REPRODUCTIVE BIOLOGY AND TECHNOLOGY IN MAMMALS

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Contents

Summary

The science of reproductive biology has undergone remarkable advances in the 20th century. These advances provide structural and functional details of the mammalian reproductive system at the physiological, cellular and molecular levels. The development of reproductive technologies has enabled improved reproductive efficiency and treatments for infertility. This chapter will review the general concepts in mammalian reproductive biology and present, in a brief form, some of the most common reproductive technologies used in human and animal health, biomedical research and animal production systems. Innovative biological research continues to support rapid progress in understanding the reproductive system and to improve the technological development for the control of reproduction.

1. Introduction

Reproduction is essential for the survival of organisms. Therefore, it is a high priority function of all mammalian species. Strategies used to perpetuate species vary greatly, but in general, they evolved to improve the success of survival of the offspring. Regulation of reproductive function in mammals involves integration of molecular and cellular functions among various organs in the body. The central nervous system perceives and processes internal (e.g., endocrine and metabolic status) and external cues (e.g., social interaction, photoperiod). This information is used for the regulation of hypophyseal function and release of adenohypophyseal and neurohypophyseal hormones. Gonadotropins from the adenohypophysis control gonadal hormone synthesis and gametogenesis. Gonadal hormones, particularly steroid hormones, influence the function of many reproductive and non-reproductive organs. In females, the uterus has a primary role in supporting embryo and fetal development. Milk synthesis by the mammary gland and nursing are essential for new born and infant nutrition. Although the reproductive biology of mammalian species can be viewed in general terms, the mechanisms regulating molecular, cellular and physiological aspects are quite diverse among mammals. Therefore, generalizations derived from a single or few species should be considered with caution. In this chapter, generalizations that may represent mammalian reproductive biology will be made and details particular to one species or group will be included as appropriate.

A better understanding of the biological processes regulating reproductive function provides opportunities to intervene in the reproductive process of mammals. Reproductive technology has emerged as a field with tremendous societal impact. Improvements in reproductive efficiency of food-producing animals, treatment of infertility in humans and non-humans, use of contraceptives to control human and wild

populations, and preservation of endangered mammalian species are examples of areas in which reproductive technology has been used successfully to promote health and well-being. The fundamentals of common reproductive technologies currently in use will be presented herein. However, the continued generation of knowledge for the understanding of biological systems and the development of new and improved technologies changes the field rapidly. It is fascinating to think what the future may bring.

2. Overview of Reproductive Biology in Mammals

2.1. Anatomy and Physiology of the Reproductive System

The mammalian reproductive system is organized by an intimate communication between the sex organs and their regulatory centers. The gonads and genitalia constitute the primary sex organs. The hypothalamus and the preoptic area, regions located in the base of the brain, and the hypophysis (or pituitary gland), an endocrine gland connected to the hypothalamus, are the primary regulatory centers. The hypothalamus can be subdivided in several functional regions and examples of regions associated with the control of reproduction include the anterior hypothalamus, supraoptic nucleus, suprachiasmatic nucleus, ventromedial and dorsomedial nuclei, arcuate (or infundibular) nucleus and median eminence (or infundibulum), which connects the hypothalamus to the hypophysis. The hypophysis can also be subdivided in functional units: the adeno- (or anterior) and neuro- (or posterior) hypophysis (Figure 1).
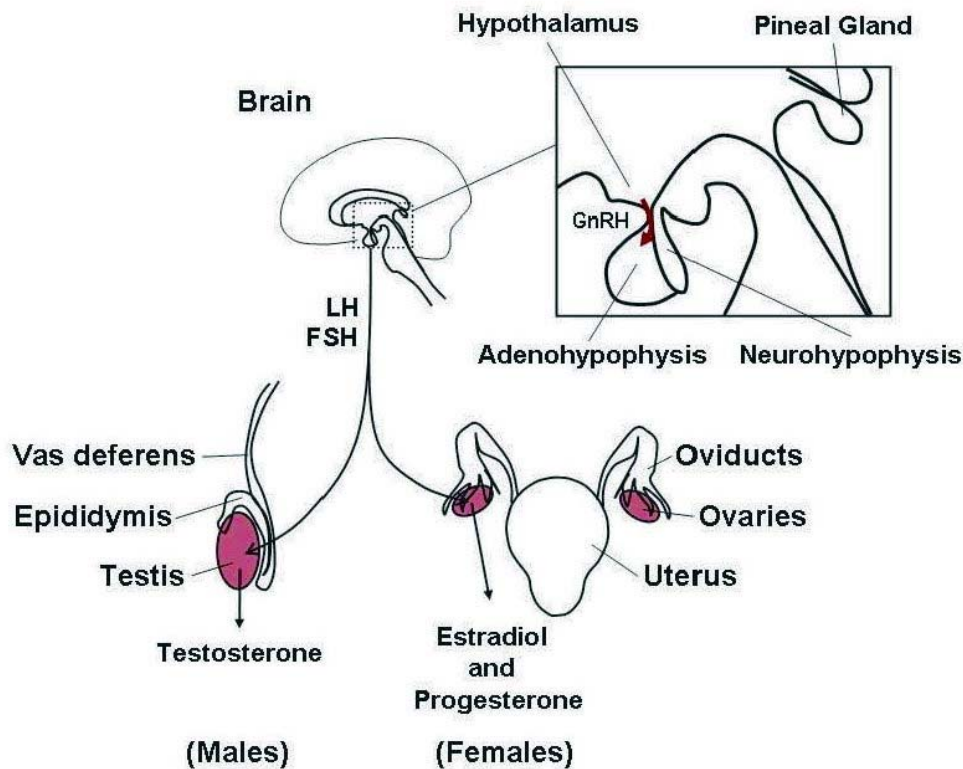

Figure 1. Diagram illustrating the reproductive endocrine axis in mammals.

Gonadotropin-releasing hormone (GnRH; enlarged diagram on the right) released by hypothalamic neurons reach the adenohypophysis via the hypothalamic-hypophyseal-portal circulation. Gonadotropes in the adenohypophysis release gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), in response to GnRH stimulation. Gonadotropins reach the gonads (testis in males and ovaries in females) via the blood circulation, and stimulate gametogenesis and steroid hormone synthesis and release. Gonadal steroid hormones (testosterone in males, and estradiol and progesterone in females) act by feedback mechanism and regulate the release of GnRH and gonadotropins.

The male reproductive tract is comprised of testes (male gonads), the duct system (epididymis and vas deferens), penis, scrotum, and accessory glands (prostate, seminal vesicles and the bulbourethral gland). The female reproductive tract consists of ovaries (female gonads), oviducts (Fallopian tubes), uterus, cervix, vagina, clitoris, vulva and vestibular glands. During gestation, the placenta forms in the uterus to maintain the pregnancy and support fetal development. In addition to serving as a site for exchange of nutrients, metabolites and gases from the mother to the developing fetus and vice versa, the placenta serves also a source of hormones and growth factors. After birth, neonate and infants of mammalian species are highly dependent upon nourishment provided to them for survival. Milk produced by the mammary gland is a major source of nutrients during the infantile period. Because of its role in milk synthesis, the mammary gland is essential for survival of the offspring in mammals.

2.1.1. Hypothalamic-Hypophyseal Axis

Hypophysiotropic neuro hormone	Action	Adenohypophyseal cell type	Adenohypophyseal hormone	Physiological function
Gonadotropin-releasing hormone (GnRH)	Stimulation	Gonadotropes	Gonadotropins Luteinizing hormone (LH) Follicle-stimulating hormone (FSH)	FSH Female: stimulates folliculogenesis Male: stimulates androgen-binding protein synthesis LH Female: follicular maturation, ovulation and maintenance of corpus luteum function Male: testosterone synthesis
Thyrotropin-releasing hormone (TRH)	Stimulation	Thyrotropes	Thyrotropin (TSH)	Stimulates thyroid hormone synthesis

Corticotropin-releasing hormone (CRH)	Stimulation	Corticotropes	Adrenocorticotropin (ACTH)	Stimulates adrenal cortex hormone synthesis
Growth hormone-releasing hormone (GHRH)	Stimulation	Somatotropes	Growth hormone (GH, somatotropin)	Stimulates insulin-like growth factor synthesis, bone growth, protein synthesis
Dopamine	Inhibition	Lactotropes	Prolactin (PRL)	Stimulates milk synthesis
Somatostatin	Inhibition	Somatotropes	Growth hormone (GH)	

Table 1. Neurohormones and adenohypophyseal hormones of the hypothalamic-hypophyseal axis.

The hypothalamus and the hypophysis are closely associated anatomically and functionally. Hypophysiotrophic neurons in the hypothalamus project to a specialized vasculature in the infundibulum – the hypothalamo-hypophyseal portal blood system, where releasing and inhibiting neurohormones are released. These neurohormones reach the adenohypophysis via the portal circulation. At the adenohypophysis, hypophysiotrophic hormones bind to specific receptors on the surface of cells, and stimulate the synthesis and release of adenohypophyseal hormones. In addition to stimulating hormones, substances with inhibitory actions on the secretion of adenohypophyseal hormones are also released into the hypothalamic-hypophyseal portal vasculature. Examples are somatostatin, which inhibits the release of growth hormone (GH) from somatotropes (GH-secreting cells), and dopamine, which inhibits the release of prolactin from lactotropes (prolactin-secreting cells). Major adenohypophyseal hormones and their regulatory hypothalamic neurohormones are shown in table 1.

Of the neurohormones presented in Table 1, gonadotropin-releasing hormone (GnRH) is considered the primary releasing hormone regulating reproductive function in mammals. Gonadotropin-releasing hormone stimulates gonadotropin synthesis and release. The cell bodies of GnRH neurons are scattered throughout the preoptic area and hypothalamus. In mice, rats, guinea pigs and rabbits, GnRH neurons concentrate in the preoptic area and anterior hypothalamus. In sheep, cattle, horses, pigs and primates GnRH neurons are also found throughout the mediobasal hypothalamus. The proportion of GnRH neurons in the mediobasal hypothalamus vary among species. Independent of the location of GnRH cell bodies, a common pathway for the axonal projections of these neurons is the infundibulum, where neuronal terminals are observed near the capillary loops of the hypothalamic-hypophyseal portal system. Neurohormones released in the infundibulum are collected into the adenohypophyseal vessels and distributed through the adenohypophysis.

In addition to the hypophysiotropic neurons, two other hypothalamic neuron populations should be noted: the vasopressin- and oxytocin-synthesizing neurons. These neurons project along the infundibulum and terminate around capillary vessels of the neurohypophysis. Vasopressin is involved in osmoregulation and the regulation of blood pressure and volume. Neurohypophyseal oxytocin has major roles in reproductive

physiology. In the uterus, oxytocin causes uterine muscle contraction that is intensified during parturition for expulsion of the fetus and fetal membranes. In the mammary gland, oxytocin causes milk letdown by stimulating contraction of the myoepithelial cells. Oxytocin released from the neurohypophysis is also involved in regulating ovarian function.

2.1.2. Adenohypophysis and Adenohypophyseal Hormones

The adenohypophysis is a mosaic of various cell types. The major endocrine cell types are the somatotropes, corticotropes, gonadotropes, thyrotropes and lactotropes (table 1). Follicle-stellate cells, presumably nonsecretory cells, are also present in the adenohypophysis and may modulate adenohypophyseal hormone secretion. In general, each adenohypophyseal hormone is synthesized primarily by a particular cell type; however, exceptions do occur. Gonadotropes in some species are observed to contain both gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). However, subpopulations of gonadotropes may contain only LH or FSH.

Growth hormone and prolactin are structurally-related protein hormones that regulate growth and development. Growth hormone has anabolic functions and stimulates somatic growth by stimulating insulin-like growth factor-1 production in the liver. In addition to somatic growth-promoting actions, growth hormone and insulin-like growth factor-1 have direct actions on reproductive organs as well. In mammals, prolactin stimulates growth of the mammary gland and lactogenesis. In addition, prolactin contributes to the control of testicular function in males. Adrenocorticotropin hormone, derived from the precursor proopiomelanocortin, stimulates the adrenal cortex to synthesize adrenal steroid hormones (cortisol and corticosterone) that regulate carbohydrate metabolism.

Thyrotropin , LH and FSH are glycoprotein hormones that contain covalent bounds to carbohydrate moieties. They are composed of alpha and beta subunits. The alpha subunit is common to all three hormones. In contrast, the beta subunit is unique and confers biological specificity to each hormone. Thyrotropin is important for stimulating synthesis of thyroxine and triiodothyronine from the thyroid gland, hormones with important role in thermogenesis and metabolic activity. Luteinizing hormone and FSH are hormones with major role in supporting gonadal hormone production and gametogenesis. Because of the importance of LH and FSH on the control of reproduction, their function in reproductive process will be discussed in more details in the following sections.

2.1.3. Gonads and Gonadal Function

In both sexes, the gonads have two major functions: gametogenesis and sex hormone production. The gonads are paired organs that vary in size depending on the species. In males, the testes support spermatogenesis and androgen (male hormone) synthesis. In females, the ovaries support folliculogenesis and female hormones (estrogen and progesterone) synthesis.

Each testis is enclosed by a connective tissue sheath consisting of three layers: the

tunica vaginalis (an outpocket of the peritoneum), the tunica albuginea (layer containing collagen and fibroblasts, and in some species it also contains smooth muscle cells with contractile function) and the tunica vasculosa (rich in blood vessels). The testis is formed by a collection of convoluted tubes named seminiferous tubules. The seminiferous tubules converge into the tubuli recti and rete testis, which opens to the ductuli efferentes and then to the epididymis. The epididymis consists of a caput (head), corpus (body) and cauda (tail), which connects to the vas deferens. Spermatogenesis takes place within the epithelium of the seminiferous tubules. Germ cells of various stages of development (from spermatogonia to spermatozoa) are embedded within supporting cells – the Sertoli cells. Sertoli cells provide nutrients and other factors necessary for sperm development and maturation. The interstitial cells (Leydig Cells) are clustered between the seminiferous tubules and are the source of androgens within the testes. Blood and lymphatic vessels, nerves, immune cells (e.g. lymphocytes and macrophages) and fibroblasts are embedded within the interstitial space.

Spermatogenesis occurs in waves along the seminiferous tubules. Spermatogonial stem cells proliferate to maintain a pool of cells that undergo into various stages of differentiation to form mature spermatozoa. During the process of spermatogenesis, spermatogonia, which contain two sets of chromosomes (2N), undergo meiosis to form spermatocytes I (4N), spermatocytes II (2N), spermatides (N) and finally the spermatozoa (N). During meiosis, the sex chromosomes (X and Y) are segregated; thus, mature spermatozoa containing one set of chromosomes will have either the X or Y chromosome. The time required for successive differentiation of a germ cell, called the spermatogenic cycle, varies among mammalian species. It is approximately 35 days in the mouse, 54 days in the bull and 74 days in the man.

The ovaries in females are intra-abdominal and are suspended in the abdominal cavity by the ovarian ligament. Morphologically, the ovaries are observed to contain a medulla, where nerve, blood and lymph supplies enter the organ, and the cortex, where oocytes and follicles in various stages of development are present. The ovaries serve as the site for storage and development of female gametes, the oocytes. In contrast to males which have continued replenishment of stermatogonial stem cells during their reproductive life, it is generally believed that female mammals acquire a definite number of oocytes during fetal development or around the time of birth. Once the female acquires reproductive maturity, the major functional structures observed in ovaries of cycling females include follicles, which contain the oocyte, and the corpus luteum.

Folliculogenesis is the process of follicular growth, beginning with its emergence from the pool of primordial follicles to either ovulation or atresia. Primordial follicles contain an oocyte surrounded by a layer of granulosa cells. During folliculogenesis, granulosa cells surrounding the oocyte multiply, and the oocyte enlarges and forms a layer of glycoproteins that surrounds the oocyte's plasma membrane. This glycoprotein layer is called zona pellucida. As follicles continue to grow and mature, a fluid-filled cavity, the antrum, forms, and the oocyte within the follicle acquires the ability to resume meiosis and undergo cellular divisions upon fertilization. Depending on the species, a single to several follicles become dominant and ultimately, ovulate. Species with typically one or two dominant follicles during each ovarian cycle include primates (including humans),

cattle, sheep, horses and goats. In pigs, dogs, cats and laboratory rodents, development of multiple dominant follicles and multiple ovulation is common. The fate of most follicles, however, is to undergo into atresia during the recurrent reproductive cycles and only a small proportion of follicles are ovulated during the reproductive life of a mammalian female. After ovulation, cells of the ovulated follicle(s) are transformed into luteal cells that form the corpus luteum.

2.2. Sex Determination and Differentiation

2.2.1. Genetic and Phenotypic Sex

In mammals, genetic sex is determined at fertilization when the male (containing either the X or Y chromosome) and female (containing the X chromosome) gametes fuse. Fertilized egg containing two X chromosomes (XX) differentiates towards the female gonad (ovaries), while fertilized eggs containing the X and Y chromosome (XY) differentiates towards the male gonad (testis) during fetal development. Hormones secreted by the developing gonad then promote differentiation of internal and external reproductive organs, and secondary sexual characteristics into male or female phenotype. The male gonad is determined by expression of a Y-chromosome gene – the SRY (sex determining region of the Y chromosome). Testis determining factor (TDF), a product of the SRY gene, induces development of the testis. Leydig cells within the testis produce male sex hormones (testosterone) that support Wolffian duct proliferation. Sertoli cells of the testis secrete Müllerian inhibitory factor (MIF) that causes the regression of the Müllerian ducts, that otherwise would develop into part of the female reproductive tract. During the undifferentiated, bipotential-gonad stage of development, the primitive gonads of male and female embryos are composed of primordial germ cells, mesenchyme of the genital ridge and an epithelium. In males, the sex cords develop into seminiferous tubules, which branch and form the rete testis and epididymis. The epithelial cells of the seminiferous tubules differentiate into Sertoli cells. The Sertoli cells embed the germ cells. The interstitial cells develop into the Leydig cells. In females, sex cords develop towards the mesenchyme and carry the primordial germ cells with them, forming the primordial follicles.

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Biographical Sketches

Marcel Amstalden is Assistant Professor in the Department of Animal Science at Texas A&M University, US. He received a degree in Veterinary Medicine from São Paulo State University, Botucatu, Brazil, in 1992, and a M.S. (2000) and a Ph.D. (2003) in Physiology of Reproduction, both from Texas A&M University. He was a Postdoctoral Research Fellow in Neuroendocrinology at the University of Cincinnati College of Medicine before joining the faculty at Texas A&M University in 2006. His research focuses on investigating the neuroendocrine mechanisms and pathways that regulate the establishment and maintenance of reproductive cycles in mammalian species.

Paul G. Harms is Professor Emeritus (Physiology of Reproduction) in the Animal Science Department at Texas A&M University. He received a B.S. in Agriculture (Dairy Science) and a M.S. in Physiology of Reproduction from the University of Illinois. After completing his Ph.D. in Physiology of Reproduction from Purdue University in 1969, he served as a Captain in the U.S. Air Force (Staff Research Physiologist) at the U.S. Air Force School of Aerospace Medicine, Brooks AFB, Texas. He was a Postdoctoral Research Fellow in Neuroendocrinology at the University of Texas Southwestern Medical School in Dallas Texas before joining the faculty at Texas A&M University in 1974. At Texas A&M, he advanced to Professor and served as Leader of the Physiology of the Reproduction Section. Paul has taught both undergraduate and graduate courses in physiology of reproduction with a focus on reproductive technologies. His research focused on the neural control of ovarian function. He was recognized for his teaching, research and service to numerous professional societies including as President of the Southern Section of the American Society of Animal Science.