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REVIEW ARTICLE

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The Sertoli cell: one hundred fifty years of beauty and plasticity

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SUMMARY

It has been one and a half centuries since Enrico Sertoli published the seminal discovery of the testicular 'nurse cell', not only a key cell in the testis, but indeed one of the most amazing cells in the vertebrate body. In this review, we begin by examining the three phases of morphological research that have occurred in the study of Sertoli cells, because microscopic anatomy was essentially the only scientific discipline available for about the first 75 years after the discovery. Biochemistry and molecular biology then changed all of biological sciences, including our understanding of the functions of Sertoli cells. Immunology and stem cell biology were not even topics of science in 1865, but they have now become major issues in our appreciation of Sertoli cell's role in spermatogenesis. We end with the universal importance and plasticity of function by comparing Sertoli cells in fish, amphibians, and mammals. In these various classes of vertebrates, Sertoli cells have quite different modes of proliferation and epithelial maintenance, cystic vs. tubular formation, yet accomplish essentially the same function but in strikingly different ways.

INTRODUCTION

The 150th year anniversary of the publication reporting the discovery of the Sertoli cell is to be celebrated (Sertoli, 1865). Although Enrico Sertoli (Fig. 1) went on to explore numerous other subjects, including physiology of blood proteins, tissue carbonic acid in the respiratory system and smooth muscle contraction, he was given the highest honor for his study of the human testis, when Von Ebner first called these cells, 'the Sertoli cells' (Ebner, 1888). Research on the Sertoli cell started out slowly, with only about 25 manuscripts published up to 1950. However, rapid advancement in our knowledge of this unique cell was soon realized with the invention of the electron microscope and discoveries of DNA, RNA and new methods of biochemistry, which permitted the incorporation of histochemistry and immunochemistry in molecular biology studies (see Table 1 for timeline of advances). Now there are nearly 500 papers published each year describing the intricate relationships established by Sertoli cells in the testis. Presented here are highlights that emphasize the superb scientific beauty and intrinsic plasticity of the cell, to which three books and numerous reviews have been devoted (Russell & Griswold, 1993b; Skinner & Griswold, 2005; Griswold, 2015b) (See Table 2 and Supplement Table S1 for lists of important reviews and books). In this brief review, we first present the basic structure of the Sertoli cell and then show how molecular biology has given us insight into the complicated mechanisms involved in its nurse cell function. We then discuss how this information is applied to current ongoing studies on the role of Sertoli cells in the spermatogonial stem cell niche and in the maintenance of testicular immune privilege. Finally, a comparative view across vertebrate species is summarized to show common but also divergent pathways that have developed to allow the same cell to establish its germ cell interactions in both cystic and tubular modes of organization. Our review cannot be totally inclusive but will highlight the passionate pursuits of the authors in the hope that we can stimulate even more interest in the complexities and importance of the Sertoli cell.

MORPHOLOGY OF THE SERTOLI CELL

Morphological studies of the Sertoli cell have gone through three phases of investigation (Fig. 2), beginning with routine light microscopy (LM), which lasted until about 1960. The next phase began after the invention of the electron microscope

Figure 1 Photo of Professor Enrico Sertoli, published in honor of his retirement after 36 years of teaching and research (Negrini *et al.*, 1908) and drawings (Sertoli, 1865) from Sertoli's original publication (Fig. 1A–D).

Table 1 Significant Sertoli cell milestones

1865	Enrico Sertoli's first publication (Sertoli, 1865)
1888	The branched cells first called 'Sertoli cells' (Ebner, 1888)
1899	Sertoli and germ cells thought to have common origin (Regaud, 1899)
1901	Sertoli cells thought to be syncytial (Regaud, 1901)
1901	Sertoli cells phagocytize degenerative germ cells (Regaud, 1901; Clermont & Morgentaler, 1955; Russell & Clermont, 1977)
1902	Sertoli cells do not divide in adult testis (Ebner, 1888)
1942	Sertoli cell tumors or tubular adenomas described (Innes, 1942)
1948	'Sertoli cell only' syndrome first described (Heller *et al.*, 1948)
1949	Sertoli cells produce estrogen (Teilum, 1949; Armstrong *et al.*, 1975)
1950	Cyclical changes in Sertoli cell morphology (Elftman, 1950; Leblond & Clermont, 1952; Brökelmann, 1963; Kerr & De Kretser, 1975)
1952	Description of changes in Sertoli cell nucleus by Stage (Leblond & Clermont, 1952)
1956	First Electron Microscopic description; Sertoli cells are not syncytial (Fawcett & Burgos, 1956)
1963	Silver method used to show changes in Sertoli cell cytoplasm morphology by Stage (Elftman, 1963)
1964	First primary cultures of Sertoli cells (Steinberger *et al.*, 1964; Steinberger & Steinberger, 1970)
1965	Sertoli unique nucleolus and heterochromatin (Monesi, 1965; Jean *et al.*, 1983)
1967	Junctional Specializations described (Flickinger & Fawcett, 1967)
1967	Blood–testis barrier defined physiologically (Setchell, 1967)
1968	Sertoli cell as a major factor in testicular secretions (Setchell *et al.*, 1968; Setchell, 1974)
1969	Sertoli cell role in spermiation ultrastructure (Fawcett & Phillips, 1969; Russell, 1984)
1970	Sertoli cell nucleolus used as a constant for counting cells (Bustos-Obregon, 1970)
1970	Blood–testis barrier defined ultrastructurally between Sertoli cell junctions; basal and adluminal compartments described (Dym & Fawcett, 1970)
1970	Sertoli cell toxicant described (Kierszenbaum, 1970)
1970	Sertoli cell secretion of fluid (Setchell, 1970; Setchell *et al.*, 1978; Wilson & Griswold, 1979)
1972	Transillumination allowed first biochemical studies of the cyclic activities (Parvinen & Vanha-Perttula, 1972)
1975	First detailed review of Sertoli cell ultrastructure (Fawcett, 1975)
1975	FSH regulation of Sertoli cells (Tung *et al.*, 1975; Fritz *et al.*, 1976; Means *et al.*, 1976; Griswold, 1993a)
1975	Sertoli cell production of androgen-binding protein (Sanborn *et al.*, 1975; Tindall *et al.*, 1975; Fritz *et al.*, 1976)
1976	Tubulobulbar complex described (Russell & Clermont, 1976)
1977	Sertoli ectoplasmic specialization junctions named (Russell, 1977)
1979	Golgi of Sertoli cell in 3-D (Rambourg *et al.*, 1979)
1979	Sertoli cell production of inhibin (Sertoli cell factor) (Chowdhury *et al.*, 1978; Labrie *et al.*, 1978; Demoulin *et al.*, 1979; Steinberger, 1979)
1980	Sertoli cell volume is enormous (Roosen-Runge, 1955; Cavicchia & Dym, 1977; Weber *et al.*, 1983; Wong & Russell, 1983; Russell *et al.*, 1986, 1990b; Kerr, 1988a,b; Sinha Hikim *et al.*, 1989)
1980	Sertoli cell production of transferrin (Skinner & Griswold, 1980)
1981	Sertoli cell production of proteins is Stage specific (Lacroix *et al.*, 1981; Parvinen, 1982; Ritzen *et al.*, 1982; Mather *et al.*, 1983; Wright *et al.*, 1983)
1982	Sertoli cell proliferation peaks just before birth and ceases at puberty (Orth, 1982)
1983	Three-dimensional reconstruction of the Sertoli cell (Russell *et al.*, 1983; Weber *et al.*, 1983; Wong & Russell, 1983)
1983	Sertoli cell numbers may change in human and horse species (Johnson & Thompson, 1983; Johnson *et al.*, 1984)
1983	Importance of Sertoli cell microtubules and other cytoskeletal elements in germ cell transport and attachment (Vogl *et al.*, 1983a,b, 1993, 2008; Vogl & Soucy, 1985; Russell *et al.*, 1989)
1984	Sertoli cell production of anti-Mullerian hormone (Picard & Josso, 1984)
1984	Sertoli cell interaction with peritubular myoid cell (Tung *et al.*, 1984)
1984	FSH increases Sertoli cell proliferation (Orth, 1984)
1986	Sertoli cell production of Cyclic Protein-2 (CP-2) (Wright & Luzarraga, 1986)
1987	Vitamin A deficiency and stage synchronization (Morales & Griswold, 1987)
1987	Sertoli cell production of testibumin (Cheng *et al.*, 1987)
1988	Autoantigenic germ cells located outside blood–testis barrier suggesting Sertoli cells secrete immunoregulatory factors (Yule *et al.*, 1988)
1989	Sertoli cell production of growth factors (Skinner *et al.*, 1989)
1990	Sertoli cell production of α2-macroglobulin (Cheng *et al.*, 1990)
1990	Discovery of SRY (Koopman *et al.*, 1990)
1991	Sertoli cell expresses Wilms' tumor gene WT1 (Pelletier *et al.*, 1991)
1993	Transplanted allogeneic Sertoli cells survive and protect islet allografts (Selawry & Cameron, 1993)
1993	Thyroid hormone contributes to terminal differentiation of Sertoli cells and testis size (Hess *et al.*, 1993; van Haaster *et al.*, 1993)
1994	GATA1 transcription factor in Sertoli cells (Yomogida *et al.*, 1994)
1996	Sertoli cell production of SOX9 (Kent *et al.*, 1996)
1998	GATA4 transcription factor in Sertoli cells (Viger *et al.*, 1998; Ketola *et al.*, 1999)
1998	Identification of DMRT1 (Raymond *et al.*, 1998, 1999)
2000	Sertoli cell production of GDNF regulates spermatogonial stem cells (Meng *et al.*, 2000)
2004	Sertoli cell based gene therapy is proposed (Dufour *et al.*, 2004)
2006	Wt1 is required for Sertoli cell expression of Sox9 (Gao *et al.*, 2006)
2008	Establishment of the SRY/SOX9 axis (Sekido & Lovell-Badge, 2008)
2009	Sertoli cell junctional complex internalization hypothesis (Young *et al.*, 2009, 2012; Du *et al.*, 2013; Vogl *et al.*, 2013, 2014; Lyon *et al.*, 2015)
2012	Movement of germ cell syncytium across Sertoli cell tight junction (Smith & Braun, 2012)
2013	INSR and IGF1R are required for FSH-mediated SC proliferation (Pitetti *et al.*, 2013)
2015	Retinoic acid initiation of spermatogenesis and the cycle (Griswold, 2015a)

(TEM), which was used to record higher resolution images of Sertoli cell organelles and membranes, and lasted until about 2000. The final phase has been immunohistochemistry and immunofluorescence (Hess & Vogl, 2015), which began prior to 2000, but has gradually become the major tool for localizing specific proteins in the testis (Hogarth & Griswold, 2013) and three-dimensional imaging of Sertoli–germ cell interactions (Fig. 3).

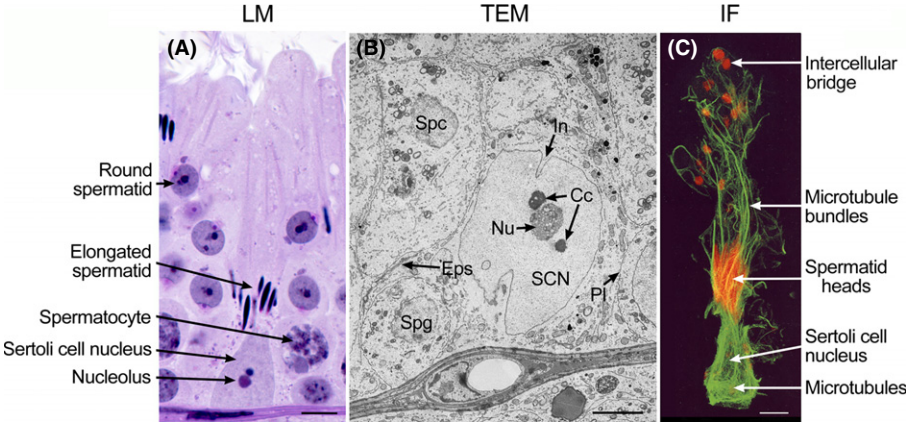
Table 2 Major reviews of the Sertoli cell

Sertoli Cell Books[a]	
1993	The Sertoli Cell (Russell & Griswold, 1993b)
2005	Sertoli Cell Biology I (Skinner & Griswold, 2005)
2015	Sertoli Cell Biology II (Griswold, 2015b)
Reviews of the Sertoli Cell	
1865	Enrico Sertoli published the 'cellule ramificate' (Sertoli, 1865)
1865–1965	Sertoli cell named (Ebner, 1888)
	Sertoli cell morphology (Regaud, 1899; Heller *et al.*, 1948)
	Sertoli cell development (Walker & Embleton, 1906; Montgomery, 1911)
	Sertoli cell and the cycle (Elftman, 1963)
1966–1980	Sertoli cell ultrastructure (Fawcett, 1975)
	Blood–testis barrier (Setchell, 1974; Setchell & Main, 1975)
	Sertoli–germ cell interactions; ectoplasmic specialization (Russell, 1980; Russell *et al.*, 1980)
	Sertoli cell and FSH (Means *et al.*, 1976; Means *et al.*, 1980)
1981–1990	Sertoli cell morphology; cell junctions; cytoskeleton; spermiation (Tindall *et al.*, 1983; Vogl *et al.*, 1983a; Russell, 1984; Russell & Peterson, 1985; Clermont *et al.*, 1987; Russell *et al.*, 1987; Kerr, 1988b; Vogl, 1989)
	Sertoli cell physiology; hormonal control (Burger & de Kretser, 1981; Ritzén *et al.*, 1981; Fritz, 1982; Mather *et al.*, 1983; Rich & de Kretser, 1983; Tindall *et al.*, 1985; Sanborn *et al.*, 1987; Skinner, 1987; Bardin *et al.*, 1988; Griswold, 1988; Griswold *et al.*, 1988; Sharpe, 1988; Ewing & Robaire, 1989; Robaire & Bayly, 1989; Wright *et al.*, 1989; Jegou, 1991)
	Sertoli cell through the cycle (Parvinen *et al.*, 1986; Wrobel & Schimmel, 1989; de Kretser, 1990; Ueno & Mori, 1990)
	Sertoli cell development (Steinberger & Steinberger, 1987; Magrek & Jost, 1991)
	Sertoli cell in vitro (Russell & Steinberger, 1989; Jegou, 1992b)
	Sertoli cell pathology and toxicology (Boekelheide *et al.*, 1989)
1991–2004	General review (Jegou, 1992a; Clermont, 1993; Jegou, 1993; Kerr, 1995; Griswold, 1998)
	Sertoli Cell Biochemistry and the Cycle (Toppari *et al.*, 1991; Morales & Clermont, 1993; Parvinen, 1993)
	Sertoli cell morphology (Pelletier & Byers, 1992; Russell, 1993a; Schulze & Holstein, 1993a; Mulholland *et al.*, 2001)
	Sertoli cell cytoskeleton and germ cell translocation (Vogl *et al.*, 1991; Vogl *et al.*, 1993)
	Blood–testis barrier (Pelletier & Byers, 1992; Grier, 1993; Lui *et al.*, 2003; Wong & Cheng, 2005)
	Sertoli-germ cell communication (Jegou, 1991, 1993; Mullaney & Skinner, 1991; Vogl *et al.*, 1991, 2000; Byers *et al.*, 1993a,b; Russell, 1993b,c; McGuinness & Griswold, 1994; Griswold, 1995; Cheng & Mruk, 2002; Mruk & Cheng, 2004a,b)
	Sertoli cell physiology (Jegou, 1992a; Josso, 1992; Sharpe, 1992; Dorrington & Khan, 1993; Griswold, 1993a,c; Heckert & Griswold, 1993; Hinton & Setchell, 1993; Kim & Wang, 1993; Sar *et al.*, 1993; Sharpe, 1993; Skinner, 1993b; Sylvester, 1993; Dym, 1994; Andersson, 2001; de Kretser *et al.*, 2001; Silva *et al.*, 2002; Walker, 2003b)
	Sertoli cell secretions (Fritz *et al.*, 1993; Griswold, 1993b; Skinner, 1993a; Sylvester, 1993; McKinnell *et al.*, 1995)
	Sertoli cell in vitro (Jegou, 1992b; Djakiew & Onoda, 1993; Steinberger & Jakubowiak, 1993; Dym, 1994)
	Sertoli cell development (Gondos & Berndston, 1993; Pelliniemi *et al.*, 1993; Orth *et al.*, 2000; Walker, 2003a; Brehm & Steger, 2005)
	Sertoli cell pathology and toxicology (Boekelheide, 1993; Schulze & Holstein, 1993b; Jegou *et al.*, 2000; Sharpe *et al.*, 2003; Toyama *et al.*, 2003)
	Species comparison (Bartke *et al.*, 1993; Hinsch, 1993; Pudney, 1993; Guraya, 1995; McKinnell *et al.*, 1995)
	Sertoli cell and immune system (Dufour *et al.*, 2003a)
2005–2015	Sertoli cell morphology (Hess & França, 2005; Kerr *et al.*, 2006; O'Donnell *et al.*, 2011; Vogl *et al.*, 2013; Berruti & Paiardi, 2014; Hess & Vogl, 2015; Lyon *et al.*, 2015)
	Sertoli cell biochemistry and the Cycle (Hermo *et al.*, 2010; Griswold, 2015a; Wright, 2015)
	Sertoli cell numbers (Johnson *et al.*, 2008)
	Sertoli cell intracellular trafficking (Cheng & Mruk, 2009; Xiao *et al.*, 2014b)
	Sertoli cell, extracellular matrix, and polarity(Siu & Cheng, 2009; Wong & Cheng, 2009)
	Sertoli cell, cytoskeleton, and germ cell interactions (Vogl *et al.*, 2008; Cheng & Mruk, 2010, 2015; Cheng *et al.*, 2010; Hermo *et al.*, 2010; Kopera *et al.*, 2010; Lie *et al.*, 2010; Mruk & Cheng, 2010; O'Donnell *et al.*, 2011; Su *et al.*, 2013; O'Donnell & O'bryan, 2014; Qian *et al.*, 2014; Xiao *et al.*, 2014a)
	Blood–testis barrier (Mital *et al.*, 2011; Pelletier, 2011; Cheng & Mruk, 2012; Lie *et al.*, 2013; Jiang *et al.*, 2014; Li *et al.*, 2015; Mruk & Cheng, 2015)
	Sertoli cell physiology (Brehm & Steger, 2005; Walker & Cheng, 2005; Cheng & Mruk, 2010; Rato *et al.*, 2010; Lucas *et al.*, 2011; Smith & Walker, 2014; Smith *et al.*, 2015)
	Sertoli cell and transcriptional regulation (Griswold & McLean, 2005; Lui & Cheng, 2008, 2012; Cheng *et al.*, 2010; Fok *et al.*, 2014; Cheng & Mruk, 2015; Chojnacka & Mruk, 2015; Heckert & Agbor, 2015; Hogarth, 2015; Wright, 2015; Yan, 2015)
	Sertoli cell and microRNAs (Ramaiah & Wilkinson, 2015)
	Sertoli cell pathology and toxicology (Wong & Cheng, 2011; Brunocilla *et al.*, 2012; Johnson, 2014; Murphy & Richburg, 2014; Gao *et al.*, 2015; Reis *et al.*, 2015)
	Sertoli cell and spermatogonial stem cells (Oatley & Brinster, 2012; Garcia & Hofmann, 2013; Hai *et al.*, 2014; de Rooij, 2015)
	Sertoli cells and immune system (Mital *et al.*, 2010; Franca *et al.*, 2012; Kaur *et al.*, 2012, 2014a, 2015)
	Sertoli cells and cancer (Oliveira *et al.*, 2015)
	Sertoli cell and meiosis (Griswold, 2016)
	Sertoli cell and development (Gassei & Schlatt, 2007; Sekido & Lovell-Badge, 2008; Barrionuevo *et al.*, 2011; Jakob & Lovell-Badge, 2011; Nicholls *et al.*, 2012; Tarulli *et al.*, 2012; Escott *et al.*, 2014; Dong *et al.*, 2015; Loveland & Hedger, 2015; Yang & Oatley, 2015; Yao *et al.*, 2015; Young *et al.*, 2015)
	Species comparison (Schulz *et al.*, 2010; França *et al.*, 2015)

[a]See Table S1 for complete listing.

Sertoli cell morphology represents one of the most complex, three-dimensional structures in cell biology and yet Enrico Sertoli made his historical observations without the benefit of a good fixative, thin sections of embedded testicular tissues and histological stains that are now common laboratory tools. Nevertheless, he was able to describe unique branches of the cell's cytoplasm that supported germ cell development and the nucleus with a large nucleolus (an important morphological feature that is used for cellular recognition today). Sertoli suggested that these cells were individual, but others claimed that they

Figure 2 Three phases observed in morphological studies of the Sertoli cell. (A) Light microscopy (LM). The image is from mouse seminiferous epithelium, Stage IV (Periodic acid-Schiff's stain). The Sertoli cell nucleus is euchromatic with a large nucleolus and a single satellite chromocenter. An intimate association of germ cells with the Sertoli cell is displayed with pachytene spermatocytes adjacent to the Sertoli cell cytoplasm and heads of elongated spermatids that are pulled deep into the Sertoli cell crypts and lying next to the apical region of its nucleus. Round spermatids are found near the lumen. Bar = 12 μm. (B) Transmission electron microscopy (TEM). The tissue is from human testis, showing the Sertoli cell resting on the basement membrane and surrounded by germ cells. Spermatogonia (Spg); Spermatocyte (Spc). The Sertoli cell has a highly euchromatic nucleus (SCN), a large nucleolus (Nu) with two satellite chromocenters (Cc) and an indentation of the nuclear membrane (In). Plasmalemma (Pl); Ectoplasmic specialization (Eps) at the blood–testis barrier. Bar = 5 μm. (C) Immunofluorescence microscopy. The Sertoli cell was isolated in vitro with attached elongated spermatids and labeled for somatic cell-specific tubulin (green) and filamentous actin (red). Actin is labeled at the ectoplasmic specialization and the intercellular bridges. The microtubules are continuous with the apical regions and around the basal area of the nucleus and directly adjacent to the ectoplasmic specializations, attached to the spermatid heads that are drawn deep into the Sertoli cell crypts. Bar = 10 μm. Original illustration provided by Dr. A. Wayne Vogl, Department of Cellular and Physiological Sciences, Faculty of Medicine, University of British Columbia, Vancouver, Canada (Vogl *et al.*, 1995). Modified and reprinted with permission of the Copyright © holder Elsevier.



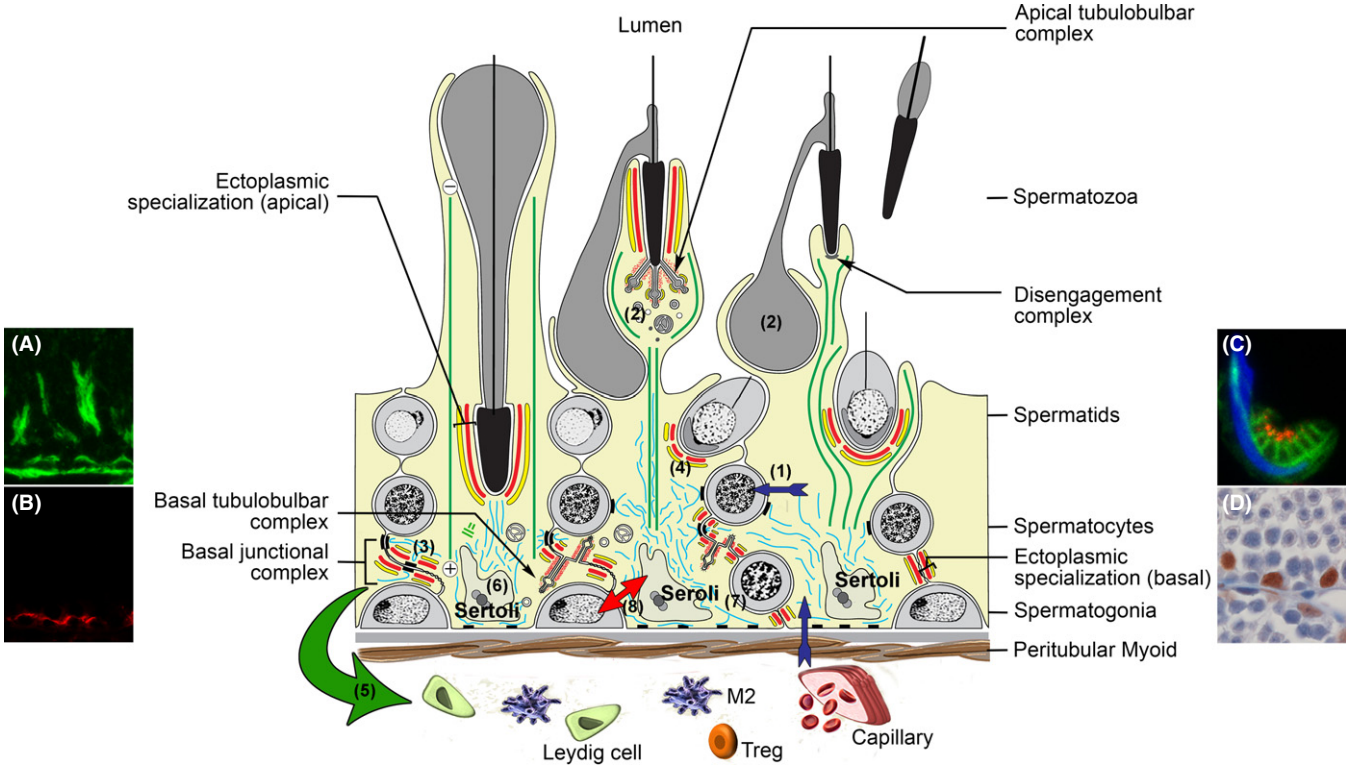
were a syncytium (Ebner, 1888), which became a standing controversy that was not settled until 1955, when electron microscopy was able to reveal cellular membranes and junctional complexes (Burgos & Fawcett, 1955; Zebrun & Mollenhauer, 1960; Fawcett, 1975). Numerous reviews of Sertoli cell morphology (Table 2) have provided unique insights into the cell's interactions within the seminiferous epithelium (Figure S1), particularly focusing on the following structures: shape of the nucleus, the thin cytoplasmic arms, the intricate physical association with germ cells, the spermatid disengagement complex and the changes observed in these features over the course of the cycle of the seminiferous epithelium (Heller *et al.*, 1948; Elftman, 1963; Flickinger & Fawcett, 1967; Fawcett, 1975; Russell, 1980, 1984, 1993a,b; Wright *et al.*, 1983; Kerr, 1988b; Russell *et al.*, 1990a; Ueno & Mori, 1990; Morales & Clermont, 1993; Mruk & Cheng, 2004b, 2010; Hess & França, 2005; Wong & Cheng, 2005; Vogl *et al.*, 2008; O'Donnell *et al.*, 2011; Xiao *et al.*, 2014a; Hess & Vogl, 2015). These morphological features have contributed significantly to the overall beauty of this 'cellule madri' or 'mother cell.'

The Sertoli cell nucleus is one of its most distinguishable organelles (Hess & França, 2005; Hess & Vogl, 2015). It is large, euchromatic (Fig. 2), and capable of changing shape throughout the cycle of the seminiferous epithelium, often exhibiting deep invaginations (Figure S2) of the nuclear membrane that is surrounded by vimentin intermediate filaments. The nucleolus is very large and stains intensely (Schulze *et al.*, 1976), with three distinct parts (tripartite), with most nucleoli have two satellite chromocenters in rodent species, although three satellite structures are found occasionally in a small percentage of mice (Kushida *et al.*, 1993; Guttenbach *et al.*, 1996) and rat Sertoli cells (Hess & Vogl, 2015). In some species, the satellite chromocenters form donut shapes, but these structures often are out of the plane of section. The nucleus is usually described as residing near the basement membrane (Russell *et al.*, 1990a); however, in some species the nucleus can be located higher in the epithelium near the lumen, as is common in stages surrounding spermiation in rodents. When staining for Sertoli cell nuclear proteins, the more apical nuclei are easily recognized, as seen with the androgen receptor (Fig. 3). However, when a nuclear protein is present in both Sertoli and spermatogonial germ cells, such as the E2f family of transcription factors (El-Darwish *et al.*, 2006), care must be taken, as stages immediately following spermiation have fewer spermatogonia and recognition of the Sertoli cells may require an evaluation of nuclear shape as well as the presence of a large nucleolus.

Major immunohistochemical markers for the Sertoli cell nucleus that are commonly used for morphology include the following: androgen receptor (AR) (Sar *et al.*, 1990); SOX9 (SRY-box containing gene 9) (Frojdman *et al.*, 2000); Wilms tumor protein-1 (WT1) (Sharpe *et al.*, 2003; Wang *et al.*, 2013); GATA-binding protein 1 (GATA1) (Chen *et al.*, 2005); GATA-binding protein 4 (GATA4) (McClusky *et al.*, 2009); and cyclin-dependent kinase inhibitor 1B ($p27^{kip1}$) (Sharpe *et al.*, 2003). Age-specific expression of these markers is an important consideration (Hess & Vogl, 2015), as SOX9 is strong prenatal but decreases dramatically post birth (Frojdman *et al.*, 2000), whereas WT1, is present in the Sertoli cell nucleus throughout all developmental ages and AR shows increasing expression after the onset of puberty (Sharpe *et al.*, 2003). GATA4 is expressed throughout development (Kyronlahti *et al.*, 2011) and does not vary with the cycle of the seminiferous epithelium in the adult. In addition, GATA4 is not inhibited by the presence of germ cells, which is a problem with GATA1 expression (Yomogida *et al.*, 1994).

Deep indentations or clefts of the nuclear envelope (Dym, 1973), which are signs of Sertoli cell maturation, are absent

Figure 3 Schematic illustration of the Sertoli cell's interaction with germ cells at different stages during spermatogenesis and other key functions, including: (1) transport of micronutrients across the junctional complex; (2) management of waste and recycled leftover cytoplasm during germ cell development; (3) maintenance of the blood–testis barrier (BTB); (4) establishment of germ cell adhesions and communication; (5) inhibition of immune reactions and maintenance of immune privilege; (6) initiation and response to endocrine signaling pathways; (7) initiation and regulation of the cycle of the seminiferous epithelium; and (8) maintenance of stem cell homeostasis. Most autoimmunogenic germ cells are sequestered within the adluminal compartment of the seminiferous epithelium behind the BTB, where Sertoli cells surround them. Sertoli cells secrete immunoregulatory factors (5) that modify the immune response and induce regulatory immune cells such as macrophages (M2) and T cells (Tregs). (A) Actin filaments (green) are seen along the basal Sertoli/Sertoli tight junctions but also lining the heads of elongated spermatids; (B) Claudin-11 (red) stains only the basal junctional complex; (C) Actin (green), Rab5 (red) and DAPI (blue for nucleus) show the intricate relationship of these proteins to the tubulobulbar complex; (D) Androgen receptor (brown) stains only the Sertoli cell nucleus in the hamster seminiferous epithelium. Original illustration provided by Dr. Wayne Vogl (Hess & Vogl, 2015). Modified with approval of the Copyright © holder for Sertoli Cell Biology, 2nd edition, Elsevier Academic Press.



during development and often in tubules following the destruction of the germ cells and in patients with impaired fertility (Schulze *et al.*, 1976; Hess & França, 2005). The clefts are difficult to observe in light microscopy without ultrathin sections (Hess & França, 2005) and are rarely observed with immunohistochemical staining. Little is known regarding the function of these nuclear modifications but they may provide a unique site for nuclear targeting of specific proteins (Rothbarth *et al.*, 2001). Other unique aspects of the Sertoli cell nucleus include a high density of nuclear pores that varies depending on the stage of spermatogenesis (Cavicchia *et al.*, 1998) and heavy vesiculation of the nucleoplasm in some species (Pawar & Wrobel, 1991).

The long cytoplasmic arms (branches as described by Sertoli) wrap the germ cells with very thin structures having widths often less than 50 nm (Hess & Vogl, 2015). These thin processes, which form cup-like areas to hold and nurture the germ cells through their differentiation (Russell, 1993a), are best illustrated by a plastic three-dimensional model built by Lonnie Russell (Russell, 1993a) and based upon the electron microscopic serial sections of a single Sertoli cell (Fig. 4). Surface area of the Sertoli cell plasma membrane is increased dramatically by the extension of these arms, up to 16,000 μm^2, showing tremendous stage-dependent variation that involves the translocation of numerous organelles, the expression of hundreds of different classes of proteins for specific functions, and requiring the transport of these proteins to specific regional positions throughout the cycle of the seminiferous epithelium (Parvinen, 1982, 1993; Ritzen *et al.*, 1982; Mather *et al.*, 1983; Wright *et al.*, 1983, 1989; Parvinen *et al.*, 1986; Kaipia *et al.*, 1991; Toppari *et al.*, 1991; Johnston *et al.*, 2008; Hess & Vogl, 2015; Wright, 2015). Without an understanding of such complex form, it would have been impossible to comprehend the numerous functional interactions that depend on the Sertoli cell plasmalemma, such as tight junctional complexes that comprise the blood–testis barrier, as well as sperm disengagement (spermiation) and phagocytosis of the residual body of leftover spermatid cytoplasm (Vogl *et al.*, 2013; Hess & Vogl, 2015; Lyon *et al.*, 2015).

The intricate physical association that Sertoli cells have with germ cells begins first with the Sertoli–Sertoli tight junction (ScTj), which contributes to the blood–testis barrier (Fig. 5). The barrier is considered to be very tight (Pelletier, 2011), but differences have been found between in vivo and in vitro conditions, which were thought to be because of the peritubular myoid and germ cells contributing to an increase in transepithelial resistance (Mruk & Cheng, 2015). One of the most surprising morphological activities of the seminiferous epithelium is the

Figure 4 A three-dimensional drawing of a Stage V rat Sertoli cell taken from a photograph of a plastic model created from 675 micrographs of 372 serial electron microscopic sections. Cellular processes and cup-like hollows show the intimate relationship with adjacent germ cells (Wong & Russell, 1983). Reprinted with approval of the Copyright © holder John Wiley & Sons, Inc.

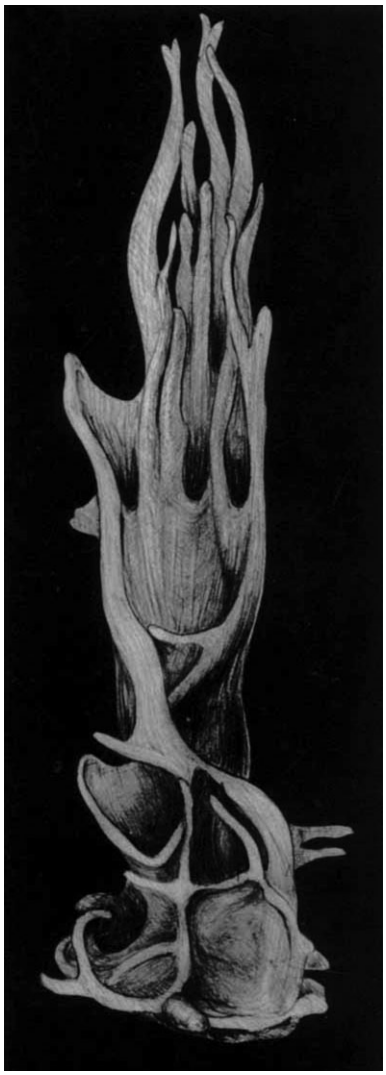
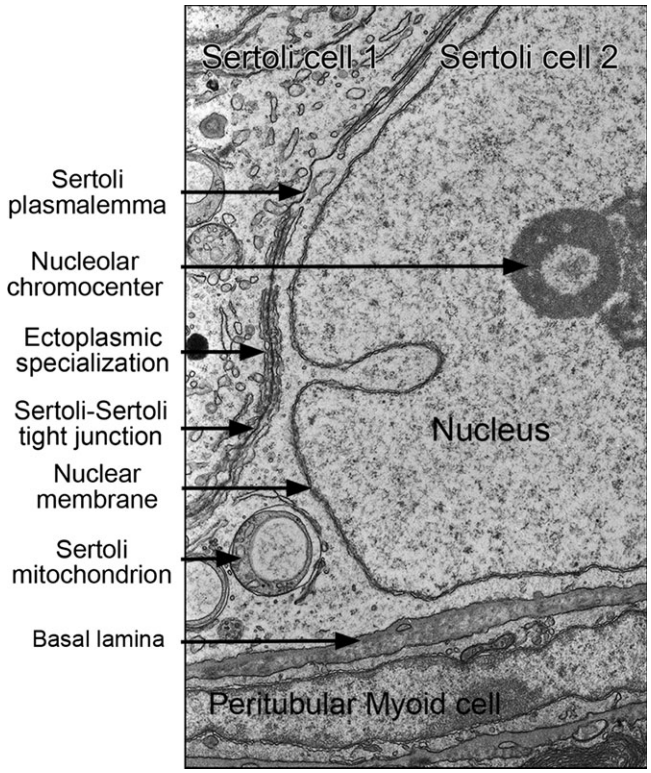


Figure 5 Electron microscopy of adjacent Sertoli cells showing the tight junctional complex (Sertoli–Sertoli tight junction) and associated basal ectoplasmic specialization. The Sertoli cell plasmalemma is seen between the two cells, which sit on the basal lamina and a peritubular myoid cell. One nucleolar chromocenters is noted in the large, euchromatic nucleus.

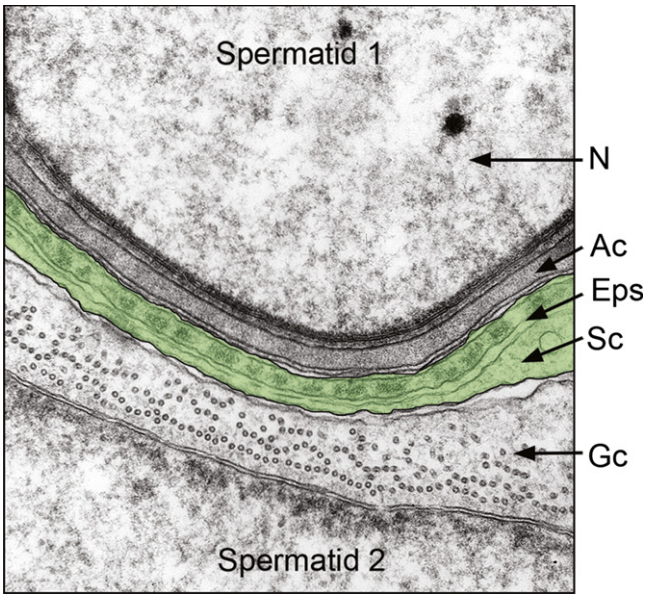


passage of preleptotene and leptotene spermatocytes through the ScTj, performing a complex transit from the basal to adluminal compartment (Smith & Braun, 2012). Passage of hundreds of syncytial germ cells occurs without disrupting this important barrier, requiring a very unique coordination, similar to that of canal locks opening and closing around a ship. However, the germ cells remain connected via cytoplasmic bridges that pass through the tricellular junctions formed by three adjacent Sertoli cells (Smith & Braun, 2012). The regulation of ScTj proteins and structure is multifaceted, involving hormones such as androgens and FSH, cytokines (i.e., TNFα and TGFβ), the presence of germ cells, actin nucleating protein N-WASP (neuronal Wiskott-Aldrich syndrome protein), and phosphorylation of key proteins such as the claudins (Mruk & Cheng, 2015). In addition, a basal tubulobulbar complex has been identified morphologically as a potential component of the assembly and disassembly that is required for passage of the early spermatocytes through the ScTj (Du *et al.*, 2013; Lyon *et al.*, 2015). The current hypothesis is that this activity provides a remodeling of the intercellular junctions and disengagement of junctional molecules in the plasma membrane, followed by endocytosis and intracellular trafficking (Du *et al.*, 2013; Lyon *et al.*, 2015; Mruk, 2015).

Lastly, another unique structural phenomenon of the physical association of Sertoli and germ cells is the positioning and transport of the elongating spermatids within the seminiferous epithelium throughout the cycle (Fig. 3). Early steps of elongated spermatids are attached in the more apical crypts (Figs 2 & 4) of the Sertoli cell (Meistrich & Hess, 2013) and lengthen perpendicular to the basement membrane. As the spermatids elongate, the germ cells are transported into deep indentations of the Sertoli cell, with their heads nearly touching the Sertoli cell nucleus (Hess, 1990). Finally, the Sertoli cell transports the late step spermatids toward the lumen where fully developed spermatozoa are released during spermiation. This dynamic mobilization of elongated spermatids is orchestrated by the Sertoli cell through the use of parallel microtubule tracts and motor proteins attached to the endoplasmic reticulum component of the ectoplasmic specialization (Vogl, 1988; Vogl *et al.*, 1991; Beach & Vogl, 1999).

Sertoli–Sertoli cell and Sertoli–germ cell attachments are some of the most elegant and dynamic structures observed with electron microscopy. Soon after the electron microscope became popular, it was recognized that the ScTj was unique (Brökelmann, 1963; Flickinger & Fawcett, 1967; Nicander, 1967), first being called 'junctional specialization' by Flickinger and Fawcett. Ten years later, Lonnie Russell coined the term

Figure 6 Electron microscopy showing the thin arm of a Sertoli cell (light green area) containing the ectoplasmic specialization (Eps) and Sertoli cell cytoplasm (Sc) adjacent to the germ cell cytoplasm showing the manchette microtubules of spermatid 2 and the acrosome (Ac) that covers the nucleus (N) of spermatid 1.



'ectoplasmic specialization' (ES), linking the ScTj with a narrow band of actin filaments and endoplasmic reticulum on both sides of the adjoining cell membranes (Russell, 1977). This exclusive structure displayed its plasticity by also appearing as a Sertoli cell component of the Sertoli-spermatid germ cell junctional attachment site, forming apical ectoplasmic specializations facing the germ cell at the edge of the very narrow cytoplasmic arms that surrounded forming spermatid heads (Fig. 6) (Xiao *et al.*, 2013; Gungor-Ordueri *et al.*, 2014; Hess & Vogl, 2015; Li *et al.*, 2015; Mruk & Cheng, 2015). The basal ES is part of the blood–testis barrier, along with the proteins and structures associated with the junctional complex (tight, gap and desmosome). Actin filament proteins are probably the most abundant and easily visualized component of the ES (Fig. 3) with an abundance of binding, adaptor, and linking proteins that coordinate this unique plasma membrane structure, whereas vimentin filaments attach to the desmosomes (Mruk & Cheng, 2015).

Another fascinating structure that participates in the Sertoli–germ cell physical juncture is the tubulobulbar complex, first described by Lonnie Russell (Russell & Clermont, 1976) as an anchoring device. Tubulobulbar complexes are elongated tubular extensions of one cell into corresponding invaginations of the adjacent cell plasma membrane, terminating with a bulb that is associated with cisternae of endoplasmic reticulum (Vogl *et al.*, 2013). These intimate structures are located at both the basal ES between adjacent Sertoli cells (as discussed with the ScTj above) and at the apical tips of Sertoli cell cytoplasm, within the concave area of the heads of late spermatids (Fig. 3; Figure S1). More recent discoveries have supported an important role for these complexes in the disengagement of mature spermatids and removal of excess germ cell cytoplasm during spermiation, as well as the recycling of junctional molecules at both locations (Young *et al.*, 2009; Du *et al.*, 2013; Vogl *et al.*, 2013, 2014; Lyon *et al.*, 2015).

The uniqueness of these specialized organelles, which support Sertoli–germ cell interactions and transport within the seminiferous epithelium, has been clearly observed morphologically over the past 50 years. However, it is the amalgamation of molecular and biochemical data with histology through immunolocalization (Fig. 3) and other imaging technologies that is now providing new explanations for the complex physiology of the Sertoli cell, which is required for the maintenance of continuous sperm production throughout life in the adult male (Vogl *et al.*, 2013, 2014; Xiao *et al.*, 2014a; Hess & Vogl, 2015; Lyon *et al.*, 2015).

MOLECULAR BIOLOGY AND MECHANISMS

The original notion of the Sertoli cell as a 'nurse cell' was a direct result of its morphological relationships with the developing germ cells. While the morphology has been elegantly reported, even after 150 years of research with new and better technological tools the Sertoli cell has retained many of its molecular secrets. The ability to independently maintain primary Sertoli cells from rodents in relatively pure cell culture led to the first molecular studies in the 1970s and 1980s (Dorrington & Armstrong, 1975; Steinberger, 1975). This approach and the availability of specific antibodies and improvements in microscopy have revealed several gene products that are unique to the Sertoli cell.

Some of the first known products of Sertoli cells included metal transport proteins such as transferrin and ceruloplasmin (Skinner & Griswold, 1980; Skinner & Griswold, 1982; Skinner & Griswold, 1983). It was proposed that these products represented the true 'nurse cell' function (Fig. 5) by providing mechanisms to transport micronutrients across the blood–testis barrier to support germ cell development (Sylvester & Griswold, 1994). A model was proposed where Sertoli cells at the basal surface can take up iron bound to serum transferrin, transfer it to a newly synthesized transferrin molecule and secrete it on the apical side of the blood–testis barrier to be used by the developing germ cells. This model provided a mechanism for moving iron (Fe^{+3}) across the blood–testis barrier for use primarily in meiotic and mitotic cell divisions in spermatogonia and spermatocytes and mitochondriogenesis in spermatids. Iron in this form is required for many cellular functions but because of its very low solubility it must be transported bound to specific transport proteins such as transferrin. Recently, with the current knowledge about additional components of the iron transport pathway and the availability of antibodies this model has been expanded (Leichtmann-Bardoogo *et al.*, 2012). The localization and regulation of a number of proteins involved in iron transport, storage, and export were examined in mouse testes and it was determined that there is an autonomous internal iron cycle within the seminiferous tubules. The cycle consists of primary spermatocytes loading with iron from the Sertoli cells, maintaining those iron stores to support mitosis, meiosis, and mitochondriogenesis through the elongated spermatid stage and then returning the bulk of the iron to the Sertoli cells in the ingested residual bodies. The Sertoli cells then recycle the ingested iron back to the primary spermatocytes. In this model, the Sertoli cells function as 'nurse cells' by providing the iron required for germ cell development but also remove and recycle the potential toxic accumulation of iron in the residual bodies. The 'nurse cell' function in this case is equivalent to emptying the bedpans!

There are likely several such mechanisms that are part of the nurse function(s) of Sertoli cells and they have been referred to as the 'recycling and waste management' functions (Yan *et al.*, 2008; Leichtmann-Bardoogo *et al.*, 2012; Young *et al.*, 2012; Vogl *et al.*, 2014; Yan, 2015).

The products of Sertoli cells can inhibit immune reactions (Dufour *et al.*, 2005; Doyle *et al.*, 2012) and provide structural features (Russell *et al.*, 1983; Russell, 1993a) under a broad classification of 'bioprotective and structural' functions. Adjacent Sertoli cells synthesize the components of the tight junction complexes (Figs 3 & 5) and contribute to the basement membrane and participate in the formation of desmosomes, gap junctions and some unique forms of junctions with germ cells. The nature of spermatogenesis results in the production of enormous numbers of gametes and therefore these structural elements must be extremely dynamic and involve the spatiotemporal expression of many genes.

Sertoli cells also play key roles in signaling in the testis by serving as the targets for FSH and testosterone and by transducing those endocrine signals and other cellular cues into paracrine regulation of germ cells (Griswold, 1993a; Johnston *et al.*, 2001). Again, both the response of gene expression to FSH and testosterone and the expression of growth factors or other signaling molecular vary with testis development and with the stage of the cycle of the seminiferous epithelium revealing the requirement for a complex and carefully controlled gene network. An example of paracrine signaling can be found in the initiation of spermatogenesis in the mouse testis. Shortly after birth the A undifferentiated spermatogonia undergo the transition to A1 differentiating spermatogonia. This transition results in a carefully timed commitment of those cells to meiosis and is absolutely dependent on retinoic acid synthesized by the Sertoli cells. In the mouse this transition occurs in patches along the tubule that lead to asynchronous entry into meiosis and the spermatogenic wave (Hogarth & Griswold, 2010; Snyder *et al.*, 2010).

Gene expression that results in the formation of the testis in the embryo occurs within the Sertoli cells. Genes encoding transcription factors such as Sry and Sox9 expressed in the Sertoli cells alter the transcriptome such that germ cells are enclosed in seminiferous tubules resulting in the formation of the testis (for a review see DiNapoli & Capel, 2008; Svingen & Koopman, 2013). In addition, Cyp26B1 expressed in Sertoli cells breaks down any retinoic acid and prevents the germ cells from entering the pathway to meiosis (Svingen & Koopman, 2013). Recently it has been shown that loss of the DMRT1 transcription factor in mouse Sertoli cells, even in adults, activates another transcription factor, Foxl2, and reprograms Sertoli cells into granulosa cells. These studies have shown that Dmrt1 in Sertoli cells is essential for sustained mammalian testis determination (Matson *et al.*, 2011). So, a dynamic transcriptome with precise spatiotemporal expression of genes in the Sertoli cells begins early in the embryo, has a unique program with the onset of spermatogenesis and then varies with the cycle of the seminiferous epithelium throughout the reproductive lifetime of the organism.

The production of spermatozoa by in vitro differentiation of germ cells has been reported for several species. If these reports are accepted at face value it means that the male germ cells have an autonomous program leading to sperm formation (de Winter *et al.*, 1993; Kerkis *et al.*, 2007; Aflatoonian *et al.*, 2009; Sato *et al.*, 2011b). However, even the most successful of the protocols in these reports are extremely inefficient at sperm production when compared with spermatogenesis in the testis. In 1993 and 1994, it was suggested in two separate reviews that Sertoli cells might be primarily 'permissive' in nature (Russell & Griswold, 1993a; Sharpe, 1994). This view would suggest that Sertoli cells provide the environment, the signaling and the structure to allow the efficient autonomous differentiation of male germ cells into spermatozoa.

Recently the use of the RiboTag mouse that allows the genetic tagging of polysomes in Sertoli cells in vivo has resulted in a relatively comprehensive description of gene expression in the postnatal Sertoli cells (Sanz *et al.*, 2013; De Gendt *et al.*, 2014; Evans *et al.*, 2014). These approaches have resulted in lists of genes that are overexpressed in Sertoli cells relative to the other cell types in the testis. Results from one study showed that the genes most overexpressed in postnatal and adult Sertoli cells involved glutathione metabolism, cytochrome P450 enzymes, drug metabolism pathways, peptidase, and enzyme inhibitor pathways (De Gendt *et al.*, 2014). Expression of these types of genes is consistent with a role in 'recycling and waste management'. Information on which genes are expressed in Sertoli cells can lead to a couple of outcomes. If the product of the gene is a protein with a known function then the role of that protein in the function of Sertoli cells may become apparent. However, it is often the case that the gene product has an unknown function or the known function is not easily reconciled with the presumed role of Sertoli cells in spermatogenesis. Many investigators have attempted to list the various functions of Sertoli cells and generally these lists imply that Sertoli cells participate in nearly all aspects of spermatogenesis (Griswold, 1988, 1995; Griswold *et al.*, 1988; Sharpe, 1994). In addition, the many roles of the Sertoli cells vary during development and across the cycle of the seminiferous epithelium in the adult and require active and well-controlled transcriptional programs. Very few highly differentiated cell types have a lifetime requirement for this type of plasticity in gene expression.

SERTOLI CELL AND THE SPERMATOGONIAL STEM CELL NICHE

The spermatogonial stem cell niche

Adult stem cells are essential for the maintenance, repair and regeneration of many organs, and proper regulation of their fate is therefore critical to maintain tissue homeostasis. Accumulating evidence suggests that stem cell self-renewal and differentiation depend on specialized microenvironments called niches (Spradling *et al.*, 2001; Scadden, 2006) and that in turn stem cells influence their environment (Baraniak & McDevitt, 2010; Mosher *et al.*, 2012; Sowa *et al.*, 2012). In the mammalian testis, spermatogonial stem cells (SSCs) reside on the basement membrane of the seminiferous tubules and are in intimate contact with the Sertoli cells. The SSC niche is made of a complex interplay of growth factors provided by Sertoli and interstitial cells, the basement membrane and stimuli from the vascular network (Chiarini-Garcia *et al.*, 2001; Chiarini-Garcia *et al.*, 2003; Kanatsu-Shinohara *et al.*, 2007; Oatley & Brinster, 2012). Because of its physical association with germ cells, the Sertoli cell is arguably the most important contributor of the niche, by providing paracrine and juxtacrine signals and secreting components of the basement membrane. In addition, experimental increase in the

number of Sertoli cells leads to additional niches that can be colonized by SSCs after transplantation (Oatley *et al.*, 2011). It is also evident that the niche must provide different sets of signals to the SSCs depending on the timing of testis development. For example, in the neonatal testis active SSC self-renewal takes place to establish the stem cell pool, and studies have demonstrated that neonatal niches are more efficient than adult niches to regenerate spermatogenesis from transplanted SSCs (Shinohara *et al.*, 2001). In contrast, SSC self-renewal in the adult may only occur during certain stages of the seminiferous cycle (Johnston *et al.*, 2011; Grasso *et al.*, 2012).

Sertoli cell factors controlling SSC maintenance and self-renewal

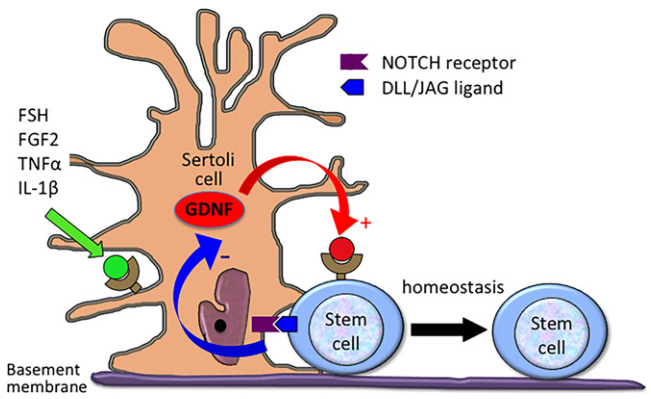
A number of soluble factors produced by Sertoli cells have been recently discovered that are critical for maintenance of pro-spermatogonia in the fetus and self-renewal of SSCs after birth. The most extensively studied niche factor is glial cell line-derived neurotrophic factor (GDNF). Mutant mice with GDNF haploinsufficency have severe fertility defects and disrupted spermatogenesis (Meng *et al.*, 2000). Similarly, ablation of GDNF and its receptors *Ret* and *Gfra1* at the surface of SSCs result in loss of the stem cells and their progeny (Naughton *et al.*, 2006). GDNF is used by SSCs throughout life; however, as its production decreases with age SSC numbers decline, which illustrates the fact that the stem cell pool is dependent upon GDNF for its maintenance (Ryu *et al.*, 2006). Conversely, ubiquitous overexpression of GDNF in transgenic mice leads to overproduction of undifferentiated spermatogonia and sharp decrease in more differentiated germ cells (Meng *et al.*, 2000). In vitro studies also demonstrated that GDNF is critical for SSC maintenance and self-renewal in short- and long-term cultures (Nagano *et al.*, 2003; Kubota *et al.*, 2004; Chen *et al.*, 2005).

During mouse development, low levels of GDNF are already detectable in the bipotential gonad at embryonic day 11 (Beverdam & Koopman, 2006) and its expression increases steadily to reach a maximum at post-natal day 3 when the SSC population starts to expand (Tadokoro *et al.*, 2002; Shima *et al.*, 2004; Miles *et al.*, 2012). In older animals, the levels of GDNF expression vary between the stages of the seminiferous epithelial cycle, but differences between species have been observed (Sato *et al.*, 2011a; Caires *et al.*, 2012a; Grasso *et al.*, 2012). In the rat, the highest levels of GDNF mRNA was observed at stages XII to III, when undifferentiated spermatogonia proliferate (Johnston *et al.*, 2011), and lowest at stages VII and VIII when most cells are quiescent and the majority of $A_{aligned}$ spermatogonia transition to the differentiating A1-A4 cells. These observations indicate that GDNF levels are cyclic, and that its dosage is crucial for the regulation of perinatal germ cell fate and stage-specific proliferation of undifferentiated spermatogonia. Although the mechanisms responsible for this cyclic expression are not understood, it is evident that GDNF production must be modulated by positive or negative stimuli. For example, follicle-stimulating hormone (FSH) is a major positive regulator of GDNF expression by Sertoli cells in vivo and in vitro (Tadokoro *et al.*, 2002). GDNF expression by cultured Sertoli cells is also stimulated by FGF2, TNFα, and IL-1β (Simon *et al.*, 2007). Mechanisms that down-regulate the production of GDNF by Sertoli cells also exist. Such a role is fulfilled by NOTCH signaling as constitutive activation of this pathway in Sertoli cells results in sharp downregulation of GDNF, a complete loss of germ cells around birth and a Sertoli cell-only phenotype (Garcia *et al.*, 2013). Conversely, ablation of RBPJ, a downstream effector of the NOTCH pathway, increases GDNF expression and results in a significant increase in SSCs and overall germ cell numbers (hyperplasia) (Garcia *et al.*, 2014). Because the NOTCH ligand JAG1 is highly expressed in undifferentiated spermatogonia and drives the expression of downstream targets of NOTCH signaling in Sertoli cells, it is assumed that germ cells use JAG1 to activate the NOTCH pathway in Sertoli cells, therefore downregulating GDNF and controlling their own numbers (Fig. 7). This mechanism would ensure proper gem cell homeostasis and sperm output, and is in accord with the observations of Johnston and colleagues who suggested that spermatogenic cell density seem to limit GDNF production by Sertoli cells (Tadokoro *et al.*, 2002; Ryu *et al.*, 2006; Johnston *et al.*, 2011).

While GDNF is certainly a major component of the stem cell niche, in vitro culture experiments demonstrated that it is not the only factor needed for maintenance and long-term renewal of SSCs. Depending on the genetic background of the mice, fibroblast growth factor (FGF2) and epidermal growth factor (EGF) in addition to GDNF are critical for long-term support (Van Dissel-Emiliani *et al.*, 1996; Kubota & Brinster, 2008; Kubota *et al.*, 2004). Production of FGF2 by Sertoli cells has been previously demonstrated and is stimulated by FSH (Smith *et al.*, 1989; Mullaney & Skinner, 1992). FGF2 promotes self-renewal independently of GDNF, through activation of the transcription factors ETV5 and BCL6B, but is less efficient (Ishii *et al.*, 2012; Takashima *et al.*, 2015).

Another Sertoli cell factor that appear to contribute to the SSCs niche is leukemia inhibitory factor (LIF) (Piquet-Pellorce *et al.*, 2000). LIF production in Sertoli cells depends on TNFα and is widely used in cultures of primordial germ cells, prospermatogonia and SSCs from several species (Pesce *et al.*, 1993; Nikolova *et al.*, 1997; Kanatsu-Shinohara *et al.*, 2003; Aponte *et al.*, 2008). While LIF maintains SSC survival and is useful to initiate long-term cultures, it is not promoting self-renewal (Kanatsu-Shinohara *et al.*, 2007). Another niche factor of interest is platelet-derived growth factor (PDGF). PDGF is specifically

Figure 7 Model depicting the possible role of NOTCH signaling in Sertoli cells after birth. Previous studies have shown that FGF2 and FSH induce GDNF expression by Sertoli cells. Recent data suggest NOTCH signaling is a negative regulator of GDNF, which might balance the effects of FGF2 and FSH. Overactivation of NOTCH signaling suppresses the expression of GDNF and leads to sterility, whereas ablation of NOTCH signaling induces germ cell hyperplasia.



produced by Sertoli cells and induces the proliferation of prospermatogonia after birth (Loveland *et al.*, 1995; Li *et al.*, 1997) probably in cooperation with estrogen (Thuillier *et al.*, 2010). Disruption of cross-talks between PDGF and estrogen-triggered signaling pathways has been suggested to take place upon exposure to xenoestrogens in the environment, which could lead to alteration of prospermatogonial behavior and preneoplastic states (Thuillier *et al.*, 2003). Also, because WNT signaling is critical for stem cell self-renewal in a variety of tissues (Clevers *et al.*, 2014), this pathway has been recently investigated in the testis (Golestaneh *et al.*, 2009; Yeh *et al.*, 2011). WNT5A is produced by Sertoli cells and promotes SSCs survival through a CTNNB1-independent mechanism that activates JNK (Yeh *et al.*, 2011), but it does not per se induce self-renewal. Indeed, CTNNB1 ablation in germ cells leads to spermatogenesis disruption but not to SSC loss (Kerr *et al.*, 2014; Rivas *et al.*, 2014). Finally, although vascular endothelial growth factor A (VEGFA) family members and their receptors are all produced by germ cells, Sertoli cells, and interstitial cells (Nalbandian *et al.*, 2003; Lu *et al.*, 2013) only the pro-angiogenic isoform VEGFA164 promotes SSC self-renewal, as determined by the SSC transplantation assay (Caires *et al.*, 2012b).

Niche factors controlling migration and homing

To remain in the basal part of the seminiferous epithelium, SSCs need to generate adhesion molecules that attach them to the basement membrane provided in part by Sertoli cells. The basement membrane mainly contains laminin, fibronectin and collagen IV, therefore attachment of SSCs is mediated at least in part by integrins, mainly ITGA6 and ITGB1 (Shinohara *et al.*, 1999). However, SSCs must migrate out of the niche to differentiate, and they also move to open niches from the apical to the basal part of the seminiferous epithelium after germ cell transplantation. It has therefore been proposed that their migration depends on chemokines or other chemotactic factors. Earlier work demonstrated that Sertoli cells produce chemokines that are regulated by the transcription factor ETV5 (Chen *et al.*, 2005) and that the chemokine CCL9, ligand of the receptor CCR1 at the surface of undifferentiated spermatogonia, is able to specifically attract these cells toward Sertoli cells (Simon *et al.*, 2010). Another chemokine of importance is SDF1 (CXCL12) expressed in the genital ridges, which is guiding CXCR4-positive primordial germ cells toward them (Doitsidou *et al.*, 2002; Ara *et al.*, 2003; Molyneaux *et al.*, 2003). In the postnatal testis SDF-1, expressed by Sertoli cells, attracts CXCR4-positive germ cells, and this activity is crucial for proper homing of SSCs after transplantation (Kanatsu-Shinohara *et al.*, 2012; Yang *et al.*, 2013). SDF-1 expression in Sertoli cells depends on the nuclear co-repressor Sin3a (Payne *et al.*, 2010). However, no SDF-1 concentration gradient has been demonstrated yet in the seminiferous epithelium. In addition, because both Sertoli cells and germ cells express CXCR4 (Johnston *et al.*, 2008; Wright, 2015), additional investigations are needed to fully understand this system.

SERTOLI CELLS AND TESTIS IMMUNE PRIVILEGE

The unique immunoregulatory status of the testis has been recognized for over two centuries; based initially on the prolonged survival of testicular tissue after transplantation into genetically disparate recipients (reviewed in Kaur *et al.*, 2013). Sertoli cells play a central role in creation of this unique immunoregulatory environment where they provide immune protection to the developing germ cells, which if exposed to the immune system can invoke a humoral and cellular immune response. Spermatocytes and spermatids first appear after immunological self-tolerance has been established and thus express novel proteins that if detected by the immune system would result in immunologic attack. This was demonstrated by the lysis of spermatocytes and spermatids after exposure to sera collected from rodents that had been previously immunized with whole semen (O'Rand & Romrell, 1977; Tung & Fritz, 1978). The immunogenicity of the germ cells is further supported by autoimmune orchitis studies where an autoimmune reaction against the germ cells resulted in loss of spermatogenesis and infertility (Jacobo *et al.*, 2011; Silva *et al.*, 2014).

The blood testis or Sertoli cell barrier

Given that the autoimmunogenic germ cells described in these studies were spermatocytes and spermatids and located within the adluminal compartment of the seminiferous epithelium, historically the lack of an autoimmune response against these germ cells was attributed to their sequestration behind the blood–testis barrier (BTB) or Sertoli cell barrier (SCB). Supporting this idea, the BTB/SCB is a physical barrier formed around puberty and composed of Sertoli cell–Sertoli cell junctions and the Sertoli cell body, which surrounds the developing germ cells (Mital *et al.*, 2011). These junctions are tight junctions (zonula occludens) comprised of occludin, claudins and junctional adhesion molecules (Mruk & Cheng, 2010). Adherens junctions, gap junctions, and desmosome-like junctions also contribute to the function of the BTB/SCB (Cheng *et al.*, 2011). The tight junctions are located along the basal region of the Sertoli cell and separate the seminiferous epithelium into adluminal and basal compartments. This barrier separates the advanced germ cells located in the adluminal compartment from the blood supply, allowing the Sertoli cells, by expressing various transporters, to control the passage of molecules across the barrier; creating a unique microenvironment for germ cell development (Mital *et al.*, 2011). The barrier also prevents the passage of leukocytes (immune cells) and molecules, including antibodies from crossing the seminiferous epithelium (Johnson & Setchell, 1968; Johnson, 1972; Dym & Romrell, 1975; Wang *et al.*, 1994; Rival *et al.*, 2006). The peritubular myoid cells form a semi-permeable barrier that also inhibits the entry of leukocytes into the seminiferous tubules (Dym & Fawcett, 1970; Fawcett *et al.*, 1970).

The primary contributors to the BTB/SCB are occludin, claudin-3, -5, and -11, zonula occludens (ZO) 1,2 and 3 and JAM-A and -B, with occludin and claudin-11 being most important for barrier integrity. Male mice lacking claudin-11 or occludin were infertile (Gow *et al.*, 1999; Saitou *et al.*, 2000). However, despite the persistence of degenerating germ cells, an autoimmune reaction was not observed. In claudin-11 knockout (KO) mice degenerating germ cells are present within the lumen of the seminiferous tubules whereas spermatozoa are absent (Gow *et al.*, 1999). Testicular autoantibodies were not detected in serum or within the adluminal compartment of the seminiferous epithelium and CD4 + T cell infiltrate was not detected within the testis. In mice treated with a mutant occludin peptide, the integrity of the BTB/SCB is disrupted and permeable to inulin, resulting in germ cell loss (Wong *et al.*, 2007). Nevertheless, antisperm antibodies were not detected in serum.

Claudin-5 KO mice all died within 10 days of birth because of a defect in blood–brain barrier function (Nitta *et al.*, 2003). Gene deletion of transcription factor ets variant 5 (ETV5) resulted in decreased claudin-5 expression in the testis and disruption of the BTB/SCB suggesting a role for claudin-5 in BTB/SCB integrity, although this has not been tested directly (Morrow *et al.*, 2009). Claudin-3 was localized to spermatocytes and newly forming tight junctions in Sertoli cells (Smith & Braun, 2012; Chihara *et al.*, 2013). Fifty percent reduction in claudin-3 mRNA expression after claudin-3 siRNA injection into testes of mice did not alter BTB/SCB integrity (Chihara *et al.*, 2013) and instead delayed spermatocyte migration across barrier. Consistently, claudin-3 KO mice were fertile and had an intact barrier (Chakraborty *et al.*, 2014).

JAMs are transmembrane proteins important for tight junction formation and cell adhesion. Deletion of JAM-A resulted in subfertility because of a defect in spermatozoa motility (Shao *et al.*, 2008), whereas male and female mice with JAM-B gene disruption were fertile (Sakaguchi *et al.*, 2006). Both JAM-A and –B KO mice appeared to have normal testicular morphology, however, the integrity of the BTB was not directly tested. ZOs are adaptor proteins that anchor claudins and occludin to the actin cytoskeleton. Knock-out of ZO-3 had no apparent phenotype (Xu *et al.*, 2009), whereas knock outs of ZO-1 and -2 were embryonic lethal (Xu *et al.*, 2009). Rescue of ZO-2 KO embryos resulted in reduced male fertility because of germ cell loss and increased permeability of the BTB to a lanthanum tracer (Xu *et al.*, 2009). However, the effect on immune function is not known, as the immunological function was not examined in these mice.

Additional support that the BTB/SCB is involved in immunological protection of the advanced germ cells has been provided by SCARKO (Sertoli Cell Androgen Receptor Knock Out) mice, which have Sertoli cell-specific deletion of the androgen receptor (Meng *et al.*, 2005; Meng *et al.*, 2011). These mice have increased permeability of the BTB/SCB and evidence of an autoimmune response (Meng *et al.*, 2005; Meng *et al.*, 2011). Thirty minutes after injection of these mice with a biotin tracer, biotin was detected within the adluminal compartment of the seminiferous tubules. In addition, germ cell autoantibodies that recognize advanced germ cells (round and elongated spermatids) were present in the serum. An increase in the number of macrophages, neutrophils, and eosinophils within the interstitial space but not within the seminiferous tubules was also observed. SCARKO mice have decreased expression of claudin-3, which was originally attributed to these observations. However, the more recent claudin-3 KO studies did not support this conclusion and suggested that androgens in Sertoli cells are performing other additional functions related to immune regulation.

Testis immune privilege is more that the BTB/SCB

While regulating access of the immune system to germ cell autoantigens is no doubt an important aspect in controlling the immune response, immunological protection of the developing germ cells is more complex and involves Sertoli cell modulation of the immune response. As a result, the whole testis is immune privileged. Evidence that the whole testis is immune privileged is provided by several studies. For instance, when foreign tissue such as allogeneic or xenogeneic pancreatic islets, skin fragments or parathyroid grafts are transplanted into the testis, they enjoy prolonged graft survival when compared with their survival after transplantation to nonimmune privileged sites even though the cells transplanted in the testis are located in the interstitial space, outside of the BTB/SCB (Barker & Billingham, 1977; Setchell, 1990; Selawry, 1994; Mital *et al.*, 2009).

Moreover, successful allogeneic spermatogonial stem cell transplantation, resulting in germ cell colonization of the basal compartment of the seminiferous tubules, has been performed in a wide range of species including birds, fish, goats, pigs, cattle, sheep, rodents, dogs, and cats without immunosuppression (reviewed in Kaur *et al.*, 2013). This is consistent with an earlier study, which demonstrated that spermatogonia and preleptotene spermatocytes, located within the basal compartment of the seminiferous epithelium, were outside of the BTB/SCB, expressed auto-immunogenic antigens (Yule *et al.*, 1988) and yet, an immune response was normally not generated against these germ cells.

In humans, fine-needle biopsies (causing local injury to the seminiferous epithelium) are performed routinely and normally do not lead to autoimmune orchitis (Mallidis & Baker, 1994). Furthermore, in seasonal breeders, during the nonbreeding cycle the BTB/SCB is disrupted. Nevertheless, meiotic spermatocytes develop normally even in the absence of a complete, impermeable BTB/SCB (Pelletier, 1986). Therefore, other mechanisms in addition to the BTB/SCB are required to create the immunologically privileged environment of the testis.

One possible downside to testis immune privilege is the potential for an increase in infections or tumors. The testis is a major site for relapse of acute lymphoblastic leukemia (ALL) (Hedger, 2015). However, overall the testis is no more susceptible to testicular tumors when compared with other tissues and infections are rare (Hedger, 2015). This has been attributed to activation of innate immunity, which could theoretically prevent infections and tumors without activating the adaptive immune response (Hedger, 2015).

Evidence for Sertoli cells in testis immune regulation

Evidence that Sertoli cells manipulate the immune response comes from transplantation studies where Sertoli cells not only survive when transplanted across immunological barriers as allografts or xenografts but also provide immune protection for co-grafted cells such as pancreatic islets to treat diabetes, adrenal chromaffin cells for neurodegenerative diseases, hepatocytes, and skin grafts (Kaur *et al.* 2015.). These unique immunomodulatory properties suggest that Sertoli cells are not only important for the overall protection and development of germ cells, they have therapeutic potential beyond the testis where they can protect co-grafted cells and even be engineered to express clinically relevant proteins like insulin to treat diabetes or neurotrophin-3 to treat spinal cord injury (Pelletier, 1986; Halley *et al.*, 2010; Kaur *et al.*, 2014b, 2015). Sertoli cells create this immune privileged environment by expressing immunoregulatory factors that actively suppress innate, humoral and cell-mediated immune responses while at the same time inducing regulatory immune cells (regulatory T cells and M2 macrophages). Sertoli cells express apoptosis inhibitors (SERPINA3N, SERPINB9), complement inhibitors (serping1, DAF or CD55, MCP or CD46, clusterin), immunomodulatory factors (IDO, galectin-1), anti-inflammatory cytokines (TGFB1), and chemokines (CCL27) that act together to modify the immune response and induce tolerance to protect the germ cells (Wang

et al., 1994; Guazzone *et al.*, 2009; Meinhardt & Hedger, 2011; Doyle *et al.*, 2012).

Interestingly, the timing for when immune privilege first develops is not clear. The majority of Sertoli cell co-transplantation studies were performed using Sertoli cells isolated from pubertal or adult testes, indicating, as expected, that these Sertoli cells had immune protective abilities (Mital *et al.*, 2010). The age of these Sertoli cells corresponded with the development of the autoimmunogenic germ cells. However, neonatal porcine Sertoli cells also survive when transplanted as xenografts (Dufour *et al.*, 2003b) and testes transplanted from fetal and early postnatal (up to 15 days) rats survived better than adult testis transplants (Statter *et al.*, 1989).

Overall Sertoli cells protect the developing auto-antigenic germ cells by forming the BTB/SCB (Fig. 3), which limits access by the immune system to the advanced germ cells, whereas at the same time modulating the immune response by secreting immunoregulatory factors that modify the immune response and induce regulatory immune cells to create a local tolerogenic environment and along with the peritubular myoid cells restricting the immune cells to the interstitial space. It is amazing that 150 years ago, when Enrico Sertoli first described Sertoli cells, he was able to intuitively suggest, based on Sertoli cell morphology, the importance of Sertoli cells in the protection of the germ cells.

COMPARATIVE SERTOLI CELL BIOLOGY

In metazoans, spermatogenesis relies on the somatic cells environment for its completion. Therefore, in order to guarantee fertility during the animal reproductive lifetime, the somatic cells named Sertoli cells in vertebrates are crucial for facilitating germ cells survival and development in such a manner that spermatozoa is usually produced in very high numbers. However, Sertoli cell structure and function in anamniotes (fish and amphibians), which present a cystic type arrangement of gametogenesis (Schulz *et al.*, 2010), shows several important differences when compared with amniotes (mammals, birds, and reptiles) (Fig 8). These particularities may provide new insights into Sertoli cell physiology and will be addressed in this section.

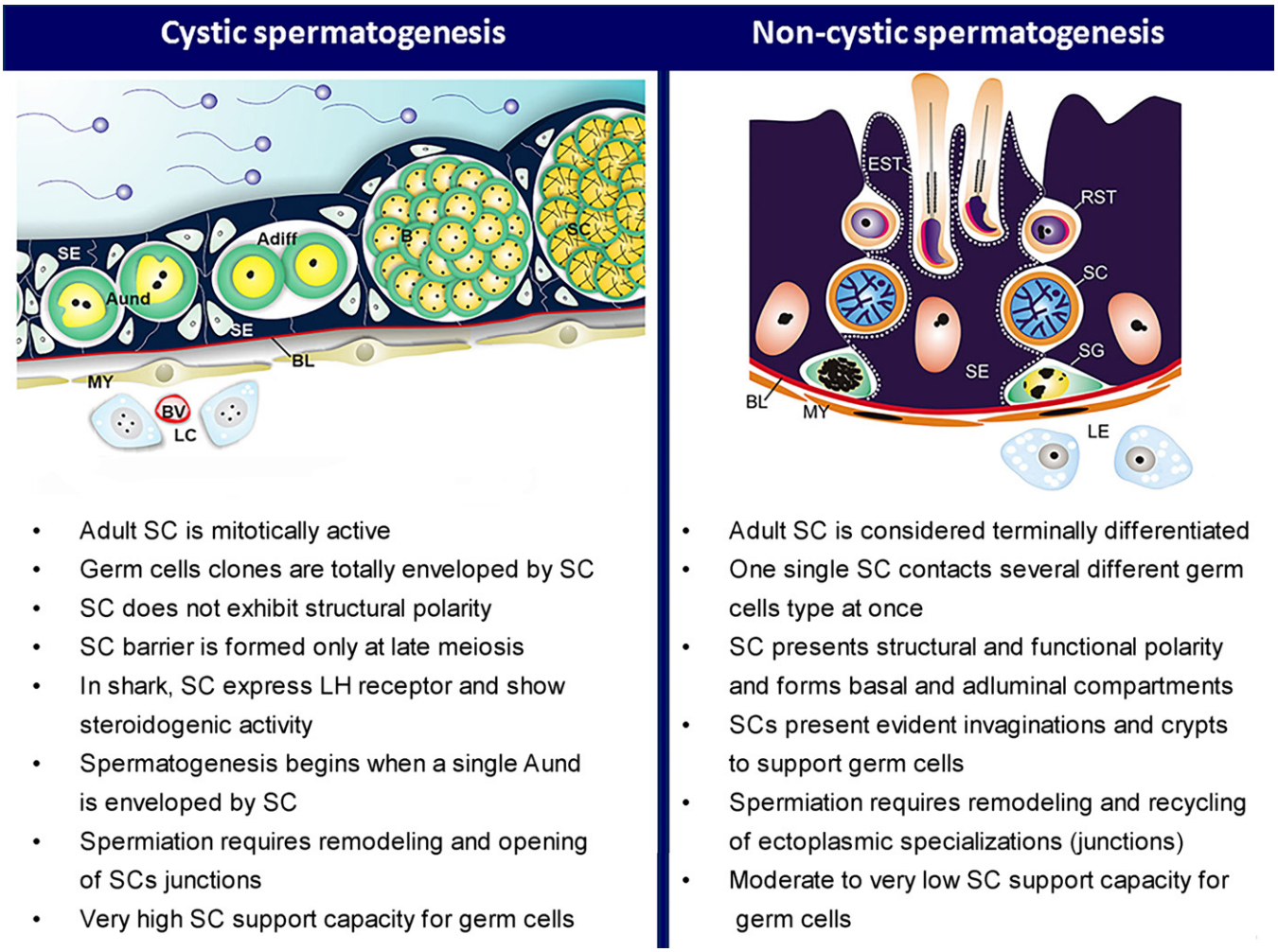
A very important particularity is that, different from amniotes, Sertoli cells remain mitotically active in fish and amphibians after they become sexually mature and two modes of proliferation that could overlap each other are observed in fish (França *et al.*, 2015) (Suppl. Fig. 3). In the first mode, Sertoli cell progenitors – that are regulated by FSH, thyroid hormone, estrogens, and insulin-like growth factor – proliferate to provide space for new niches that will be occupied by SSCs or single spermatogonia, forming therefore new spermatogenic cysts (Morais *et al.*, 2013; França *et al.*, 2015). In this instance, similar to rodents (Dovere *et al.*, 2013), paracrine factors (i.e., GDNF) produced by Sertoli cells in fish may stimulate SSC self-renewal divisions or attract SSCs from other areas (Lacerda *et al.*, 2013; Nakajima *et al.*, 2014). However, in contrast to rodents (Cooke *et al.*, 2005), thyroid hormones (thriiodothyronine-T3) in zebrafish increase the mitotic activity of Sertoli cells via Igf signaling system (*igf3* gene). This is particularly true for Sertoli cells not yet associated with germ cells or in contact with type A spermatogonia. Therefore, Igf also stimulates the proliferation of undifferentiated spermatogonia in a sex steroid independent manner (Morais *et al.*, 2013).

The second mode of Sertoli cell proliferation is under the regulation of FSH, androgens, and progestins. In this mode, Sertoli cells within the existing cysts divide to accommodate the expanding germ cell clones, according to the respective reproductive strategy and distribution of spermatogonial cells in the testis parenchyma of each species (Billard & Breton, 1978; Almeida *et al.*, 2008; França *et al.*, 2015). Although solid scientific evidence is still lacking for this mode, the existence of a Sertoli progenitor or stem cell population seems quite plausible and deserves careful investigation based upon the following observations: the long-term capacity of Sertoli cell division in successive reproductive cycles, the fully functional sex reversal in adults (Shibata & Hamaguchi, 1988; Kobayashi *et al.*, 2009), and the natural sexual plasticity observed in sequentially hermaphroditic fish species (Kobayashi & Nagahama, 2009).

It seems that in anamniotes, Sertoli cells enveloping a germ cell cyst are only terminally differentiated after meiosis is complete, because this functional status correlates with the formation of tight junctions between Sertoli cells (Leal *et al.*, 2009; França *et al.*, 2015). Therefore, considering their proliferating activity and the establishment of tight junctions, Sertoli cells seem to behave similarly throughout vertebrates. In this regard, evaluation of an individual spermatogenic cyst in anamniotes will reveal that the number of Sertoli cells increases steadily during the mitotic phase, stabilizing upon completion of meiosis/start of spermiogenesis (Matta *et al.*, 2002; Schulz *et al.*, 2005; Leal *et al.*, 2009).

Compared with mammals, the number of spermatogonial mitotic cycles in anamniotes is usually quite high, whereas much lower numbers of apoptotic germ cells (30–40% loss from the theoretically expected number) are observed in spermatogenic cysts (Vilela *et al.*, 2003; Leal *et al.*, 2009; França *et al.*, 2015). Therefore, hundreds of more advanced germ cells (meiotic and post meiotic) are usually present in a cyst in association with low number of Sertoli cells. It means that, despite having little or no direct contact (junctions) with germ cells, Sertoli cells efficiency in lower vertebrate is quite high. Although this issue is very complex and deserves further evolutionary investigation, reproductive efficiency is clearly related to the number of gametes required for a particular mode of reproduction. It is at great cost to the organism that gametes are produced so it is likely that evolution carefully monitors the efficiency so that sufficient numbers of gametes are produced to ensure the continuation of the species while increasing the efficiency of fertilization and survival of the offspring. In particular, the number of spermatids per Sertoli cell, which is considered species-specific, varies greatly during vertebrate evolution and decreases strikingly from more than one-hundred in fish to less than ten in most mammalian species already investigated, reaching about four in humans (Assis *et al.*, 2015; França *et al.*, 2015) (Figure S4). This quite illustrative figure allows us to speculate that perhaps humans will not produce sperm in the future. As anamniote Sertoli cells present very high support capacity for germ cells, a careful and comprehensive investigation on these somatic cells may provide important clues regarding their regulatory mechanisms during evolution. An important aspect that deserves consideration is the fact that in the vast majority of fish species spermatozoa has no acrosome, requiring therefore a very high number of gametes for reproduction through external fertilization.

Figure 8 Schematic representation of the main differences between Sertoli cells (SC in the legend) in cystic and non-cystic spermatogenesis. A_{diff}, type A differentiated spermatogonia; A_{und}, type A undifferentiated spermatogonia; B, type B spermatogonia; BL, basal lamina; BV, blood vessel; EST, elongated spermatid; LE or LC, Leydig cells; MY, peritubular myoid cells; RST, round spermatid; SC, spermatocytes; SE, Sertoli cell; SG, spermatogonia. Modified from previous publications with permission of the Copyright © holder of Sertoli Cell Biology, 2nd edition, Elsevier Academic Press (Schulz *et al.*, 2010; França *et al.*, 2015).



In fish both Sertoli and Leydig cells express receptors for FSH and LH that directly stimulate steroidogenesis. It is worth mentioning that sharks do not have steroidogenic Leydig cells in the interstitial compartment. Therefore, unlike higher vertebrates, in addition to regulating Sertoli cells activities and proliferation (Schulz *et al.*, 2012), Fsh in fish is also a potent steroidogenic hormone (Prat *et al.*, 1996; Campbell *et al.*, 2003; França *et al.*, 2015) and is associated with spermatogonial proliferation and differentiation (Skaar *et al.*, 2011; Assis *et al.*, 2015; Melo *et al.*, 2015; Nóbrega *et al.*, 2015).

New evidence from electron microscopy studies (França *et al.*, 2015) has shown that Sertoli cells seem to be in contact with different type of germ cells clones in different phases of cystic spermatogenesis (i.e., spermatogonial and spermiogenic), an important aspect that will open new possibilities for investigating germ–Sertoli cells signaling pathways. Particularly the mechanisms related to the structural and functional aspects of Sertoli–germ cell interactions that may contribute to the strikingly anamniote Sertoli cell efficiency need to be investigated. Comprehensive studies investigating the biology of SSCs and their niche have been pivotal in this scenario (Nóbrega *et al.*, 2010). As acting mainly on Sertoli cells, FSH plays a pivotal role in fish testis function and gametogenesis through several different growth factors (i.e., AMH, androgens, progestins, thyroid hormones, Igf3) that regulate SSCs renewal and differentiation (Nóbrega *et al.*, 2010, 2015; Skaar *et al.*, 2011; Chen *et al.*, 2013; Morais *et al.*, 2013; Assis *et al.*, 2015; França *et al.*, 2015; Melo *et al.*, 2015). Through a nuclear estrogen receptor, eel Sertoli cells also regulate SSC renewal via the expression of platelet-derived endothelial cell growth factor (Pdecgf) that is considered a SSC renewal factor (Miura *et al.*, 2003). Moreover, under the influence of progestin, trypsin expression (Miura *et al.*, 2009), and taurine biosynthesis (Higuchi *et al.*, 2012) were observed in eel Sertoli cells, leading to germ cells expression of a solute carrier gene (*slc6a6*) and their subsequent entry into meiosis (Higuchi *et al.*, 2013).

In higher vertebrates, the derivatives of mesonephric tissue form the efferent ducts and sperm storage tissues. Considering that in most fish species spermatozoa are stored in the tubular lumen and that after the spawning season the residual spermatozoa are very efficiently phagocytized by SCs, fish represent an interesting model for investigating both the 'recycling and waste

management' functions of Sertoli cells and spermatogonial kinetics. Finally, based on several important aspects mentioned in this section, we hope that we have convincingly demonstrated that Sertoli cells in lower vertebrates are highly dynamic and plastic cells. In contrast to mammals, Sertoli cells in fish are able to provide an adequate environment for spermatogenesis progression and sperm formation after xenogenic germ cell transplantation from phylogenetically distant species (Lacerda *et al.*, 2013; Lacerda *et al.*, 2014) and this is certainly another very important illustration of the amazing plasticity of anamniote Sertoli cells.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Sertoli Cell Books and Biographies of Enrico Sertoli.
Figure S1. Key Morphological Features of Sertoli Cell are listed with representative examples illustrated.
Figure S2. Electron microscopy of the Sertoli cell nucleus (N) from a human testis showing a large nucleolus (Nu) and deep indentation (In) of the nuclear membrane (Nm).
Figure S3. Schematic representation of Sertoli cell (SC) proliferation in relation to endocrine and paracrine regulation of fish spermatogenesis.
Figure S4. Number of spermatids per Sertoli cell (SC), based on the available literature, for different vertebrate groups.

