental influences—Given the findings that ambient temperature influences gonadal sexual differentiation in amphibians and reptiles [38], it is interesting to consider the potential role of various environmental factors on sexual differentiation in mammals. For example, exposure to environmental chemicals has been investigated with some substances, mostly in the pesticide category, identified as endocrine disruptors [39]. A primary route of action for these endocrine disruptors is their binding to estrogen and androgen receptors and subsequent mimicking of natural hormones.

An unfortunate lesson was learned about artificial manipulations of fetal estrogens via pharmacological manipulations when women were prescribed the drug diethylstilbestrol (DES) to reduce the risk of spontaneous abortion in the 1940s–60s. After about 25 years and millions of fetuses exposed to this drug, causal connections were made between the drug and effects on the reproductive organs in females, including deformed uteri and increased risk for vaginal cancer (as well as immune and behavioral adverse effects). Consequently, DES was banned in 1972 [40]. In fact, evidence from both animal and human studies suggest an epigenetic effect for offspring of individuals exposed to DES during fetal development [41].

Outside of the pharmacological realm, environmental chemicals have also been found to have an impact on sexual development [14]. Bisphenol A, a chemical released from polycarbonate products such as water and baby bottles is an estrogen receptor ligand and, consequently, considered an endocrine-disrupting chemical [40]. In females, fetal exposure to this chemical results in mammary gland and vaginal alterations as well as accelerated puberty and, in males, increased prostate weight [42]. Other chemicals known as alkyl phenols, found in products such as paints, detergents, and herbicides also bind to estrogen receptors as well as exert a weak androgenic effect, have been found to reduce the synthesis of testosterone and size of testes and masculinize females by interrupting steroid feedback sensitivity [43–45]. Certain chemicals found in natural sources such as plants also have effects on sexual development; specifically, isoflavones have been reported to feminize male-typical behavior and masculinize gonadal, endocrine, and behavioral sex-typical responses in females [46–48].

Finally, natural variations in the uterine environment have been found to influence sexual differentiation in rodents. For example, the specific position of fetuses in litter-bearing mammals such as rats and mice may influence sexual development. Research confirms that having a uterine position between male and female fetuses differentially exposes the fetuses to varied levels of gonadal steroids and affects sexual differentiation, especially in females [42]. Considering that during sexual differentiation male fetuses produce high levels of testosterone that can pass into adjacent fetuses, the fetus positioned next to the male fetuses experiences supplemental testosterone levels. Thus, a female positioned between two male fetuses experiences a different fetal hormonal milieu than in the same uterus as a female positioned between two female fetuses [49]. In humans, however, uterine exposure to gonadal steroid levels during fetal development varies according to variables such as maternal age, reproductive history, and diet [50].

Natural genetic and endocrine variations have both short-term and long-term effects on sexual differentiation. In humans, case studies have been monitored to characterize the general effects of these naturally varied conditions. One of the most extensively studied medical conditions affecting sexual differentiation is the genetic condition congenital adrenal hyperplasia (CAH), a spectrum of related conditions resulting from exposure to higher than normal androgen levels during gestation due to enzymatic disruptions in steroid hormone production in the adrenal glands [51]. This condition often results in ambiguous-looking genitalia in females prompting early hormone therapy following birth. Researchers have followed these cases throughout childhood and adulthood to determine long-term effects of high levels of androgens during