Physiology of Testicular Function

2

Gerhard F. Weinbauer, Craig Marc Luetjens,
Manuela Simoni, and Eberhard Nieschlag

Contents

G. F. Weinbauer (✉)
Research and Safety Assessment, Covance Laboratories GmbH, Kesselfeld 29, D-48169 Münster,Germany
e-mail: gerhard.weinbauer@covance.com

2.1 Functional Organization of the Testis

The testes produce the male gametes and the male sexual hormones **(androgens).** The term **spermatogenesis** describes and includes all the processes involved in the production of gametes, whereas **steroidogenesis** refers to the enzymatic reactions leading to the production of male steroid hormones. Spermatogenesis and steroidogenesis take place in two compartments morphologically and functionally distinguishable from each other. These are the tubular compartment, consisting of the seminiferous tubules **(tubuli seminiferi)** and the interstitial compartment **(interstitium)** between the seminiferous tubules (Figs. 2.1 and 2.2). Although anatomically separate, both compartments are closely connected with each other. For quantitatively and qualitatively normal production of sperm the integrity of both compartments is necessary. The function of the testis and thereby also the function of its compartments are governed by the hypothalamus and the pituitary gland **(endocrine regulation).** These endocrine effects are mediated and modulated at the testicular level by local control mechanisms **(paracrine and autocrine factors).**

2.1.1 Interstitial Compartment

The most important cells of this compartment are the Leydig cells. These cells are the source of testicular testosterone and of **insulin-like factor 3** (INSL3). Aside from Leydig cells, the interstitial compartment also contains immune cells, blood and lymph vessels, nerves, fibroblasts and loose connective tissue. In experimental animals this compartment comprises

E. Nieschlag et al. (eds.), *Andrology*,
DOI: 10.1007/978-3-540-78355-8_2,

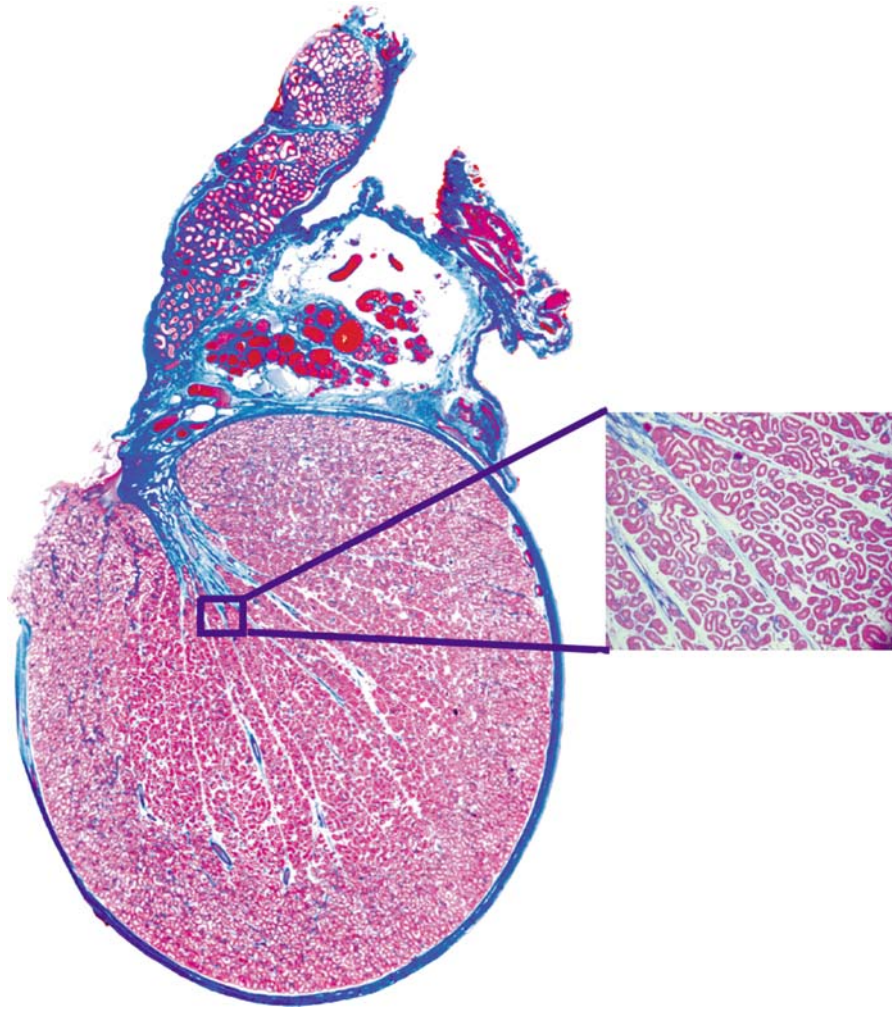

Fig. 2.1 Section of an entire human testis cut transversally. The preparation also includes parts of the efferent ducts and the epididymis. The lobular architecture of the testis is evident (Courtesy of Prof. Dr. A.F. Holstein, Institute of Anatomy, University of Hamburg)

about 2.6% of the total testicular volume. In the human testis the interstitial compartment represents about 12–15% of the total testicular volume, 10–20% of which is occupied by **Leydig cells.** Human testes contain approximately 200×10^6 Leydig cells.

2.1.1.1 Leydig Cells

These cells were first described in 1850 by Franz Leydig (1821–1908). Leydig cells produce and secrete the most important male sexual hormone, testosterone. From the developmental, morphological and functional viewpoint different types of cells can be distinguished: stem Leydig cells as founder cell, progenitor Leydig cells as a committed stem cell, fetal Leydig cells as a terminally differentiated cell in the fetus, and adult Leydig cells as the terminally differentiated Leydig cell (Ge and Hardy 2007). Fetal Leydig cells become neonatal Leydig cells at birth and degenerate thereafter or regress into immature Leydig cells (Prince 2007). Fetal Leydig cells produce testosterone. Immature Leydig cells that mainly produce androstane-3α, 17β-diol instead of testosterone have also been described.

Adult Leydig cells are rich in smooth endoplasmic reticulum and mitochondria with tubular cristae. These physiological characteristics are typical for steroid-producing cells and are very similar to those found in other steroidogenic cells, such as those in the adrenal gland and in the ovary. Other important cytoplasmic components are lipofuscin granules, the final product of endocytosis and lyosomal degradation, and lipid

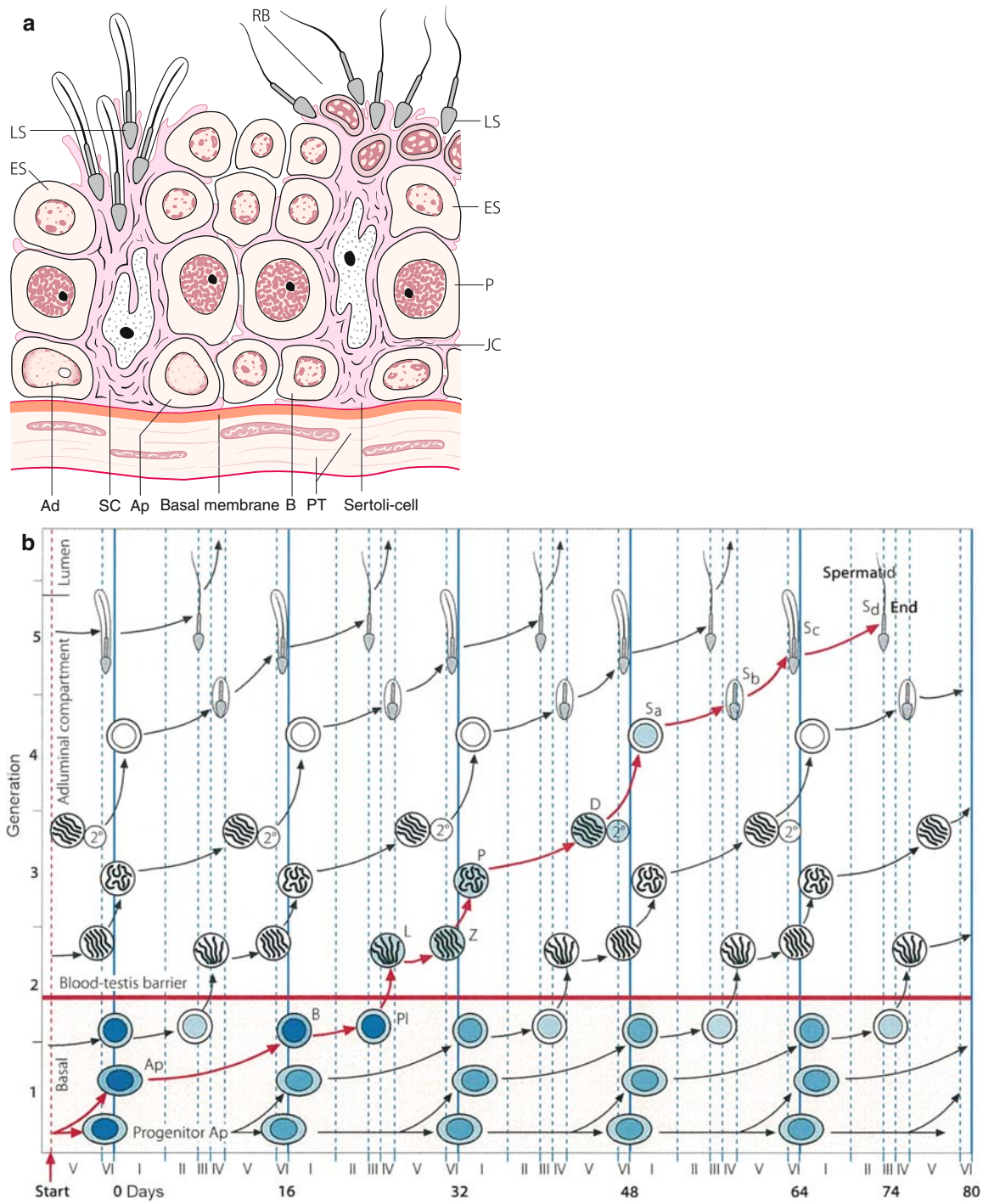

Fig. 2.2 (**a**) Schematic representation of the architecture of the human seminiferous epithelium. Note that the tubular wall is composed of several layers of peritubular cells (PT) and a basal lamina (BL). RB = Residual Body, LS = Late/elongating and elongated spermatids, ES = Early/round spermatids, P = Spermatocytes, Ad = A-dark-type spermatogonia (testicular stem cells), Ap = A pale-type spermatogonia; B = B-type spermatogonia, SC = Sertoli cells, JC = junctional complexes constituting the blood-testis barrier built by interconnected Sertoli cells. Modified from Ross (1985)

(**b**) Depicts the kinetics of the human seminiferous epithelium. Ap spermatogonia are the progenitor stem cells that enter the spermatogenic cycle (color-coded). All descendents of this progenitor cell represent a single clone of germ cells. Ad = A-dark-type spermatogonia, B = B-type spermatogonia, Pl = prelepototene spermatocyte; L = leptotene spermatocyte; Z = zygotene spermatocyte; D = diplotere spermatocyte. It takes 4–4.6 cycles ("generation" on the y-axis, denoted as Start and End) until a sperm (Sd) has developed from a progenitor cell (Sa, Sb, Sc) 2° = 2nd meitotic division. (Modified from Amann 2008)

droplets, in which the preliminary stages of testosterone synthesis take place. Special formations, called Reinke's crystals, are often found in the adult Leydig cells. These are probably subunits of globular proteins whose functional meaning is not known. The proliferation rate of the Leydig cells in the adult testis is rather low and is influenced by LH. The ontogeny of Leydig cells is not entirely clear and mesonephros, neural crest and coelomic sources have been involved. In the adult testis, Leydig cells develop from perivascular and peritubular mesenchymal-like cells and the differentiation of these cells into Leydig cells is induced by LH but also by growth factors and differentiation factors derived from Sertoli cells.

2.1.1.2 Macrophages. Lymphocytes and Nerve Fibers

Besides Leydig cells, the interstitial compartment also contains cells belonging to the immune system: macrophages and lymphocytes. For every 10–50 Leydig cells one macrophage is to be found. The macrophages probably influence the function of the Leydig cells, in particular their proliferation, differentiation and steroid production, through the secretion of cytokines. Macrophages secrete stimulators and inhibitors of steroidogenesis. Proinflammatory cytokines, reactive oxygen species, **nitric oxide** and **prostaglandins** can inhibit Leydig cell function (Hales 2007). There is evidence for an involvement of neurotransmitters and related signalling factors during regulation of Leydig cell function (Mayerhofer et al. 1999). The immunological meaning of these cells for testicular physiology will be discussed under Sect. 2.5.

2.1.2 Tubular Compartment

Spermatogenesis takes place in the tubular compartment. This compartment represents about 60–80% of the total testicular volume. It contains the germ cells and two different types of somatic cells, the **peritubular cells** and the **Sertoli cells.** The testis is divided by septa of connective tissue into about 250–300 lobules (Fig. 2.1), each one containing 1–3 highly convoluted seminiferous tubules. Overall, the human testis contains about 600 seminiferous tubules. The length of individual seminiferous tubules is about 30–80 cm. Considering an average number of about 600 seminiferous tubules per testis and an average length of the tubuli seminiferi of about 60 cm each, the total length of the tubuli seminiferi is about 360 m per testis, i.e., 720 m of seminiferous epithelium per man.

2.1.2.1 Peritubular Cells

The seminiferous tubules are covered by a lamina propria, which consists of a **basal membrane,** a layer of collagen and the **peritubular cells (myofibroblasts).** These cells are stratified around the tubulus and form up to concentrical layers that are separated by collagen layers (Fig. 2.1). These characteristics differentiate the human testicle from the majority of the other mammals, whose seminiferous tubules are surrounded only by 2–4 layers of myofibroblasts. Peritubular cells produce several factors that are involved in cellular contractility: **panactin, desmin, gelsolin, smooth muscle myosin and actin** (Holstein et al. 1996). These cells also secrete extracellular matrix and factors typically expressed by connective tissue cells: **collagen, laminin, vimentin, fibronectin, growth factors, fibroblast** protein and adhesion molecules (Albrecht et al. 2006; Schell et al. 2008). The latter work established a human peritubular cell culture system demonstrating secretion of nerve growth factor and pro-inflammatory molecules, e.g., **Il-1β** and **cyclooxygenase-2** under the influence of **TNF-α** (Schell et al. 2008). Myofibroblasts are poorly differentiated myocytes with the capacity of spontaneous contraction. Mature sperm are transported towards the exit of the seminiferous tubules by contraction of these cells and several regulators of cell contractions are reported, e.g., **oxytocin,** oxytocin-like substances, **prostaglandins,** androgenic steroids, **endothelins,** endothelin converting enzymes and endothelin receptors. Peritubular contractility is mediated by endothelin and this effect is modulated by the relaxant peptide **adrenomedullin** produced by Sertoli cells (Romano et al. 2005). Mice with selective peritubular cell androgen receptor deficiency revealed defects in contractility-related genes, e.g., endothelin-1 and endothelin receptor A and B, adrenomedullin receptor and **vasopressin** receptor 1a (Zhang et al. 2006).

Disturbances of testicular function and decreased or absent spermatogenic activity are associated with a

thickening of the layer of collagen fibres and of the material present between the peritubular cells. When this is the case, the tubular wall becomes **fibrotic** or – based on the histological appearance – **hyalinized.** The decrease of testicular volume involves folding of the wall along the length of the tubuli seminiferi, thereby causing an enlargement of the tubular diameter. This becomes particularly evident when fluid is injected into regressed seminiferous tubules. Tubular diameter increases and tubular wall thickness decreases (Schlatt et al. 1999). An interaction between testicular mast cells and peritubular cells leading to fibrotic changes of the seminiferous tubular wall has been suggested (Albrecht et al. 2006). Peritubular and interstitial fibrosis incidence correlated progressively with spermatogenic damage in testis from vasectomized men (Raleigh et al. 2004).

2.1.2.2 Sertoli Cells

Sertoli cells are somatic cells located within the germinal epithelium. In adulthood these cells are mitotically inactive. They are named after Enrico Sertoli (1842–1910), the Italian scientist who first described these cells in 1865 and, due to their prominent cytoplasmatic projections and ramifications called them "cellulae ramificate". These cells are located on the basal membrane and extend to the lumen of the tubulus seminiferus and, in a broad sense, can be considered as the **supporting structure of the germinal epithelium.** Along the cell body, extending over the entire height of the germinal epithelium, all morphological and physiological differentiation and maturation of the germinal cell up to the mature sperm take place. Special ectoplasmic structures sustain alignment and orientation of the sperm during differentiation. About 35–40% of the volume of the germinal epithelium is represented by Sertoli cells. The intact testis with complete spermatogenesis contains 800–1200 × 10^6 Sertoli cells (Zhengwei et al. 1998a) or approximately 25 × 10^6 Sertoli cells per gram testis (Raleigh et al. 2004).

Sertoli cells synthesize and secrete a large variety of factors: proteins, cytokines, growth factors, opioids, steroids, prostaglandins, modulators of cell division etc. The morphology of Sertoli cells is strictly related to their various physiological functions. Cytoplasm contains endoplasmic reticulum both of the smooth **(steroid synthesis)** and rough type **(protein synthesis)**, a prominent Golgi apparatus **(elaboration and transport of secretory products)**, lysosomal granules **(phagocytosis)** as well as microtubuli and intermediate filaments (**adapation of the cell shape** during the different phases of germ cell maturation). It is generally assumed that Sertoli cells coordinate the spermatogenic process topographically and functionally. On the other hand, more recent data support the contention that germ cells control Sertoli cell functions. At least the time pattern of germ cell transitions and development during the spermatogenic cycle seem to be autonomous as suggested from heterologous **germ cell transplantation** studies (Nagano et al. 2001). One spermatogenic cycle lasts about 8 days in mice and 12–13 days in rats. Notably, the cycle duration of rat germ cells transplanted into mouse testis remained 12–13 days whereas that of the host germ cells was maintained at 8 days (Franca et al. 1998).

Another important function of Sertoli cells is that they are responsible for final **testicular volume** and **sperm production** in the adult. Each individual Sertoli cell is in morphological and functional contact with a defined number of sperm. The number of sperm per Sertoli cell depends on the species. In men we observe about 10 germ cells or 1.5 spermatozoa per each Sertoli cell (Zhengwei et al. 1998a). In comparison, every macaque monkey Sertoli cell is associated with 22 germ cells and 2.7 sperm (Zhengwei et al. 1997, 1998b). This suggests that within a certain species a higher number of Sertoli cells results in a greater production of sperm and testis size, assuming that all the Sertoli cells are functioning normally. In contrast, as determined by flow cytometry, testicular cell numbers were very similar across several primate species, suggesting that testis size is the main determinant of total germ cell output (Luetjens et al. 2005).

Stereological investigations suggest that the number of Sertoli cells in men increases until the 15th year of life. In the prepubertal cynomolgus monkey and the rhesus monkey, Sertoli cells exhibit little mitotic activity, whereas some proliferative activity of A-type spermatogonia occurs in the quiescent testis. Sertoli cell proliferation is markedly activated when exposed to gonadotropin activity (Plant et al. 2005; Schlatt et al. 1995). Both Sertoli cell number and expression of markers of cell division are stimulated by these hormones. The division of Sertoli cells ends when the first germ cells undergo meiotic division and Sertoli cells have built tight junctions between each other, the

so-called blood-testis-barrier (see Sect. 2.5). Lack of **connexin-43,** a predominant gap-junction protein, prevents Sertoli cell maturation associated with continued division of Sertoli cells and spermatogenic arrest beyond spermatogonial development (Brehm et al. 2007; Sridharan et al. 2007). Expression of Sertoli cells markers such as **transferrin, androgen-binding protein** and junctional proteins such as **N-cadherin,** connexin-43, gelsolin, laminin-γ3, **occludin, testin, nectin, zyxin** and **vinculin** is androgen-dependent (Zhang et al. 2006). It appears that several of these components are involved in establishing the blood-testis-barrier but also in the release of sperm and subsequent remodelling of the Sertoli cell-germ cell junctions (Yan et al. 2008). In the rat, the experimental prolongation of the division phase of Sertoli cells, produced for example by a deprivation of thyroid hormones, results in an increase of testicular weight and sperm production by about 80%. On the other hand, the decrease of Sertoli cell numbers such as that produced by an antimitotic substance leads to a reduction of testicular volume and sperm production. Patients with Laron dwarfism suffer from a disturbance of thyroid function and growth hormone/IGF-I deficiency, and often have testicles larger than normal.

Through the production and secretion of tubular fluid Sertoli cells create and maintain the patency of the tubulus lumen. More than 90% of Sertoli cell fluid is secreted in the tubular lumen. Special structural elements of the blood-testis barrier prevent reabsorption of the secreted fluid, resulting in pressure that maintains the patency of the lumen. Sperm are transported in the tubular fluid, the composition of which is known in detail only in the rat (Setchell 1999). Unlike blood, the tubular fluid contains a higher concentration of potassium ions and a lower concentration of sodium ions. Other constituents are bicarbonate, magnesium and chloride ions, inositol, glucose, carnitine, glycerophosphorylcholine, amino acids and several proteins. Therefore, the germ cells are immersed in a fluid of **unique composition**.

The basolateral aspect of neighboring Sertoli cells comprises membrane specializations forming a band sealing the cells to each other and obliterating the intracellular space (occluding tight junctions). The physiological function of the blood-testis barrier has been proven in experiments showing that dyes or lanthanum applied outside the barrier could diffuse only up to the tight junctions without reaching the lumen of the seminiferous tubules. The closure of the blood-testis barrier coincides with the beginning of the first meiosis in the germinal cells (preleptotene, zygotene) and with the arrest of proliferation of Sertoli cells. Through the blood-testis-barrier the seminiferous epithelium is divided into two regions which are anatomically and functionally completely different from each other. Early germ cells are located in the **basal region** and the later stages of maturing germ cells in the **adluminal region.** During their development germ cells are displaced from the basal to the adluminal compartment. This is accomplished by a synchronized dissolution and reassembly of the tight junctions above and below the migrating germ cells.

Two important functions are postulated for the blood-testis-barrier: the physical isolation of haploid and thereby antigenic germ cells to prevent recognition by the immune system (prevention of autoimmune orchitis, see Sect. 2.5) and the preparation of a special milieu for the meiotic process and sperm development. In certain seasonal breeders the opening and closure of the barrier depends much more on the activity of the Sertoli cells than on the developmental phase of the germinal epithelium. The constitution of the blood-testis-barrier and its selectivity in excluding certain molecules means that the cells localized in the adluminal compartment have no direct access to metabolites deriving from the periphery or from the interstitium. Therefore, these cells are completely dependent on Sertoli cells for their maintenance. This "nourishing function" could be exercised through different mechanisms: selective transport and transcytosis as well as synthesis and vectorial secretion.

2.1.2.3 Germinal Cells

Spermatogenesis starts with the division of stem cells and ends with the formation of mature sperm (Figs. 2.3 and 2.4). The various germ cells are arranged in typical cellular associations within the seminiferous tubules known as spermatogenic stages (Fig. 2.5) and the entire spermatogenic process can be divided into four phases:

1. **Mitotic** proliferation and differentiation of diploid germ cells (spermatogonia) **(spermatogoniogenesis)**
2. **Meiotic** division of tetraploid germ cells (spermatocytes) resulting in haploid germ cells (spermatids)
3. Transformation of spermatids into testicular sperm **(spermiogenesis)**
4. Release of sperm from the germinal epithelium into the tubular lumen **(spermiation)**.

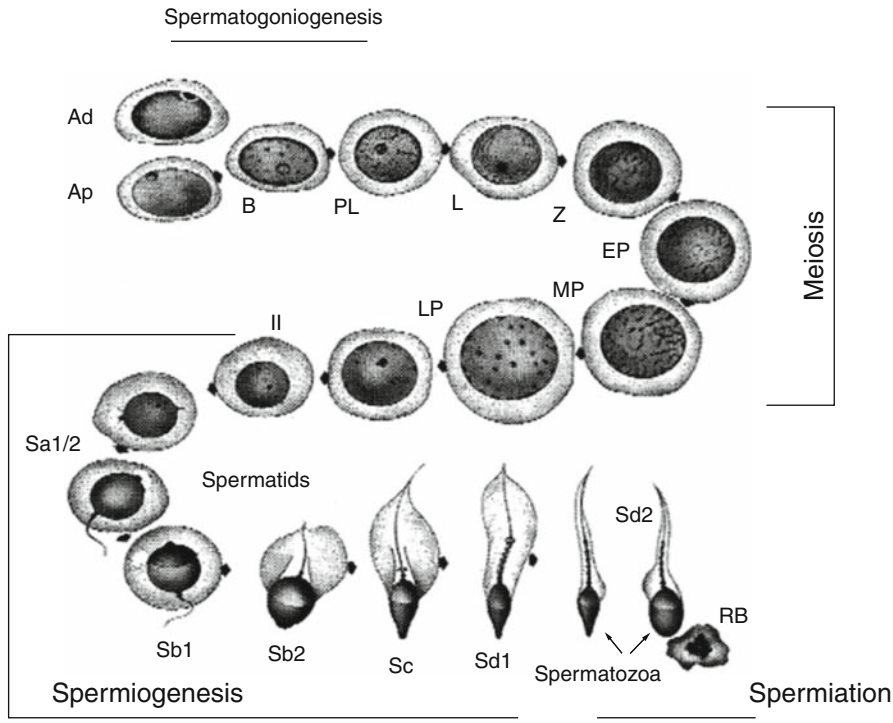

Fig. 2.3 Schematic representation of all germ cell types that occur in the human seminiferous epithelium. Ap spermatogonia enter the spermatogenic process (arrow on the cell indicates direction of germ cell development). Ad spermatogonia are believed to constitute the testicular stem cells. Ad = A-dark spermatogonium, Ap = A-pale spermatogonium, B = B spermatogonium, Pl = preleptotene spermatocytes, L = leptotene spermatocytes, EP = early pachytene spermatocytes, MP = mid pachytene spermatocytes, LP = late pachytene spermatocytes, II = 2nd meiotic division, RB = residual body, Sa1 – Sd2 = developmental stages of spermatid maturation

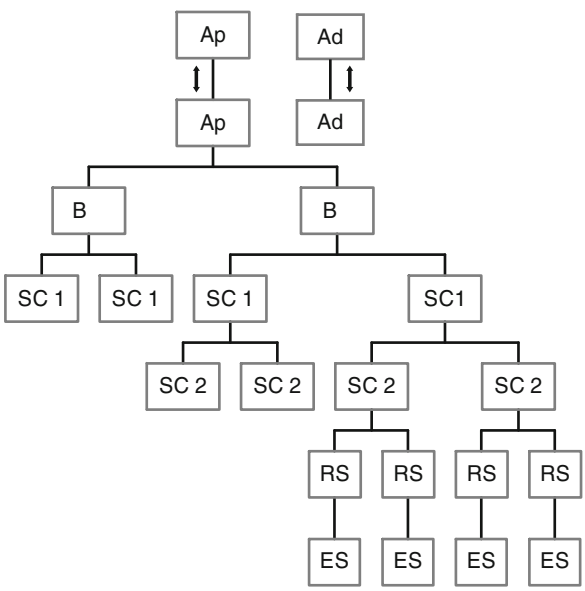

Fig. 2.4 Schematic representation of the proliferative kinetics of human gametogenesis. For the sake of clarity, complete development of only one spermatogonium is shown. The human testis contains about 1 billion sperm and releases around 25,000 sperm every minute (Amann 2008). One Ap spermatogonium can be the progenitor for 16 elongated spermatids. Since the human seminiferous epithelium contains only one generation of B-type spermatogonia, the final germ cell number produced is lower than in species with multiple spermatogonial divisions. Ad = A-dark spermatogonium (testicular stem cells, divides rarely), Ap = A-pale spermatogonium (self-renewing and progenitor cell for spermatogenesis), B = B spermatogonium, SC1 = primary spermatocyte, SC2 = secondary spermatocyte, RS = round spermatid, ES = elongated spermatid

Spermatogonia lie at the base of the seminiferous epithelium and are classified as type A and type B spermatogonia. Two types of A spermatogonia can be distinguished, originally from a cytological and now also from a physiological point of view: the Ad (dark) spermatogonia and the Ap (pale) spermatogonia. The Ad spermatogonia do not show proliferating activity under normal circumstances and are believed to divide only rarely (Ehmcke and Schlatt 2006). These spermatogonia are considered to represent **testicular stem cells** (Ehmcke et al. 2006). These germ cells, however, undergo mitosis when the overall spermatogonial population is drastically reduced, for example due to radiation (de Rooij 1998). In contrast, the Ap spermatogonia divide, renew themselves and differentiate into two B spermatogonia. Detailed studies in non-human primates led to a revised model for spermatogonial expansion in men (Ehmcke and Schlatt 2006): only Ap spermatogonia divide and give rise to Ap spermatogonia (to replenish this cell pool) as well as to B-type spermatogonia for further development. In contrast to the earlier model of Clermont, Ad spermatogonia are not the source of Ap spermatogonia during regular spermatogenic cycles. From B spermatogonia the preleptotene spermatocytes are derived directly before the beginning of the meiotic division. The latter germ cells commence DNA synthesis. Mother and daughter cells remain in close contact with each other through intercellular bridges (Alastalo et al. 1998). This "clonal"

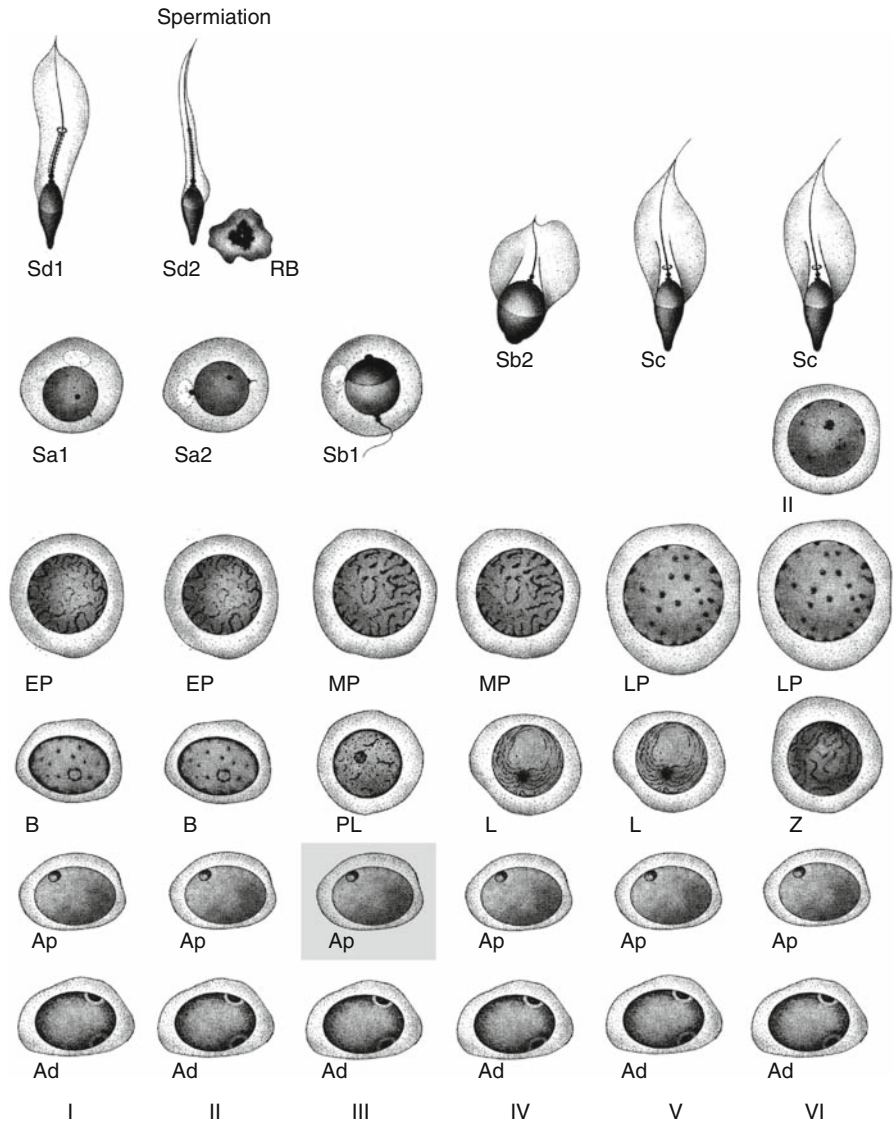

Fig. 2.5 Representation of the specific stages of spermatogenesis of the human testis using the six-stage system. A tubular cross-section contains typical germ cell associations that are denoted as stages of spermatogenesis. The six stages (I–VI) in the human last altogether 16 days. Since a spermatogonium has to pass through minimally four cell layers, the complete duration of spermatogenesis in men is at least 64 days. The complete duration of the human spermatogenic process is still not entirely clear (Amann 2008). Ad = A-dark spermatogonium (testicular stem cells, divides rarely), Ap = A-pale spermatogonium (self-renewing and progenitor cell for spermatogenesis), B = B spermatogonium, Pl = preleptotene spermatocytes, L = leptotene spermatocytes, EP = early pachytene spermatocytes, MP = mid pachytene spermatocytes, LP = late pachytene spermatocytes, II = 2nd meiotic division, RB = residual body, Sa1–Sd2 = developmental stages of spermatid maturation

mode of germ cell development – also confirmed for primates (Ehmcke et al. 2005) – is possibly the basis and at the same time probably the prerequisite for the coordinated maturation of gametes in the seminiferous epithelium.

Tetraploid germ cells are known as **spermatocytes** and go through the different phases of the meiotic division. The pachytene phase is characterized by intensive RNA synthesis. **Haploid germ cells,** the spermatids, result from the meiotic division. The meiotic process is a critical event in gametogenesis, during which recombination of genetic material, reduction of chromosome number and development of spermatids have to be accomplished. Secondary spermatocytes are derived from the first meiotic divison. These germ cells contain a haploid chromosomal set in duplicate form. During the second meiotic division spermatocytes are divided into the haploid spermatids. The prophase of the first meiosis lasts 1–3 weeks, whereas the other phases of the first meiosis and the entire second meiosis are concluded within 1–2 days.

Spermatids are derived from the second meiotic division and are round mitotically inactive cells which undergo a remarkable and complicated transformation leading to the final production of differentiated elongated spermatids and sperm. These processes include

condensation and structural shaping of the cell nucleus, the formation of a **flagellum** and the expulsion of a large part of cytoplasm. The overall process is called **spermiogenesis** and, from a qualitative point of view, is identical in all species. It is useful to divide spermiogenesis into four phases: Golgi, cap, acrosomal and maturation phases.

During the **Golgi phase** acrosomal bubbles and craniocaudal symmetry appear. In the **cap phase** the spermatids become elongated and the acrosome develops, covering the cranial half to two-thirds of the spermatid. During the fertilization process enzymes are released by the acrosome, allowing the sperm to penetrate the egg (see Chap. 3).

In the **acrosomal phase** the cell nucleus becomes further condensed and elongation of the cell continues. During condensation the majority of histones are lost and gene transcription stops. Nuclear chromatin is now extremely condensed, implying that the proteins necessary for spermiogenesis have to be transcribed before this timepoint and justifying the finding of RNA species with very long half-life and RNA binding proteins. This is the case for transition proteins and protamines. The mRNA translational control mechanisms are just being unravelled and RNA-binding proteins seem to play an important role. The **flagellum** is now mature.

The principal event during the maturation phase of the spermatids is the extrusion of the rest of the cytoplasm as the so-called **residual body.** Residual bodies are phagocytosed by Sertoli cells and have a regulatory role. Elongated spermatids and their residual bodies influence the secretory function of Sertoli cells (production of tubular fluid, inhibin, androgen-binding protein and interleukin-1 and 6). In parallel with degradation of the residual bodies, a new spermatogenic cycle begins.

The release of sperm into the tubular lumen is designated as **spermiation.** This event is influenced by plasminogen activators and possibly also by thimet oligopeptidases. This process can be particularly affected by hormonal modifications, temperature and toxins. The reasons for this sensitivity are, however, not yet known. Sperm that are not released are phagocytosed by Sertoli cells. Round and elongated spermatids already contain all the information necessary for fertilization; since introduction of intracytoplasmatic injection of testicular sperm and even round spermatids it has become possible to induce pregnancies successfully (see Chap. 3 for details).

2.1.2.4 Kinetics of Spermatogenesis

The complex process of division and differentiation of germ cells follows a precise pattern. All germ cells pass through several stages characterized by particular cellular associations. Recognizing that acrosome development is stage-dependent was crucial for the understanding of germ cell maturation. The number of stages of spermatogenesis depends on morphological criteria. For the rat 14 stages (I–XIV) are used and 12 stages (I–XII) in macaques, For men, originally 12 spermatid maturation steps were described but a six-stage (I–VI) approach is currently used. More recently, the six-stage system has also been applied to Old World and New World primate species (Wistuba et al. 2003). The succession of all stages along time is called the spermatogenic cycle.

The duration of the spermatogenic cycle depends on the animal species and lasts between 8–17 days in mammals. **One human spermatogenic cycle requires 16 days.**

For the development and differentiaton of an Ap spermatogonium into a mature sperm at least four spermatogenic cycles are necessary. It can be deduced that the **overall duration of spermatogenesis** is calculated as around 50 days in the rat, 37–43 days in different monkey species and at least 64 days in man. It must be pointed out, however, that a recent review recommends 74 days by including time for spermatogonial renewal (Amann 2008). Investigations carried out in the 1960s led to the conclusion that the duration of spermatogenesis is genetically determined, does not vary throughout life and cannot be influenced experimentally. However, many indirect experimental findings oppose this hypothesis. For example, the first spermatogenic cycle during puberty proceeds faster than in the adult age. It has also been demonstrated in the rat that the duration of germ cell maturation can actually be manipulated by exogenous factors. In contrast, endocrine factors do not alter the duration of spermatogenic cycles (Aslam et al. 1999).

The spermatogenetic stages appear well orchestrated, not only in **time** but also in **space**. In the rat, serial transversal sections through the seminiferous tubules show that stage I is always followed by stage II, stage III always by stage IV and so on. This is known as the **spermatogenic wave.** In contrast, in the

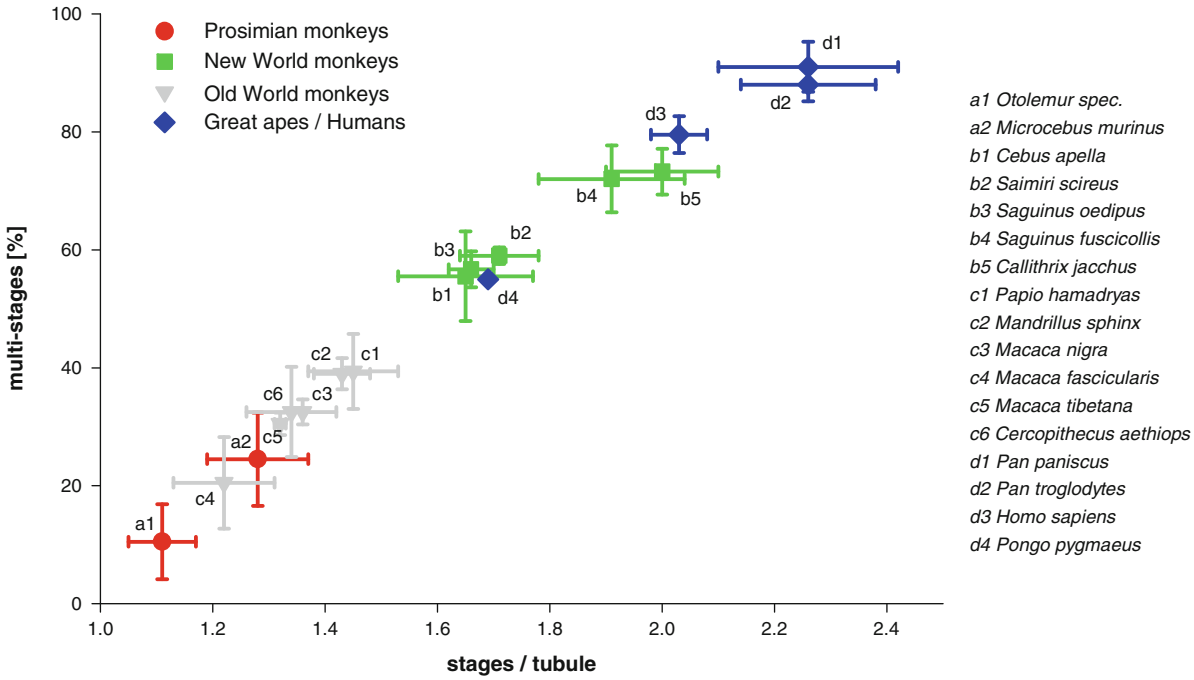

Fig. 2.6 Frequency (%) of seminiferous tubules containing more than one spermatogenic stage versus the number of stages per tubular cross-section across the primate order. a1–a2: prosimians, b1–b5: New World monkeys, c1–c6: Old World monkeys, d1, d2 and d4: great apes, d3: men. Note the clustering of multi-stage distribution and the increased number of stages in New World monkeys, great apes and humans. The incidence of multi-stage versus single-stage tubules was not related to germ cell production (Luetjens et al. 2005; Fig. 2.7)

entire human testis and in parts or whole testis of various monkey species, tubular sections show different stages simultaneously (Fig. 2.6). While this was initially considered to be an irregular arrangement, quantitative analysis of the germ cell population suggested a helical topography of spermatogenic stages with several helices being spaced apart with spermatogonia at their basis and elongated spermatids at their apical part (Schulze and Rehder 1984; Zannini et al. 1999)). Other investigations of human spermatogenesis confirmed the principle of **helical patterns** but not the presence of a complete spermatogenic wave, i.e., the complete succession of all stages (Johnson et al. 1996). At the most, 2–4 consecutive stages could be found on serial sections. Since the topographical distribution of the stages could be reproduced by assigning random numbers, the arrangement of the human spermatogenic stages along the seminiferous tubule might be at random. On the other hand, germ cell transplantation revealed that one spermatogenic stage represents a single clone of germ cells (Nagano et al. 2001). Therefore, variation in clonal size could lead to the appearance of several stages per cross-section (Wistuba et al. 2003 for further discussion) and that species differences with regard to number of spermatogenic stages are related – at least in part – to clonal size. A comparative and quantitative analysis of the incidence of tubules with one or more spermatogenic stages in 17 primate species yielded that in men, great apes and New World monkeys, multi-stage tubules are more common, whereas in prosimians and Old World monkeys single-stage tubules predominate (Luetjens et al. 2005; Fig. 2.6).

Human germ cell production results in comparatively low sperm numbers per Sertoli cell (see Sect. 2.1.2.2). When expressed in millions of sperm per g/testis in 24 h, the rat has values of 10–24, non-human primates values of 4–5 and men values of 3–7. Since earlier work also suggested that about 50% of germ cells are lost during the meiotic divisions human spermatogenesis has been considered inefficient. However, studies using contemporary stereological approaches failed to detect meiotic germ cell losses in primates including men (Zhengwei et al. 1997, 1998a, b). More

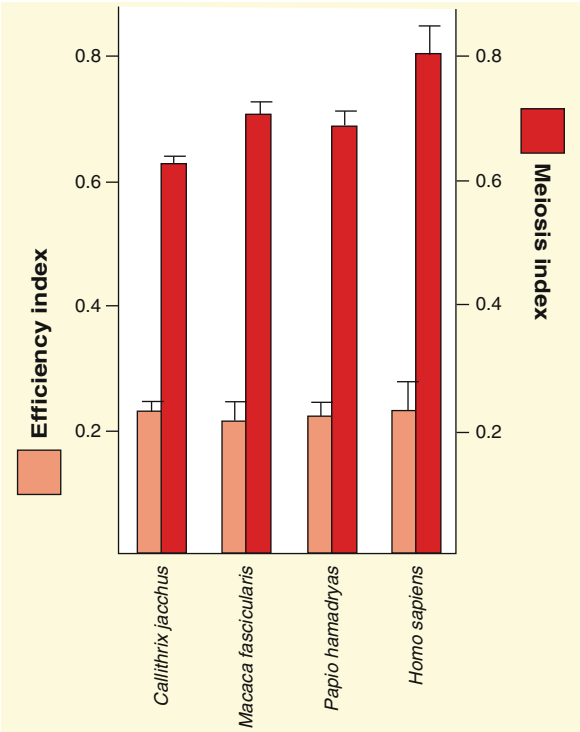

Fig. 2.7 Spermatogenic efficiency index (mean ± SEM) and meiosis indices for New World monkeys (*Callithrix jacchus* = marmoset, n = 4), Old World monkeys (*Macaca fascicularis* = cynomolgus monkey, n = 5; *Papio hamadryas* = *Hamadryas* baboon, n = 6) and man (*Homo sapiens*, n = 9) based upon flow cytometric analyses of testicular tissue. The efficiency index is defined as the number of elongated cells divided by total cell number. The meiosis index is defined as the number of haploid cells divided by total cell number. Note that efficiency and meiosis indices are comparable between human and other primates (Based on Wistuba et al. 2003 and Luetjens et al. 2005)

recent work using flow cytometric quantitation of testiclar cell numbers including meiotic cells and spermatids showed that human germ cell yields are comparable to other primates (Luetjens et al. 2005; Fig. 2.7). Hence it is obvious that human spermatogenesis is more efficient than assumed earlier. Differences in germ cell number per cell or tissue unit are rather related to the number of spermatogonial divisions (Ehmcke et al. 2005) with men considered to have only a single generation of B-type spermatogonia (Fig. 2.4), whereas macaques can have four such generations.

Primate sperm production is controlled through the number of spermatogonia entering meiosis.

2.1.2.5 Apoptosis and Spermatogenesis

Programmed cell death **(apoptosis)** comprises a coordinated sequence of signalling cascades leading to cell suicide. Unlike necrosis, this form of cell death occurs under physiological conditions (spontaneous apoptosis) but can also be induced by exposure to toxicants, disturbances of the endocrine milieu, etc. In the human testis spermatogonia, spermatocytes and spermatids undergoing apoptosis have been detected and ethnic differences in the incidence of testicular apoptosis have been suggested. Blockade of apoptotic events in the mouse model leads to accumulation of spermatogonia and infertility underpinning the need for germ cell death via apoptosis as a physiological process. Endocrine imbalance or heat treatment induced testicular apoptosis via the intrinsic and extrinsic pathways in non-human primates (Jia et al. 2007). In a non-human primate model, differentiation of Ap spermatogonia into B-type spermatogonia was found to be gonadotropin-dependent (Marshall et al. 2005). Recent human data suggest that gonadotropins may act as cell survival factors for spermatogonia rather than as stimulators of cell proliferation (Ruwanpura et al. 2008).

2.2 Hormonal Control of Testicular Function

The endocrine regulation of testicular function, i.e., the production of sperm and of androgens is well investigated. Understanding the hormonal interactions has important clinical consequences, presented in the following paragraphs. Figure 2.8 offers an overview of the systems involved, of the endocrine factors and of their physiological effects.

2.2.1 Functional Organization of the Hypothalamo-Pituitary System

The **gonadotropins luteinizing hormone (LH)** and **follicle-stimulating hormone (FSH)** are produced and secreted by the gonadotropic cells of the **anterior pituitary.** Their designation is derived from the function

exerted in females. In males, they control steroidogenesis and gametogenesis in the testis. Pituitary gonadotropes are the central structure controlling gonadal function and in turn, are regulated by the hypothalamic gonadotropin-releasing hormone (GnRH). Since GnRH secretion is pulsatile, gonadotropin release also occurs in discrete peaks, more evident in the case of LH, due to its shorter half-life in circulation compared to FSH. In turn, GnRH secretion depends on the activation of the GPR54 receptor, located on the surface of the GnRH neurons and stimulated by the peptide kisspeptin.The pituitary function is also under the control of gonadal steroids and peptides that influence its activity both directly and through the hypothalamus (Fig. 2.8). Due to their very strict anatomical and functional connection, hypothalamus and pituitary gland have to be considered as an unique functional unit.

The hypothalamus is the rostral extension of the brain stem reticular formation. It contains, among others, the cellular bodies of neurons that project their axon terminals toward the **median eminence** (ME), a specialized region of the floor of the third ventricle from which the pituitary stalk originates. The **hypothalamus** is classically subdivided into three longitudinal zones, periventricular, medial and lateral, the latter functioning as the connecting area between limbic and brain stem regions, whereas the former two contain most of the nuclei controlling neuroendocrine and visceral functions. The ME is the ventral bulge of the hypothalamus and is the site where the axon terminals of the neurosecretory neurons make contact with the capillary plexus, giving rise to the hypophyseal portal circulation. The nerve terminals form buttons on the capillaries and release the neurohormones into the portal blood by diffusion through the basal membrane. The ME is outside the blood-brain barrier and thereby freely accessible to the regulatory influences of hormones and substances present in the systemic circulation and mediating the release of neurohormones in portal blood. The blood supply of the ME is provided by the superior hypophyseal arteries. The long portal hypophyseal vessels originate from the confluence of capillary loops which supply the anterior pituitary gland with the highest blood flow of any organ in the body. In humans, the perikarya of neurons stained positive for GnRH are especially found in the ventral part of the mediobasal hypothalamus, between the third ventricle and the ME, scattered throughout the periventricular infundibular region.

The **pituitary** gland lies in the sella turcica, beneath hypothalamus and optic chiasm, covered with the sellar diaphragm. Thus, pituitary tumors can result in visual impairment by exerting pressure on the optical nerves. Gonadotropic cells are localized in the adenohypophysis, the most ventral part of the gland, of ectodermic origin from Rathke's pouch. The adenohypophysis consists of the anterior lobe (or pars distalis, the anatomically and functionally most important part), the pars intermedia and pars tuberalis.

The pars distalis is of pivotal importance for pituitary function. Gonadotropin-producing cells constitute approximately 15% of the adenohypophyseal cell population, are scattered in the posteromedial portion of the pars distalis and are basophilic and PAS-positive. Although the secretion of LH and FSH can be partially dissociated under certain circumstances, the same cell type is believed to secrete both gonadotropins. About 80% of the gonadotropic cells in men contain both LH and FSH. The cells have a very well developed RER, a large Golgi complex and are rich in secretory granules. In normal men, the pituitary contains approximately 700 IU of LH and 200 IU of FSH. Following gonadectomy or in primary hypogonadism the cells become vacuolated and large (castration cells). Finally, pituitary gonadotropes are often found in close connection with prolactin cells, suggesting a paracrine interaction between the two cell types.

2.2.2 The Kisspeptin-GPR54 System

GnRH secretion is under the control of the kisspeptin-GPR54 system. Kisspeptin is the product of the *KISS1* gene, located on chromosome 1q32.1. The name of the *KISS1* gene derives from the chocolate "kisses" of Hershey, Pennsylvania, the city in which the gene was identified. *KISS1* was originally described as a human tumor suppressor gene for its ability to inhibit the growth of melanoma and breast cancer metastasis. Later on it was shown that kisspeptin (also known as metastin) is the natural ligand of the orphan receptor GPR54 and has an important role in initiating GnRH secretion at puberty.

The product of the *KISS1* gene is a 145-amino-acid peptide, which is cleaved into a 54-amino-acid peptide known as kisspeptin-54. Shorter peptides (kisspeptin 10, −13, and −14), sharing a common C-terminal,

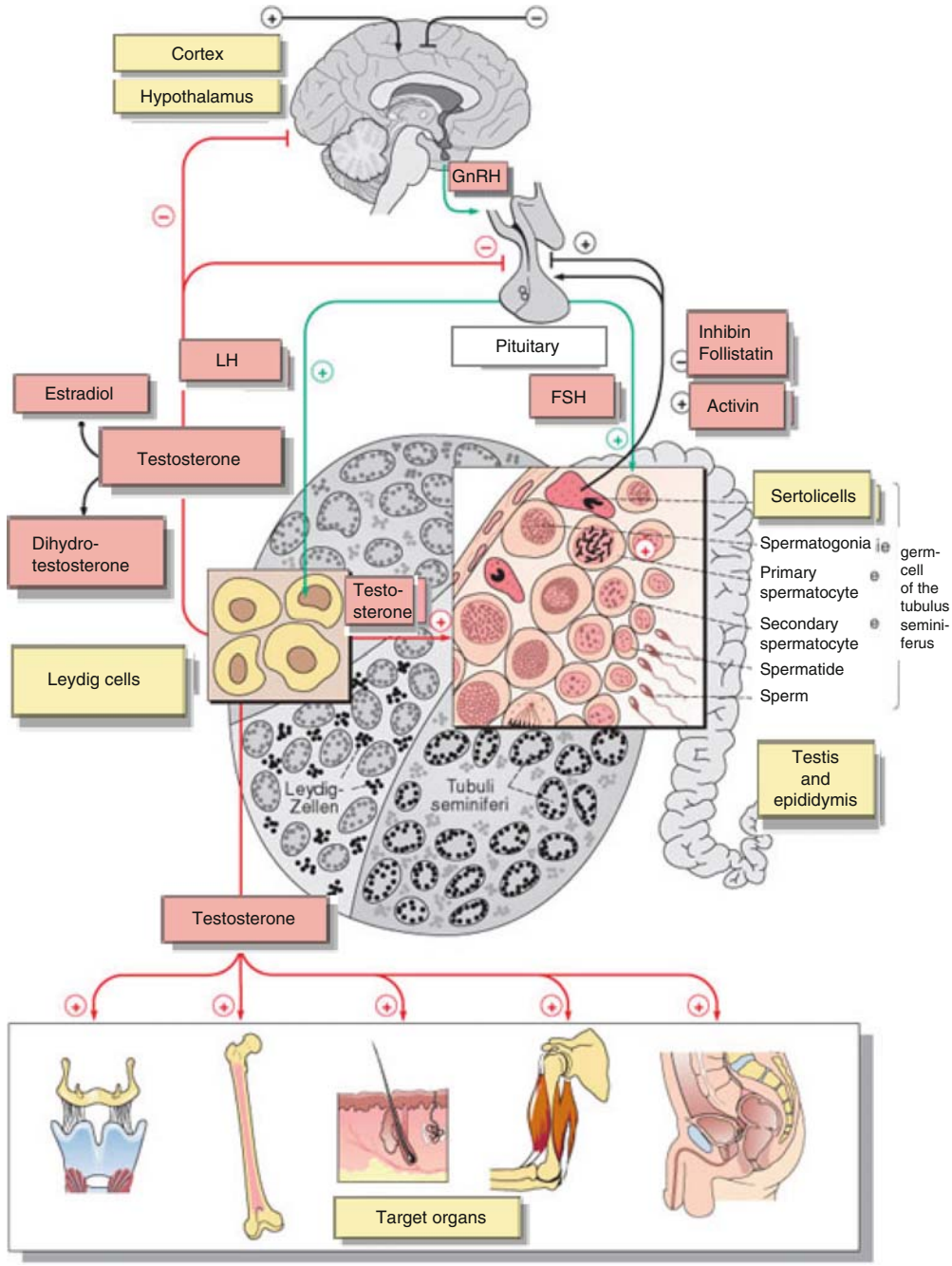

Fig. 2.8 Hormonal regulation of the testicular function and effects of androgens. Key hormones are luteinizing hormone (LH) and follicle-stimulating hormone (FSH), synthesized and secreted under hypothalamic control of gonadotropin-releasing hormone (GnRH). Leydig cells are located between the seminiferous tubules and synthesize and secrete testosterone under the control of LH. Testosterone stimulates the maturation of germ cells in seminiferous tubules. FSH acts directly on the seminiferous tubules. In the germinal epithelium only Sertoli cells possess receptors for testosterone and FSH. It is therefore believed that the trophic effects of testosterone/FSH on gametogenesis are mediated via somatic Sertoli cells. The testis and the hypothalamo-pituitary system communicate through steroids and protein hormones. Testosterone inhibits the secretion of GnRH and gonadotropins. Inhibin B and follistatin suppress selectively the release of FSH from the pituitary gland, while activin stimulates this process. Beside the effects on gametogenesis, testosterone plays an important role in hair growth, bone metabolism, muscle mass and distribution, secondary sexual characteristics and function of the male reproductive organs. (Nieschlag et al. 2008)

RF-amidated motif with kisspeptin-54, are probably degradation products (Popa et al. 2008). Kisspeptin-expressing neurons are located in the anteroventral periventricular nucleus (AVPV), in the periventricular nucleus, in the anterodorsal preoptic nucleus and in the the arcuate nucleus (ARC). Outside the nervous system, the *KISS1* gene is expressed in placenta, testis, pancreas, liver and intestine.

When injected icv or iv to rodents and primates, kisspeptin stimulates LH secretion, an effect mediated by the interaction with its receptor, GPR54, located on the surface of the GnRH-secreting neurons. This receptor was first identified as an orphan G protein coupled receptor in the rat in 1999. The *GPR54* gene is located on chromosome 19p13.3. In 2003 it was discovered that loss-of-function mutations of *GPR54* in the human cause failure to progress through puberty and hypogonadotropic hypogonadism (De Roux et al. 2003; Seminara et al. 2003). Therefore, the kisspeptin-GPR54 system is essential to initiate gonadotropin secretion at puberty and to maintain normal androgenization in adulthood. In fact, kisspeptin neurons located in ARC and AVPV send projections to the medial preoptica area, a region rich in GnRH cell bodies, providing the anatomical evidence of a direct relationship between kisspeptin fibers and GnRH neurons which, in turn, express *GPR54*. The indispensable role of the kisppeptin-GPR54 system for gonadotropin secretion is proven also by the hypogonadotropic hypogonadal phenotype of mice bearing targeted null mutations of the *kiss1* or *gpr54* gene (Seminara et al. 2003; d'Anglemont de Tassigny et al. 2007).

GPR54 signals through a Gq-type of G protein. Experimentally, kisspeptin stimulates phosphatidylinositol (PI) turnover, calcium mobilization and arachidonic acid release in GPR54 – expressing cells and induces phosphorylation of mitogen-activated protein (MAP) kinases. Continuous infusion of kisspeptin results in rapid increase in LH secretion after 2 h, followed by a decrease to the basal levels by 12 h of infusion due to desensitization of GPR54, since GnRH is still able to elicit LH secretion under these conditions. This suggests that endogenous, pulsatile kisspeptin release is physiologically responsible for pulsatile GnRH and LH secretion.

Kisspeptin is sensitive to steroid levels within the circulation and is the mediator of the negative and positive (in the female) feedback regulation of gonadotropin secretion. In fact, although androgens, estrogens and progesterone suppress gonadotropin secretion through androgen receptor (AR)-, estrogen receptor (ER)α-, and progesterone receptor (PR)-dependent mechanisms, respectively, none of these sex steroids affect GnRH secretion by direct action on GnRH neurons. On the contrary, kisspeptin neurons in the ARC are direct targets of sex steroids in all species and should be viewed as the site of the negative feedback control of GnRH production. In addition, kisspeptin produced by the AVPV, a sexually dimorphic nucleus rich in steroid-sensitive neurons in the female, mediates the positive feedback effects of estrogen on GnRH secretion (Popa et al. 2008). Finally, kisspeptin neurones seem to be involved in the regulation of the reproductive axis by metabolic signals sensing the energy balance of the organism, e.g., leptin. Reproductive hormones are inhibited during starvation and kisspeptin mediates some of leptin's effects on reproduction. According to the current model, leptin and perhaps other adiposity and satiety factors stimulate *KISS1* expression, which results in stimulation of GnRH release. When levels of adiposity and satiety factors decrease or when such factors are not detected, the expression of *KISS1* (and presumably its secretion) decreases, thus reducing excitatory input to GnRH neurons. Among the other metabolic factors influencing gonadal function, the growth hormone secretagogue ghrelin should be considered as a possible modulator of kisspeptin neurones (Tena-Sempere 2008). The regulation of GnRH and gonadotropin secretion by kisspeptin is shown in Fig. 2.9.

2.2.3 GnRH

2.2.3.1 Structure of GnRH

Two forms of GnRH, termed GnRH-I (or GnRH) and GnRH-II, encoded by separate genes have been identified. The two forms are structurally very similar but show a significantly different tissue distribution and regulation of gene expression (Cheng and Leung 2005). GnRH-I, the peptide involved in gonadotropin regulation, is a decapeptide produced in the GnRH neurons of the hypothalamus. Unique among neurons producing hypothalamic neurohormones, they originate from olfactory neurones and during embryonic development migrate toward the basal forebrain along branches of the terminal and vomeronasal nerves,

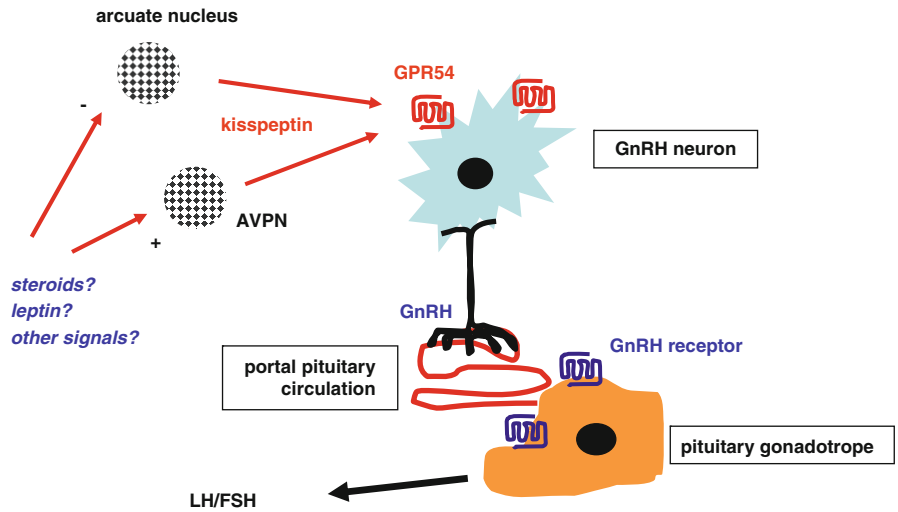

Fig. 2.9 Current model of regulation of gonadotropin secretion. Kisspeptin produced by the arcuate nucleus and in the anteroventral periventricular nucleus (AVPV) stimulates GnRH release by acting on the G protein–coupled receptor GPR54. GnRH release results in increased gonadotropin secretion from the pituitary gland, which stimulates LH and FSH production. Peripheral sex steroids (e.g., androgens, estrogens and progesterone) as well as metabolic signals (e.g., leptin) regulate Kiss1 expression and signaling to GnRH neurons

across the nasal septum. This event is regulated by a number of factors that influence the migration of different portions of the GnRH neuronal population at different steps along the route and the formation of the olfactory bulb (Tobet and Schwarting 2006). The importance of such factors is demonstrated by mutations in the respective coding gene in patients with Kallmann syndrome. In about 10% of patients with Kallmann syndrome and anosmia due to a hypoplasia of the bulbus olfactorius, mutations or deletions of the KAL1 gene on the X chromosome were detected. This gene was the first implicated in Kallmann syndrome and encodes for anosmin-1 which is produced in the bulbus and in other tissues, and which is transiently expressed as an extracellular matrix and basal membrane protein during organogenesis and interacts with heparan sulphate. Other genes implicated in GnRH neuron migration and Kallmann syndrome are those encoding the fibroblast growth factor receptor 1 (*FGFR1*) and its ligand fibroblast growth factor 8 (*FGF8*), as well as prokineticin 2 (*PK2*) and its receptor (*PKR2*) (Falardeau et al. 2008; Kim et al. 2008).

In primates, the main locations of GnRH neurons are the medio-basal hypothalamus and the arcuate nucleus, but they are found also in the anterior hypothalamus, preoptic area, septum and other parts of the forebrain. GnRH neurons are synaptically connected with terminals stained positive for pro-opiomelanocortin-related peptides and enzymes involved in the metabolism of catecholamines and γ-aminobutyric acid (GABA). Furthermore, GnRH-positive neurons of the nucleus arcuatus are connected to neuropeptide Y (NPY) neurons in the preoptical area and in the eminentia mediana. All these substances are known to influence GnRH secretion (Evans 1999; Li et al. 1999).

The gene encoding GnRH is localized at the chromosomal site 8p21-p11.2. GnRH is produced by successive cleavage stages from a longer precursor, called preproGnRH, transported along the axons to the ME and there released into portal blood. Phylogenetically GnRH is a rather ancient hormone, highly conserved among different species with 80% sequence identity between mammals and fish. In the precursor with a length of 92 amino acids, GnRH is preceded by a signal peptide consisting of 24 amino acids, and followed by a stretch of 56 amino acids forming the GnRH-associated peptide (GAP). PreproGnRH is processed in the rough endoplasmic reticulum and in the Golgi complex, the first step being the removal of the signal peptide and the cyclization of the aminoterminal Gln residue to pyroGlu. At the junction between GnRH and GAP a Gly-Lys-Arg sequence provides a processing signal important for the cleavage of GAP and C-terminal amidation of the last Pro residue. Mature GnRH is therefore a single chain decapeptide cyclized at the N-terminus and amidated at the C-terminus and assumes a folded conformation as the result of a ß-II type bend involving the central Tyr-Gly-Leu-Arg residues that brings the N- and C termini in close proximity (Millar et al. 2008).

GnRH has a very short half-life (<10 min) and is mostly retained and degraded in the pituitary gland immediately after secretion by several peptidase systems. Deciphering the GnRH sequence earned Andrew

Schally the 1977 Nobel prize, and enabled the development of analogs with agonistic or antagonistic properties. As the generation of synthetic analogs of GnRH has shown, the amino acids in position 6–10 are important for high affinity binding of the neuropeptide, whereas positions 1–3 are critical for biological activity and positions 5–6 and 9–10 are involved in enzymatic degradation. The discovery of the amino acid sequence of GnRH permitted the design of GnRH analogs exerting agonistic or antagonistic action relative to the endogenous GnRH.

2.2.3.2 Secretion of GnRH

GnRH is released into the portal blood in discrete pulses. Although this event cannot be directly demonstrated in vivo, all the experimental data accumulated up to now demonstrate that each LH peak is induced by a GnRH pulse. It is the frequency of GnRH pulses and the amplitude of its secretory episodes that determine the type of LH and FSH secretion from the pituitary gland (Fig. 2.10). GnRH is the sole releasing factor for both gonadotropins, but modulating its frequency results in preferential release of LH or FSH (Hayes and Crowley 1998).

The pulsatile nature of GnRH secretion is partly an intrinsic characteristic of the GnRH neurons, since isolated immortalized GnRH neurons have a spontaneous pulsatile secretory activity in vitro. However, in vivo GnRH is under the control of the kisspeptin/GPR54 system, which mediates the effects of the peripheral steroids on GnRH secretion and is involved in the control of GnRH pulsatility. The pulse generator is under the continuous tonic inhibition of peripheral steroids and, e.g., gonadectomy results in an immediate increase of frequency and amplitude of gonadotropin secretion. Thus, in the absence of steroids the pulse generator becomes free-running (Lopez et al. 1998).

In man, the major hormone controlling GnRH secretion is testosterone, which inhibits gonadotropin secretion via negative feedback both at the hypothalamic and pituitary level (Fig. 2.8). Testosterone can act as such or after metabolism to DHT or estradiol. The effects of testosterone and its metabolites appear to vary depending on the experimental model but, in general, we can assume that both T and DHT act mainly at the hypothalamic level by decreasing the frequency of GnRH pulsatility, whereas estrogens depress gonadotropin secretion by reducing the amplitude of LH and FSH peaks at the pituitary level (Hayes and Crowley 1998). Progesterone inhibits gonadotropin release at least in part via arcuate nucleus dopaminergic and NPY neurons (Dufourny et al. 2005). The negative feedback action of androgens and progestins is most important for the development of a male fertility control regimen (Chap. 29).

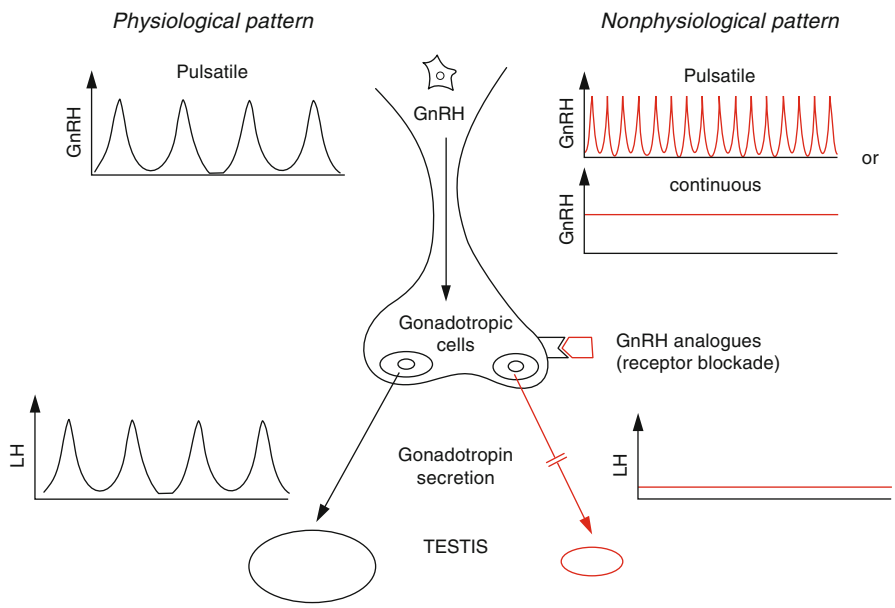

Fig. 2.10 Importance of the pulsatile pattern of GnRH secretion for gonadotropin secretion and testicular function. Unphysiologically high GnRH pulse frequencies or continuous administration of GnRH inhibit gonadotropin secretion and testicular function (red). Similarly, blockade of GnRH receptors by means of GnRH analogs results in suppression of testicular function

Among the neurotransmitters and neuromodulators that might influence GnRH secretion, the noradrenergic system and NPY show stimulatory activity, whereas interleukin-1, dopaminergic, serotoninergic and GABAergic systems are inhibitory. Opioid peptides seem to modulate the negative feedback of gonadal steroids. Finally, leptin has been shown to stimulate gonadotropin secretion. Leptin is produced by the fat cells of the body and influences the interactions concerning the control of body mass and gonadotropin secretion. This effect is probably mediated by the hypothalamus via NPY-, POMC- and especially kisspeptin-containing neurons with numerous receptors for leptin (Popa et al. 2008).

2.2.3.3 Mechanism of GnRH Action

GnRH acts through interaction with a specific receptor. The GnRH receptor belongs to a family of G-coupled receptors linked by the typical seven-membrane domain structure. This group also encompasses the receptors for LH, FSH and TSH. With 328 amino acids, it is the smallest G protein-coupled receptor known up to now and possesses a rather short extracellular domain (Fig. 2.11). The intracellular C-terminus is practically absent and the signal transduction is probably carried out by the intracytoplasmic loops connecting the seven membrane spanning segments, especially the third one which is unusually long. The receptor contains two glycosylation sites, projecting into the extracellular space, of uncertain function.

The conformation of the binding site is unknown. The gene encoding the human GnRH receptor includes three exons separated by two introns. The 5′ flanking region contains multiple TATA transcription initiation sites and several cis-acting regulatory sequences which confer responsiveness to cAMP, glucocorticoids, progesterone, thyroxin, PEA-3, AP-1, AP-2 and Pit-1-sensitive sequences. The GnRH receptor is specifically expressed in the gonadotropic cells within the pituitary. An orphan receptor, steroidogenic factor 1(SF-1) is involved in the expression of human GnRH (Ngan et al. 1999). Transcription factors such as SF-1, Pit-1 and Pro-Pit-1 are generally needed for the development and maturation of the hypothalamo-hypophyseal-gonadal axis. SF-1-deficient mice and patients bearing a mutation in the Pro-Pit-1 gene exhibit

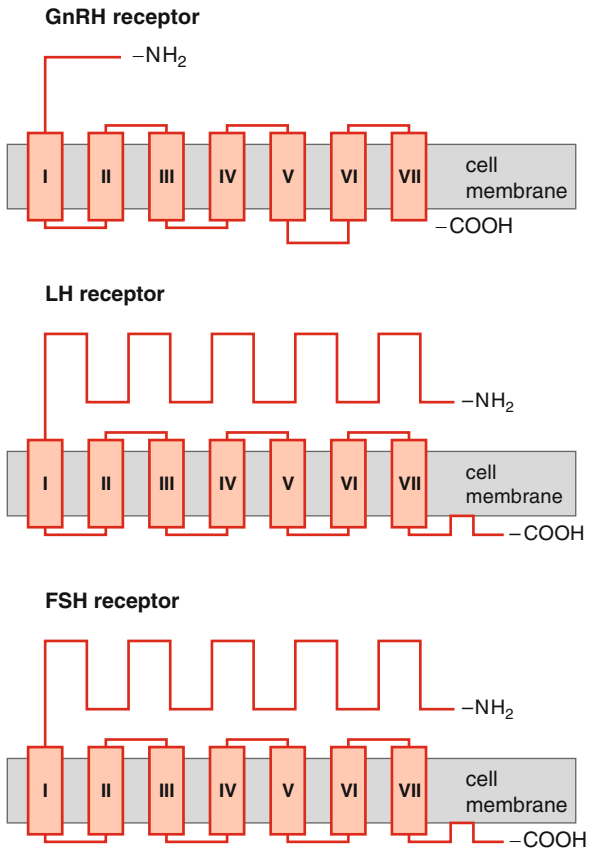

Fig. 2.11 Schematic representation of the receptors for GnRH (*upper panel*), LH (*middle panel*) and FSH (*bottom panel*). These receptors belong to the family of G protein-coupled receptors. They possess an extracellular domain, a transmembrane domain with seven membrane spanning segments and an intracellular domain. The GnRH receptor is characterized by a very short extracellular domain and practically no intracellular domain. On the contrary, the extracellular domain is very large in the gonadotropin receptors, where it plays a crucial role in hormone binding. The intracellular domain is important for signal transduction

pronounced alterations of gonadotropin secretion. Recently, a second GnRH receptor gene was identified in non-human primates (type II GnRH receptor) which is structurally and functionally distinct from the classical, type I receptor. The GnRH type II receptor, however, is not functional in the human and many other species and its role is yet unknown (Millar 2005). Both GnRH-I and GnRH-II were shown to signal through the GnRH type I receptor (ligand-selective-signaling). While GnRH-I regulates gonadotropins, GnRH-II appears to be a neuromodulator and stimulates sexual behavior.

Following GnRH-receptor interaction, a **hormone receptor complex** is formed. This results in the interaction with Gq protein, hydrolysis of phosphoinositide and production of diacylglycerol and inositol trisphosphate, which leads to calcium mobilization from the intracellular stores and influx of extracellular calcium into the cell. Diacylglycerol and calcium then activate protein kinase C (PKC), inducing protein phosphorylation and further activation of calcium channels. The increase in intracellular calcium results in prompt gonadotropin release by exocytosis and, with time, more sustained gonadotropin synthesis and secretion. Thereafter, the hormone-receptor complex is internalized by endocytosis and undergoes degradation in lysosomes.

GnRH is capable of **modulating number and activity** of its own **receptors** and the effects depend on the secretory pattern and dose of neurohormone. The receptor expression is higher when GnRH is given in a pulsatile manner and the withdrawal of GnRH during the interpulse intervals leads to increase of GnRH binding sites just before the next pulse occurs (self priming). Conversely, continuous exposure to GnRH results in an initial rise in response followed by desensitization. This property of the GnRH receptor is exploited in therapy with GnRH agonists that, owing to their prolonged and sustained stimulatory activity, cause slow receptor desensitization and decrease of gonadotropin secretion. The molecular mechanism of receptor desensitization is not completely understood. Owing to the lack of an intracellular domain, GnRH agonists cannot induce phosphorylation and rapid desensitization of the receptor.

2.2.4 Gonadotropins

2.2.4.1 Structure of Gonadotropins

LH and FSH are glycoprotein hormones secreted by the pituitary gland that control development, maturation and function of the gonad. Like the related thyroid stimulating hormone (TSH) and human chorionic gonadotropin (hCG), they consist of two polypeptide chains, α and β, bearing carbohydrate moieties N-linked to asparagine (Asn) residues. The α subunit is common to all members of the glycoprotein hormone family, whereas the β subunit, although structurally very similar, differs in each hormone and confers specificity of action.

The subunits are encoded by separate genes localized on different chromosomes but structurally related. The gene encoding for the α subunit is composed of four exons and three introns, whereas the β genes consist of three exons separated by two introns. The FSH β gene is located on chromosome 11 and differs from the other glycoprotein hormone β subunit genes in possessing a rather long 3' untranslated region probably involved in RNA stability. The LH β gene belongs to an extraordinary complex cluster of genes also including at least seven nonallelic hCGβ-like genes arranged in tandem on chromosome 19. The regulation of gene expression of LH and FSH has been extensively studied in experimental animals, expecially rodents, and involves a complex interplay between hypothalamic GnRH and gonadal steroids and peptides acting at the hypothalamic and pituitary level (Burger et al. 2004).

The common α subunit contains two **glycosylation** sites, at position 52 and 78. The glycosylation sites of FSH ß are 7 and 24, whereas LH is glycosylated only at position 30. In mammals the α subunit is also produced by the placenta and, conversely, the pituitary gland has been shown to contain and secrete trace amounts of hCG. α and β subunits are non-covalently linked and the probable tertiary structure of pituitary gonadotropins can be approximated and deduced by analogy with its cognate hCG, whose crystal structure has been resolved.

LH and hCGβ subunits are structurally very similar and, in fact, LH and hCG act on the same receptor. A peculiar feature of hCGβ is a carboxyl-terminal extension containing four O-linked sugar residues that remarkably reduces the rate of metabolism and increases the half-life of the hormone. This peculiarity of hCGß has been recently exploited for the production of a synthetic gonadotropin hybrid containing a similar C-terminal extension in the ß subunit which resulted in a conspicuous increase of the gonadotropin half-life. A prolongation of the half-life of hCG was achieved by producing a chimera containing fusioned α and β chains (Boime and Ben-Menahem 1999), while a synthetic chimeric gonadotropin containing a β subunit derived from both hCGβ and FSHβ displays the biological properties of both gonadotropins (Garone et al. 2006).

The oligosaccharide structure consists of a central mannose core, bound to an Asn residue through two residues of N-acetyl-glucosamine, and terminal extensions of tetrasaccharide branches, bi- or triantennary, terminating with sialic acid (FSH) or sulfate (LH) residues. These carbohydrate structures can be more or less extended in length and are rich in sugar terminals, constituting the molecular basis of the gonadotropin heterogeneity evident after chromatographic separation.

Having a different terminal glycosylation, LH and FSH also have a different half-life. LH is rich in N-acetyl-glucosamine sulfate and is quickly removed from the circulation after interaction with specific liver receptors that recognize sulfate terminals. This rapid removal of sulfate LH from the blood results in rapid clearance of a relevant amount of the LH discharged in each secretory episode and "amplifies" the pulsatile features of LH in circulation. Conversely, FSH is predominantly sialylated and thereby protected from immediate capture and metabolism in the liver. As a result, LH and FSH half-lives are about 20 min and 2 h, respectively. Therefore, although both gonadotropins are secreted simultaneously from the pituitary gland following a GnRH pulse, LH appears to be highly pulsatile and FSH much less so (Moyle and Campbell 1995).

The importance of **sugar residues** on gonadotropin activity has been investigated in vitro using glycosylation-deficient hormones. It was found that glycosylation is not critical for receptor binding but is important for receptor activation

The use of recombinant variants of hCG and FSH defective in sialic acid or truncated at the mannose ramification revealed that glycosylation at position α 52 is necessary for the steroidogenic and cAMP response. Isoforms completely devoid of carbohydrates cannot be secreted by the producing cells and behave as competitive antagonists of the wild type. Overall, the current view is that glycosylation is fundamental for gonadotropin secretion and bioactivity, and strongly influences the half-life in circulation and in vivo biopotency.

It was found recently that two polymorphic variants of LH are present in the normal population. One of them has two amino acid exchanges in positions 8 and 15 of the β chain leading to a second glycosylation site in position 13. Approximately 12% of Europeans produce this allelic variant. Under in vitro conditions, this variant displays increased bioactivity and shortened half-life. A difference in immunoactivity can be detected when certain monoclonal antibodies are used (Huhtaniemi et al. 1999). Polymorphic variants of the FSHβ gene exist as well and are associated with serum FSH levels in men (Grigorova et al. 2008). Such a variant was found to be associated with a significant reduction in free testosterone and testes volume, but on the contrary, in an increase of semen volume, sex hormone-binding globulin, serum testosterone and estradiol.

2.2.4.2 Secretion of Gonadotropins

After the synthetic process is completed, LH and FSH are stored in different secretion granules, ready to be released upon stimulation with GnRH. A portion of molecules, however, is not stored in secretory granules, i.e., does not enter the regulated pathway of secretion and is, instead, constitutively secreted. FSH especially follows the latter route. Storage in separate granules and the natural propensity to follow one of the two secretory pathways are the main reasons why the same GnRH stimulus can, under certain conditions, preferentially release one of the two gonadotropins. Low GnRH pulse frequency causes preferential release of FSH probably due to differential expression of the FSH receptor (Ferris and Shupnik 2006).

LH and FSH are measurable in the pituitary gland as early as the 10th week of gestation and during the 12th week in peripheral blood. In fetal life and in infancy FSH is predominant over LH and the FSH/LH ratio is higher in females than in males. The relative abundance of the two gonadotropins changes during development.

It is noteworthy that before birth both male and female fetuses grow in an environment extraordinarily rich in potent, maternal estrogens.

It is testosterone that determines the initial phase of testicular migration and the development of male external genitalia. Testosterone is already produced by the fetal testicle during the 10th week of gestation, under the stimulation of fetal LH and maternal hCG.

The role of maternal hCG in this crucial phase of gonadal development is suggested by the fact that a mutation of the LH ß chain leading to a biologically inactive gonadotropin is associated with normal sexual differentiation (Huhtaniemi et al. 1999). Conversely, inactivating mutations of the LH receptor produce a clinical syndrome resembling complete androgen insensitivity, with a phenotype of female external genitalia (Themmen et al. 1998).

During infancy gonadotropins in serum are very low. The pulsatile secretion of gonadotropins becomes evident at the time of puberty, when LH and FSH pulses in serum are detected first during sleep, at night, and then progressively also during the day. Before puberty, gonadotropin levels are very low and GnRH secretion appears to be extremely limited, even in the presence of negligible steroid production by the gonads. High sensitivity of the hypothalamus to negative steroid feedback is believed to suppress GnRH production before puberty, but certainly other factors such as body mass, leptin and signals from the central nervous system are important to maintain the hypothalamo-pituitary-gonadal axis silent before the programmed time.

The steroid regulation of gonadotropin gene expression, synthesis and secretion is rather complex and shows many facets depending on the experimental model. In general, however, it is currently accepted that gonadal steroids exert their negative control on gonadotropins mainly at the hypothalamic level, depressing the release of GnRH most probably via the **kisspeptin/** GPR54 system. The steroid effect at the pituitary level is more complex, but there is considerable evidence that estrogens inhibit GnRH-stimulated gonadotropin synthesis and secretion at this level. In rodents, testosterone has a specific stimulatory effect on FSH gene expression, synthesis and secretion directly at the pituitary level. In primates, however, the effects of testosterone are always inhibitory. Testosterone is the main testicular product suppressing FSH and LH secretion in men.

FSH secretion is, obviously, also under the control of some other factor(s) related to the efficiency of spermatogenesis, since oligoazoospermia is often accompanied by selective increase of serum FSH in the presence of normal testosterone levels. New assays for **inhibin B** permitted analysis of the relationship between **FSH and inhibin secretion** in man. A pronounced inverse correlation was established between serum concentrations of inhibin B and serum levels of FSH, testis size and sperm numbers. Clearly, inhibin B is the physiologically relevant form of inhibin in men (von Eckardstein et al. 1999). It appears at present that the serum levels of inhibin B directly reflect the integrity of the germinal epithelium and of the Sertoli cells (Boepple et al. 2008).

2.2.4.3 Mechanism of Action of Gonadotropins

LH and FSH exert their function via specific receptors (Simoni et al. 1997). The gonadotropin receptors also belong to the family of the G protein-coupled receptors and are characterized by a very large extracellular domain to which the hormone binds specifically, the usual membrane-spanning domain including 7 hydrophobic segments connected to each other through three extracellular and three intracellular tracts, and an intracellular carboxyterminal domain (Fig. 2.11).

The genes for LH and FSH receptors are localized on chromosome 2 and consist of 11 and 10 exons, respectively. The last exon encodes a small portion of the extracellular domain, the entire transmembrane domain and the intracellular C-terminus. The extracellular domain contains the high-affinity hormone binding site and is rich in leucine repeats. The 5′-flanking region of the two genes contains no conventional promoter and has multiple transcription start sites. In addition, the human LH receptor was recently shown to contain a cryptic exon (denominated exon 6A) which plays a very important role in the intracellular processing of the mature receptor protein and can be mutated in rare cases of LH resistance (Kossack et al. 2008). Several alternatively spliced transcripts of LH and FSH receptors have been described, lacking one or more exons, but presently it is not known whether these RNA **isoforms** are translated into proteins of any physiological function. Single nucleotide polymorphisms give rise to **allelic variants** with different biological activity in vitro and/or in vivo (Gromoll and Simoni 2005; Piersma et al. 2007).

The **mature receptor proteins** are glycosylated at several points, a process that does not seem to be involved in receptor activation and signal transduction but probably necessary for receptor folding and transport to the cell membrane. Recently, the partially deglycosylated complex of human FSH bound to the extracellular hormone-binding domain of its receptor was crystallized, showing that binding specificity is mediated by key interaction sites involving both the common alpha- and hormone-specific beta-subunits.

On binding, FSH undergoes a concerted conformational change that affects protruding loops implicated in receptor activation. The FSH-FSHR complexes form dimers in the crystal important for transmembrane signal transduction (Fan and Hendrikson 2005) resulting in activation of the G protein, cAMP production and activation of protein kinase A. Gonadotropins act mainly through stimulation of intracellular cAMP. More recently it has been shown that LH and FSH can also induce an increase of Ca^{++} influx in target cells, but the physiological importance of this mechanism is still unknown. cAMP remains, therefore, the main signal transducer and calcium could possibly act as a signal amplification or modulating mechanism. Following the hormone-receptor interaction there is an increase in cAMP concentrations and subsequent activation of protein kinaseses which, in turn, phosphorylate existing proteins such as enzymes, structural and transport proteins and transcriptional activators. Activating and inactivating mutations of the gonadotropin receptors have been identified. The biological consequences of these mutations are described below and in Chap. 13.

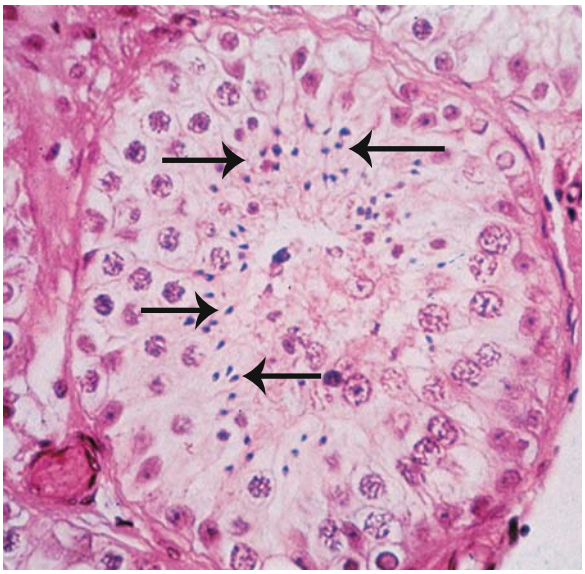

Fig. 2.12 Testicular histology of a 5.2-year old boy with an activating mutation of the LH receptor. Note complete spermatogenesis (arrows). (Courtesy of Prof. Dr. W. Rabl, Pediatric Clinic of the Technical University Munich)

2.2.5 Endocrine Regulation and Relative Importance of LH and FSH for Spermatogenesis

The primary functions of the testis, androgen production and gamete development, are regulated by the brain, e.g., hypothalamus and hypophysis via GnRH and gonadotropins. Importantly, the hypothalamo-hypophyseal circuit is subject to **negative feedback regulation** mediated by testicular factors (Fig. 2.8). Testosterone inhibits the secretion of LH and FSH. For FSH, the protein hormone **inhibin B** plays an important role (Boepple et al. 2008).

For the interpretation of hormonal regulation and hormonal effects on spermatogenesis, the following terminology should be remembered:

1. **Initiation:** First complete cycle of spermatogenesis during puberty
2. **Maintenance:** Hormonal requirements of intact spermatogenesis in the adult
3. **Reinitiation:** Hormonal requirements for the restimulation of gametogenesis after transitory interruption
4. **Qualitatively normal spermatogenesis:** All germ cells are present although in subnormal numbers
5. **Quantitatively normal spermatogenesis:** All germ cells are present in normal numbers

Considerable efforts were undertaken to unravel the relative importance of LH/testosterone and FSH for qualitative and quantitative initiation, maintenance and reinitiation of spermatogenesis (Fig. 2.12). It is generally assumed that either testosterone and FSH alone are able to initiate, maintain and reinitiate spermatogenesis but only to a qualitative extent (Weinbauer et al. 2004). In order to achieve quantitative effects on germ cell production and sperm numbers, at least under physiological conditions, both LH and FSH activities are needed.

These assertions are based upon controlled studies in non-human primate models and volunteers, and on studies in patient populations and case reports. The latter has provided interesting information but can also confound interpretation of findings owing to the variable endocrine and medical history of the patients. Complete spermatogenesis is seen in the vicinity of testosterone-producing Leydig cell tumors and in patients with activating mutations of the LH receptor, suggesting that pharmacologically high local

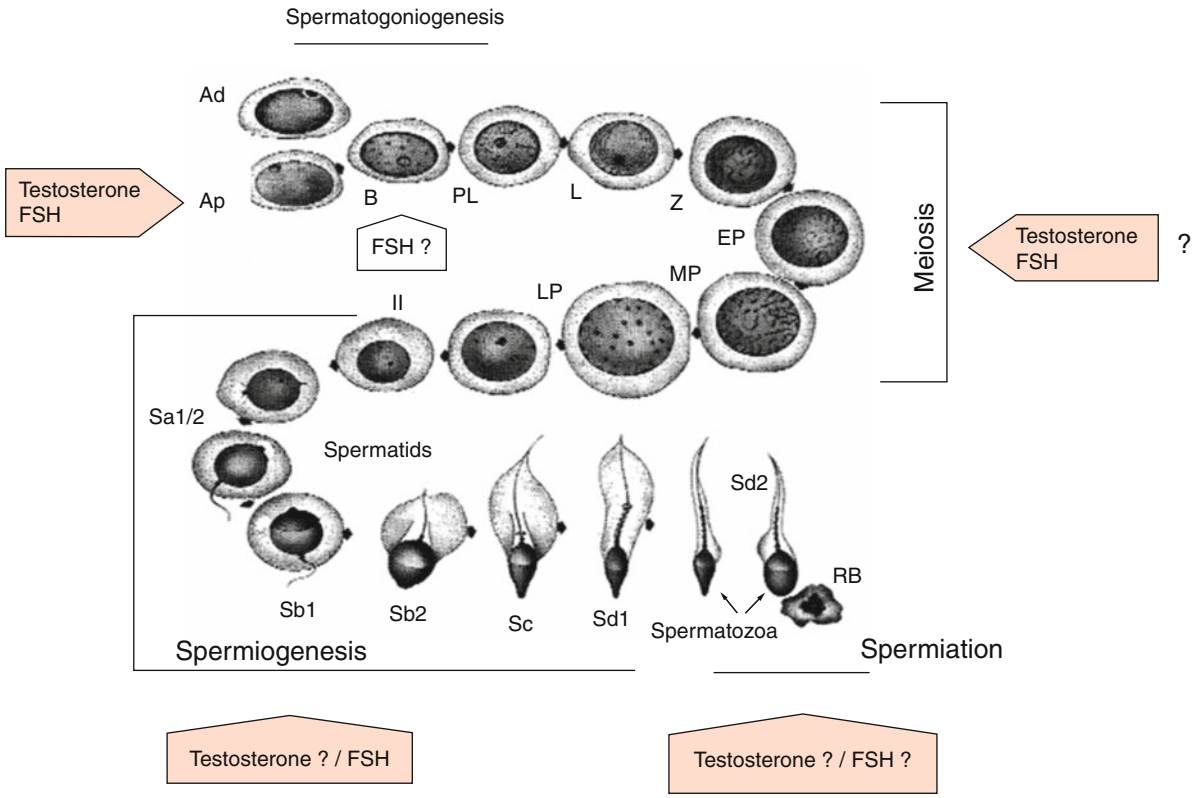

Fig. 2.13 Sites of action of testosterone and FSH on the spermatogenic process in primates. (?) denotes unresolved questions. Ap spermatogonia enter the spermatogenic process (arrow on the cell indicates direction of germ cell development). Ad spermatogonia are believed to constitute the testicular stem cells. The majority of available data indicate that testosterone and FSH act on spermatogenesis via increasing numbers of type A-pale spermatogonia followed by an increase of subsequent germ cell populations. These endocrine factors might act via stimulation of proliferation and/or prevention of cell death. Meiotic transitions appear to be independent of testosterone/FSH action. FSH has been reported to play a role in chromatin condensation during spermiogenesis. Whether testosterone is needed for spermiogenesis has not been studied yet. Spermiation is affected by gonadotropin sufficiency but it is currently unclear whether this is related to diminished actions of testosterone or FSH or both. Ad = A-dark spermatogonium (testicular stem cells, divides rarely), Ap = A-pale spermatogonium (self-renewing and progenitor cell for spermatogenesis), B = B spermatogonium, Pl = preleptotene spermatocytes, L = leptotene spermatocytes, EP = early pachytene spermatocytes, MP = mid pachytene spermatocytes, LP = late pachytene spermatocytes, II = 2nd meiotic division, RB = residual body, Sa1–Sd2 = developmental stages of spermatid maturation

testosterone concentrations induce sperm formation (Fig. 2.13). The aim of treatment is to obtain sufficiently high intratesticular testosterone concentrations, which are crucial. This is normally pursued clinically by giving hCG, which contains high LH activity, together with FSH. On the other hand, patients bearing a defective FSH β subunit, presented with azoospermia (Lindstedt et al. 1998; Phillip et al. 1998). One of these patients was normally virilized, suggesting the need of FSH for complete initiation of spermatogenesis in man. Conversely, patients with Pasqualini syndrome, a disorder with selective LH deficiency, can have complete spermatogenesis, indicating the ability of FSH to initiate the entire male germ cell development cascade.

Exogenous provision of supranormal doses of testosterone or of gestagenic compounds suppresses gonadotropin secretion through the negative feedback mechanism and leads to a drastic decrease of sperm numbers in the ejaculate. In primates – unlike in rodents – it is essential that complete suppression of FSH secretion is achieved despite inhibition of LH secretion (Narula et al. 2002; Weinbauer et al. 2001). In the latter study, albeit LH bioactivity had been completely eliminated, a slight and transient rebound of FSH secretion provoked an escape of spermatogenic suppression. In gonadotropin-suppressed men, either FSH or LH maintained spermatogenesis (Matthiesson et al. 2006). The importance of FSH is also evident

from a hypophysectomized patient in whom an activating mutation of the FSH receptor coexisted with normal spermatogenesis in the absence of LH (Gromoll et al. 1996). Conversely, inactivating mutations of FSH action do not necessarily lead to a complete block of spermatogenesis (Huhtaniemi 1996). Although either hormone on its own has the potential to elicit the entire spermatogenic process, this is not always the case in patients receiving androgen/hCG therapies. In case of failure of hCG, however, the addition of FSH has been shown to permit completion of spermatogenesis in hypogonadotropic men with azoospermia (Bouloux et al. 2003).

In certain animal species, e.g., Djungarian hamsters, FSH is the only hormone responsible for spermatogenesis, while LH and testosterone stimulate the development of androgen-dependent organs and sexual behavior. Conversely, in primates, both gonadotropins are necessary for spermatogenesis. The biological meaning of this **dual regulation system** is not clear yet (Weinbauer et al. 2004).

From a clinical viewpoint it is concluded that the synergistic action of LH/testosterone and FSH is necessary for the initiation, maintenance and also for reinitiation of normal spermatogenesis.

2.2.6 Local Regulation of Testicular Function

As described above, the regulation of testicular function is primarily controlled by central structures. The complexity of the testicular cell types and architecture also mandates a variety of local control and regulatory mechanisms. The categories of local interactions and communication can be classified as **paracrine,** referring to factors acting – mainly by diffusion – between neighboring cells; **autocrine,** referring to factors which are released from the cell and work back on the same cell and **intracrine,** referring to factors and substances which never leave the cell and whose site of production and action is the same cell. The term "paracrinology" has inadvertently been used earlier to characterize all types testicular cell interactions which seem better described by **"local interaction"** (Weinbauer and Wessels 1999). In addition, the interplay between the different testicular compartments are also subsumed under local interactions.

Many local factors have been identified and for several of them, gene-targetting in mice either confirmed or challenged their pivotal role for somatic and germ cell development. This approach in mice has been strengthened by the ability of conditional and cell-specific gene targetting. In contrast and for obvious experimental and ethical reasons, the identification of essential local factors for human testicular function has been limited.

It is evident that the endocrine mechanisms play the central role in the regulation of testicular function and **factors produced locally** are important for the **modulation of hormone activity** and local factors could thus be seen as **mediators of hormone action and** intra-**/intercellular communication.** From this point of view both gametogenesis and endocrine function of the testis are under local control. An example for this might be the earlier report of stage-specific expression of androgen receptor in the human testis (Suarez-Quian et al. 1999). While Sertoli cells were viewed as coordinators and regulators of germ cell development and maturation for a long time, these cells are now believed to be influenced by germ cell products that can influence the secretory activity of Sertoli cells. Hence, Sertoli cells are under the local control of germ cells having varying requirements for metabolic substances depending on the spermatogenic cycle phase (Franca et al. 1998).

A plethora of factors with potentially local testicular activity has accumulated, e.g., **growth factors, stem cell factors, immunological factors, opioids, oxytocin** and **vasopressin, peritubular cell modifying substance, renin** and **angiotensin, GHRH, CRH, ACTH, GnRH, calmodulin, ceruloplasmin, transport proteins, glycoproteins, plasminogen activator, metalloproteases, dynorphin, PACAP,** etc. Moreover, it can be reasonably assumed that other, still unidentified protein factors mediate the communication between interstitial and tubular compartments, between Sertoli cells and germ cells and between germ cells.

2.2.6.1 Steroid Hormones

Testosterone is the main secretory product of the testis, along with 5α-dihydrotestosterone (DHT), androsterone, androstenedione, 17-hydroxyprogesterone, progesterone and pregnenolone. The role of androsterone, progesterone and 17-hydroxyprogesterone in the testis

is unknown but progesterone receptors have been found in some peritubular cells and on spermatozoa (Luetjens et al. 2006; Modi et al. 2007). Using a derivative of progesterone, norethisterone enanthate, no direct effects on testicular/epididymidal function were found (Junaidi et al. 2005).

For testosterone, a classic endocrine factor, compelling evidence is available as a pivotal local regulator of spermatogenesis. Rodent data demonstrated that selective elimination of Leydig cells, interruption of testicular testosterone transport and specific Sertoli cell androgen receptor knockout models provoked profound alterations of germ cell maturation (Takaimya et al. 1998). Selective peritubular cell androgen receptor knockout mice exhibited specific Sertoli cell and peritubular cell defects (Zhang et al. 2006). Spermatogenesis was present in boys with testosterone-producing Leydig cell tumors but only in seminiferous tubules adjacent to the tumor and not in tumor-free areas. Similarly, activating mutations of the LH receptor prematurely induced qualitatively normal spermatogenesis.

In fertile men testicular testosterone concentrations exceed that of SHBG/ABP by about 200-fold (Jarow et al. 2001), indicating a substantial surplus of testosterone in the testis. Relative to serum, testicular testosterone concentrations were >80-fold higher (Coviello et al. 2005). Testosterone is metabolized to DHT by testicular 5α-reductase activity and to estradiol by testicular aromatase activity. To what extent these metabolic activities are essential for spermatogenesis besides testosterone itself is not entirely clear. Treatment of volunteers with the 5α-reductase-inhibitor finasteride did not alter spermatogenesis (Kinniburgh et al. 2001; Overstreet et al. 1999), whereas more recently, a mild decrease in semen parameters was reported for finasteride and dutasteride (Amory et al. 2007b). Addition of dutasteride to a contraceptive steroid regimen (testosterone and levonorgestrel) did not augment suppression of spermatogenesis (Matthiesson et al. 2005a). With regard to estradiol, aromatase activity and estrogen receptor-β are present in human Sertoli cells and germ cells (Carreau et al. 2006 for review; Berensztein et al. 2006). Administration of aromatase inhibitor in a non-human primate model with seasonal reproduction (bonnet monkey) resulted in impairment of spermiogenesis and altered sperm chromatin condensation (Shetty et al. 1997, 1998). The clinical evidence for a causal role of estrogens for testicular functions is ambiguous since isolated cases with estrogen receptor deficiency or aromatase deficiency did not reveal a consistent picture, with some patients being ill and having cryptorchidism (O'Donnell et al. 2001; Maffei et al. 2004).

Although it is established beyond doubt that testosterone is an essential local regulator of spermatogenesis, it has been surprisingly difficult to demonstrate a clear-cut relationship between testicular testosterone concentrations and germ cell production. In non-human primates, no correlation between testicular androgen levels and germ cell production/spermatozoal number was observed (Narula et al. 2002; Weinbauer et al. 2004 for review). Similarly, contraceptive studies in volunteers failed to demonstrate a correlation between intratesticular steroids and germ cell numbers (Matthiesson et al. 2005b).

In the non-human primate testosterone induces the formation of smooth muscle actin in the peritubular cells during prepubertal testicular maturation (Schlatt et al. 1993). Peritubular cells express the androgen receptor. The testosterone effect is significantly reinforced by FSH. Since FSH receptors are found only in Sertoli cells it follows that FSH influences androgen action indirectly through factors arising in the Sertoli cell. This indicates that as an endocrine factor FSH can also induce the formation of physiologically relevant, locally acting factors in the primate testis. Interestingly, recombinant FSH stimulates testosterone production in men (Levalle et al. 1998) and in patients with selective FSH deficiency (Lofrano-Porto et al. 2007), lending further support to the importance of local interactions between Sertoli cells, Leydig cells and peritubular cells in connection with the actions of androgens and gonadotropins. In-vitro studies using monkey Sertoli cells exposed to testosterone and FSH, revealed that estradiol was produced in the presence of testosterone but not of FSH (Devi et al. 2006). This is surprising since based upon rodent data, Sertoli cell aromatase activity is regulated by FSH.

Testosterone acts both as an endocrine and local (paracrine and autocrine) factor within the testis.

2.2.6.2 Insulin-like Factor 3

Insulin-like factor 3 (INSL3) is a relaxin-like proteohormone produced by Leydig cells (Foresta et al. 2004) and signals through a G-coupled receptor (LGR8) that is expressed in Leydig cells and meiotic/

postmeiotic testicular human germ cells but not in peritubular and Sertoli cells (Anand-Ivell et al. 2006). Compelling evidence is available to indicate that INSL3 is a marker of Leydig cell differentiation and of entry into male puberty (Ferlin et al. 2006; Wikström et al. 2006). Levels of INSL3 are influenced by hCG/LH but this effect appears uncoupled from the steroidogenic effects of LH on testosterone synthesis (Bay et al. 2005, 2006). Since receptors for INSL3 are present on advanced germ cells it is tempting to assume a local role for INSL3 during spermatogenesis. A retrospective analysis of several male contraceptive studies suggested that circulating INSL3 levels were lower in azoospermic subjects compared to non-azoospermic subjects and a weak correlation between INSL3 levels and sperm number was reported (Amory et al. 2007a). More data is needed to reach a firm conclusion on the relevance of INSL3 as a local regulator of human spermatogenesis.

2.2.6.3 Growth Factors

Growth factors bind to surface receptors and induce cell-specific differentiation events via specific signal transduction cascades. Among those factors participating in the local regulation of spermatogenesis are **transforming growth factor** (TGF)-α and -β, **inhibin** and **activin, nerve growth factor** (NGF), **insulin-like growth factor I** (IGF-I), fibroblast growth factor (FGF) and **epidermal growth factor** (EGF).

Inhibin and activin have been detected not only in Sertoli cells, but also in Leydig cells of primates. Inhibins and activins are structurally related proteins. The heterodimer inhibin consists of an α subunit and a βA or βB subunit whereas activins are homodimers (βAβA or βBβB). Generally, activins are considered to stimulate spermatogonial proliferation whereas inhibins exert inhibitory actions. Of considerable clinical interest are recent discoveries that serum concentrations of **inhibin** are correlated with spermatogenic activity, testis size and sperm production. This growth factor can actually be used as an endocrine indicator of local spermatogenic defects (Boepple et al. 2008; Meachem et al. 2001).

In vitro studies have suggested that the local function of inhibin and activin could be a modulation of steroidogenic activity in Leydig cells. Activins inhibit or stimulate Leydig cell steroidogenesis in a species-dependent manner. Generally, IGF-I and TGF-α exert a stimulatory activity in the testis, while TGF-β acts as an inhibitor. In the rat, the development of Leydig cells is sustained by an interplay between TGF-α and TGF-β and LH activity is modulated by IGF-I. In the human Leydig cell the steroidogenic activity is also stimulated by EGF. This growth factor directly influences spermatogenesis: IGF-I concentrations are positively related to the number of pachytene spermatocytes. In man, IGF-I shows the highest expression in these spermatocytes and stimulates DNA synthesis in mitotic germ cells. Administration of IGF-I to patients increased testis size but this was also associated with increased gonadotropin secretion and testosterone (Laron and Klinger 1998). An important role of NGF for the structural organization of the human seminiferous tubules is postulated, since the culture of seminiferous tubules can be successful only in the presence of NGF. NGF has been localized in peritubular cells by immunocytochemistry. In the rat, NGF is an important regulator of meiotic division. Fibroblast growth factor has been involved in mitosis and Sertoli-germ cell interactions.

2.2.6.4 Immune System Factors

Cells of the immune system and the blood-testis-barrier provide a special environment for the development of otherwise antigenic germ cells (see Sect. 2.5). It appears, however, that immune factors may also play a more direct role during testicular steroidogenesis and gametogenesis and might have a relationship to male infertility (Albrecht et al. 2005; Fijak and Meinhardt 2006; Hedger 2002). These factors involve leucocyte, macrophage and mast cell products. For example, cytokines such as interferon, tumor necrosis factor (TNF), interleukins, leukemia inhibing factor (LIF), stem cell factor (SCF), macrophage migration inhibiting factor (MIF) that bind to cell surface receptors and provoke cell proliferation and differentiation. TNF and LIF are suspected to play a role in Sertoli cell-germ cell interactions and in the autocrine control of Sertoli cell proliferation. MIF is produced specifically by Leydig cells and is found in Sertoli cells, basal germ cells and peritubular cells following elimination of Leydig cells, indicating that compensatory mechanisms maintain testicular MIF production. Unlike interleukins, SCF and its receptor (c-kit) are

clearly essential local factors that govern germ cell migration during ontogenesis and spermatogonial differentiation in the adult testis. SCF is synthesized and secreted by Sertoli cells whereas the receptor is expressed on spermatogonial surfaces. The SCF/c-kit system is important for spermatogonial differentiation and development.

2.3 Testicular Descent

The incidence of positional anomalies of the testis is over 3% and ranges among the most common congenital defects (reviewed in Virtanen et al. 2007a). These defects are associated with spermatogenic disturbances such as fewer **spermatogonial stem cells** at birth compared with normal boys (Virtanen et al. 2007b) and increased risk of **testicular tumor** development. Testicular descent is multifactorial with two distinct phases. First a **descending phase** from the lower kidney pole to the pelvic cavity **(transabdominal phase of descent)** controlled by the swelling of the gubernaculum. The shortening of the gubernacular cord and the outgrowth of the gubernacular bulb controlled by the genitofemoral nerve is independent of androgens. The gubernaculum deposits extracellular matrix, rich in glycosaminoglycans and hyaluronic acid, and forms a cone-like structure at the caudal end of the gonad, anchoring the developing testis close to the inguinal region during fetal growth. The second phase, the descent into the scrotum **(inguino-scrotal phase of descent)** is controlled by androgen action (Shono 2007). In the 26th gestational week the gubernaculum begins to grow through the inguinal canal and reaches the scrotum by gestational week 35, pulling the testis in its path before the gubernaculum shrinks to a fibrous remnant The intra-abdominal pressure and the shrinkage of the gubernaculum may force the testis through the inguinal canal. At birth, the testes reach at the bottom of the scrotum and in 97% of boys testicular descent is completed within another 12 weeks (Fig. 2.14).

The physiological and endocrine mechanisms that govern testicular descent are not known in detail. Several factors besides androgen such as calcitonin gene-related peptide, epidermal growth factor (EGF) and fibroblast growth factor (FGF) family are candidates for inducing the testicular descent (Nightingale et al. 2008). Hoxa-10 and insulin-like factor 3 (INSL3) are potential regulators of these phases as suggested by the fact that in gene knockout mice maldescended testes remain located in the abdominal cavity. INSL3 produced by the Leydig cells together with androgen induces the gubernaculum growth and is therefore needed in the early phase (Emmen et al. 2000). INSL3 knockout mice have their testes high in the abdominal cavity. In Hoxa-10 knockout mice, testes remain close to the scrotum after the initial descending phase. Estrogens or environmental endocrine disruptors have also been suspected to induce a down-regulated INSL3 expression and thus disturb testicular descent (Toppari et al. 2006). Genetic analysis in men revealed several functionally deleterious mutations in both INSL3 and

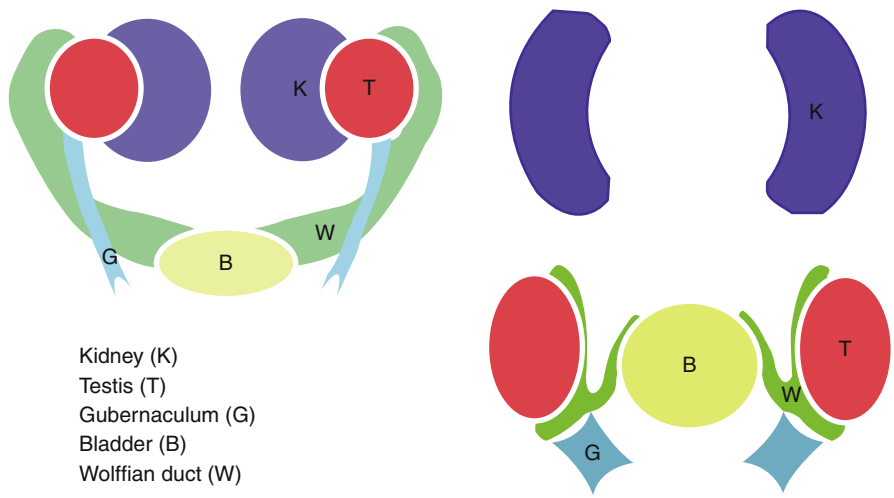

Fig. 2.14 Testicular descent. At an early stage the gonad precursor organ is located next to the kidney (K). During the first testicular descendent phase between week 8 (left image) and week 17 (right image), the testis is anchored by the swollen gubernaculum close to the inguinal region. The bud-like growth of the gubernaculum is regulated by INSL-3 and androgens

its receptor GREAT/LGR8 gene. Although some mutations were found only in patients with maldescended testes, the causative link between the presence of mutations in INSL3 or GREAT/LGR8 and the undescended testes remains to be demonstrated (Feng et al. 2006; Yamazawa et al. 2007).

2.4 Vascularization, Temperature Regulation and Spermatogenesis

Vascularization of the testis has two main roles: **transport and mobilization** of endocrine factors and metabolites, as well as regulation of **testicular temperature.** The arterial supply of the testicular parenchyma follows the lobular division of the seminiferous tubules. Each lobule is supplied by one artery from which segmental arteries originate at a distance of about 300 µm from each other, supplying blood to the lateral regions of the lobuli (Ergün 1994a, b). Segmental arteries and capillaries become branched between the Leydig cells and finally give rise to the venous system.

In men, testicular temperature is about 3–4°C below core body temperature and about 1.5–2.5°C above the temperature of scrotal skin. For the maintenance of a physiologically lower temperature the testis relies on two **thermoregulatory systems.** Heat can be transferred to the external environment through the scrotal skin, as the scrotal skin is very thin, possesses hardly any subcutaneous fat tissue and has a very large surface.

The second regulatory system is the pampiniform plexus. In this system, the convoluted testicular artery is surrounded by several veins coiling around the artery several times. Arterial blood arriving at the testis is thereby cooled down by the surrounding venous blood. The usual explanation for the pampiniform plexus here is to efficiently maintain the optimal temperature which is below body temperature. Recently, a new theory has been put forward hypothesizing that the process of spermatogenesis results in a large amount of heat which has to be regulated. Testes are located in the scrotum in order to maintain lower than body temperatures (Skandhan and Rajahariprasad 2007). Some mammals' testes remain functional inside the body, e.g., elephants, but these animals lack sweat glands and are closely related to aquatic ancestors which have to compensate for the chilling effects of the heat-conducting environment, namely water. Human scrotal skin is devoid of subcutaneous fat and the presence of high sweat gland density enables heat transmission. Upon exposure to cold temperatures, the scrotal surface is minimized by contraction for preventing temperature loss and cremaster muscles retract the testes closer to the abdomen for temperature maintenance.

In case of **varicoceles,** defined as abnormally dilated scrotal veins, scrotal temperature is increased (Lerchl et al. 1993). Approximately 15% of normal men have a varicocele, with 40% of them having fertility problems. Venous reflux and testicular temperature elevation appear to play an important role in testicular dysfunction, although the exact pathophysiologic mechanisms involved are not yet completely understood. An increase of testicular temperature results in damage of the spermatogenic function of the testis (Jung and Schuppe 2007). If testicular temperature is increased in adults, reversible spermatogenic damage can be induced. Most importantly, however, substantial increase of temperature must be achieved. Scrotal temperature elevations by 0.8–1°C over a period of 52 weeks in healthy volunteers had no adverse effect on the number and quality of spermatozoa (Wang et al. 1997).

Treatment with heat leads to sustained activation of both mitogen-activated protein kinase MAPK13 and MAPK14. Activation of MAPK13 and MAPK14 is accompanied by an increase in B-cell leukemia/lymphoma 2 (BCL2) levels in both cytosolic and mitochondrial fractions of testicular lysates, leading to apoptosis mediated by cytochrome c and DIABLO release (Jia et al. 2007). Heat also results in inactivation of Bcl2, an anti-apoptotic protein, through phosphorylation at serine 70, thereby favoring the apoptotic pathway. Both the protein kinases and the anti-apoptotic factor Bcl2 help balance the death pathway in male germ cells. However, although testicular temperature is lower than body temperature in the majority of mammals, this difference is not obligatory in every species.

2.5 Immunology of the Testis

Gonocytes migrate to the testis even during prenatal development, but spermatogonia begin to differentiate into spermatozoa only at puberty after the immune system has matured and a systemic self-tolerance is

developed. As the spermatogonia proliferate and differentiate into spermatocytes, many new surface and intracellular proteins are expressed which are unique in the body, especially for the immune system. These proteins are also novel antigens which have to be tolerated by the immune system. At the same time, adjacent Sertoli cells form complex networks of specialized tight junctions (zonula occludens) that cause isolation of the tubular contents from the blood vascular compartment. This barrier contains several integral membrane proteins, comprising various components such as junctional adhesion molecules, claudin family proteins, and occludin. The **blood-testis-barrier** provides a separation between immune cells and haploid germ cells with antigenic properties. This barrier was thought to provide the basis of the systemic tolerance to newly developing auto-antigens. Transplantation of spermatogonia into testis tubuli can restore spermatogenesis, even across species borders (Schlatt et al. 1999), demonstrating that the seminiferous tubules are an immunprivileged site.

Notwithstanding this, auto-antigens are also expressed in cells located outside the inter-Sertoli cell tight junctions, e.g., in the basal compartment of the seminiferous epithelium and are recognized as foreign (Pöllänen and Cooper 1994)). Experiments with allo- and xenografts placed directly into the testicular interstitial space can lack signs of degeneration caused by a graft-versus-host reaction, indicating absent or reduced immune reactions (Gores et al. 2003; Isaac et al. 2005). However, the testis is capable of a normal inflammatory response, demonstrated by its effective reaction to viral and bacterial infections, especially in the rete testis where testicular sperm enter the efferent ducts of the epididymis (Naito and Itoh 2008). This transition zone has a special arrangement of Sertoli-like cells, forming a valve with many macrophages patrolling the tissue. The blood-testis-barrier is terminated in this area; subsequently, spermatozoa are no longer protected, whereas later on, an **epididymidal-blood-barrier** is present (Dube et al. 2007). This status is confirmed by the observation that certain forms of autoimmune orchitis are first manifested in the rete testis. Studies demonstrated that the blood-testis-barrier at this transition point is incomplete against humoral antibodies and also intravenously injected horseradish peroxidase. The immune privilege of the testis is not only due to the blood-testis-barrier, but also depends on a specific

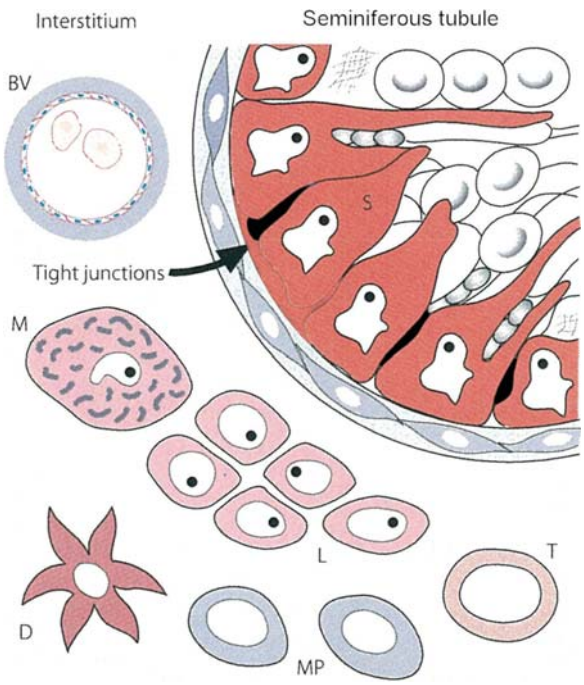

Fig. 2.15 Immunological compartments of the testis. Sertoli cells (S) traverse the testicular tubules, keeping in close contact with the germ cells. Together with the peritubular cells, they form the seminiferous epithelium. The blood–testis barrier (tight junctions) is built by tight junctions between neighboring S, dividing the seminiferous tubules into a basal and adluminal compartment. The interstitial space contains the Leydig cells (L) and the immune cells such as macrophages (MP), dendritic cells (D), mast cells (M), and T cells as well as blood vessels (BV) with migrating leukocytes

intratesticular regulation of the immune system function (Fig. 2.15). The predominant interstitial cell type is the Leydig cell. In transplantation studies, rats pretreated with estrogen to suppress Leydig cell testosterone production promptly rejected intratesticular allografts, indicating that high intratesticular testosterone concentrations seem to play an important role in the maintenance of testicular immune privilege. However, it remains unknown how testosterone may mediate an anti-inflammatory effect. In fact, the interstitium contains a variety of immunocompetent cells e.g., leukocytes, macrophages, monocytes, dendritic cells, T and B lymphocytes, and mast cells.

Testicular macrophages are found as early as week 7 of gestation and probably originate from hematopoietic precursor cells that migrate to the testis. Macrophages proliferate in the testis during postnatal life, probably under pituitary control, since hCG is

able to increase the mitotic index of testicular macrophages in rats. In the adult, human testis macrophages represent about 25% of all interstitial cells. Morphologically and biochemically they are similar to macrophages resident in other tissues. Testicular macrophages have a reduced capacity to excrete some cytokines such as IL-1β and TNF-α compared to macrophages from other tissues (Hayes and Crowley 1998). Furthermore, lipopolysaccharides, resembling the surface of bacteria, given to immature and mature mice resulted in enhanced levels of the testicular cytokine IL-6 and constitutively elevated the production of other anti-inflammatory mediators (Elhija et al. 2005; Isaac et al. 2005). Interestingly, the expression of pro-inflammatory cytokines such as IL-1β and TNF-α by testicular macrophages demonstrates the testicular capability of an inflammatory response. Up to now two macrophage types have been distinguished in the adult testis that differ in the expression of markers and inflammatory mediators. In the rat, the $ED2^+$ expressing macrophages do not participate in promoting inflammatory processes. They may take part in maintaining the immune privilege as an immunoregulatory team player. However, the $ED1^+$ $ED2^-$ macrophages are involved in testicular inflammatory responses. During acute and chronic inflammation the influx of $ED1^+$ monocytes change the equilibrium of the macrophage population. The number of mononuclear cells increases in case of testicular disease.

In about 5% of testicular biopsies from infertile men lymphoid cells surround the tubules with markedly higher spermatogenic damage. Similarly, mononuclear infiltrates are often associated with carcinoma in situ. Seminomas generally show a very conspicuous infiltration of immunocompetent cells. Diseases such as mumps can be complicated by a severe testicular inflammation in 35% of cases.

The contribution of mast cells to the immune system has often been underestimated, but recently the complexity of these cells was shown and their involvement in the innate and adaptive immune system (Gilfillan and Tkaczyk 2006; Stelekati et al. 2007). Mast cells can release factors that act as mediators and as such are capable of influencing disease induction and progression. In the brain these cells can change vascular permeability through factor release, thereby opening the blood-brain-barrier, allowing the entry of activated T lymphocytes and increased inflammatory cell traffic. In men the testicular mast cell presence changes with age, with numbers increasing slightly during infancy, decreasing during childhood, and increasing again at puberty. Their major released product, a serine protease tryptase, is a mitogen for fibroblasts, enhancing the synthesis of collagen, resulting in fibrosis, thickening, and hyalinization of the tubuli walls.

Histological features of spermatogenic pathologies are associated with increased numbers of mast cells and are often found in men with infertility problems (Apa et al. 2002; Sezer et al. 2005). Mast cell inhibitors are beneficial in the treatment of idiopathic oligozoospermia and oligoasthenozoospermia too and can prevent mast cell activation (Cayan et al. 2002; Hibi et al. 2002). Human peritubular cells express receptors for the mast cell products histamine and tryptase (Albrecht et al. 2006). This may be helpful in the maintenance of an immunosuppressive phenotype not only in the testis.

2.6 Testicular Androgens

Androgens are essential for the development and function of testes, maturation of secondary sexual characteristics, masculinization of the bone-muscle apparatus, libido, and stimulation of spermatogenesis. Physiological effects of androgens depend on different factors such as number of androgen molecules, distribution of androgens and their metabolites inside the cell, interaction with the receptors, polyglutamine number of the amino acid sequence in the androgen receptor and receptor activation (Palazzolo et al. 2008). In turn, androgen concentrations in the blood depend on the synthesis rate, balanced by metabolic conversion and excretion. Androgens also exert rapid non-genomic effects contributing to the physiological actions (Lösel et al. 2003). Whereas genomic effects take hours or days to produce their actions, rapid steroid effects are activated within seconds or minutes. These non-genomic actions are not removed by inhibition of transcription or translation and are often activated by membrane-impermeant steroid conjugates. However, rapid pathways of androgen action can modulate transcriptional activity of androgen receptors or other transcription factors (Rahman and Christian 2007).

In men, **testosterone** is by far the most important and abundant androgen in blood. More than 95% of

the existing androgens derive from the testis, which synthesizes about 6–7 mg testosterone per day. Besides the testes the remaining contribution to androgen production derives mainly from the adrenals. The site of androgen production in the testis is the Leydig cell. Both synthesis and secretion are under regulation of pituitary LH and local factors (Lei et al. 2001; Sriraman et al. 2005).

Since Leydig cells cannot store androgens, de novo biosynthesis takes place continuously. The starting point for androgen synthesis is cholesterol, a fundamental substance of metabolism, with the typical steroid ring conformation energetically compatible with the transformation into androgens. Unlike most cells that use cholesterol primarily for membrane synthesis, Leydig cells have additional requirements for cholesterol, because it is the essential precursor for all steroid hormones. LH as the central regulatory factor controls both steroidogenesis and Leydig cell cholesterol homeostasis in vivo. Cholesterol can either be incorporated by the cell through receptor-mediated endocytosis from low-density lipoproteins (LDL), or can be synthesized de novo within the Leydig cell starting from acetyl-coenzyme A. In addition testosterone signaling regulates lipid homeostasis in Leydig cells. It also affects the synthesis of steroids and modulates the expression of genes involved in de novo cholesterol synthesis (Eacker et al. 2008). Cholesterol is stored in cytoplasmic lipid droplets. The number of lipid droplets is inversely related to the rate of androgen synthesis in the Leydig cell, i.e., a high synthesis rate leads to a low content of lipid droplets and vice versa.

2.6.1 Synthesis of Androgens

Androgen synthesis requires the **conversion of cholesterol to testosterone** (Fig. 2.16). This transformation goes through five different enzymatic steps in which the side chain of cholesterol is shortened through oxidation from 27C to 19C. The steroidal A-ring assumes a keto configuration at position 3. The starting point for the transformation of cholesterol into testosterone is the shortening of the side chain through C 22 and C 20 hydroxylases, followed by cleavage of the bond between C 20 and C 22, leading to production of pregnenolone. The steps following pregnenolone formation occur in the endoplasmic reticulum either through the Δ4 or through the Δ5 pathway.

The designation Δ4 or Δ5 refers to the localization of the double bond in the steroid. The **Δ5 pathway** is predominant over the Δ4 in human steroid synthesis. Along the **Δ4 pathway,** pregnenolone is dehydrated to progesterone, a key biological substance. The Δ4 pathway proceeds to the intermediate 17α-hydroxyprogesterone. If the side chain is removed at this stage, the intermediate androstene-3, 17-dione is produced, which, through further reduction at position C 17, is then transformed into testosterone. In the Δ5 synthesis pathway, testosterone synthesis occurs through the intermediates 17-hydroxypregnenolone and dehydroepiandrosterone.

Cholesterol is the starting point for biosynthesis of steroids, oxysterols and bile acids. After cholesterol, an insoluble molecule is de novo synthesized or taken up via the LDL receptor into the cell. Cholesterol for steroidogenesis is stored in an ester form in lipid droplets, which are hydrolyzed by LH activation of cholesterol ester hydrolase. It has to be transported within the cell to the mitochondria where it is imported into the cristae of the mitochondria.

The discovery of the Steroidogenic Acute Regulatory protein (StAR) and related proteins containing StAR-related lipid transfer domains have helped much to understand this limiting step of testosterone synthesis. StAR mRNA expression is triggered by endocrine stimuli and is rapidly and widely distributed in steroidogenic tissues including the adrenals and corpora lutea. StAR moves cholesterol from the outer to the inner mitochondrial membrane, but acts exclusively on the outer membrane. The precise mechanism by which StAR's action stimulates the influx of cholesterol remains unclear, but when StAR connects to cholesterol it performs a conformational change that opens a cholesterol-binding pocket (Miller 2007). After a phosphorylation StAR interacts with voltage-dependent anion channel 1 (VDAC1) on the outer membrane, which processes the phospho-StAR to a smaller intermediate. If VDAC1 is lacking, phospho-StAR is degraded by cysteine proteases preventing the mitochondrial membrane transport (Bose et al. 2008). The physiological importance of StAR is highlighted by the phenotype of patients with an inactivating mutation of the StAR gene. These patients suffer from life-threatening congenital adrenal hyperplasia as they are unable to produce the necessary amounts of steroids.

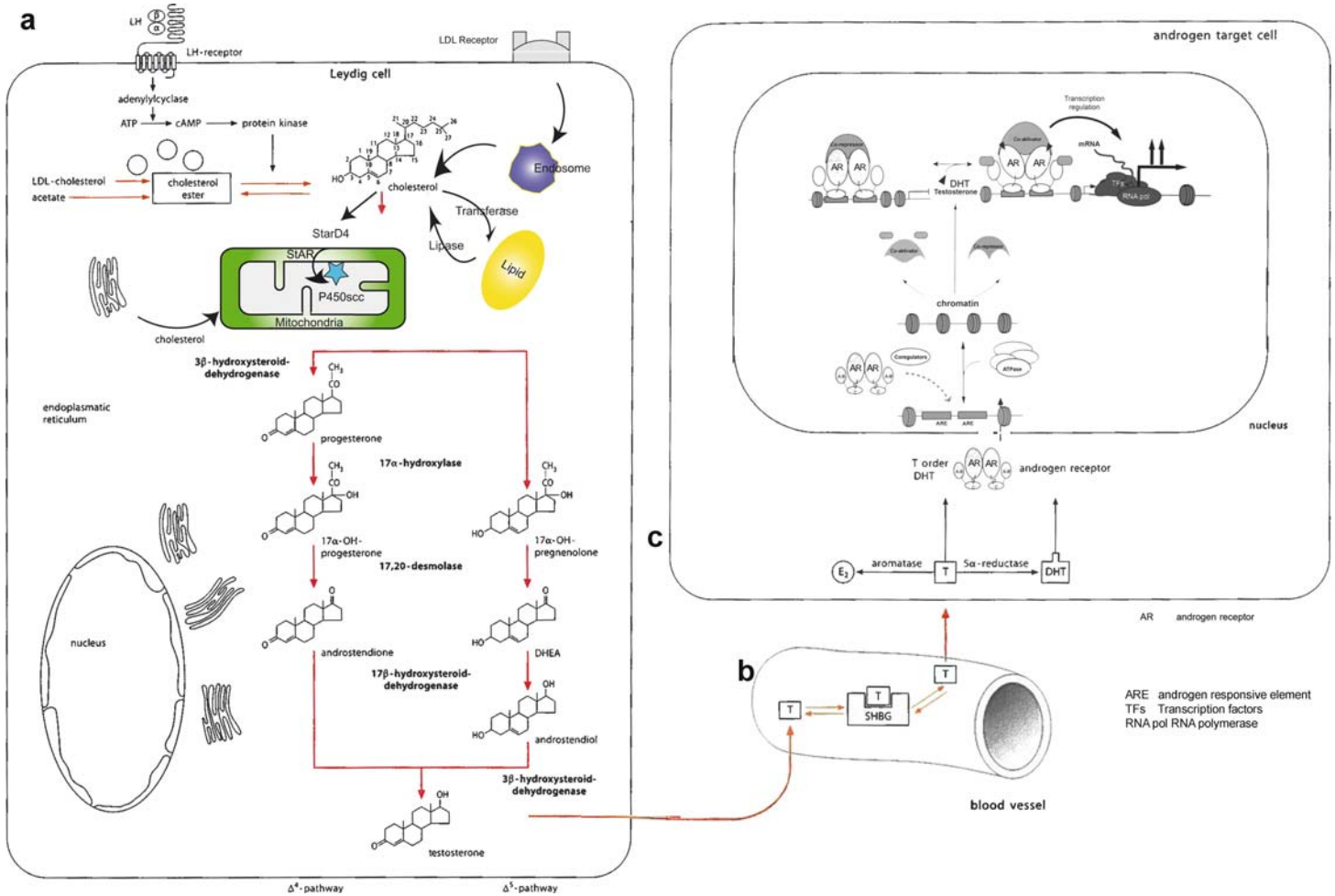

Fig. 2.16 (**a**) Steroid biosynthesis in the Leydig cell. Steroid biosynthesis is induced by LH through activation of adenylyl cyclase. Starting material is cholesterol or acetate. The C atoms of cholesterol are numbered in order to follow better the different enzymatic modifications and their localization. StAR (Steroidogenic acute regulatory protein) plays a key role during steroidogenesis. StAR is localized to the inner mitochondrial membrane and governs cholesterol transport. (**b**) Cholesterol uptake and transport to the inner mitochondrial membrane. (**c**) Normally human steroidic cells take up circulating low-density lipoproteins (LDL) through receptor-mediated endocytosis, directing the cholesterol to endosomes. It may be esterfied by acyl-CoA cholesterol transferase and stored in lipid droplets as cholesterol esters. Free cholesterol, set free by the hormone-sensitive lipase, is probably bound by StarD4 for transcytoplasmic transport to the mitochondrial membrane. StAR is responsible for the rapid movement of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where it is converted by P450scc to pregnenolone

At the inner mitochondrial membrane site cytochrome P450ssc (ssc = side chain cleavage) catalyzes the conversion of cholesterol into pregnenolone. The enzyme cytochrome P450ssc is responsible for the different enzymatic reactions leading to the production of pregnenolone. Like other steroid synthetic enzymes, it belongs to the group of monooxygenases, containing a prosthetic hemogroup as in hemoglobin and is localized on the internal membrane of mitochondria. This reaction consists of three consecutive monooxygenations requiring two electrons to activate molecular oxygen; a 22-hydroxylation, 20-hydroxylation and the cleavage of the C 20–C 22 bond, yielding pregnenolone and isocaproic aldehyde. Pregnenolone diffuses across the mitochondrial membranes and is transformed into testosterone through the enzyme cytochrome P450 C 17, also belonging to the group of monooxygenases and located in the endoplasmic reticulum. The overall enzymatic system, however, is not capable of transforming every molecule of pregnenolone into testosterone so that several intermediates are produced.

Testosterone is the main secretory product of the testis, along with **5α-dihydrotestosterone (DHT),** androsterone, androstenedione, 17-hydroxyprogesterone, progesterone and pregnenolone. The transformation of testosterone into DHT takes place principally in the target organs, e.g., prostate. Androstenedione is important as a precursor for the production of extratesticular estrogens. Biologically active **estradiol** can be produced as a result of extratesticular aromatization of androstenedione to estrone that is subsequently reduced to estradiol in peripheral tissues. Only a very small portion of the testosterone produced is stored in the testis and the androgen is mainly secreted in blood.

Testosterone concentrations in the testicular lymphatic circulation and in the venous blood are very similar, but there are essential differences in the flow rate and velocity of both systems. Therefore, transport of testosterone in the general blood circulation occurs mainly through the spermatic vein. Androgens diffuse into interstitial fluid and then enter testicular capillaries or enter capillaries directly from Leydig cells that are in direct contact with the testicular microvasculature. The mechanism for testosterone transport from the Leydig cell into the blood or lymph is not completely known. Probably lipophilic steroids distributed within cells or small cell groups are released through passive diffusion.

2.6.2 Testosterone Transport in Blood

During transport in plasma, testosterone is mainly bound to albumin or to **sex hormone binding globulin (SHBG)** which is produced by hepatocytes. A protein, the androgen binding protein (ABP), with similar steroid-binding characteristics was found to be produced in the testis. SHBG is a β-globulin consisting of different protein subunits. In rats it is expressed in Sertoli cells and is secreted preferentially into the seminferous tubules, migrates into the caput epididymis where it is internalized by epithelial cells regulating androgen-dependent mechanisms of sperm maturation. Testicular SHBG isoforms are found in sperm and released from these during the capacitation reaction. Plasma SHBG is about 95 kDa in molecular weight, 30% of which is represented by carbohydrate, and possesses one androgen binding site per molecule. Human testicular SHBG transcripts are expressed in the germ cells and contain an alternative exon 1 sequence, appearing to encode an SHBG isoform that is 4–5 kDa smaller than plasma SHBG. The testosterone binding capacity is also much lower compared to the plasma SHBG (Selva et al. 2005).

In normal men, only 2% of total testosterone circulates freely in blood, while 44% is bound to SHBG and 54% to albumin. The binding affinity of testosterone to albumin is about 100 times lower compared to SHBG. However, since albumin concentration is much higher than that of SHBG, the binding capacity of both proteins for testosterone is about the same. The ratio of testosterone bound to SHBG over free SHBG is proportional to SHBG concentration. A direct measurement of free testosterone is impractical in routine practice, so that several equations are used to estimate the free testosterone concentration in serum.

The main dissociation of testosterone from binding proteins takes place in capillaries. The interaction of binding proteins with the endothelial glycocalyx leads to a structural modification of the hormonal binding site and thereby to a change in affinity. As a result testosterone is set free and can diffuse freely into the target cell or binds together with SHBG to **megalin,** a cell importer protein (Hammes et al. 2005). Megalin is expressed in sex-steroid target tissues and is a member of the low density lipoprotein receptor superfamily of endocytic proteins. In the serum 98–99.5% of the sex steroids are protein-bound and endocytosis is quantitatively more relevant for tissue delivery of biologically active steroid

hormones than free diffusion. Until now several different ways have been described how steroids can enter the target cells and it is still under debate which of these are the most relevant pathways to take up all kinds of steroids.

SHBG not only binds testosterone but also estradiol. The type of binding is influenced by the different SHBG isoforms, but generally testosterone binds threefold higher than estradiol to SHBG. For example, it could be demonstrated that post-translational changes in the carbohydrate structure of SHBG can lead to different binding affinity of the protein to testosterone or estradiol. SHBG concentration in serum is under hormonal regulation and primarily regulated through opposing actions of sex steroids on hepatocytes, estrogen stimulates and androgen inhibits SHBG production. Other hormones such as thyroid hormones are also potent stimulators of SHBG production. SHBG concentration in men is about one third to one half of the concentration found in women. In normal, healthy men with an intact hypothalamo-pituitary-testicular axis, an increase in plasma concentrations of SHBG leads to an acute decrease of free testosterone and simultaneous stimulation of testosterone synthesis, persisting until achievement of normal concentrations. SHBG concentrations can be elevated in hypogonadal men.

2.6.3 Extratesticular Metabolism of Testosterone

Testosterone is a precursor of two important hormones: through 5α-reduction it gives rise to the highly biologically (three to sixfold compared to testosterone) active hormone **5α-dihydrotestosterone (DHT),** and through aromatization to **estradiol.** The **half-life of testosterone** in plasma is **only about 12 min.** Estrogens influence testosterone effects by acting either synergistically or antagonistically. Moreover, estrogens have other specific effects which were originally described to be typical of testosterone. It has been found that inactivating mutations of the estrogen receptor or aromatase, preventing estrogen action on the bones, result in continuous linear growth and lack of epiphyseal closure.

Low levels of bioavailable estrogen and testosterone are strongly associated with high bone turnover, low bone mineral density and high risk of osteoporotic fractures. In aromatase-knockout and estrogen receptor-knockout male mice an association between impaired glucose tolerance with insulin resistance and lack of estrogens with elevated testosterone concentrations have been found (Takeda et al. 2003). It also seems that aromatase deficiency in men is associated with the occurrence of insulin resistance and diabetes mellitus type 2 during high-dose testosterone treatment (Maffei et al. 2004). The impairment of the estrogen to testosterone ratio is thought to be responsible for the development of impaired glucose tolerance and insulin resistance in aromatase deficiency patients receiving testosterone replacement therapy. An imbalance in the ratio of estrogen to androgen tissue levels is also postulated as a major cause in the development of gynecomastia. Furthermore recent investigations have shown a local neuroprotective effect of newly aromatized estradiol on the brain.

Reduction of testosterone to DHT occurs in the endoplasmic reticulum through the enzyme 5α-reductase which is in the microsomes of the cell. Both testosterone and DHT bind to the same intracellular androgen receptor to regulate gene expression in the target tissue. Although they interact with the same androgen receptor, testosterone and DHT produce distinct biological responses and the molecular mechanisms are still under debate. Two isoforms of 5α-reductase could be identified in humans by NADPH-dependent enzymes reducing the double bond at the four to five position in C 19 steroids as well as C 21 steroids. The gene for 5α-reductase type I is located on chromosome 5 encoding for a protein with 259 amino acids, while the gene for the 5α-reductase type II is on chromosome 2 encoding for a slightly shorter protein with 254 amino acids.

The two isoforms are very similar to each other, but show different biochemical properties. Type I enzyme works optimally at an alkaline pH, while the optimal pH for type II is acidic. Also, the tissue distribution of the two forms is different. Type I 5α-reductase has been localized in the non-genital skin, liver, brain prostate, ovary and testis, while type II is mainly active in classical androgen-dependent tissues, such as the epididymis, gentital skin, seminal vesicle, testis and prostate but also in liver, uterus, breast, hair follicles and placenta.

At the cellular level, DHT sustains differentiation and growth and is particularly important for normal sexual development and virilization in men. It also affects the muscle mass and the deepening of the voice.

DHT transactivates the androgen receptor leading to gene transcription in the prostate and its growth.

Overall, testosterone effects result from influences of the hormone itself and of its metabolites estradiol and DHT. Changes in the property of type II 5α-reductase due to mutation can result in *c*omplete *a*ndrogen *i*nsensitivity *s*yndrome (CAIS) or *p*artial *a*ndrogen *i*nsensitivity *s*yndrome (PAIS). DHT is eliminated by type 3α-HSD (aldo-keto reductase (AKR) 1C2) which reduces 5α-DHT to 3α-androstanediol. Studies in men have demonstrated that 3α-androstanediol can be converted back to 5α-DHT to stimulate growth of the prostate by a short-chain dehydrogenase/reductase called RoDH like 3α-HSD (Penning 2000). In human tissues five aldo-keto reductase isoforms (AKR) exist with varying reductase activity on the 3-, 17- and 20-ketosteroid position with isoform (AKR1C2) predominately converting 5α-DHT to 3α-diol. The inactivated metabolites are excreted in the urine.

Some androgen metabolites are excreted in free form, others are glucuronated by the liver before excretion. Recently excreted metabolites have been used in screening assays to uncover doping with exogenous testosterone esters in high-performance athletics (Saudan et al. 2006). The 17-glucuronidation of the DHT metabolite androstane-3α, 17ß-diol is correlated with several metabolic risk factors in men. The ratio of 17G to DHT is associated with total fat mass, its distribution, intrahepatic fat, disturbed lipid profile, insulin resistance, and diabetes (Vandenput et al. 2007).

2.6.4 Mechanism of Androgen Action

Testosterone dissociates from SHBG at the target organ and diffuses into the cells. The conversion of testosterone into DHT is organ-dependent. This occurs, for example, in the prostate, where DHT is the main biologically active androgen. Hydroxysteroid dehydrogenases regulate ligand access to the androgen receptor in the human prostate. The first step in androgen action is binding to the androgen receptor which belongs to the family of steroid hormone receptors (Fig. 2.17). Together with RoDH the hydroxysteroid dehydrogenases are involved in the pre-receptor regulation of androgen action. The mechanism through which the androgen receptor and other nuclear receptors act as transcriptional factors has a general mechanism in which they bind to their ligand cytosolicly, inducing conformational changes, loss of chaperones, dimerization, and nuclear translocation. In the nucleus both (ligand and nuclear receptor) bind to specific sequences of genomic DNA and induce stimulation of RNA synthesis. Chromatin remodeling such as modification of histones plays a role in gene transcription, and many nuclear receptor interacting co-regulators perform significant roles in gene transcription. Mineralocorticoid, glucocorticoid, thyroid hormone, retinol, fatty acid metobolite, estrogen and progesterone receptors also belong to this family. Currently 48 nuclear receptors have been identified in humans [reviewed in (Kishimoto et al. 2006)]. These receptors share substantial functions and are thought to have evolved from a single ancestral gene (Bertrand et al. 2004). Orphan nuclear receptors have also been found for which no ligand has yet been identified. Members of this receptor family possess an N-terminal domain, a DNA-binding domain, a hinge region and a hormone-binding domain (Fig. 2.18).

Nuclear receptors have several functional domains, a constitutionally activating function domain, a highly conserved DNA-binding domain, a nuclear localization signal domain and a ligand binding domain. Steroid receptors show high homology with the corresponding DNA-binding and ligand-binding domains in the mineralocorticoid, glucocorticoid and progesterone receptors. In contrast, at the N-terminal domain little similarity with these receptors remains. The nuclear receptors are subdivided into two subfamilies depending on their ligand partners' forming homodimers such as the androgen receptor and other steroid receptors; another subfamily form heterodimers with only one ligand, such as the thyroid hormone receptor. In most nuclear receptors the binding in a pouch to their ligand at the C-terminal end made of 12 α-helices, leads to a shift of the most C-termianl α-helix and to a change of the activation activity. Inside the 12 helices a hydrophobic cave is induced to change its formation and angle in which the helix 12 changes. This angle is associated with transactivation status of the receptor.

An important characteristic of the N-terminal domain of the androgen receptor is the presence of short tandem repeats (STRs) CAG, coding for polymorphic polyglutamine, TGG repeats coding for polyproline and GGC repeats for polyglycine. In normal

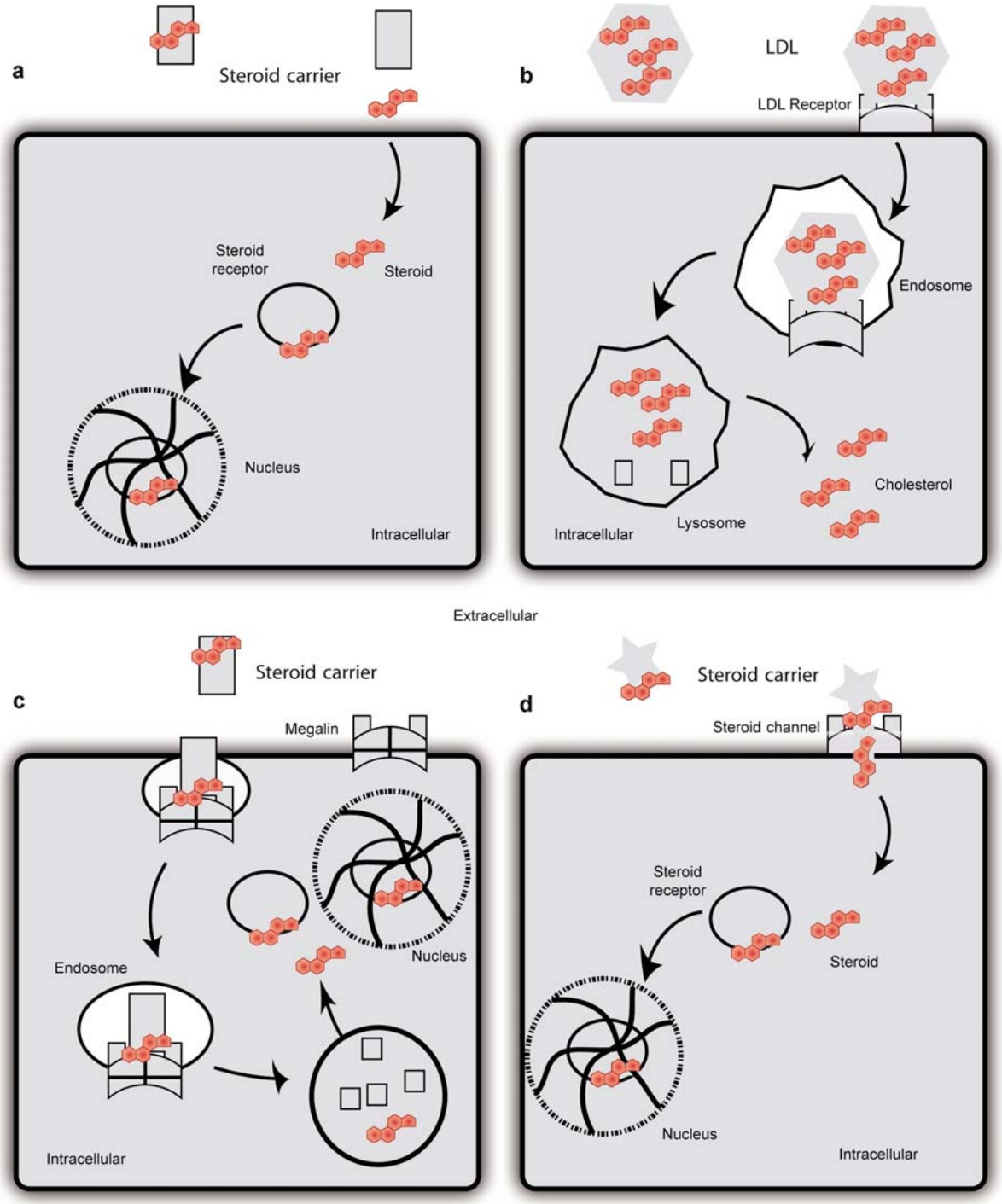

Fig. 2.17 Mechanisms by which steroidal hormones can enter cells. (**a**) The steroid hormone diffuses freely across the cell membrane, binds to an intracellular receptor and enters the nucleus to regulate gene expression. (**b**) Receptor-mediated endocytosis of steroid containing lipophilic molecules. The lipoprotein LDL binds its receptor and is taken up, degraded in lysosomes and the steroid cholesterol can enter different metabolic pathways. (**c**) Receptor-mediated endocytosis of steroids. The entire hormone-carrier is incorporated by endocytosis after binding to a carrier protein. Following intracellular degradation of the carrier, the ligand hormone is released into the free cytoplasm. (**d**) Transport-mediated uptake of molecules through the membrane. The steroid-carrier is recognized by a membrane receptor and the ligand is transported into the cell

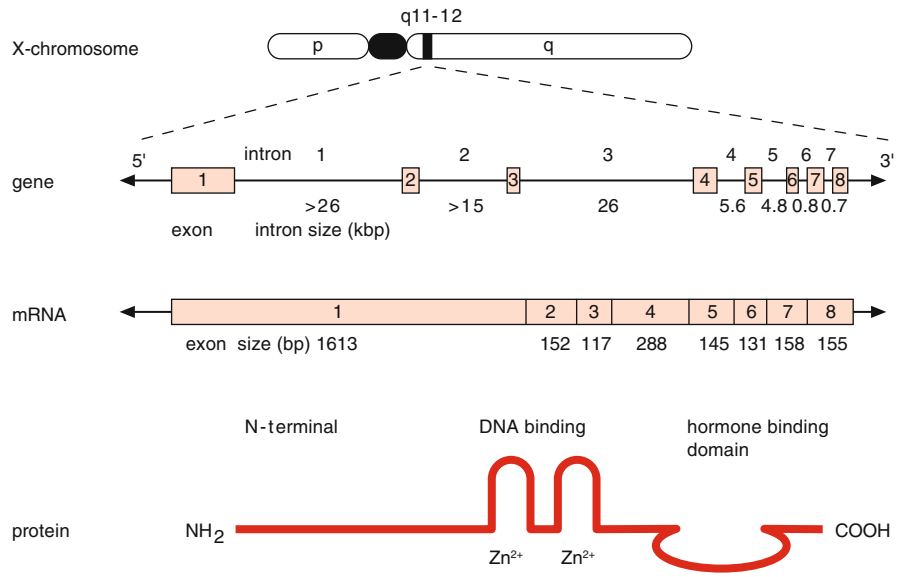

Fig. 2.18 Organization of the androgen receptor and chromosomal localization of the androgen receptor and structure of the androgen receptor gene and protein. *Upper* figure: the gene for the androgen receptor is localized in the pericentromeric region of chromosome X, at the site Xq11–12. Middle figure: the entire gene encompasses a region of about 90 kilobase pairs (kbp) on genomic DNA. Different exons are represented by boxes 1–8, with corresponding intron sizes reported below. Exon 1 encodes the N-terminal region, exon 2–3 the DNA binding domain and exon 4–8 the steroid binding domain. *Lower* figure: schematic representation of the androgen receptor protein with the different functional domains. The genome spans more than 80 kb of chromosome X with eight exons. In exon 1 short tandem repeat regions (Gln and Gly). The protein structure consists of a transcription regulation domain, a DNA-binding domain, a hinge region and the androgen binding region

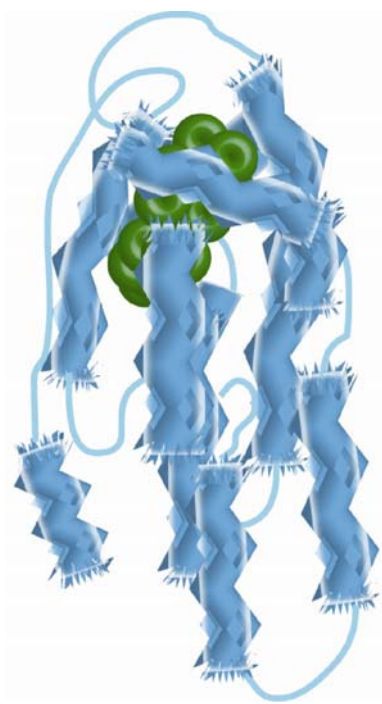

Fig. 2.19 Model of the androgen receptor binding pouch. Twelve α-helices (blue) open up a gap into which the steroid (green) can slip. Thereby one helix turns its angle and upregulates the activity of the entire receptor

men, about 17–29 glutamine repeats and 13–17 glycine repeats are present. Alleles of small GGC size have been associated with esophageal cancer, while in patients with Kennedy disease, a disease with degenerating motoneurons, up to 72 such glutamine repeats are present. Furthermore, in the androgen receptor long CAG and GGC alleles are associated with decreased transactivation function and have been associated with cancers in women. In the androgen receptor a low size CAG (<19 repeats) and GGC (<15 repeats) alleles result in higher receptor activity, and have been associated with earlier age of onset, and a higher grade and more advanced stage of prostate cancer at the time of diagnosis. The number of glutamine repeats of the androgen receptor has been associated with azoospermia or oligozoospermia but no clear association was found (Asatiani et al. 2003).

The androgen receptor gene (Fig. 2.18) encoding for two isoforms is located on the X-chromosome and spans about 90 kb and codes for a 2,757-base pair open reading frame within a 10.6-kb mRNA. The location of the androgen receptor gene on the X chromosome is preserved in evolutionary distant animals, such as marsupials and monotremes, and may reflect a developmentally significant association of the androgen

receptor gene with other systemic genes. The coding sequence, containing the sequence of nucleotides translated into amino acids, consists of eight exons. The N-terminal domain, the transcriptional regulatory region of the protein, is fully encoded by exon 1, while DNA-binding domain is encoded by exon 2 and 3 and steroid-binding domain by exons 4–8.

Similar to all the other steroid receptors, the DNA-binding domain of the androgen receptor contains two zinc fingers. This domain is about 70 amino acids long and is localized between the N-terminal and the androgen-binding domain. In this part of the sequence, eight cysteines are spatially arranged such that four sulphurs keep one zinc in place, giving rise to the typical structure of two overlapping helices. Exon 2 and 3 of the androgen receptor encode for the DNA-binding domain. The first zinc finger, encoded by exon 2, is important for the specific binding of the androgen receptor to the second and fifth nucleotide pairs in the first androgen-response element repeat GGTACA of the DNA. The second zinc finger, encoded by exon 3, stabilizes DNA receptor binding by hydrophobic interactions with the first finger and contributes to specificity of receptor DNA binding, leading to dimerization of two receptor molecules. In immediate proximity to the DNA binding domain, a short amino acid sequence is responsible for the transport of the receptor into the nucleus.

The androgen-binding domain of the androgen receptor encompasses about 30% of the overall receptor (the DNA-binding domain about 10%) and is responsible for the specific binding of androgens. This domain forms a lipophilic pocket based upon 24 amino acids that enables the binding of testosterone or other androgens (Fig. 2.19). Experimental deletion of this domain results in increased gene transcription in vitro.This part of the receptor is necessary for inducible and regulated gene transcription. Regulation of steroid hormone receptor action occurs, in part, by posttranslational modifications, such as phosphorylation. The androgen receptor is phosphorylated at a number of sites in response to agonist binding that results in nuclear localization, but usually not in response to antagonists. It has also been shown that phosphorylation is regulated by steroid hormones or forskolin and phorbol esters at different sites. DHT binds to the androgen receptor with higher affinity than testosterone, mainly because testosterone more quickly dissociates from the receptor again. Other steroids such as androstenedione, estradiol and progesterone bind to the androgen receptor with much lower affinity than testosterone. The phosphorylation in the hinge region required for full transcriptional activity is involved in the signal transduction mediated by cyclic AMP and protein kinase C.

Chaperones such as heat shock protein 90 (HSP 90) are responsible for the maintenance of the receptor in the inactive state and are released from the complex. Without its hormone ligand the androgen receptor can bind to homologous or heterologous ligands in the cytoplasm and exert inhibitory effects on cell death processes. The loss of this protein has uncovered functional domains of the receptor and is necessary for nuclear transport. The nuclear transport is mediated by import receptors through a pathway that employs two proteins, importin-α and importin-β. In this nuclear-protein-import pathway, the positively charged nuclear localization signal of the androgen receptor is recognized in the cytoplasm by importin-α that serves as an adaptor to the nuclear transport factor importin-β. The importin-α–importin-β androgen receptor complex then moves through nuclear-pore complexes and dissociates mediated by a GTPase protein, thus releasing the androgen receptor inside.

The actual interaction with the DNA occurs through the two zinc fingers in the DNA-binding domain of the androgen receptor. The androgen receptor interacts with DNA in a homodimeric form consisting of two identical hormone receptor complexes. These homodimers bind to DNA sequences known as androgen responsive elements which contain typical palindromic sequences, i.e., DNA tracts with a nucleotide sequence independent of the reading direction. Before it can bind to the DNA, covalent modifications of the DNA trapping proteins, the histones, have to occur first. At the N-terminal ends of histones various covalent modifications can be carried out, such as acetylation, phosphorylation, ubiquitination and methylation. Depending on the histone modifications, genes will be either expressed or silenced and the methylation of lysines or arginines has been linked to either transcriptional activation or repression. Lysine residues of the histone tails can be monomethylated, dimethylated or trimethylated. The differentially methylated lysine residues serve as binding sites for various effector proteins which may be co-repressor or co-activators.

The lysine-specific demethylase 1 (LSD1) has the function to silence genes if no nuclear receptor is present but in the opposite case to demethylate histones if such a ligand-activated receptor is docking onto the site. During this step, LSD1 forms a chromatin-associated complex with the ligand-activated androgen

receptor. Another co-activator is needed to start the actual gene expression of the LSD1 chromatin-associated ligand-activated androgen receptor complex. This is again a demethylase (JHDM2A) which is only associated with the DNA in combination with the above-metioned complex. Two different mechanisms involving demethylases in the transcriptional regulation of the androgen receptor are needed to express androgen target genes (Metzger et al. 2006). The time sequence of events is not known. Up to now many methylation/demethylation enzymes have been found which all may be needed for fine tuning of gene expression. The transcription complex induces mRNA synthesis of androgen-dependent genes and, after mRNA translation, synthesis of new androgen-dependent proteins such as prostate specific antigen (PSA).

The regulation of androgen receptor expression at the transcriptional and translational level is complex and depends on factors such as age, cell type and tissue. Generally, androgens have a positive effect on stabilization of the receptor protein, so that androgen administration leads to inhibition of receptor degradation and thereby to an increase in androgen receptor protein levels. The effects of androgens on the androgen receptor mRNA are opposite. In this case, androgen administration leads to down-regulation of the androgen receptor mRNA by shortening of the mRNA half-life. Current and future activities concentrate on **selective androgen receptor modulators (SARMs),** analogous to the SERMs for the estrogen receptor. This work leads to a general understanding of structure-activity relationships of new pharmacophors of nonsteroidal SARMs by structural modification of nonsteroidal antiandrogens.

SARM pharmacophores can be classified into four categories so far: aryl-propionamide, bicyclic hydantoin, quinoline, and tetrahydroquinoline analogs. A characteristic of these molecules is that they are not substrates for aromatase or 5α-reductase. The functions of these androgen receptor ligands are intended to act as agonists in anabolic organs (e.g., muscle and bone) but neutral in androgenic tissues (e.g., prostate and seminal vesicles). Other possible molecular mechanisms related to the tissue selectivity of SARMs include ligand-dependent changes in androgen receptor conformation, differential interaction with the promoter context of target tissue genes, and the differential recruitment of coregulators in these tissues. The development stages of these new compounds are now in preclinical animal trials or already in phase I clinical trials. The hope is that SARMs are of benefit for the treatment of primary or secondary hypogonadism, anemias, osteopenia or osteoporosis, frailty, BPH, and may be also of help in designing a hormonal male contraception method.

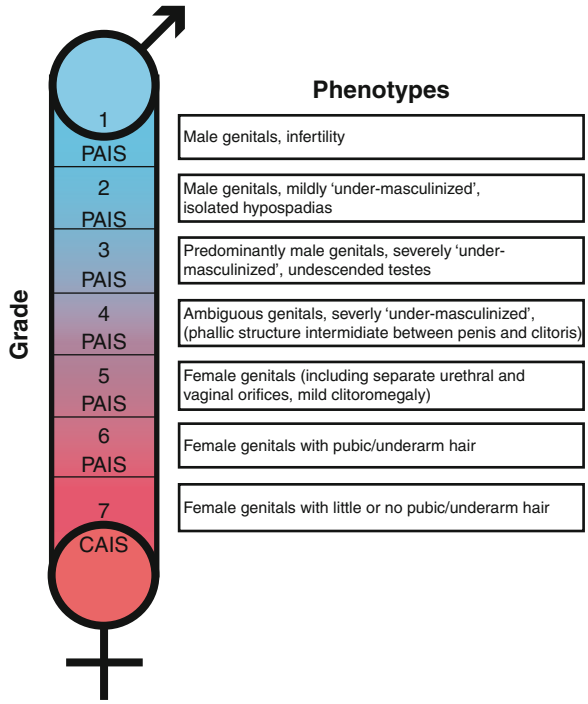

Fig. 2.20 Grading of the different severities of androgen insensitivity. The highest grade (7) is named CAIS (complete) while the lowest form is PAIS grade 1 (partial) (Adapted from Rajender et al. 2007)

Androgen receptor defects such as deletions or inactivating mutations can profoundly alter receptor function. The resulting phenotype is highly variable ranging from undervirilization to testicular feminization. Inactivating mutations of the androgen receptor gene in a 46 XY male with testes resulted in a female phenotype owing to the complete lack of all androgen activity (Fig. 2.20). However, there is no uterus and only a partially formed vagina, and during puberty pubic and axillary hair is scant or absent. This syndrome of complete androgen insensitivity (AIS) was earlier called testicular feminization.

Similar clinical consequences are also typical for mutations which severely damage the function of the androgen receptor, such as those in the DNA-binding or androgen-binding domain. **Partial AIS (PAIS)** is due to mutations in the androgen receptor gene and over 800

mutations have been reported (http://www.androgendb.mcgill.ca). Furthermore, mutations which involve co-activators or co-repressors can also lead to PAIS of different severity. Moreover, mutations in the N-terminal domain of the androgen receptor, which can lead to elimination of the androgen receptor function, plays a minor role in male idiopathic infertility (Zuccarello et al. 2008). The mentioned subtle differences in the number of repeats e.g., CAG, or TGG, GGC of ARgen have also been tested for spermatogenic effects. In spite of many efforts to demonstrate that the number of CAG triplets influences the transcriptional activity of the androgen receptor, no clear relationship to disturbances of spermatogenesis has been found in a wide variety of human ethnics (Rajender et al. 2007). Some oligozoospermic and azoospermic men bearing mutations in the ligand-binding domain have also been identified (Zuccarello et al. 2008). Over 120 mutations in the androgen receptor are associated with prostate carcinoma; both in the primary tumor tissue and in metastases and in very few other cases they have been associated with other cancers, such as testicular carcinoma, or liver and breast cancer.

2.6.5 Biological Actions of Androgens

In primates, the androgen receptor can be found not only in the classical androgen-dependent organs, such as muscles, prostate, seminal vesicles, epididymis and testes, but also in almost every tissue, e.g., hypothalamus, pituitary, kidney, spleen, heart, salivary glands. Hence, testosterone exerts a variety of actions on many body targets (Fig. 2.21)

In the testis, the androgen receptor is expressed in Sertoli cells, peritubular cells, and Leydig cells, while the germ cells seem not to express it. Studies in cell-specific androgen receptor knockout mice demonstrated that the Sertoli cells require androgen for the

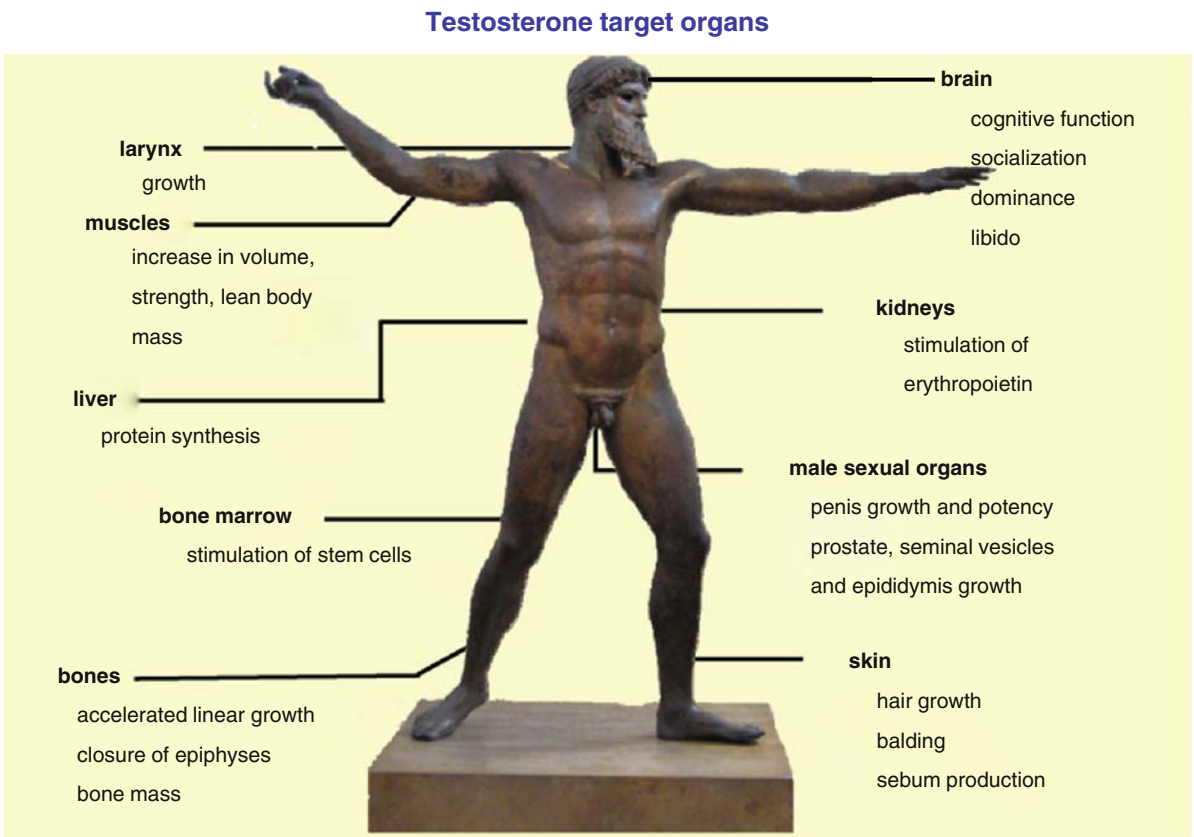

Fig. 2.21 Targets of testosterone action in the male

maintenance of complete spermatogenesis, and that spermatocyte and spermatid development depends on androgens. The peritubular myoid cells maintain their cell contractility, ensuring normal spermatogenesis and sperm output. A functional androgen receptor in Leydig cells is essential to maintain spermatogenesis and testosterone production, and is required for normal male fertility (Chang et al. 2004; Zhang 2006; Xu et al. 2007).

Androgens are important in every phase of human life. During the embryonal stage, testosterone determines the differentiation of the sexual organs, during puberty, the further development toward the adult male phenotype which is then maintained along with important anabolic functions. DHT is the main androgen acting on epididymis, vas deferens, seminal vesicles and prostate, originating from testosterone through 5α-reductase. These tissues are particularly dependent on continuous androgen action. In addition, testosterone aromatization to estrogens plays an important role in prostate growth. Estrogen concentrations in prostate stromal tissue are clearly increased in case of benign prostate hyperplasia (BPH). Estrogens, acting in synergy with androgens and the estrogen receptor ß, are required to regulate the proliferative and antiproliferative changes that occur during normal prostate development and differentiation. In the epididymis, the seminal vesicles and the vas deferens a lack of testosterone can result in regression of the secretory epithelia, eventually leading to aspermia (ejaculation failure). The androgen effects in these organs are mediated through testosterone, DHT and estradiol.

Both testosterone and DHT are necessary for normal penis growth, which is positively correlated with the increasing testosterone concentrations during puberty. The masculinization of Wolffian ducts is primarily caused by testosterone, whereas the transformation of the external genitalia, urethra, and prostate is primarily due to DHT. However, androgen receptors are no longer expressed in the penis of adult men and any androgen deficiency after puberty results in only minor decrease of penis size. Similarly, testosterone administration to adults is not capable of increasing penis size.

Testosterone is the main androgen present in muscles, which have very low 5α-reductase activity. Skeletal muscles are capable of converting circulating dehydroepiandrosterone (DHEA) to testosterone and estrogen. Furthermore it is discussed whether skeletal muscle cells even synthesize DHT which activates the glucose metabolism-related signaling pathway. Testosterone has direct anabolic effects both on smooth and striated muscles with an increase of muscular mass and hypertrophy of the fibers. In modern sports these effects have led to an abuse of these steroids to increase the muscle mass in both sexes. The number of muscular fibers, however, does not change. Loss of testosterone can lead to muscular atrophy. As a consequence of testosterone action, mRNA synthesis and glycogen synthesis increase in the striated muscles. Testosterone also has an anabolic effect the heart, increasing mRNA synthesis.

Both androgens and estrogens induce an increase of bone density by stimulating mineralization, while the lack of these steroids results in osteoporosis. The skeleton develops distinctly in males and females, particularly at the periosteal surface. Sex differences in skeletal morphology and physiology occur at or around puberty, with little effect of gonadal steroids prior to puberty. At the beginning of puberty, the increase in linear growth of bones is directly correlated with increasing testosterone concentrations. It is discussed that gender differences, particularly with respect to ‘bone quality’ and architecture (i.e., predominantly bone width) are modulated by the balance of the sex steroids estrogen and androgen. At the end of puberty, depending on the presence of testosterone, epiphyseal closure occurs, an event that can be consistently delayed in the presence of low testosterone concentrations. Low testosterone is associated with increased risk of fracture, particularly with hip and nonvertebral fractures. It is clear now that the androgen action on bone metabolism is mediated through estradiol. Serum estradiol and testosterone contribute to bone turnover rate, with testosterone increasing bone formation, and estradiol suppressing both bone formation and resorption. Conversely, the administration of high testosterone doses can induce precocious epiphyseal closure. This effect can be used therapeutically in cases of excessively tall stature. Moreover, serum SHBG seems to be an independent positive predictor of bone turnover rate (Valimaki et al. 2004).

The effect of androgens on skin and dependent organs vary in the different cutaneous districts and are mediated by testosterone and, probably, DHT. Depending on testosterone, the growth of sebaceous glands can be stimulated and **sebum production** in the face, upper part of the back and in the skin of the chest can be induced. Testosterone contributes to the development of acne vulgaris, while estrogens can diminish

sebum concentration. The effects of DHT and testosterone on the **hair** are influenced by the androgen sensitivity of the hair follicle. DHT stimulates the expression of nitric oxide synthase in human dermal papilla cells, suggesting that nitric oxide works as a signaling molecule and implys androgen-mediated nitric oxide production to be involved in the regulation of hair follicle activity. While **axillary hair** and the lower part of **pubic hair** start growing even in the presence of low androgen concentrations, much higher androgen levels are necessary for the growth of **beard,** upper part of the pubic hair and chest hair. The hairline is determined both by genetic factors and individual distribution of the androgen receptor and depends on the androgen milieu. High 5α-reductase activity has been observed in bald men, while in patients with 5α-reductase deficiency or hypogonadism there is no regression of the hair line. Since the growth of the scalp hair is related to increased 5α-reductase activity, increased activity of this enzyme with consequences for hair loss could be an expression of the precocious aging of the hair follicles. Androgens stimulate hair follicles to alter hair color and size via the hair growth cycle and seem to reduce alopecia (Randall et al. 2008).

During puberty, there is a testosterone-dependent growth of the length of the *larynx* of about 1 cm. This size increase, together with the length and mass of the vocal cords, leads to a lowering of vocal register. To maintain a high soprano voice, young males in the sixteenth–nineteenth century were castrated before puberty. A deep voice is directly related to androgens so that a lower register can also be induced in women by testosterone treatment. The depth of voice in a man is correlated with the duration of the pubertal phase after which the androgen receptors are lost. Once reached, register remains unchanged and no modification of the voice can be obtained after puberty in hypogonadal patients. The gender-specific change of the vocal register is correlated with the degree of mineralization in human thyroid cartilage. This modification is linked with the expression of the enzyme alkaline phosphatase. Few chondrocytes near the mineralization front are positive for the androgen receptor and also for alkaline phosphatase, suggesting an involvement in androgen mediated thyroid cartilage mineralization (Claassen et al. 2006).

In the **central nervous system** (CNS) testosterone can be either aromatized or reduced to DHT. The individual activities of the different enzymes and distribution of receptors are not homogeneous in the CNS, but rather vary according to the brain region. During the intrauterine period a boy's brain develops in the male direction induced by testosterone, and in a girl in the female direction through its absence. The gender identity, the sexual orientation, and other CNS controlled behaviors are programmed at this early period of development. Androgens are also important for other male characteristics such as aggressive behavior, initiative and concentration capacities. Women have different mechanisms to exert these character traits. A connection with spatial orientation and mathematical and composition skills are still under discussion. There is a close relationship between androgen milieu and normal corporeal and spiritual performance and activity as well as good general mood and self-confidence. The frequency and presence of sexual phantasies, morning erections, frequency of masturbation or copulation and sexual activity are related to blood testosterone concentrations in the normal-to-subnormal range. Conversely, androgen deficiency is often accompanied by loss of interest, lethargy, depressive mood, loss of libido and sexual inactivity. Furthermore in the adult, testosterone in the brain is neuroprotective. Although the role of testosterone in the CNS is still poorly understood, evidence suggests that testosterone could be helpful in the treatment of cognitive diseases, including dementia and may influence motor neuron regeneration in adulthood. Testosterone appears to activate a distributed cortical network and addition of testosterone may improve spatial cognition in younger and older hypogonadal men. As already mentioned reduced testosterone is associated with depressive disorders and depends upon the androgen receptor genotype (Zitzmann 2006).

The function of the **liver** is also influenced by steroid hormones. A sexual dimorphism is known both for protein synthesis in the liver and for many liver enzyme systems. This is reflected by the existence of different reference ranges for hepatic enzymes depending on the sex. As far as protein production is concerned, estrogens and testosterone/DHT have often antagonist effects, e.g., on SHBG synthesis. Testosterone and estrogens, however, can also have synergistic effects, for example on α-1-antitrypsin.

The influence of androgens on the **hematopoietic system** is twofold. Through the androgen-dependent, receptor-mediated erythropoietin synthesis there is a

stimulation of erythrocyte production. Androgens also directly affect the hematopoietic stem cells and lead to increased synthesis of hemoglobin. These effects can also be demonstrated in vitro on the granulopoietic and thrombopoietic stem cells, although the role of androgens in this field is still unclear.

There is substantial evidence that androgens may play a role in determining sex-specific **blood pressure.** It is well known that sclerosis of the coronary arteries is one of the most frequent causes of death in Western industrialized countries and that the risk of contracting this disease is twice as high in men compared to women. For this sex-dependent disease, differences in hormone secretion between sexes are supposed. Androgen binding sites can be found in the area postrema and in the preoptic region, suggested to regulate in part blood pressure and heart rate. Therefore, sex-dependent differences in blood pressure might partly result from a sexually dimorphic pattern of the brain which determines central blood pressure regulation.

Suppression of testosterone concentrations in serum leads to a subsequent increase in high density lipoprotein (HDL) concentrations. Influences of testosterone and estradiol on coagulation and fibrinolysis are also discussed. Androgen deficiency is associated with an elevation of the levels of plasminogen activator type 1 which, in turn, can lead to decreased fibrinolysis. While testosterone treatment seems to decrease vascular tone but the long-term effects appear to be vasoconstriction via upregulation of thromboxane A2 expression, norepinephrine synthesis, angiotensin II expression, and endothelin-1 action. Furthermore, androgens are discussed to affect atherosclerosis, vascular remodelling and induce renal prohypertensive processes involving the renin-angiotensin-aldosterone system. The effects of sex hormones on different parts of the renal-vascular system are often contradictory.

Metabolic syndrome is associated with weight gain, diabetes type II and androgen deficiency in men. Cross-sectional studies have found that between 20% and 64% of men with diabetes have hypogonadism, with higher prevalence rates found in aging men. The mechanisms leading to these diseases include change in body composition, androgen receptor polymorphisms, glucose transport, and reduced antioxidant effects. Conversely, diabetes and the metabolic syndrome are also risk factors for hypogonadism through similar mechanisms, body weight change, decreased sex hormone binding globulin levels, suppression of testosterone production, and increased aromatase activity contributing to relative estrogen excess.

2.6.6 Androgen Secretion and Sexual Differentiation

Sexual dimorphism precedes gonadal development, in a pregonadal stage. Sex determination is primarily under genetic control and is mediated by the "sex determining gene on the Y chromosome" (Sry) and other transcription factors (Fig. 2.21). It has been shown that the Sry gene and others are already expressed at the one-cell stage and also in the blastocyst stage, enabling these genes to trigger the sex determination cascade. Reproductive development is driven by the production of hormones in the male, or their absence in the female. These factors govern testicle formation and male germ cell development but this gene is neither necessary nor sufficient for testis induction. There is growing evidence supporting the hypothesis that the gene SOX-9 (SRY HMG-box-related gene 9) is necessary as the testis inducer gene. 46XY individuals with the condition of camptomelic syndrome caused by haploinsufficiency of SOX-9 show a partial sex reversal. Duplication of SOX-9 is coupled to masculinization in an XX fetus, and mouse XX Sox-9 transgenics develop as males. SOX-9 regulates Fgf9 expression and Fgf9 is likely to be required to promote the male pathway and suppress the female pathway. Contrary to what was long believed, female development does not occur by default while male development is induced. In fact, a female sex-determining gene is predicted (Fig. 2.22).

Sexual differentiation refers to the hormonal induction and regulation of the maturation of the secondary reproductive organs. During the 1st weeks of gestation, the external genitalia are indistinguishable between the sexes and have the potential of developing in the female or in the male direction. Under the influence of DHT, differentiation of the bipotent structures starts at the 8th week of gestation towards the male phenotype. Hormonal production of differentiated gonads is relevant for differentiation of the internal and external genitalia during fetal life. Antimullerian hormone (AMH) secreted by Sertoli cells inhibits the development of female internal genitalia (tube, uterus,

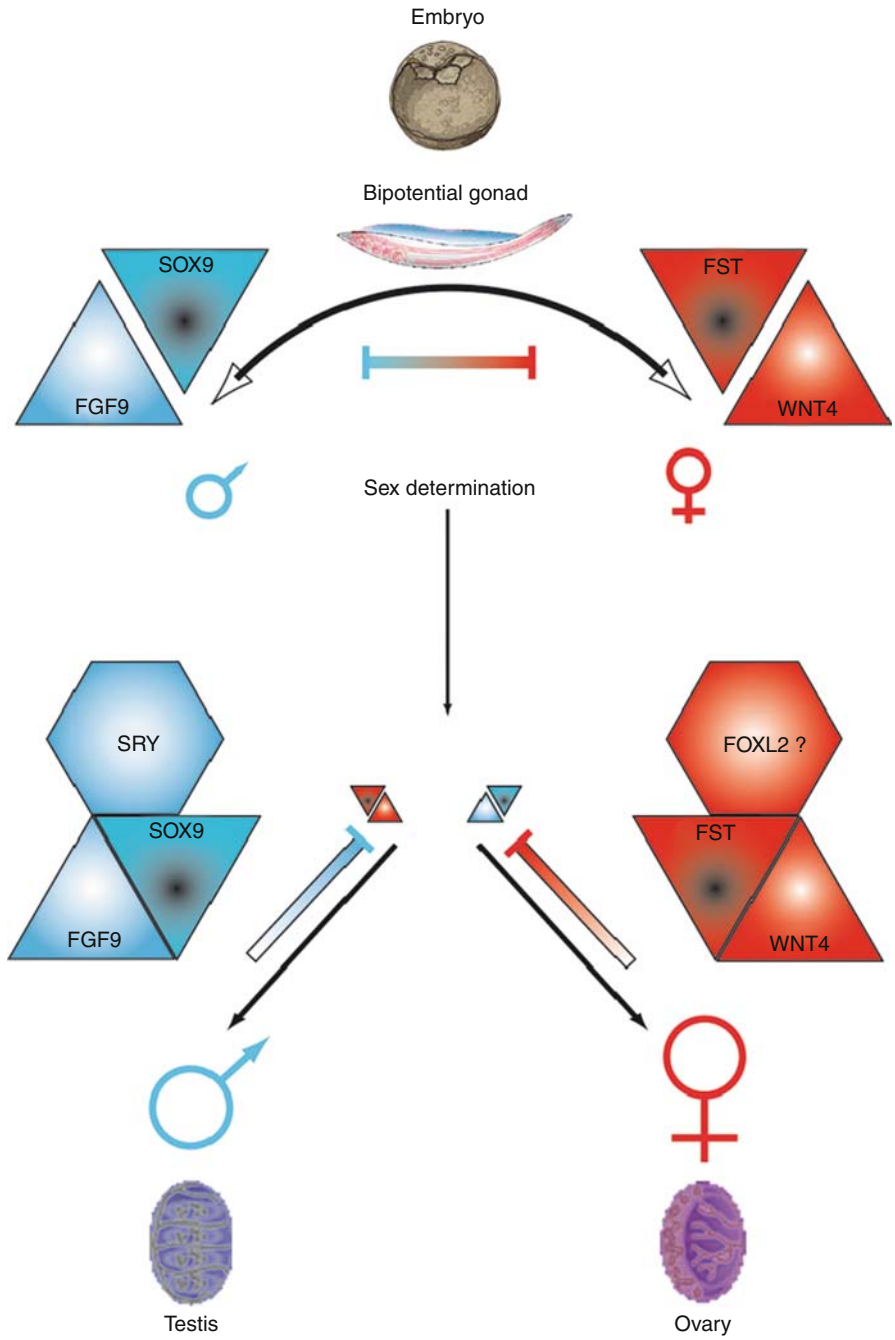

Fig. 2.22 Mammalian sex determination. The male-promoting (SOX9 and FGF9) and female-promoting (WNT4 and possibly FST) genes keep the gonad in a bipotential state. In the presence of SRY expression the balance to the male pathway changes and out-competes the female signals.In the absence of SRY and possibly the presence of FOXL2, the female-promoting signals shut down these signals and ovarian differentiation is induced

upper part of vagina). Testosterone secreted by Leydig cells induces stabilization of Wolffian ducts and development of internal male genitalia. Differentiation of external male genitalia and the prostate requires the transformation of testosterone to dihydrotestosterone by 5-α-reductase type 2 expressed in genital skin and the urogenital sinus. DHT stimulates prostate differentiation from this sinus genitalis. After the end of the differentiation process, at about week 14 of gestation, DHT influences the continuous growth of the external sexual organs, in particular the penis. Fetal testosterone production is maximal at the moment of differentiation of the external sexual organs. This occurs between weeks 9 and 14 of gestation, after

which testosterone production decreases. The production of testicular testosterone seems to be mainly induced by hCG, but after the second gestation week, fetal LH assumes the stimulation of the Leydig cells.

In the male newborn, serum testosterone concentrations are comparable to those found in normal adults, decrease then at the end of the 1st week of life, increase again in the 2nd month of life to a new height and fall by the 6th month of life to very low levels such as those found in female babies. Up to the 7th year of life, androgen concentrations in serum are very low, although there is a remarkable increase of free testosterone levels. During this phase, Leydig cells can be stimulated to produce testosterone through the exogenous administration of hCG.

At the age of about 7 years androgen production starts increasing, first due to increased secretion of dehydroepiandrosterone by the adrenal gland (adrenarche). At the age of about 10 years gonadotropin secretion starts, first with pulsatile discharge of LH by the pituitary only during sleeping hours. This nocturnal LH pulsatility becomes progressively evident, also during the day, in the course of pubertal maturation until the pattern typical for the adult man is reached. FSH also starts increasing, along with basal LH and testosterone serum concentrations. The clinical modifications during puberty follow the hormonal modifications in a characteristic sequence.

Normal testosterone concentrations of young men in peripheral serum range between 12 and 30 nmol/l. Testosterone concentrations in blood follow a circadian rhythm with higher levels in the morning hours and about 25% lower levels in the evening. In the aging man a decline of free serum testosterone concentrations occurs but simultaneously the SHBG levels increase. In the case of a strong decrease of testosterone some men develop symptoms of late-onset hypogonadism with clinical consequences such as frailty, changes in body composition, cardiovascular disease, sexual dysfunction and osteoporosis.

References

Alastalo TP, Lonnstrom M, Leppa S, Kaarniranta K, Pelto-Huikko M, Sistonen L, Parvinen M (1998) Stage-specific expression and cellular localization of the heat shock factor 2 isoforms in the rat seminiferous epithelium. Exp Cell Res 240:16–27

Albrecht M, Frungieri MB, Gonzalez-Calvar S, Meineke V, Köhn FM, Mayerhofer A (2005) Evidence for a histaminergic system in the human testis. Fertil Steril 83:1060–1063

Albrecht M, Rämsch R, Köhn FM, Schwarzer JU, Mayerhofer A (2006) Isolation and cultivation of human peritubular cells: A novel model for investigation of fibrotic processes in the human testis and male infertility. J Clin Endocrinol Metab 81:1956–1960

Amann RP (2008) The cycle of the seminiferous epithelium: A need to revisit? J Androl 29:469–487

Amory JK, Page ST, Anawalt BD, Coviello AD, Matsumoto AM, Bremner WJ (2007a) Elevated end-of-treatment serum INSL3 is associated with failure to completely suppress spermatogenesis in men receiving male hormonal contraception. J Androl 28:548–554

Amory K, Wang C, Swerdlof RS, Anawalt BD, Matsumoto AM, Bremner WJ, Walker SE, Haberer LJ, Clark RV (2007b) The effect of 5α-reductase inhibition with dutasteride and finasteride on semen parameters and serum hormones in healthy men. J Clin Metab Endocrinol 92:1659–1665

Anand-Ivell RJK, Relan V, Balvers M, Coiffec-Dorval I, Fritsch M, Bathgate RAD, Ivell R (2006) Expression of the insulin-like peptide 3 (INSL3) hormone-receptor (LGR8) system in the testis. Biol Reprod 74:945–953

Apa DD, Cayan S, Polat A, Akbay E (2002) Mast cells and fibrosis on testicular biopsies in male infertility. Arch Androl 48:337–344

Asatiani K, von Eckardstein S, Simoni M, Gromoll J, Nieschlag E (2003) CAG repeat length in the androgen receptor gene affects the risk of male infertility. Int J Androl 26: 255–261

Aslam H, Rosiepen G, Krishnamurthy H, Arslan M, Clemen G, Nieschlag E, Weinbauer GF (1999) The cycle duration of the seminiferous epithelium remains unaltered during GnRH antagonist-induced testicular involution in rats and monkeys. J Endocrinol 161:281–288

Bay K, Ivell R, Schumacher M, Jürgensen D, Jorgensen N, Holm M, Skakkebaek NE, Andersson AM (2005) Insulin-like factor 3 serum levels in 135 normal men and 85 men with testicular disorders. Relationship to the luteinizing hormone-testosterone axis. J Clin Endocrinol Metab 90: 3410–3418

Bay K, Matthiesson KL, McLachlan RI, Andersson AM (2006) The effects of gonadotropin suppression and selective replacement on insulin-like factor 3 secretion in normal adult men. J Clin Endocrinol Metab 91:1108–1111

Berensztein EB, Baquedano MS, Gonzalez CR, Saraco NI, Rodriguez J, Ponzio R, Rivarola MA, Belgorosky A (2006) Expression of aromatase, estrogen receptor α and β, androgen receptor, and cytochrome P-450 in the human early prepubertal testis. Ped Res 60:740–744

Bertrand S, Brunet FG, Escriva H, Parmentier G, Laudet V, Robinson-Rechavi M (2004) Evolutionary genomics of nuclear receptors: From twenty-five ancestral genes to derived endocrine systems. Mol Biol Evol 21:1923–1937

Boepple PA, Hayes FJ, Dwyer AA, Raivio T, Lee H, Crowley WF Jr, Pitteloud N (2008) Relative roles of inhibin B and sex steroids in the negative feedback regulation of follicle-stimulating hormone in men across the full spectrum of seminiferous epithelium function. J Clin Endocrinol Metab 93:1809–1814

Boime I, Ben-Menahem D (1999) Gycoprotein hormone structure-function and analog design. Recent Prog Horm Res 54: 271–288

Bose M, Whittal RM, Miller WL, Bose HS (2008) Steroidogenic activity of StAR requires contact with mitochondrial VDAC1 and PCP. J Biol Chem 283:8837–8845

Bouloux PMG, Nieschlag E, Burger HG, Skakkebaek NE, Wu FCW, Handelsman DJ, Baker GHW, Ochsenkühn R, Syska A, McLachlan RI, Giwercman A, Conway AJ, Turner L, van Kuijk JHM, Voortman G (2003) Induction of spermatogenesis by recombinant follicle-stimulating hormone (Puregon) in hypogonadotropic azoospermic men who failed to respond to human chorionic gonadotropin alone. J Androl 24:604–611

Brehm R, Zeiler M, Rüttinger C, Herde K, Kibschull M, Winterhager E, Willecke K, Guillou F, Lecureuil C, Steger K, Konrad L, Biermann K, Failing K, Bergmann M (2007) A sertoli cell-specific knockout of connexin43 prevents initiation of spermatogenesis. Am J Pathol 171:19–31

Burger LL, Haisenleder DJ, Dalkin AC, Marshall JC (2004) Regulation of gonadotropin subunit gene transcription. J Mol Endocrinol 33:559–584

Carreau S, Delalande C, Silandre D, Bourguiba S, Lambard S (2006) Aromatase and estrogen receptors in male reproduction. Mol Cell Endocrinol 246:65–68

Cayan S, Apa DD, Akbay E (2002) Effect of fexofenadine, a mast cell blocker, in infertile men with significantly increased testicular mast cells. Asian J Androl 4:291–294

Chang C, Chen YT, Yeh SD, Xu Q, Wang RS, Guillou F, Lardy H, Yeh S (2004) Infertility with defective spermatogenesis and hypotestosteronemia in male mice lacking the androgen receptor in Sertoli cells. Proc Natl Acad Sci USA 101:6876–81

Cheng CK, Leung PC (2005) Molecular biology of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and their receptors in humans. Endocr Rev 26:283–306

Claassen H, Monig H, Sel S, Werner JA, Paulsen F (2006) Androgen receptors and gender-specific distribution of alkaline phosphatase in human thyroid cartilage. Histochem Cell Biol 126:381–388

Coviello AD, Matsumoto AM, Bremner WJ, herbst KL, Amory JK, Anawalt BD, Sutton PR, Wright WW, Brown TR, Yan X, Zirkin BR, Jarow JP (2005) Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. J Clin Endocrinol Metab 90:2595–2602

d'Anglemont de Tassigny X, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG, Zahn D, Franceschini I, Caraty A, Carlton MB, Aparicio SA, Colledge WH (2007) Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. Proc Natl Acad Sci USA 104:10714–10719

de Rooij DG (1998) Stem cells in the testis. Int J Exp Pathol 79:67–80

De Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci USA 100:10972–10976

Devi YS, Sarda K, Stephen B, Nagarajan P, Majumdar SS (2006) Follicle-stimulating hormone-independent functions of primate Sertoli cells: Potential implications in the diagnosis and management of male infertility. J Clin Endocrinol Metab 91:1062–1068

Dube E, Chan PTK, Hermo L, Cyr DG (2007) Gene expression profiling and its relevance to the blood-testis-barrier in the human epididymis. Biol Reprod 76:1034–1044

Dufourny L, Caraty A, Clarke IJ, Robinson JE, Skinner DC (2005) Progesterone-receptive dopaminergic and neuropeptide Y neurons project from the arcuate nucleus to gonadotropin-releasing hormone-rich regions of the ovine preoptic area. Neuroendocrinology 82:21–31

Eacker SM, Agrawal N, Qian K, Dichek HL, Gong EY, Lee K, Braun RE (2008) Hormonal regulation of testicular steroid and cholesterol homeostasis. Mol Endocrinol 22:623–635

Ehmcke J, Schlatt S (2006) A revised model for spermatogonial expansion in man: lessons from non-human primates. Reproduction 132:673–680

Ehmcke J, Luetjens CM, Schlatt S (2005) Clonal organization of proliferating spermatogonial stem cells in adult males of two species of non-human primates, *Macaca mulatta* and *Callithrix jacchus*. Biol Reprod 72:293–300

Ehmcke J, Wistuba J, Schlatt S (2006) Spermatogonial stem cells: Questions, models and perspectives. Hum Reprod Update 12:275–282

Elhija MA, Potashnik H, Lunenfeld E, Potashnik G, Schlatt S, Nieschlag E, Huleihel M (2005) Testicular interleukin-6 response to systemic inflammation. Eur Cytokine Netw 16:167–172

Emmen JM, McLuskey A, Adham IM, Engel W, Grootegoed JA, Brinkmann AO (2000) Hormonal control of gubernaculum development during testis descent: Gubernaculum outgrowth in vitro requires both insulin-like factor and androgen. Endocrinology 141:4720–4727

Ergün S, Stingl J, Holstein AF (1994a) Segmental angioarchitecture of the testicular lobes in man. Andrologia 26: 143–150

Ergün S, Stingl J, Holstein AF (1994b) Microvasculature of the human testis in correlation to Leydig cells and seminiferous tubules. Andrologia 26:255–262

Evans JJ (1999) Modulation of gonadotropin levels by peptides acting at the anterior pituitary gland. Endocr Rev 20:46–67

Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N (2008) Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest [Epub ahead of print]

Fan QR, Hendrickson WA (2005) Structure of human follicle-stimulating hormone in complex with its receptor. Nature 433:269–277

Feng S, Bogatcheva NV, Truong A, Engel W, Adham IM, Agoulnik AI (2006) Over expression of insulin-like 3 does not prevent cryptorchidism in GNRHR or HOXA10 deficient mice. J Urol 176:399–404

Ferlin A, Arredi B, Zuccarello D, Garolla A, Selice R, Foresta C (2006) Paracrine and endocrine roles of insulin-like factor 3. J Endocrinol Invest 29:657–664

Ferris HA, Shupnik MA (2006) Mechanisms for pulsatile regulation of the gonadotropin subunit genes by GNRH1. Biol Reprod 74:993–998

Fijak M, Meinhardt A (2006) The testis immune privilege. Immunol Rev 213:66–81

Foresta C, Bettella A, Vinanzi C, Dabrilli P, Meriggiola MC, Garolla A, Ferlin A (2004) A novel circulating hormone of testis origin in humans. J Clin Endocrinol Metab 89: 5952–5958

Franca LR, Ogawa T, Avarbock MR, Brinster RL, Russell LD (1998) Germ cell genotype controls cell cycle during spermatogenesis in the rat. Biol Reprod 59:1371–1377

Garone LM, Ammannati E, Brush TS, Fischer DJ, Tos EG, Luo J, Altobello KL, Ciampolillo C, Ihley TM, Kurosawa E, Tiebout A, McKenna S (2006) Biological properties of a novel follicle-stimulating hormone/human chorionic gonadotropin chimeric gonadotropin. Endocrinology 147:4205–42012

Ge R, Hardy MP (2007) Regulation of Leydig cells during pubertal development. In: Payne AH, Hardy MP (eds) The Leydig cell in health and disease. Humana Press, Totowa, pp 55–70

Gilfillan AM, Tkaczyk C (2006) Integrated signalling pathways for mast-cell activation. Nat Rev Immunol 6:218–230

Gores PF, Hayes DH, Copeland MJ, Korbutt GS, Halberstadt C, Kirkpatrick SA, Rajotte RV (2003) Long-term survival of intratesticular porcine islets in nonimmunosuppressed beagles. Transplantation 75:613–618

Grigorova M, Punab M, Ausmees K, Laan M (2008) FSHB promoter polymorphism within evolutionary conserved element is associated with serum FSH level in men. Hum Reprod 23:2160–2166

Gromoll J, Simoni M (2005) Genetic complexity of FSH receptor function.Trends Endocrinol Metab 16:368–373

Gromoll J, Simoni M, Nieschlag E (1996) An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. J Clin Endocrinol Metab 81:1367–1370

Hales DB (2007) Regulation of Leydig cell functions as it pertains to the inflammatory response. In: Payne AH, Hardy MP (eds) The Leydig cell in health and disease. Humana Press, Totowa, pp 305–322

Hammes A, Andreassen TK, Spoelgen R, Raila J, Hubner N, Schulz H, Metzger J, Schweigert FJ, Luppa PB, Nykjaer A, Willnow TE (2005) Role of endocytosis in cellular uptake of sex steroids. Cell 122:751–762

Hayes FJ, Crowley WF Jr (1998) Gonadotropin pulsations across development. Horm Res 49:163–168

Hedger MP (2002) Marcophages and the immune responsiveness of the testis. J Reprod Immunol 57:19–34

Hibi H, Kato K, Mitsui K, Taki T, Yamada Y, Honda N, Fukatsu H, Yamamoto M (2002) Treatment of oligoasthenozoospermia with tranilast, a mast cell blocker, after long-term administration. Arch Androl 48:451–459

Holstein AF, Maekawa M, Nagano T, Davidoff MS (1996) Myofibroblasts in the lamina propria of human seminiferous tubules are dynamic structures of heterogenous phenotype. Arch Histol Cytol 59:109–125

Huhtaniemi I (1996) Ontogeny of the luteinizing hormone action in the male. In: Payne ADH, Hardy MP, Russell LD (eds) The Leydig Cell. Cache River Press, Vienna, pp 365–382

Huhtaniemi I, Jiang M, Nilsson C, Petterson K (1999) Mutations and polymorphisms in gonadotropin genes. Mol Cell Endocrinol 151:89–94

Isaac JR, Skinner S, Elliot R, Salto-Tellez M, Garkavenko O, Khoo A, Lee KO, Calne R, Wang DZ (2005) Transplantation of neonatal porcine islets and sertoli cells into nonimmunosuppressed nonhuman primates. Transplant Proc 37:487–488

Jarow JP, Chen H, rosner TW, Trentacoste S, Zirkin BR (2001) Assessment of the androgen environment within the human testis: Minimally invasive method to obtain intratesticular fluid. J Androl 22:640–645

Jia Y, Hikim AP, Lue YH, Swerdloff RS, Vera Y, Zhang XS, Hu ZY, Li YC, Liu YX, Wang C (2007) Signaling pathways for germ cell death in adult cynomolgus monkeys (*Macaca fascicularis*) induced by mild testicular hyperthermia and exogenous testosterone treatment. Biol Reprod 77:83–92

Johnson L, McKenzie KS, Snell JR (1996) Partial wave in human seminiferous tubules appears to be a random occurrence. Tissue Cell 28:127–136

Junaidi A, Luetjens CM, Wistuba J, Kamischke A, Yeung CH, Simoni M, Nieschlag E (2005) Norethisterone enanthate has neither a direct effect on the testis nor on the epididymis: A study in adult male cynomolgus monkeys (*Macaca fascicularis*). Eur J Endocrinol 152:655–661

Jung A, Schuppe HC (2007) Influence of genital heat stress on semen quality in humans. Andrologia 39:203–215

Kinniburgh D, Anderson RA, Baird DT (2001) Suppression of spermatogenesis with desogestrel and testosterone pellets is not enhanced by addition of finasteride. J Androl 22:88–95

Kim SH, Hu Y, Cadman S, Bouloux P (2008) Diversity in fibroblast growth factor receptor 1 regulation: Learning from the investigation of Kallmann syndrome. J Neuroendocrinol 20:141–163

Kishimoto M, Fujiki R, Takezawa S, Sasaki Y, Nakamura T, Yamaoka K, Kitagawa H, Kato S (2006) Nuclear receptor mediated gene regulation through chromatin remodeling and histone modifications. Endocr J 53:157–172

Kossack N, Simoni M, Richter-Unruh A, Themmen AP, Gromoll J (2008) Mutations in a novel, cryptic exon of the luteinizing hormone/chorionic gonadotropin receptor gene cause male pseudohermaphroditism. PLoS Med 5:e88

Laron Z, Klinger B (1998) Effect of insulin-like growth factor –I on serum androgens and testicular and penile size in males with Laron syndrome (primary growth hormone resistance). Eur J Endocrinol 138:176–180

Lei ZM, Mishra S, Zou W, Xu M, Foltz M, Li X, Rao CV (2001) Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. Mol Endocrinol 15:184–200

Lerchl A, Keck C, Spiteri-Grech J, Nieschlag E (1993) Diurnal variations in scrotal temperature of normal men and patients with varicocele before and after treatment. Int J Androl 16:195–200

Levalle O, Zylbersztein C, Aszpis S, Aquilano D, Terradas C, Colombani M, Aranda C, Scaglia H (1998) Recombinant human follicle-stimulating hormone administration increases testosterone production in men, possibly by a Sertoli cell-secreted nonsteroid factor. J Clin Endocrinol Metab 83: 3973–3976

Li C, Chen P, Smith MS (1999) Morphological evidence for direct interaction between arcuate nucleus neuropeptide Y (NPY) neurons and gonadotropin-releasing hormone neurons and the possible involvement of NPY Y1 receptors. Endocrinology 140:5382–5390

Lindstedt G, Nystrom E, Matthews C, Ernest I, Janson PO, Chatterjee K (1998) Follitropin (FSH) deficiency in an infertile male due to FSHbeta gene mutation. A syndrome of normal puberty and virilization but underdeveloped testicles with azoospermia, low FSH but high lutropin and normal serum testosterone concentrations. Clin Chem Lab Med 36:663–665

Lofrano-Porto A, Casulari LA, Nascimento PP, Giacomini L, Naves LA, da Motta LD, Layman LC (2007) Effects of follicle-stimulating hormone and huma chorionic gonadotropin on gonadal steroidogenesis in two sibling with a follicle-stimulating hormone beta subunit mutation. Fertil Steril [Epub ahead of print]

Lopez FJ, Merchenthaler IJ, Moretto M, Negro-Vilar A (1998) Modulating mechanisms of neuroendocrine cell activity: The LHRH pulse generator. Cell Mol Neurobiol 18: 125–146

Lösel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K, Wehling M (2003) Nongenomic steroid action: Controversies, questions, and answers. Physiol Rev 83:965–1016

Luetjens CM, Weinbauer GF, Wistuba J (2005) Primate spermatogenesis: New insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. Biol Rev 80:475–488

Luetjens CM, Didolkar A, Kliesch S, Paulus W, Jeibmann A, Böcker W, Nieschlag E, Simoni M (2006) Tissue expression of the nuclear progesterone receptor in male non-human primates and men. J Endocrinol 189:529–539

Maffei L, Murata Y, Rochira V, Tubert G, Aranda C, Vazquez M, Clyne CD, Davis S, Simpson ER, Carani C (2004) Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: Effects of testosterone, alendronate, and estradiol treatment. J Clin Endocrinol Metab 89:61–70

Marshall GR, Ramaswamy S, Plant TM (2005) Gonadotropin-independent proliferation of the pale type A spermatogonia in the adult rhesus monkey (*Macaca mulatta*). Biol Reprod 73:222–229

Matthiesson KL, Amory JK, Berger R, Ugoni A, McLachlan RI, Bremner WJ (2005a) Novel male hormonal contraceptive combinations: the hormonal and spermatogenic effects of testosterone and levonorgestrel comobined with 5α-reductase inhibitor or gonadotropin-relasing hormone antagonist. J Clin Endocrinol Metab 90:91–97

Matthiesson KL, Stanton PG, O'Donnell L, Meachem SJ, Amory JK, Berger R, Bremner WJ, Mclachlan RI (2005b) Effects of testosterone and levonorgestrel combined with a 5α-reductase inhibitor or gonadotropin-releasing hormone antagonist on spermatogenesis and intratesticular steroid levels in normal men. J Clin Endocrinol Metab 90:5647–5655

Matthiesson KL, McLachlan RI, O'Donnell L, Frydenberg M, Robertson DM, Stanton PG, Meachem SJ (2006) The relative roles of follicle-stimulating hormone and luteinizing hormone in maintaining spermatogonial maturation and spermiation in normal man. J Clin Endocrinol Metab 91: 3962–3969

Mayerhofer A, Frungieri MB, Fritz S, Bulling A, Jessberger B, Vogt HJ (1999) Evidence for catecholaminergic, neuronlike cells in the adult human testis: Changes associated with testicular pathologies. J Androl 20:341–317

Meachem S, Nieschlag E, Simoni M (2001) Inhibin B in male reproduction: Pathophysiology and clinical relevance. Eur J Endocrinol 145:561–571

Metzger E, Wissmann M, Schule R (2006) Histone demethylation and androgen-dependent transcription. Curr Opin Genet Dev 16:513–517

Millar RP (2005) GnRHs and GnRH receptors. Anim Reprod Sci 88:5–28

Millar RP, Pawson AJ, Morgan K, Rissman EF, Lu ZL (2008) Diversity of actions of GnRHs mediated by ligand-induced selective signaling. Front Neuroendocrinol 29:17–35

Miller WL (2007) StAR search-what we know about how the steroidogenic acute regulatory protein mediates mitochondrial cholesterol import. Mol Endocrinol 21:589–601

Modi DN, Shah C, Puri CP (2007) Non-genomic membrane progesterone receptors on human spermatozoa. Soc Reprod Fertil(Suppl 63):515–529

Moyle WR, Campbell, RK (1995) Gonadotropins. In: DeGroot JL, Besser M, Burger HG, Jameson LJ, Loriaux DL, Marshall JC, Odell WD, Potts jr JT, Rubenstein AH (eds) Endocrinology. Saunders, Philadelphia/London, pp 230–241

Nagano M, McCarrey JYR, Brinster RL (2001) Primate spermatogonial cells colonize mouse testis. Biol Reprod 64:1409–1416

Naito M, Itoh M (2008) Patterns of infiltration of lymphocytes into the testis under normal and pathological conditions in mice. Am J Reprod Immunol 59:55–61

Narula A, Gu YQ, O'Donell L, Stanton PG, Robertson DM, McLachlan RI, Bremner WJ (2002) Variability in sperm suppression during testosterone administration to adult monkeys is related to follicle stimulating hormone suppression and not to intratesticular androgens. J Clin Endocrinol Metab 87:3399–3406

Ngan ES, Cheng PK, Leung PC, Chow BK (1999) Steroidogenic factor-1 interacts with a gonadotrope-specific element within the first exon of the human gonadotropin-releasing hormone receptor gene to mediate gonadotrope-specific expression. Endocrinology 140:2452–2462

Nieschlag E, Weinbauer GF, Cooper TG, Wittkowski W, Cantz T (2008) Reproduktion. In: Speckmann E-J, Hescheler J, Köhling R (eds.) Physiologie 5. Auflage, Urban & Fischer, München pp 652–677

Nightingale SS, Western P, Hutson JM (2008) The migrating gubernaculum grows like a "limb bud". J Pediatr Surg 43:387–390

O'Donnell L, Robertson KM, Jones ME, Simpson ER (2001) Estrogen and spermatogenesis. Endocr Rev 22:289–318

Overstreet JW, Fuh VL, Gould J, Howards SS, Lieber MM, Hellstrom W, Shapiro S, Carroll P, Corfman RS, Petrou S, Lewis R, Toth P, Shown T, Roy J, Jarow JP, Bonilla J, Jacobsen CA, Wang DZ, Kaufman KD (1999) Chronic treatment with finasteride daily does not affect spermatogenesis or semen production in young men. J Urol 162:1295–1300

Palazzolo I, Gliozzi A, Rusmini P, Sau D, Crippa V, Simonini F, Onesto E, Bolzoni E, Poletti A (2008) The role of the polyglutamine tract in androgen receptor. J Steroid Biochem Mol Biol 108:245–253

Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N, Ratnam K (2000) Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: Functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem J 351:67–77

Phillip M, Arbelle JE, Segev Y, Parvari R (1998) Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone. N Engl J Med 338: 1729–1732

Piersma D, Verhoef-Post M, Berns EM, Themmen AP (2007) LH receptor gene mutations and polymorphisms: An overview. Mol Cell Endocrinol 260–262:282–A286

Plant TM, Ramaswamy S, Simorangkir D, Marshall GR (2005) Postnatal and pubertal development of the rhesus monkey (*Macaca mulatta*) testis. Ann NY Acad Sci 1061:149–162

Pöllänen P, Cooper TG (1994) Immunology of the testicular excurrent ducts. J Reprod Immunol 26:167–216

Popa SM, Clifton DK, Steiner RA (2008) The role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. Annu Rev Physiol 70:213–238

Prince FP (2007) The human Leydig cell: Functional morphology and developmental history. In: Payne AH, Hardy MP (eds) The Leydig cell in health and disease. Humana Press, Totowa, pp 71–90

Rahman F, Christian HC (2007) Non-classical actions of testosterone: An update. Trends Endocrinol Metab 18:371–378

Rajender S, Singh L, Thangaraj K (2007) Phenotypic heterogeneity of mutations in androgen receptor gene. Asian J Androl 9:147–179

Raleigh D, O'Donnell L, Southwick GJ, de Kretser DM, McLachlan RI (2004) Stereological analysis of the human testis after vasectomy indicates impairment of spermatogenic efficiency with increasing obstructive interval. Fertil Steril 81:1595–1603

Randall VA, Jenner TJ, Hibberts NA, De Oliveira IO, Vafaee T (2008) Stem cell factor/c-Kit signalling in normal and androgenetic alopecia hair follicles. J Endocrinol 197:11–23

Romano F, Tripiciano A, Muciaccia B, De Cesaris P, Ziparo E, Palombi F, Filippini A (2005) The contractile phenotype ofperitubular smooth muscle cells is locally controlled: Possible implications in male fertility. Contraception 72: 294–297

Ruwanpura SM, McLachlan RI, Matthiesson KL, Meachem SJ (2008) Gonadotrophins regulate germ cell survival, not proliferation, in normal adult men. Hum Reprod 23:403–411

Saudan C, Baume N, Robinson N, Avois L, Mangin P, Saugy M (2006) Testosterone and doping control. Br J Sports Med 40:21–24

Schell C, Albrecht M, Mayer C, Schwarzer JU, Frungieri MB, Mayerhofer A (2008) Exploring human testicular peritubular cells: Identification of secretory products and regulation by tumor necrosis factor-α. Endocrinology 149: 1678–1686

Schlatt S, Weinbauer GF, Arslan M, Nieschlag E (1993) Appearance of alpha-smooth muscle actin in peritubular cells of monkey testes is induced by androgens, modulated by follicle-stimulating hormone, and maintained after hormonal withdrawal. J Androl 14:340–50

Schlatt S, Arslan M, Weinbauer GF, Behre HM, Nieschlag E (1995) Endocrine control of testicular somatic and premeiotic germ cell development in the immature testis of the primate *Macaca mulatta*. Eur J Endocrinol 133:235–247

Schlatt S, Rosiepen G, Weinbauer GF, Rolf C, Brook PF, Nieschlag E (1999) Germ cell transfer into rat, bovine, monkey and human testis. Hum Reprod 14:144–150

Schulze W, Rehder U (1984) Organization and morphogenesis of the human seminiferous epithelium. Cell Tissue Res 237:395–407

Selva DM, Bassas L, Munell F, Mata A, Tekpetey F, Lewis JG, Hammond GL (2005) Human sperm sex hormone-binding globulin isoform: Characterization and measurement by time-resolved fluorescence immunoassay. J Clin Endocrinol Metab 90:6275–6282

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH (2003) The GPR54 gene as a regulator of puberty. N Engl J Med 349:1614–1627

Setchell BP (1999) Blood-testis barrier. In: Knobil E, Neill JD (eds) Encyclopedia of Reproduction. Academic, San Diego, CA, pp 375–381

Sezer C, Koksal IT, Usta MF, Gulkesen KH, Erdogru T, Ciftcioglu A, Baykara M (2005) Relationship between mast cell and iNOS expression in testicular tissue associated with infertility. Arch Androl 51:149–158

Shetty G, Krishnamurthy H, Krishnamurthy HN, Bhatnagar S, Moudgal RN (1997) Effect of estrogen deprivation on the reproductive physiology of male and female primates. J Steroid Biochem Mol Biol 61:157–166

Shetty G, Krishnamurthy H, Krishnamurthy HN, Bhatnagar AS, Moudgal NR (1998) Effect of long-term treatment with aromatase inhibitor on testicular function of adult male bonnet monkeys (*M. radiata*). Steroids 63:414–420

Shono T 2007 Molecular and anatomical studies of testicular descent. Hinyokika Kiyo 53:505–508

Simoni M, Gromoll J, Nieschlag E (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology and pathophysiology. Endocr Rev 18:739–773

Skandhan KP, Rajahariprasad A (2007) The process of spermatogenesis liberates significant heat and the scrotum has a role in body thermoregulation. Med Hypotheses 68: 303–307

Sridharan S, Simon L, Meling DD, Cyr DG, Gutstein DE, Fishman GI, Guillou F, Cooke PS (2007) Proliferation of adult Sertoli cells following conditional knockout of the gap junctional protein GJA1 (connexin 43) in mice. Biol Reprod 76:804–812

Sriraman V, Anbalagan M, Rao AJ (2005) Hormonal regulation of Leydig cell proliferation and differentiation in rodent testis: A dynamic interplay between gonadotrophins and testicular factors. Reprod Biomed Online 11:507–518

Stelekati E, Orinska Z, Bulfone-Paus S (2007) Mast cells in allergy: innate instructors of adaptive responses. Immunobiology 212:505–519

Suarez-Quian CA, Martinez-Garcia F, Nistal M, Regadera J (1999) Androgen receptor distribution in adult human testis. J Clin Endocrinol Metab 84:350–358

Takaimya K, Yamamoto A, Furukawa K, Zhao J, Fukumoto S, Amashiro S, Okada M, Haraguchi M, Shin M, Kishikawa M, Shiku H, Auzawa S, Furukawa K (1998) Complex gangliosides are essential in spermatogenesis of mice: possible roles in the transport of testosterone. Proc Natl Acad Sci USA 95:12147–12152

Takeda K, Toda K, Saibara T, Nakagawa M, Saika K, Onishi T, Sugiura T, Shizuta Y (2003) Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. J Endocrinol 176:237–246

Tena-Sempere M (2008) Ghrelin and reproduction: Ghrelin as novel regulator of the gonadotropic axis. Vitam Horm 77:285–300

Themmen AP, Martens JW, Brunner HG (1998) Activating and inactivating mutations in LH receptor. Mol Cell Endocrinol 145:137–142

Tobet SA, Schwarting GA. (2006) Recent progress in gonadotropin-releasing hormone neuronal migration. Endocrinology 147:1159–1165

Toppari J, Virtanen H, Skakkebaek NE, Main KM (2006) Environmental effects on hormonal regulation of testicular descent. J Steroid Biochem Mol Biol 102:184–186

Valimaki VV, Alfthan H, Ivaska KK, Loyttyniemi E, Pettersson K, Stenman UH, Valimaki MJ (2004) Serum estradiol, testosterone, and sex hormone-binding globulin as regulators of peak bone mass and bone turnover rate in young Finnish men. J Clin Endocrinol Metab 89:3785–3789

Vandenput L, Mellstrom D, Lorentzon M, Swanson C, Karlsson MK, Brandberg J, Lonn L, Orwoll E, Smith U, Labrie F, Ljunggren O, Tivesten A, Ohlsson C (2007) Androgens and glucuronidated androgen metabolites are associated with metabolic risk factors in men. J Clin Endocrinol Metab 92:4130–4137

Virtanen HE, Bjerknes R, Cortes D, Jorgensen N, Rajpert-De Meyts E, Thorsson AV, Thorup J, Main KM (2007a) Cryptorchidism: classification, prevalence and long-term consequences. Acta Paediatr 96:611–616

Virtanen HE, Cortes D, Rajpert-De Meyts E, Ritzen EM, Nordenskjold A, Skakkebaek NE, Toppari J (2007b) Development and descent of the testis in relation to cryptorchidism. Acta Paediatr 96:622–627

von Eckardstein S, Simoni M, Bergmann M, Weinbauer GF, Gaßner P, Schepers AG, Nieschlag E (1999) Serum inhibin B in combination with serum follicle-stimulating hormone (FSH) is a more sensitive marker than serum FSH alone for impaired spermatogenesis in men, but cannot predict the presence of sperm in testicular tissue samples. J Clin Endocrinol Metab 84: 2496–2501

Wang C, McDonald V, Leung A, Superlano L, Berman N, Hull L, Swerdloff RS (1997) Effect of increased scrotal temperature on sperm production in normal men. Fertil Steril 68:334–339

Weinbauer GF, Wessels J (1999) Paracrine control of spermatogenesis. Andrologia 31:249–262

Weinbauer GF, Schlatt S, Walter V, Nieschlag E (2001) Testosterone-induced inhibition of spermatogenesis is more closely related to suppression of FSH than to testicular androgen levels in the cynomolgus monkey (*Macaca fascicularis*). J Endocrinol 168:25–38

Weinbauer GF, Niehaus M, Nieschlag E (2004) The role of testosterone in spermatogenesis. In: Nieschlag E, Behre HM (eds) Testosterone: Action, deficiency, substitution. Cambridge University Press, Cambridge, pp 173–206

Wikström AM, Bay K, Hero M, Andersson AM, Dunkel L (2006) Serum insulin-like factor 3 levels during puberty in healthy boys and boys with Klinefelter syndrome. J Clin Endocrinol Metab 91:4705–4708

Wistuba J, Schrod A, Greve B, Hodges KJ, Aslam H, Weinbauer GF, Luetjens CM (2003) Organization of the seminiferous epithelium in primates: Relationship to spermatogenic efficiency, phylogeny and mating system. Biol Reprod 69:582–591

Xu Q, Lin HY, Yeh SD, Yu IC, Wang RS, Chen YT, Zhang C, Altuwaijri S, Chen LM, Chuang KH, Chiang HS, Yeh S, Chang C (2007) Infertility with defective spermatogenesis and steroidogenesis in male mice lacking androgen receptor in Leydig cells. Endocrine 32:96–106

Yamazawa K, Wada Y, Sasagawa I, Aoki K, Ueoka K, Ogata T (2007) Mutation and polymorphism analyses of INSL3 and LGR8/GREAT in 62 Japanese patients with cryptorchidism. Horm Res 67:73–76

Yan HH, Mruk DD, Wong EW, Lee WM, Cheng CY (2008) An autocrine axis in the testis that coordinates spermiation and blood-testis-barrier restructuring during spermatogenesis. Proc Natl Acad Sci USA 105:8950–8955

Zannini C, Turchetti S, Guarch R, Buffa D, Psece C (1999) Cell counting and three-dimensional reconstruction to identify a cellular wave in human spermatogenesis. Ann Quant Cytol Histol 21:358–362

Zhang C, Yeh S, Chen YT, Wu CC, Chuang KH, Lin HY, Wang RS, Chang YJ, Mendis-Handagama C, Hu L, Lardy H, Chang C (2006) Oligozoospermia with normal fertility in male mice lacking the androgen receptor in testis peritubular myoid cells. Proc Natl Acad Sci USA 103:17718–17723

Zhengwei Y, McLachlan RI, Bremner WJ, Wreford NG (1997) Quantitative (stereological) study of the normal spermatogenesis in the adult monkey (*Macaca fascicularis*). J Androl 18:681–687

Zhengwei Y, Wreford NG, Royce P, de Kretser DM, McLachlan RI (1998a) Stereological evaluation of human spermatogenesis after suppression by testosterone treatment: heterogeneous pattern of spermatogenic impairment. J Clin Endocrinol Metab 83:1284–1291

Zhengwei Y, Wreford NG, Schlatt S, Weinbauer GF, Nieschlag E, McLachlan RI (1998b) Acute and specific impairment of spermatogonial development by GnRH antagonist-induced gonadotrophin withdrawal in the adult macaque (*Macaca fascicularis*). J Reprod Fertil 112:139–147

Zitzmann M (2006) Testosterone and the brain. Aging Male 9:195–199

Zuccarello D, Ferlin A, Vinanzi C, Prana E, Garolla A, Callewaert L, Claessens F, Brinkmann AO, Foresta C (2008) Detailed functional studies on androgen receptor mild mutations demonstrate their association with male infertility. Clin Endocrinol (Oxf) 68:580–588

http://www.springer.com/978-3-540-78354-1

Andrology
Male Reproductive Health and Dysfunction
Nieschlag, E.; Behre, H.M.; Nieschlag, S. (Eds.)
2010, XVII, 629 p., Hardcover
ISBN: 978-3-540-78354-1