Chapter 9
Biology of the Sertoli Cell in the Fetal, Pubertal, and Adult Mammalian Testis

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Abstract A healthy man typically produces between 50×10^6 and 200×10^6 spermatozoa per day by spermatogenesis; in the absence of Sertoli cells in the male gonad, this individual would be infertile. In the adult testis, Sertoli cells are sustentacular cells that support germ cell development by secreting proteins and other important biomolecules that are essential for germ cell survival and maturation, establishing the blood–testis barrier, and facilitating spermatozoa detachment at spermiation. In the fetal testis, on the other hand, pre-Sertoli cells form the testis cords, the future seminiferous tubules. However, the role of pre-Sertoli cells in this process is much less clear than the function of Sertoli cells in the adult testis. Within this framework, we provide an overview of the biology of the fetal, pubertal, and adult Sertoli cell, highlighting relevant cell biology studies that have expanded our understanding of mammalian spermatogenesis.

9.1 Introduction

Sperm are produced within seminiferous tubules, the functional unit of the testis, by spermatogenesis. Spermatogenesis, which is comprised of mitosis, meiosis, and spermiogenesis, is largely under the regulation of gonadotropins, androgens, and other important biomolecules that include gonadotropin-releasing hormone (Gn-RH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (reviewed in Holdcraft and Braun 2004; Smith and Walker 2014; Walker and Cheng 2005) (Fig. 9.1). In the rat, spermatogenesis commences on postnatal day 5 (in the mouse, on postnatal day 3; in the human, between 9 and 14 years of age) when undifferentiated spermatogonial stem cells (SSCs; i.e., A_{single} spermatogonia) either self-renew into two SSCs or differentiate into two

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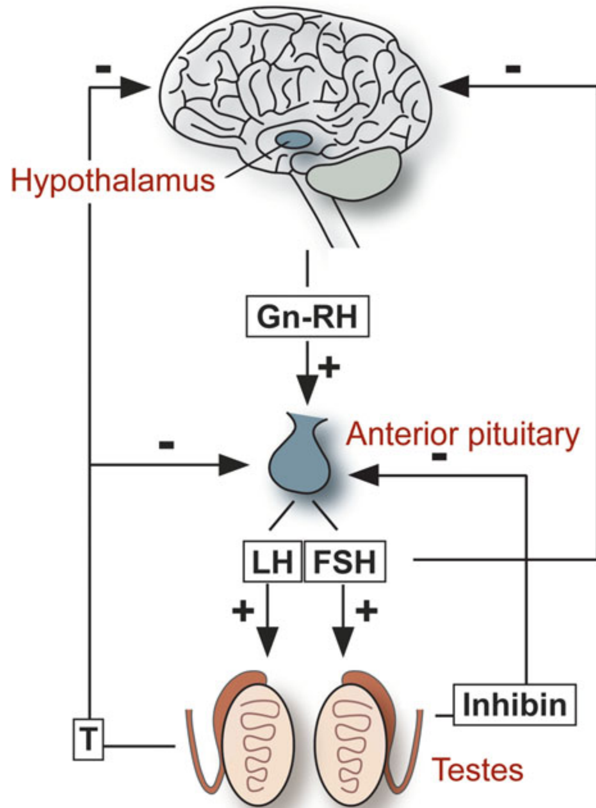

Fig. 9.1 The hypothalamic–pituitary–testicular axis. This axis, which is composed of several tightly regulated positive (+) and negative (–) feedback loops that span three organs (i.e., brain, pituitary, and testis), produces hormones, androgens, and other important biomolecules (*boxed*) that are essential for male reproductive development and function. *Gn-RH* gonadotropin-releasing hormone, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone, *T* testosterone

spermatogonia connected by cytoplasmic bridges due to incomplete cytokinesis (i.e., A_{paired} spermatogonia) (reviewed in de Rooij and Russell 2000; de Rooij and Griswold 2012; Greenbaum et al. 2011; Oatley and Brinster 2008). Spermatogonia connected by cytoplasmic bridges undergo several mitotic divisions to give rise to 4-, 8-, or 16-cell chains of spermatogonia (i.e., $A_{aligned}$ spermatogonia), before proliferation significantly decreases. Thereafter, $A_{aligned}$ spermatogonia differentiate into differentiated spermatogonia by six mitotic divisions (i.e., type A1→A2→A3→A4→intermediate→type B spermatogonia), before giving rise to primary spermatocytes. This process is followed by meiosis I and meiosis II, which produce secondary spermatocytes and spermatids, respectively. In the last phase of spermatogenesis (i.e., spermiogenesis), spermatids undergo several changes that essentially transform round spermatids into elongated spermatids, the so-called spermatozoa. Thereafter, spermatozoa detach from the seminiferous epithelium, release into the seminiferous tubule lumen at spermiation, and enter the epididymis where they will mature and acquire the ability to fertilize ova (reviewed in Clermont 1972; Hermo et al. 2010). This entire process (not including transit through the epididymis) takes 51.6 days in the rat (reviewed in de Kretser and

Kerr 1988; Kerr et al. 2006; Schlatt and Ehmcke 2014). In the mouse and human, the total duration of spermatogenesis is 34.5 and 64 days, respectively (Amann 2008; Heller and Clermont 1963; Oakberg 1956).

Throughout spermatogenesis, Sertoli cells support developing germ cells as they traverse the seminiferous epithelium. Sertoli cells are nurse-like somatic cells that extend from the base to the lumen of the seminiferous tubule, and they are essential for spermatogenesis. For mature germ cells to reach the seminiferous tubule lumen, preleptotene/leptotene spermatocytes have to cross the blood–testis barrier, which is created by Sertoli cell junctions. The movement of preleptotene/leptotene spermatocytes across the blood–testis barrier involves coordinated restructuring of these cell junctions at specific stages of the seminiferous epithelial cycle. It is well established, however, that the blood–testis barrier is never completely disassembled or assembled but in a dynamic flux of the two states. Instead, spermatocytes cross the blood–testis barrier while enclosed within a sealed intermediate compartment (Dym and Cavicchia 1977; Russell 1978; Smith and Braun 2012; reviewed in Russell 1993a). As preleptotene/leptotene spermatocytes move toward the tubule lumen, junctions in front of germ cells disassemble, while those in back of germ cells assemble. This is just one example of the important function of Sertoli cells in spermatogenesis; certainly, there are others, and they are discussed herein.

There are several stages of testis development: (1) development of the genital ridge (i.e., bipotential gonad), (2) sex determination, (3) testis differentiation (i.e., testis cord formation), (4) testis development (i.e., elongation of testis cords and seminiferous tubule formation), and (5) testis maturation and restructuring. In this perspective, we discuss these stages of testis development, while highlighting the role of the Sertoli cell in the fetal, pubertal, and adult testis. We hope that the information in this review provides a strong framework for future studies in the field.

9.2 Sex Determination and Testis Cord Formation

9.2.1 Genital Ridge Formation and Sex Determination

Genetic sex is determined at fertilization with the acquisition of either an X or a Y chromosome from the father to produce an XX or XY individual. Gonad development begins with the migration of primordial germ cells (PGCs), which are specified extragonadally, to the bipotential genital ridge (otherwise known as the gonadal ridge), the site of the future ovary or testis. Structurally, there is no difference in the bipotential genital ridge from an XX and XY embryo. The gonadal ridge, which derives from the intermediate mesoderm, is comprised of somatic cells, as well as PGCs that migrate from the allantois through the hindgut mesentery and to the genital ridge. The fate of the bipotential gonads is largely determined by the genes expressed by the somatic cells of the genital ridges. If the somatic cells are XX, then an ovary will develop; if the somatic cells are XY, then a testis will form.

Testis differentiation is largely controlled by sex determination region on Y chromosome (*Sry*, previously termed testis-determining factor), a high-mobility group (HMG)-containing transcription factor that initiates the testis differentiation program (reviewed in Kashimada and Koopman 2010; Larney et al. 2014; Tanaka and Nishinakamura 2014; Wilhelm et al. 2007c). In the XY gonad, *Sry* is expressed by somatic cells (at this developmental stage, known as pre-Sertoli cells). Somatic cells, which derive from the coelomic epithelium that overlays the bipotential gonads, give rise to the Sertoli cells, the supporting cells in the adult testis. However, in the absence of *Sry* expression by the somatic cells the gonads differentiate into ovaries (Albrecht and Eicher 2001), the Wolffian ducts (the precursors to the epididymides, vas deferens, and secondary sex glands) regress and the Müllerian ducts develop into the fallopian tubes, uterus, cervix, and anterior vagina (Fig. 9.2). Aberrant *Sry* expression or regulation, however, impedes testis development in XY embryos, whereas a loss of or a mutation within *Sry* results in ovary development (Battiloro et al. 1997; Hawkins et al. 1992; Kato et al. 2013; McElreavey et al. 1996; Nagamine et al. 1999; reviewed in Larney et al. 2014; Ostrer 2014). Most mutations within *Sry* disrupt its ability to bind to a consensus-binding motif (A/TA/TCAAA/TG) and bend DNA into its proper conformation (Gubbay et al. 1990; Mitchell and Harley 2002; Schmitt-Ney et al. 1995).

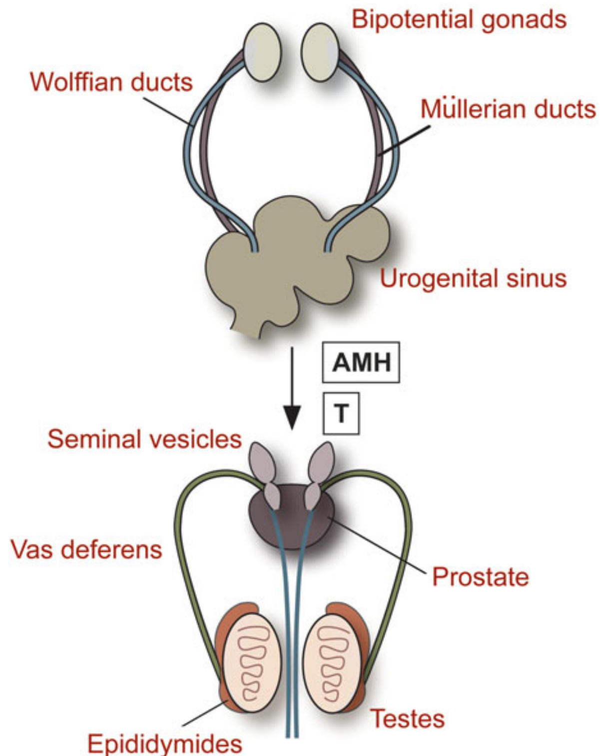

Fig. 9.2 Sex differentiation and testis development. Gonad development commences with the migration of primordial germ cells to the bipotential genital ridge, the site of the future ovary or testis. The fate of the primordial gonads is determined by the genes expressed by the somatic cells of the genital ridges. In the presence of anti-Müllerian hormone (AMH) and testosterone (T), the Müllerian ducts (the precursors to the fallopian tubes, uterus, cervix, and anterior vagina) regress and the Wolffian ducts (the precursors to the epididymides, vas deferens, and secondary sex glands) develop

Equally important, *Sry* expression, which is restricted to a very short period of testis development [*Sry* is expressed from 10.5 to 12.5 *dpc* (days *post coitum*) in the mouse (Hacker et al. 1995)], is strictly controlled by several genes (reviewed in Eggers et al. 2014; Larney et al. 2014). For instance, embryos homozygous for the boygirl (*byg*/*byg*) mutation, which renders MAPK kinase kinase (*Map3k4*, otherwise known as MAPK kinase 4) nonfunctional, exhibit sex reversal (i.e., XX embryos develop male organs and XY embryos develop female organs) due to a decrease in *Sry* expression (Bogani et al. 2009; Gierl et al. 2012; Warr et al. 2012; Wu et al. 2015). This effect is partly mediated by GADD45γ (growth arrest and DNA damage-inducible 45 gamma), a protein involved in cell differentiation, DNA repair, cell cycle control, apoptosis, and senescence (reviewed in Yang et al. 2009). Furthermore, the spatiotemporal pattern of *Gadd45γ* expression resembles that of *Sry* (Warr et al. 2012), suggesting that *Gadd45γ* and *Sry* function together in sex determination. Interestingly, C57BL/6J mice deficient for *Gadd45γ* exhibit sex reversal due to a decrease in *Sry* expression and p38 MAPK phosphorylation (Warr et al. 2012). GADD45γ, which is expressed by somatic cells in both XX and XY gonads, associates with MAP3K4 (Takekawa and Saito 1998). In addition, *Map3k4* overexpression rescues sex reversal in *Gadd45γ*-deficient embryos. Collectively, these studies demonstrate that *Map3k4* is critical for sex determination.

Other genes involved in the early stages of gonadogenesis are steroidogenic factor 1 (*Sf1*), GATA-binding protein 4 (*Gata4*), dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (*Dax-1*), Wilms' tumor suppressor 1 (*Wt1*), LIM homeobox 1 (*Lhx1*), *Lhx9*, and empty spiracles homeobox 2 (*Emx2*) (reviewed in Larney et al. 2014; Ludbrook and Harley 2004; Parker et al. 1999; Tanaka and Nishinakamura 2014; Wilhelm et al. 2013). For instance, mice null for *Sf1* show gonadal agenesis (i.e., the absence of one or both gonads), resulting from an arrest in genital ridge development (Luo et al. 1994). *Sf1* also regulates the transcription of genes that encode several hormones acting within the hypothalamic–pituitary–gonadal axis (reviewed in Kohler and Achermann 2010) (Fig. 9.1).

SRY binds SF1 to form a complex, which triggers the expression of *Sox9*, an HMG-containing transcription factor that specifies the Sertoli cell lineage (reviewed in Jakob and Lovell-Badge 2011; Jo et al. 2014; Koopman 1999). *Sox9* expression is upregulated at 11.5 *dpc* in the mouse when *Sry* expression is already highest at 10.5 *dpc*. Thereafter, *Sox9* downregulates *Sry* expression at 12.5 *dpc*, thereby leading to the cessation of its expression. To complete testis development, SOX9 recruits other proteins. Firstly, SOX9 activates lipocalin-type prostaglandin D synthase (PGDS), an enzyme that converts prostaglandin H2 into prostaglandin D2 (PGD2), and stimulates PGD2 secretion (Malki et al. 2005; Moniot et al. 2009; reviewed in Urade and Hayaishi 2000). The reverse is also true; PGDS2 promotes SOX9 activity by transforming pre-Sertoli cells and enhancing commitment to the male pathway (Wilhelm et al. 2005, 2007b). Interestingly, *Pgds* expression is induced immediately after *Sox9* expression (Adams and McLaren 2002; Wilhelm et al. 2007b). Secondly, *Sox9* activity promotes anti-Müllerian hormone (AMH), which inhibits Müllerian duct development (Behringer et al. 1994; reviewed in

Behringer 1995; Lee and Donahoe 1993) (Fig. 9.2). Collectively, these reports illustrate that SOX9 mediates pre-Sertoli cell differentiation. Similar to *Sry*, a loss of *Sox9* results in ovary development in XY embryos (Barrionuevo et al. 2006; Chaboissier et al. 2004). Sex determination is described in Chap. 3.

9.2.2 Testis Cord Formation

Testis cord formation, which occurs between 11.5 and 13.5 *dpc* in the mouse, involves the organization of pre-Sertoli cells and prospermatogonia (otherwise known as gonocytes) into testis cords, the future seminiferous tubules. Testis cord formation is composed of clustering (or coalescence), partitioning, and patterning (or remodeling) (Combes et al. 2009b). Initially pre-Sertoli cells and prospermatogonia are evenly distributed in the genital ridge, which is followed by the clustering of pre-Sertoli cells and the enclosure of germ cells by pre-Sertoli cells. During partitioning, endothelial cell precursors migrate from the mesonephros and invade the developing gonads. Thereafter, early testis cords grow, peritubular myoid cells (PMCs) surround testis cords and deposit the basement membrane together with pre-Sertoli cells, and testis cords and the vasculature mature and are remodeled (Combes et al. 2009a). At 13.5 *dpc* in the mouse, the number of testis cords that will become seminiferous tubules is established (reviewed in Cool et al. 2012).

The development of the vasculature is one of the most important processes to occur during testis cord formation. Endothelial cells detach from the arteries of the mesonephric plexus in the mesonephros, which is at the border with the gonad, and invade the XY gonad to create the distinct coelomic vessel on the surface of the testis (Brennan et al. 2002; Coveney et al. 2008; Martineau et al. 1997). Smaller vessels branch from the coelomic vessel and situate in the interstitium of the testis. Testis cord development is impeded in the absence of the vasculature with paths of migrating cells and branches of the coelomic vessel determining the shape and number of testis cords (Combes et al. 2009b; Cool et al. 2011). These results indicate that the vasculature is required for testis cord patterning. PMCs also drive testis cord development by contributing to the formation of the basement membrane, which initiates the polarization of pre-Sertoli cells (Combes et al. 2009b; Skinner et al. 1985; Tung and Fritz 1987).

Around birth, prospermatogonia migrate from the center of the testis cord to the basement membrane, which is critical for the establishment of the spermatogonial stem cell niche. Prospermatogonia, the precursors of spermatogonia, refer to germ cells from the time they inhabit the primordial gonads to the time they reach the basement membrane of the testis cords and differentiate into spermatogonia. Prospermatogonia are lost from the mouse testis a few days after birth (reviewed in Culty 2009). However, considering the molecular control of testis cord formation, testis cords do not form in the absence of *Sox9* expression (Barrionuevo et al. 2009). Other genes downstream of *Sry* and *Sox9* that are involved in testis

development are fibroblast growth factor 9 (*Fgf9*), doublesex- and mab-3-related transcription factor 1 (*Dmrt1*), and *Dax-1* (reviewed in Brennan and Capel 2004; Eggers et al. 2014; Koopman 2001; Wilhelm et al. 2007c). For instance, *Sox9* upregulates *Fgf9* expression, and *Fgf9* upregulates *Sox9* expression, which are important for male sex determination (Kim et al. 2006). Furthermore, mutations within *Fgf9* result in the loss of *Sox9* expression and partial or complete sex reversal (Colvin et al. 2001). Thus, the strong upregulation of *Sox9* by several factors inhibits female sex-determining genes and canalizes the male pathway and fate (reviewed in Piprek 2009).

9.3 Differentiation of Sertoli Cells in Fetal, Neonatal, and Prepubertal Testes

Vast changes in pre-Sertoli cell structure underlie the formation and expansion of testis cords. At 11.5–12.5 *dpc* in the mouse, pre-Sertoli cells undergo a mesenchymal-to-epithelial transition and then enclose either a single prospermatogonium or a group of prospermatogonia, resulting in the formation of testis cords (reviewed in Cool et al. 2012). The testis cords are the precursors of the seminiferous tubules that segregate germ cell development from androgen production in the adult testis. At 12.5 *dpc* in the mouse, a basement membrane surrounds testis cords. At 13.5 *dpc*, Sertoli cells position their nuclei near the basement membrane and extend their body into the center of the testis cords, which is indicative of cell polarization. Cell polarization also associates with dynamic changes in the cytoskeleton with actin filaments accumulating at the basal domain of Sertoli cells (Kanai et al. 1992). During fetal development, Sertoli cells proliferate (Orth 1982, 1984; reviewed in Sharpe et al. 2003), which is controlled by several factors that include FSH, thyroid hormone (T_3), and activin A, as well as the insulin-like growth factor 1 (IGF1) pathway (reviewed in Lucas et al. 2014; Sharpe et al. 2003). For instance, Sertoli cell proliferation increases after the administration of recombinant FSH to neonatal rats (Meachem et al. 1996). It deserves emphasis that the final number of Sertoli cells determines the number of germ cells they will support in adulthood (i.e., spermatogenic capacity increases with the number of Sertoli cells) so that these factors are crucial for testis development (Orth 1982; Orth et al. 1988; reviewed in Griswold 1995). During early postnatal development, Sertoli cells continue to proliferate, differentiate, and mature, which marks the transition of testis cords into seminiferous tubules. At the onset of puberty, however, they cease to proliferate and undergo additional changes in structure and function (Orth 1982; reviewed in Sharpe et al. 2003). While adult Sertoli cells are generally considered to be terminally differentiated, Sertoli cells in the adult human and hamster can proliferate under certain hormonal conditions (Tarulli et al. 2006, 2013; reviewed in Tarulli et al. 2012). In the human, mouse, and rat, each adult

Sertoli cell supports approximately 11, 35, and 20 developing germ cells, respectively (reviewed in Kerr et al. 2006).

At the onset of spermatogenesis, there is extensive proliferation of germ cells, which coincides with an increase in Sertoli cell size and the development of extensive Sertoli cell cytoplasmic processes that establish contact with germ cells. Sertoli cells establish the blood–testis barrier, which creates a specialized microenvironment for meiotic spermatocytes and post-meiotic spermatids throughout adulthood (reviewed in Fawcett et al. 1970; Mruk and Cheng 2015; Setchell and Waites 1975). Successful spermatogenesis relies on these early events in Sertoli cells, because abnormal Sertoli cell proliferation or differentiation can subsequently disrupt fertility (Orth et al. 1988; reviewed in Sharpe et al. 2003). Upon the appearance of spermatids in the seminiferous epithelium, Sertoli cell differentiation is complete. Interestingly, in the adult mouse testis, there are only 15–20 seminiferous tubules that add up to 2 m in length; in the human, there are 250–1000 seminiferous tubules that add up to 300–900 m (Bascom and Osterud 1925). Collectively, these studies demonstrate that Sertoli cells not only establish sex determination; they also orchestrate testis development throughout fetal, neonatal, and prepubertal development.

9.4 Cellular Organization of the Adult Mammalian Testis

9.4.1 The Seminiferous Tubule

Germ cell development occurs within seminiferous tubules under the regulation of gonadotropins, androgens, and other important factors that include Gn-RH, FSH, LH, and testosterone (reviewed in Holdcraft and Braun 2004; Sharpe 1994; Smith and Walker 2014; Walker and Cheng 2005). The basic organization of the rat testis, which is visible by light microscopy when adult testis cross sections are stained with either periodic acid–Schiff or hematoxylin and eosin, is comprised of seminiferous tubules that contain Sertoli and germ cells organized into 14 discrete associations or stages (denoted as stages I–XIV). Each stage is comprised of four or five generations of germ cells that are concentrically arranged within the seminiferous tubule. These stages run in succession so that stage I will eventually develop into stage II, and so on, thereby constituting a 12.9-day cycle in the rat. The major difference in the seminiferous epithelial cycle across different mammals is in the length of the cycle. The cycle then repeats along the entire length of the seminiferous tubule (Hess 1990; LeBlond and Clermont 1952; reviewed in Hess and Renato de Franca 2008; Russell et al. 1990).

The different stages of the seminiferous epithelial cycle are discerned by examining the relative position of the oldest generation of spermatids within each seminiferous tubule cross section. For instance, the heads of step 17 spermatids, which are narrow and defined, deeply embed within Sertoli cell crypts at stage V of

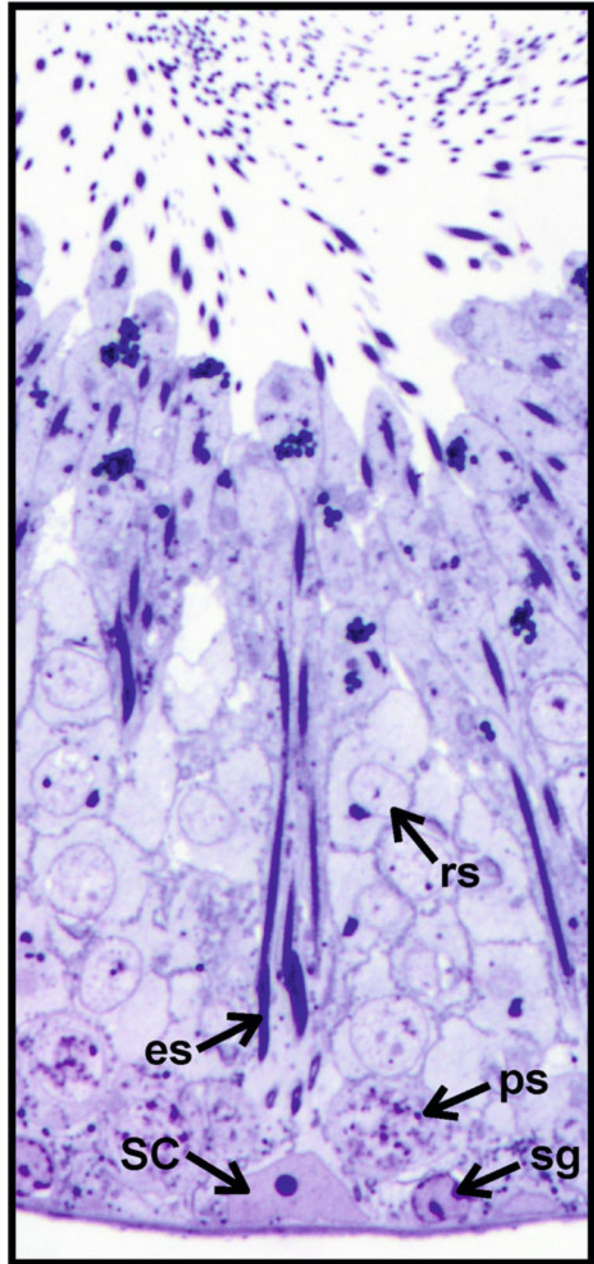

Fig. 9.3 A highly magnified image that shows a portion of a stage V seminiferous tubule from the adult rat testis. In the rat, each adult Sertoli cell (SC) supports approximately 20 developing germ cells. *sg* spermatogonium, *ps* pachytene spermatocyte, *rs* round spermatid, *es* elongated spermatid

the seminiferous epithelial cycle in the rat (Hess 1990; LeBlond and Clermont 1952; reviewed in Hess and Renato de Franca 2008; Russell et al. 1990) (Fig. 9.3). Sertoli cells in which elongated spermatids are embedded within crypts are defined

as type B; Sertoli cells without crypts are defined as type A. Additional criteria such as the presence of type B spermatogonia at stages IV–VI, which stain for dense chromatin, can be used when a stage is ambiguous (e.g., to discern stage III from stage IV). However, correct staging using this and other similar criteria calls for excellent preservation of testis morphology, which can be difficult to achieve (Russell et al. 1990).

9.4.2 The Interstitium

As previously discussed, Sertoli and germ cells reside in the seminiferous tubule, which is surrounded by PMCs. In the adult testis, PMCs mediate the contraction of seminiferous tubules, which sends spermatozoa to the epididymis (reviewed in Maekawa et al. 1996). In the rodent, a single layer of PMCs surrounds seminiferous tubules (Dym and Fawcett 1970; Gardner and Holyoke 1964; Regaud 1901). In the human, however, they are circumscribed by three or four layers of PMCs (Ross and Long 1966). In the rat (but not in other species), PMCs restrict the entry of lanthanum nitrate, a small electron-dense tracer, into approximately 85 % of seminiferous tubules (Dym and Fawcett 1970), indicating these cells partly contribute to barrier function. During development and throughout adulthood, PMCs work with Sertoli cells to deposit the basement membrane [it is largely composed of laminin, collagen IV, heparin sulfate proteoglycan, entactin, and fibronectin (Hadley and Dym 1987)] that surrounds seminiferous tubules (Skinner and Fritz 1985; Skinner et al. 1985; Tung and Fritz 1987; reviewed in Dym 1994) (Fig. 9.4), indicating that PMC–Sertoli cell interactions are critical for seminiferous tubule architecture and spermatogenesis (reviewed in Skinner et al. 1991; Verhoeven et al. 2000).

Equally important, PMCs control spermatogenesis through the androgen receptor (AR). For instance, Welsh et al. (2009) report that PMC-specific *Ar* knockout mice, which were created by crossing mice heterozygous for Cre recombinase driven by the PMC-specific promoter of the smooth muscle myosin heavy chain gene with mice homozygous for a floxed *Ar*, are azoospermic. Interestingly, this decrease in the spermatozoa count is not the result of a defect in SSC function, even though PMCs associate intimately with SSCs residing in the niche (Shinohara et al. 2001; Tegelenbosch and de Rooij 1993). SSCs, which either self-renew to replenish the SSC pool or differentiate into spermatogonia connected by cytoplasmic bridges, maintain spermatogenesis (reviewed in de Rooij and Russell 2000; Greenbaum et al. 2011; Oatley and Brinster 2008). While the significance of these interactions is not yet clear, these results support the importance of PMCs in spermatogenesis. Additional studies are needed to determine where PMCs derive from.

In the interstitium of the adult testis, Leydig cells are the major component. There are two populations of Leydig cells: fetal (FLCs) and adult Leydig cells (ALCs) (reviewed in Griswold and Behringer 2009; Habert et al. 2001; Haider

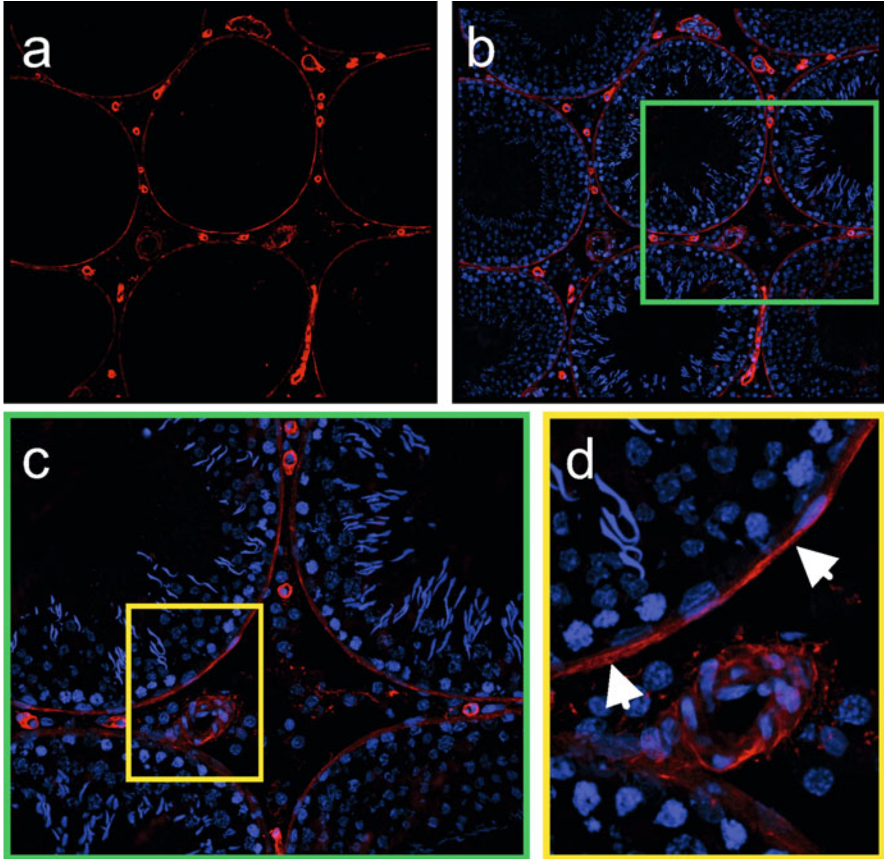

Fig. 9.4 The immunolocalization of collagen IV in the adult rat testis. Frozen testis cross sections were immunostained for collagen IV (*red* fluorescence, **a**–**d**), a protein of the basement membrane that is largely produced and deposited by peritubular myoid cells. The *green boxed area* (**b**) is magnified in the neighboring image (**c**); and the *yellow boxed area* (**c**) is magnified in the neighboring image (**d**). *Arrows* (**d**) point to collagen IV. The vasculature was also immunoreactive for collagen IV. Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI, *blue* fluorescence)

2004; Mendis-Handagama and Ariyaratne 2001; O'Shaughnessy et al. 2006; Tremblay 2015). FLCs, which do not express *Sry* (Zwingman et al. 1994), differentiate in response to Sertoli cell factors that include platelet-derived growth factor A (PDGFA) and in response to Dessert Hedgehog (DHH) and Notch signaling (Tang et al. 2008; reviewed in Barsoum and Yao 2006; O'Shaughnessy et al. 2006; Svingen and Koopman 2013). FLCs are critical for the elongation of testis cords. FLCs masculinize the reproductive tract through testosterone and induce testis descent via insulin-like growth factor 3 (IGF3), a secreted Leydig cell protein (Wilhelm et al. 2007a; reviewed in Bay et al. 2011; Hutson et al. 2013). FLCs

initially synthesize testosterone in the absence of LH. However, they subsequently express luteinizing hormone receptors (LHR) and respond to LH (O'Shaughnessy et al. 1998). Shortly after birth, FLCs undergo apoptosis. Thus, FLCs do not appear to give rise to ALCs (Ariyaratne et al. 2000). The origin and differentiation of FLCs and ALCs are described in Chap. 5.

ALCs, on the other hand, derive from Leydig stem cells, which are capable of self-renewal. Leydig stem cells develop into progenitor Leydig cells, which express 3β-hydroxysteroid dehydrogenase (3βHSD) and LHR (Dong et al. 2007), which is followed by differentiation into immature and adult cells that no longer proliferate (reviewed in Griswold and Behringer 2009; Haider 2004). ALCs produce testosterone in the presence of LH, which is essential for the establishment and maintenance of secondary sex characteristics and the continuation of spermatogenesis (reviewed in Ge et al. 2008; Smith and Walker 2014; Walker 2011). Sertoli cells are critical for the differentiation of FLCs and the preservation of ALCs. For instance, the ablation of Sertoli cells in a transgenic adult mouse model results in a marked reduction in Leydig cell number, as well as the loss of all germ cells (Rebourcet et al. 2014a, b). PMC function is also affected in these mice. Collectively, these reports illustrate that Sertoli cells are critical for Leydig cell function.

Immune cells (e.g., macrophages, T-cells, mast cells, natural killer cells, and dendritic cells) are also present in the interstitium of the adult testis where they function in innate and adaptive immune responses (reviewed in Perez et al. 2013). Of these immune cells, macrophages are the most abundant, constituting approximately 25 % of the interstitial cells in the adult rodent testis (Giannessi et al. 2005; Niemi et al. 1986). This population of macrophages associates with Leydig cells and the vasculature (Hume et al. 1984; Hutson 2006). In a recent study, DeFalco et al. (2015) describe a second population of macrophages that associates with PMCs and the vasculature, residing within the spermatogonial stem cell niche. Interestingly, the number of $A_{aligned}$, but not A_{single}, spermatogonia declines after the ablation of both populations of macrophages, indicating there is cross talk between immune cells and spermatogonia. Macrophages also establish cell junctions with Leydig cells, which facilitate cross talk between cells (reviewed in Christensen and Gillim 1969; Meinhardt and Hedger 2011; Perez et al. 2013). For example, macrophages secrete 25-hydroxycholesterol, which is used by ALCs for testosterone synthesis (Lukyanenko et al. 2001; Nes et al. 2000). Cytokines produced by activated macrophages can also modulate Leydig cell steroidogenesis (Hales et al. 1992; reviewed in Bornstein et al. 2004; Hales 2002). Collectively, these studies demonstrate that the function of macrophages in the testis goes beyond that of the immune response.

9.5 Establishment and Maintenance of the Sertoli Cell Barrier

The blood–testis barrier, a physical barrier that isolates meiotic and post-meiotic germ cells from immune and lymphatic systems, is one of the tightest tissue barriers based on studies that revealed the testis to be impenetrable to intravenously injected dyes (Ribbert 1904). It is constituted by Sertoli cell junctions that divide the seminiferous epithelium into a basal compartment, where spermatogonia and early primary spermatocytes (i.e., preleptotene/leptotene spermatocytes) reside, and an adluminal compartment, where more-mature primary spermatocytes (i.e., zygotene, pachytene, and diplotene spermatocytes), secondary spermatocytes, and spermatids dwell (Cavicchia and Dym 1977; Dym and Fawcett 1970; reviewed in Cheng and Mruk 2012; Setchell and Waites 1975). Strictly speaking, germ cells that occupy the basal compartment are not isolated from immune and lymphatic systems. To reflect its location within the seminiferous epithelium, the blood–testis barrier is more appropriately defined as the Sertoli cell barrier instead of the blood–testis barrier. However, the term "blood–testis barrier" is more commonly used in the literature. The blood–testis barrier has three main functions: it prevents the entry of unwanted substances into the adluminal compartment, regulates the passage of substances into/out of the same compartment, and sequesters meiotic and post-meiotic germ cells from immune and lymphatic systems. Thus, the blood–testis barrier is marked by anatomical, physiological, and immunological features.

There are several important differences between the blood–testis barrier and other blood–tissue barriers that include the blood–brain, blood–retinal, and blood–epididymal barriers. Firstly, the blood–testis barrier is constituted by tight junctions, basal ectoplasmic specializations, desmosomes, and gap junctions that localize to the basal domain of Sertoli cells (Dym and Fawcett 1970; reviewed in Cheng and Mruk 2012). This is different from other blood–tissue barriers that are largely comprised of tight junctions that are restricted to the apical domains of epithelial and endothelial cells (reviewed in Matter and Balda 2003). Furthermore, the localization of cell junctions at the blood–testis barrier is highly variable with desmosomes and gap junctions coexisting with tight junctions and basal ectoplasmic specializations. Secondly, the blood–testis barrier does not assemble until puberty unlike most other blood–tissue barriers that form in utero or early postnatal development (reviewed in Rizzolo 2007; Yao et al. 2014). In the rat, interstitially injected tracers permeate seminiferous tubules until postnatal day 16 (Vitale et al. 1973), indicating the blood–testis barrier assembles on postnatal day 16. A delay in blood–testis barrier formation halts meiosis (Chihara et al. 2013b; Hosoi et al. 2002; Toyama et al. 2001). Likewise, the assembly of the blood–epididymis barrier completes on postnatal day 21 in the rat (Agarwal and Hoffer 1989). Thirdly, the blood–testis barrier restructures to accommodate the passage of preleptotene/leptotene spermatocytes at stages VIII–XI (Dym and Cavicchia 1977; Russell 1978; Smith and Braun 2012; reviewed in Russell 1993a). Previous studies show that preleptotene/leptotene spermatocytes cross the blood–testis

barrier while enclosed within an intermediate compartment that is created by Sertoli cell junctions. The entry of spermatocytes into the adluminal compartment initiates when cell junctions ahead of spermatocytes disassemble, while new cell junctions assemble behind them.

The blood–testis barrier is largely comprised of tight junctions and basal ectoplasmic specializations. Basal ectoplasmic specializations are testis-specific anchoring junctions whose component proteins directly or indirectly attach to the actinomyosin cytoskeleton (reviewed in Fawcett et al. 1970; Mruk and Cheng 2004b, 2010; Vogl et al. 2008). Desmosomes and gap junctions, which coexist with tight junctions and basal ectoplasmic specializations, also contribute to blood–testis barrier function (reviewed in Lie et al. 2011a; Pointis et al. 2010). Continuous cross talk among the component proteins of these cell junctions is essential for spermatogenesis, and blood–testis barrier function is affected if cross talk is perturbed. Tight junctions are highly complex regions of close apposition between cells that divide the plasma membrane of polarized epithelial and endothelial cells into apical and basal domains (reviewed in Anderson and Van Itallie 2008; Furuse 2010). Tight junctions regulate the passage of molecules through the paracellular pathway (otherwise known as the gate function) and restrict the movement of proteins and lipids between apical and basal domains (otherwise known as the fence function) (reviewed in Madara 1998; Shin et al. 2006). There are two major types of tight junctions: bicellular tight junctions form between two cells, while tricellular tight junctions form where three cells meet. While claudins are the main structural and functional components of the bicellular tight junction, tricellulin is that for the tricellular tight junction (reviewed in Furuse et al. 2014; Tsukita and Furuse 2000). Occludin is another example of a transmembrane tight junction protein. It binds zona occludens-1 (ZO-1), a cytoplasmic protein (Fig. 9.5).

In the rodent testis, claudins (*Cldn*) 3 and 11 are best studied. In the adult mouse testis, claudin 3 expression is highest at stages VI–IX. However, it is not expressed by the rat testis (Kaitu'u-Lino et al. 2007). Claudin 3 localizes to newly assembled tight junctions behind migrating spermatocytes (Chihara et al. 2013a; Meng et al. 2005; Smith and Braun 2012), indicating that it reseals the blood–testis barrier after spermatocyte movement. Furthermore, claudin 3 expression is significantly reduced in $Ar^{\text{invflox(exl-neo)Y}}$;Tg(*Amh-Cre*) mice, which were created by crossing mice with a hypomorphic inverted floxed *Ar* with mice expressing Cre recombinase driven by the Sertoli cell-specific promoter of the *Amh* gene. These mice present for conditional androgen sensitivity and azoospermia (Meng et al. 2005), which indicates that claudin 3 is regulated by androgens. The permeability of the blood–testis barrier is also affected in these mice (Meng et al. 2005). Claudin 11 is also critical for blood–testis barrier integrity, because tight junction function is disrupted in mice in which *Cldn11* is constitutively deleted. These mice are infertile due to the inability of germ cells to differentiate beyond the spermatocyte stage (Gow et al. 1999). In addition, Sertoli cells proliferate in *Cldn11*-deficient mice (Mazaud-Guittot et al. 2010), illustrating that claudin 11 contributes to the terminal differentiation of Sertoli cells.

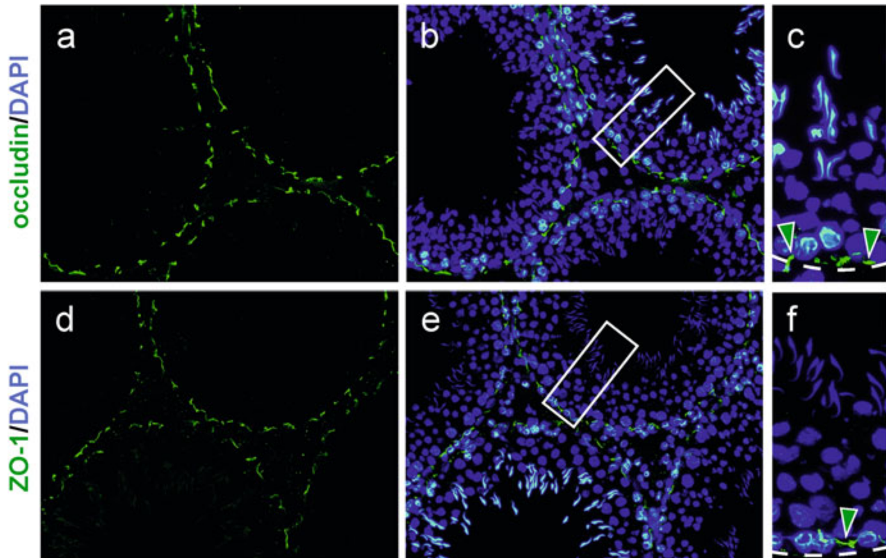

Fig. 9.5 The immunolocalization of occludin and zona occludens-1 (ZO-1) in the adult rat testis. Frozen testis cross sections were immunostained for occludin (*green* fluorescence, **a**–**c**) or ZO-1 (*green* fluorescence, **d**–**f**), component proteins of the tight junction. The *white boxed areas* (**b**, **e**) are magnified in the adjacent images (**c**, **f**), respectively. *Arrowheads* point to occludin (**c**) and ZO-1 (**f**) at the blood–testis barrier. Cell nuclei were stained with DAPI (*blue* fluorescence)

9.6 Sertoli Cells in the Adult Testis

In the adult mammalian testis, terminally differentiated Sertoli cells are characterized by the ability to (1) support the structure of the seminiferous epithelium, (2) assemble the blood–testis barrier, (3) secrete proteins and other biomolecules that are needed by developing germ cells, (4) facilitate spermatozoa detachment at spermiation, and (5) phagocytose germ cell residual bodies (reviewed in Bardin et al. 1988; Griswold 1998; Mruk and Cheng 2004b; Russell 1993b). For instance, Sertoli cells secrete approximately 15 % of the proteins that they produce, many of which are secreted stage specifically (reviewed in Bardin et al. 1988; Djakiew and Onoda 1993; Griswold 1993; Skinner 1993). Activin A (activates FSH secretion, thereby stimulating Sertoli cell proliferation) and inhibins A and B (inhibit FSH secretion) are possibly the best studied Sertoli cell proteins (reviewed in de Kretser 1990; de Kretser et al. 2004). During testis development, the production of activin A significantly decreases, which contributes to the differentiation of Sertoli cells (Barakat et al. 2008; Mithraprabhu et al. 2010).

Throughout spermatogenesis, germ cells attach to Sertoli cells via adhesion junctions, which enable spermatogonia to develop into spermatozoa with the support of somatic cells. The developmental stage of the germ cell determines whether adhesion is mediated by intermediate filament-based desmosomes or actin-

based apical ectoplasmic specializations. In the adult testis, desmosomes are found between Sertoli cells and all germ cells up to, but not including, elongating and elongated spermatids (i.e., step 8 and beyond). They are also found at the blood–testis barrier where they contribute to barrier integrity (Lie et al. 2010; Russell 1977; Russell et al. 1983; reviewed in Lie et al. 2011a; Mruk and Cheng 2011). Desmosomes are comprised of transmembrane proteins of the desmosomal cadherin family (i.e., desmoglein and desmocollin) that connect to intermediate filaments through cytoplasmic proteins of armadillo (e.g., plakoglobin, plakophilin) and plakin (e.g., desmoplakin) families (reviewed in Harmon and Green 2013; Nekrasova and Green 2013) (Fig. 9.6). Apical ectoplasmic specializations, on the other hand, are found between Sertoli cells and elongating and elongated spermatids (reviewed in Mruk and Cheng 2004a; Vogl et al. 1993, 2008). Adhesion mediated by the apical ectoplasmic specialization is robust and dynamic, and it involves cross talk between several multiprotein complexes. When the strength needed to detach germ cells from Sertoli cells in vitro is measured with a micropipette pressure transducing system, adhesion is strongest between step 8 spermatids and Sertoli cells (Wolski et al. 2005). Of the multiprotein complexes present at this structure, nectin (*Pvrl*), a Ca^{2+}-independent integral membrane protein, is critical for apical ectoplasmic specialization function, because mice null for *Pvrl2* or *Pvrl3* show defects in the apical ectoplasmic specialization, actin distribution, and sperm morphology, rendering mice infertile (Bouchard et al. 2000; Inagaki et al. 2006; Ozaki-Kuroda et al. 2002).

It is well established that both desmosomes and apical ectoplasmic specializations undergo restructuring, which facilitates germ cell movement across the seminiferous epithelium. However, it is not known how these cell junctions disassemble and reassemble throughout spermatogenesis. While component proteins of the tight junction and basal ectoplasmic specialization internalize after Sertoli cells are treated with cytokines (Lie et al. 2011b; Xia et al. 2009; Yan et al. 2008), it is not clear whether desmosomes are regulated by a similar mechanism. Keratinocytes, which use desmosomes for adhesion, maintain two distinct cell adhesion states: stable hyper-adhesion (i.e., Ca^{2+}-independent) and dynamic weak adhesion (Ca^{2+}-dependent), which are prompted by wound healing or experimental Ca^{2+} switch. At the molecular level, protein kinases such as protein kinase C, proto-oncogene tyrosine-protein kinase SRC, and epidermal growth factor receptor induce weak adhesion, resulting in the internalization of desmoglein (Aoyama and Kitajima 1999; Calkins, et al. 2006). In the testis, desmosomes lack the electron-dense midline, which is characteristic of desmosomes in keratinocytes, and they likely exhibit weak adhesion (Russell 1977). Further studies are needed in the testis, because there are apparent differences in the regulation of desmosomes across different epithelia.

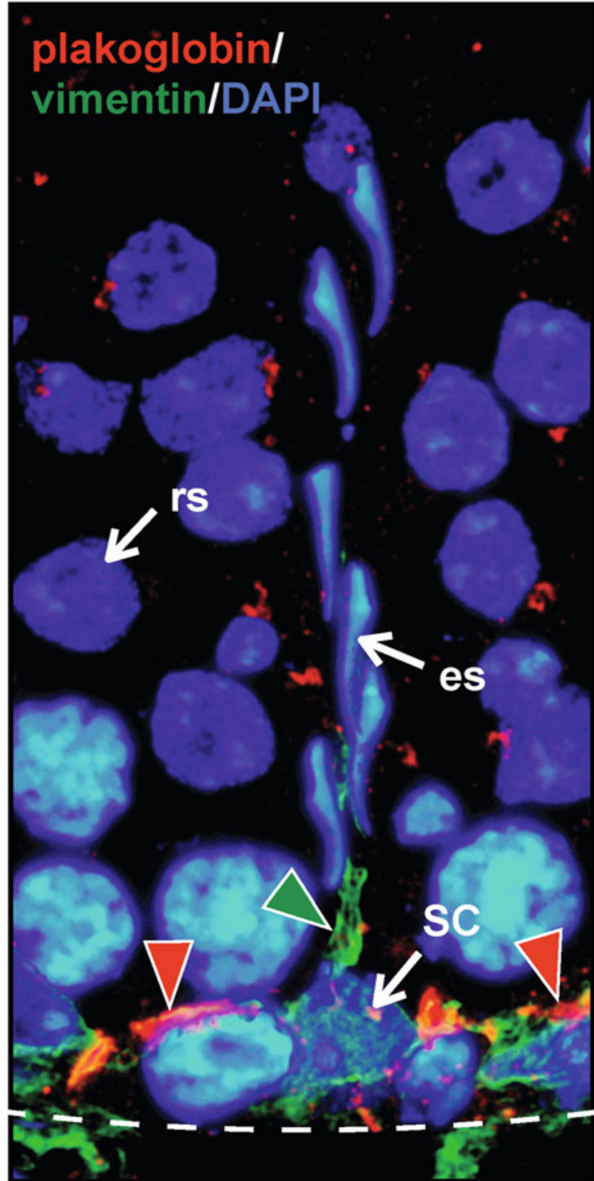

Fig. 9.6 The immunolocalization of plakoglobin and vimentin in the adult rat testis. Frozen testis cross sections were immunostained for plakoglobin (*red* fluorescence) and vimentin (*green* fluorescence), component proteins of the desmosome. *Red* and *green arrowheads* point to plakoglobin and vimentin, respectively, in a stage V seminiferous tubule. Cell nuclei were stained with DAPI (*blue* fluorescence). *SC* Sertoli cell, *rs* round spermatid, *es* elongated spermatid

9.7 Conclusion

In this perspective, we have highlighted the importance of Sertoli cells in the development of the fetal, pubertal, and adult testis. Collectively, these reports illustrate that Sertoli cells are critical for the initiation of spermatogenesis, as

well as for its maintenance throughout adulthood. Our knowledge on the Sertoli cell has mostly come from in vitro studies, because these cells are relatively easy to isolate from the testes of early pubertal rats (i.e., 18–20 days old). For instance, Sertoli cells initiate polarization, establish cell junctions, and secrete proteins into the apical and/or basal compartment when they are cultured at high density on Matrigel-coated bicameral units, similar to Sertoli cells in vivo (reviewed in Djakiew and Onoda 1993; Steinberger and Jakubowiak 1993). Thus, Sertoli cell cultures are a good system for present and future studies. Recent studies show that adult human Sertoli cells can be cultured and expanded in vitro while maintaining their primary characteristics (Chui et al. 2011; Guo et al. 2015), indicating that they may have use in reproductive medicine. Future studies should continue to investigate the proliferative ability of human Sertoli cells in vivo.

The Sertoli cell—its structure, function, and regulation—has intrigued reproductive cell biologists for more than a century, resulting in thousands of publications. The goal of this review was to briefly present the biology of the Sertoli cell, highlighting specific milestones and major achievements made by several investigators. Interested readers are strongly encouraged to refer to the references cited herein. We hope that the information in this review provides a strong framework for future studies in the field.

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