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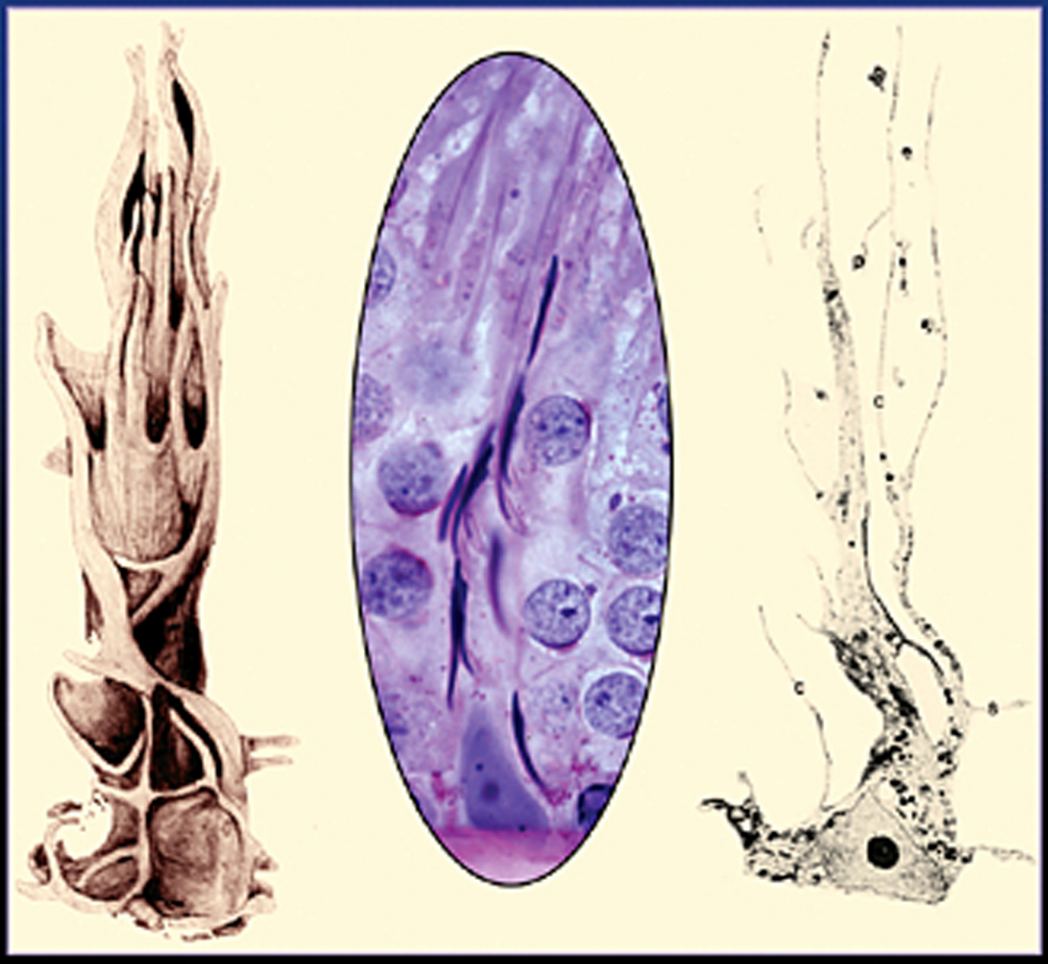

Edited by

Michael K. Skinner and Michael D. Griswold

SERTOLI CELL BIOLOGY

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SERTOLI CELL BIOLOGY

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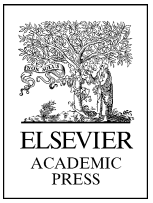

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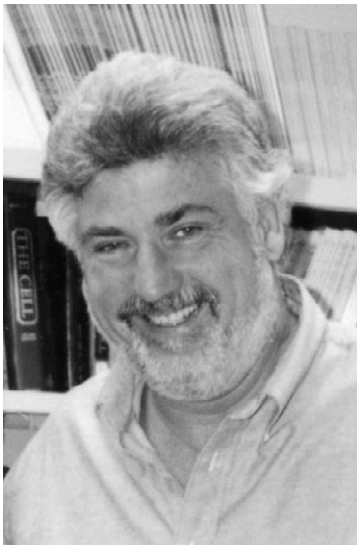

This book is dedicated to the memory of Professor Lonnie Russell of Southern Illinois University. Lonnie died in a drowning accident in Brazil in 2001 at the height of a successful research career in reproductive physiology. Lonnie used his expertise in anatomy to help define the intricacies of spermatogenesis with a focus on the function of the Sertoli cells. In 1993 Lonnie Russell and Michael Griswold edited a book titled *The Sertoli Cell*, which has served as a major resource and inspiration for investigators interested in male reproduction. It is appropriate that now—more than 10 years later—the advances in this field are summarized in this new text. It is also appropriate that this volume is a tribute to the many scientific contributions made by Lonnie Russell. It can only be hoped that this volume generates a small part of the energy and enthusiasm for science that Lonnie was able to stimulate with his wit and enquiring mind.

Michael K. Skinner
Michael D. Griswold

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Preface

In 1993 Lonnie Russell and Michael Griswold edited a book titled *The Sertoli Cell*, which has served as a major resource for investigators interested in male reproduction. It is appropriate that now—more than 10 years later—the advances in this field are summarized in this new text. It is also appropriate that this volume is a tribute to the many scientific contributions made by Professor Lonnie Russell of Southern Illinois University. Lonnie died in a drowning accident in Brazil in 2001 at the height of a successful research career in reproductive physiology. Lonnie used his expertise in anatomy to help define the intricacies of spermatogenesis with a focus on the function of Sertoli cells. He was first an anatomist and physiologist, and he used those tools to produce an enormous amount of information on the testis. His laboratory published the first and only visualization of intact Sertoli cells reconstructed from serial sections in the electron microscope. These papers allowed those of us in the field a fundamental visualization of the beauty and complexity of the Sertoli cells. It is essential for the molecular biologists and biochemists to place their findings in the context of the complex biology of the testis. Part of the goal of this current text is to make that process easier.

The editors of this text (the Mikes) have a combined nearly 50 years of experience focused on exposing the secrets of the Sertoli cells. Both of us have spent many years reporting on specific gene products of Sertoli cells and over the combined 50 years we have thoroughly investigated perhaps a dozen gene products. The biggest change in the field has been the use of expression arrays and gene knockout technologies that allow the monitoring of thousands of expressed sequences and the more laborious testing of their physiological functions. In *The Sertoli Cell*, Russell and Griswold reported that nearly 300 papers were published in 1990 that somehow involved these cells. Since the millennium there have been nearly twice that number of papers per year, suggesting these new technologies and other factors have stimulated an increased interest in the Sertoli cell.

The current text attempts to present a systems biology approach to an understanding of the Sertoli cell. A combination of molecular, cellular, and physiological aspects of Sertoli cells are presented. Due to the essential role Sertoli cells play in the process of spermatogenesis, topics such as spermatogonial stem cells and germ cell transplantation are also presented. An attempt was made to identify areas that have had significant advances over the past decade, as well as suggest important areas for the future analysis of Sertoli cell biology. Information is presented to provide the novice with basic information on the topics as well as to provide experts with new details that have advanced the field. We hope you find *Sertoli Cell Biology* a useful reference and that it provides insight for an understanding of the Sertoli cell and male reproduction.

ACKNOWLEDGMENTS

The chapters provided and hard work of the contributing authors is what made this book possible. The editors thank Ms. Mica Haley, Ms. Jacqueline Garrett and the staff at Elsevier/Academic Press for their assistance and patience. We thank Dr. Rex Hess and acknowledge his interest in the book, particularly in the design of the cover. The cover includes an electron micrograph of a Sertoli cell from Dr. Lonnie Russell and his three-dimensional reconstruction of the cell, as well as a light micrograph of the Sertoli cell provided by Dr. Hess. This book would not have been possible without the editorial and administrative support of Ms. Jill Griffin. Jill's dedication and efficiency were critical for the book and indispensable to the editors.

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PART

I

INTRODUCTION

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CHAPTER

1

History of the Sertoli Cell Discovery

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II. THE STUDENT
III. THE MICROSCOPE
IV. THE LABORATORY
V. THE DISCOVERY
VI. COMPLETING THE MANUSCRIPT
VII. AFTER GRADUATION
VIII. TRANSITION TO THE MODERN SERTOLI CELL
IX. POSTSCRIPT
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I. INTRODUCTION

The first edition of *The Sertoli Cell* was an appropriate vision of the late Professor Lonnie D. Russell, because he studied the Sertoli cell in more depth than most other modern-day scientists. He published more than 200 papers, of which nearly half were focused on the Sertoli cell, including the first book devoted to the cell, which he coedited with Michael D. Griswold [1]. Therefore, this chapter is written in honor of Lonnie because he was a fun-loving friend and visionary scientist who always used the microscope and his imagination to find new insights into complex scientific problems of the testis, and in particular the Sertoli cell. Lonnie's devotion to this cell was exemplified by the license plate that he attached to his automobile, which read "Sertol 1," and by his cat whose name was also "Sertoli." Factual events surrounding Sertoli's life were gathered from reading numerous reviews [2–9], particularly those of the distinguished scholar Brian P. Setchell [10,11], whose foreword in the first book provided a sincere and deserved admirable look at Enrico Sertoli [12].

II. THE STUDENT

Enrico Sertoli was 18 years old when he began his studies in medicine and research at the University of Pavia in Northern Italy in 1860 [3]. He studied general medical subjects at first and then after 2 years began his research studies in the laboratory of the distinguished physiologist and histologist, Professor Eusebio Oehl (1827–1903). It is interesting that Camillo Golgi, for whom the Golgi apparatus is named, was a fellow student at the university and he and Sertoli both studied under Professor Oehl [13] and graduated in the same year, 1865.

Sertoli was born on June 6, 1842, to a noble family in the small town of Sondrio, located North of Milano along the Italian–Swiss border [3]. His noble birth in all probability meant that he was expected to attend university and study medicine. Unfortunately, as Sertoli entered his teenage years, the countryside was not at peace and there was talk of war between Prussia and Austria during this period. Accordingly, Sertoli may have had the same urges of young people today to join the local forces and defend his country, but his father surely urged him to complete his medical training before entering the army, which Sertoli did when war broke out after he graduated in 1865 [12].

The University of Pavia was an old, well-established center of higher education located just south of Milano, which according to historical records was established by edict of the Emperor Lotarius in the year 825. The culture surrounding this famous university was surely one that encouraged the highest standards of achievement and bred intellectual inquiry that was capable of producing Nobel Prize–winning scientists, such as Golgi, who also studied under Bizzozero and was later nominated for the first Nobel Prize in Physiology and Medicine in 1901 and then every year until 1906, when he shared the prize with Santiago Ramón y Cajal.

III. THE MICROSCOPE

The *cellule ramificate* or *branched cell* was discovered using the personal microscope of Enrico Sertoli (Fig. 1.1). He had purchased the microscope in 1862, after he began his research studies under Professor Oehl. The Belthle microscope that he purchased from the Kellner Optical Institute in Wetzlar, Germany, was a state-of-the-art light microscope at that time. Instruments produced by the Kellner Optical Institute were compound microscopes with three or more lenses, and each came with 10× and 20× magnifier eyepieces. They also had a screw system for lowering the compound lenses to the slide, so that there was less breakage of the glass coverslips.

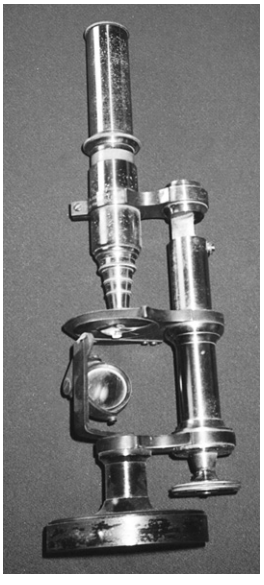

FIGURE 1.1 The Belthle microscope that Sertoli personally purchased in 1862 and the same microscope that he used to make the famous discovery of the cell that now carries his name. (Photograph kindly provided by Michi Sertoli, the great nephew of Professor Enrico Sertoli, of Milan, Italy.)

In contrast, some of the more common microscopes found in laboratories in 1862, such as the van Deyl and Beck microscopes, were no more than high-powered magnifying lenses. Such microscopes would have posed difficulties for a serious student and may have been reason enough for Sertoli to purchase one of the highest quality microscopes available, knowing that the instrument chosen would become the limiting factor in his histological research accomplishments.

We do not know the complete story behind the purchase of this microscope, but it was surely a momentous occasion. It is possible that microscope purchases were required for every medical student on entry into the histology course. Regardless, we can envision that some students were capable of purchasing a compound microscope, whereas others found it necessary to settle for the less expensive brands that provided only a magnifying lens.

The quality of the Belthle microscope and its personal importance are evidenced by the care that Sertoli devoted to it, which has permitted its survival for more than 100 years, along with a wooden storage box and the original two-page letter of guarantee that was provided by Belthle from the Kellner Optical Institute (Fig. 1.2).

IV. THE LABORATORY

To our knowledge, there are no photographs of Sertoli in his laboratory; therefore, we must assume that the room was similar to others that were photographed in the late 1800s. Professor Oehl's laboratory was likely a typical large room with a high ceiling and large windows. Because the Edison lamp did not arrive until 1890, few professors, except in the department of physics, would have had electric lights in their offices and laboratories.

The furniture would likely have been made from a hardwood and coated with dark stain. Tall wood encasings would have lined the outer walls. A bench would have run the full length of the room with hardwood shelving down the center. It would have encased chemical solutions, flasks, stopcock bottles, and other supplies common to the research lab. Natural gas was used quite extensively in Europe by the late 1860s; however, harnessing the gas was not easy, and Robert Bunsen had not as yet invented the famous "Bunsen burner." This marvelous invention in 1885 permitted the controlled burning of natural gas through a metal tubule by regulating the amount of gas and air proportions individually, which essentially allowed for increases in the temperature and in the intensity of the flame. Until this device came along, everyone

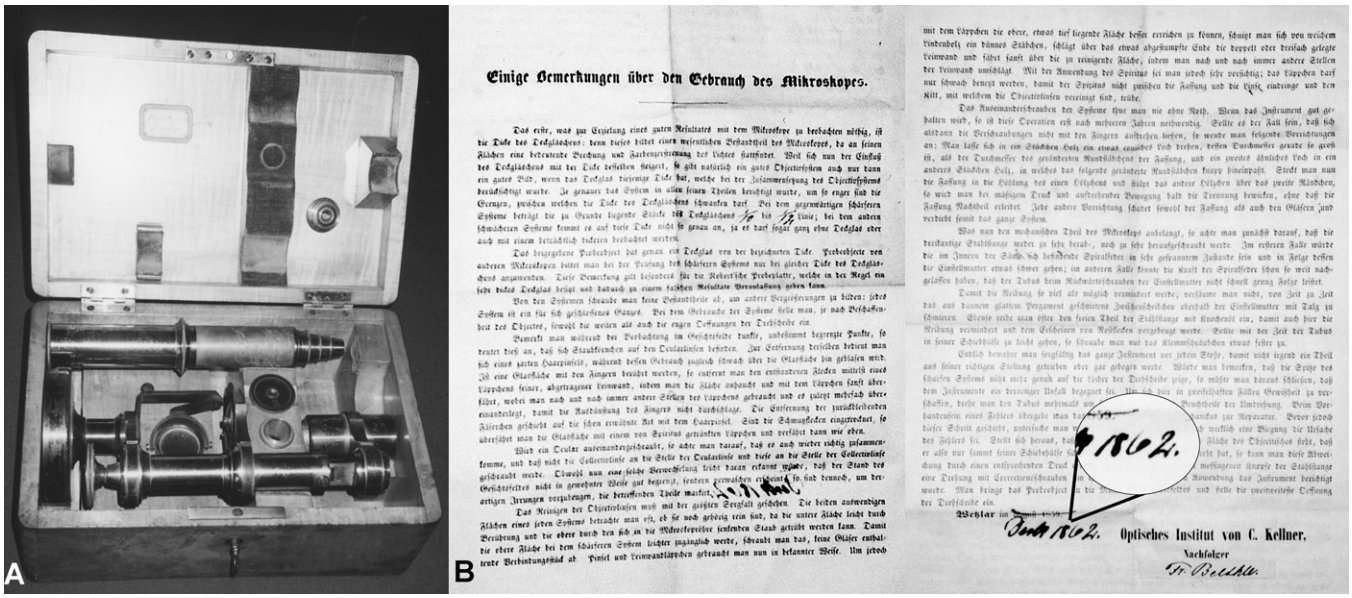

FIGURE 1.2 (A) This wooden box is the original storage box for the microscope, which Sertoli maintained so meticulously. (B) The original two-page document that was sent along with the microscope from the German company Belthle in Wetzlar. The paper is a guarantee from the Optics Institute of von C. Kellner. (The date 1862 has been magnified digitally for emphasis. Image kindly provided by Michi Sertoli, the great nephew of Professor Enrico Sertoli, of Milan, Italy.)

simply used a straight tubule with a round base and controlled the flow of gas by a single valve. This led to many accidents and explosions, but the flame was still superior to candle or alcohol lamps.

It was common to have a large blackboard and chalk in the laboratory. We can imagine the blackboard being covered with drawings of Sertoli's observations and maybe even outlines of experiments dealing with Professor Oehl's own research. On the bench may have been another typical microscope that was often used in physiology, the Cuff simple microscope, which was made by Dollond of London. This monocular-type scope was used for dissections. It was capable of magnifying with fairly good resolution up to 10× to 20×. The instrument had a tube body mounted above a stage, similar to the compound microscope, but it was strictly a magnifying lens on a stand. Thus, this crude magnifier was the precursor of the dissecting microscope. Along that same bench would have been glass jars with specimens preserved in alcohol and acids and other types of solutions that were used in histology. On the opposite side could have been stacks of histology glass slides and possibly an open copy of the first textbook of histology, written by the Swiss scientist, Albert Kölliker [14].

Like many scientists during that period, Sertoli would have worn the typical white smock-like lab coat with five buttons up the front. The coat would protect his white shirt and dark tie, the formal dress of a university student.

V. THE DISCOVERY

In anticipation of the microscope's arrival, Sertoli likely collected several pieces of human testes preserved in a sublimate solution (a precipitating solution formed by adding ammonia to mercuric chloride) that he later reported as the incubation solution of choice at that time [15]. Without being aware, Sertoli was using a very nice model to investigate the seminiferous epithelium, because in humans, unlike in mice and rats, the Sertoli cell occupies about 37% of the epithelium. In contrast, the Sertoli cell occupies about 15–20% in the rodent species. The human testis has a higher ratio of Sertoli to germ cells, due to the reduced efficiency of spermatogenesis. It appears that Sertoli worked only with human testes throughout his career. Thus, working in a medical school was an advantage to Sertoli from the very beginning.

Sertoli used several different types of preparations of testes, including microdissections of individual seminiferous tubules, thin sections of the testis after sublimate incubation, pieces of fresh tissue, and frayed sections of tubules. Like all young students in the laboratory, he probably worried that the tissue may have remained in the solution too long. With such concerns, perhaps he had numerous conversations with Golgi and other students regarding the latest methodologies that were being tested in histology to better preserve structures and improve the visualization of cells.

After the new microscope arrived, Sertoli probably spent the first few weeks working obsessively with the shiny new instrument. He no doubt spent endless hours getting the angle of the sunlight just right so that the cells would appear more clearly.

To a scientist today, the methods used by Sertoli were crude and harsh. It is astounding that such important discoveries were made during this early period of development of what we now accept as routine histological procedures. These early observations were made without the benefit of fixatives that could cross-link proteins and bind lipids in the tissue. Alcohols and acids were the primary methods for treatment of fresh samples. Although formaldehyde was discovered in 1859, it was not until after the commercial synthesis of formalin from methanol by Hoffman in 1868 that sufficient quantities became available for testing in various medical and chemical procedures. The actual use of formalin for hardening of tissue did not come about until 1892 when a chemist, Blum, was asked to test formalin as a potential antiseptic agent. Formalin hardened the skin on his fingertips, and the rest is now history for this widely used fixative.

At first, Sertoli may have called the cell a *tree-like cell* or *stringy cell* or some other description that suggested that this cell had long extensions. On the first page of his publication [15], the term *mother cells* is used, which suggests that his observations were quite perceptive and even intuitive of the true Sertoli cell function.

In 1863, it was necessary to draw observations made through a microscope. Although a photo was actually produced in 1827 on materials hardened after exposure to light for up to 8 hours [16], the word *photography* was not invented until 1839, by Sir John Herschel. This early type of photography was time consuming and quite expensive; therefore, it was not routine in the scientific laboratory, and a box for holding the film that could be used on a microscope was not invented until 1884, when George Eastman introduced flexible film and the boxed camera [16]. Therefore, Sertoli would have spent many hours drawing what he observed.

Sertoli's first drawings must have been simple (probably similar to those in Figure I of the original plate, reproduced here as Fig. 1.3). Looking through a microscope and drawing what you see is difficult even when the tissue is well preserved and well stained. Unfortunately, the corrosive solutions that Sertoli used extracted the cells and left them rather transparent. Nevertheless, he would have been very excited, as every student would be upon using for the first time a new instrument or observing for the first time a new organelle or cell. In truth, the discoveries by Sertoli were things that had never before been described by others.

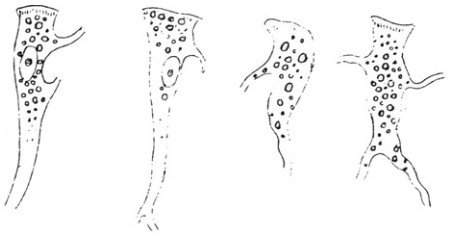

FIGURE 1.3 Drawings taken from the original paper (Fig. I a–d) of Sertoli [15].

Reproductive biology literature was scarce in the 1800s. However, Sertoli was reading the work of Kölliker [17] and referred to his work as being the "most authoritative one." But Kölliker claimed that these cells of interest to Sertoli were polygonal in shape, instead of conical or cylindrical in shape as described by Sertoli, and such statements in the early scientific literature tended to become dogma. To discover something that contradicts the published literature surely provides the ultimate excitement for a scientist, and such exhilaration may have been even greater during that period in time if we take into consideration the culture of science in Europe in 1863. Such discoveries can also be intimidating, especially when one considers that their observation may contradict a famous scientist, such as Kölliker.

One cannot help but wonder what Sertoli wrote in his notebook. Today we publish only small portions of the total data that are collected during an experiment. However, in the 1800s, every observation was novel and worthy of discussion. He probably had numerous drawings lying on the bench, and his notebooks were likely filled with drawings and intricate descriptions. We know he described the cell as having branches and blobs at the ends. Maybe the branches surrounded germ cells and maybe the branches were like those of a tree that drops its fruit as the harvest ripens. Anything was possible, because it was new. It was possible that this cell, like the great maestro of a symphony, directed the production of sperm in the testis. Maybe spermatozoa developed directly from the branched cell.

Sertoli and the other histology students on the campus, such as Golgi, may have discussed the work of Professor Waldeyer in Berlin. Professor Waldeyer had extracted a substance from logwood (*Haematoxylon campechianum*) that someone had collected from Central America. Apparently, the substance, now called *hematoxylin*, was a nonquinonoid that was soluble in water and, when readily oxidized, would stain leather. Waldeyer was the first to propose this solution for staining histological tissues, too. However, it was not

until Ehrlich formulated the extract with alum that the method worked efficiently as a counter stain to eosin [18]. Thus, Sertoli had little to work with in 1863 but he must have tried whatever solutions he could find in the laboratory or borrow from other labs. He mentioned in the paper that he tried clearing the tissue with nitric and acetic acids, and even tried staining with iodine. He wrote, as translated by Setchell [10], "Nitric acid shrivels and deforms the cells and turns the nuclei yellow. Tincture of iodine stains these structures yellow, but the nuclei even more intensely than the cells." Such testing of different methods may have improved the observations, because in some drawings, Sertoli included round germ cells, "seminiferous cells," embedded within the branched cell limbs (Fig. 1.4).

Sertoli drew with intricate detail what he observed. He was the first to recognize and report lipid droplets in this cell, and he mentioned several times that lipid could exert very important functions in the cell, a function that we still know little about today. He also drew the cell as appearing syncytial or as a branched multinucleated cell, which surely raised many questions from students such as Golgi, because such ideas were common among those who studied the brain. Sometimes he simply looked at fresh tissue (Fig. 1.5) and observed what appeared to be an epithelial lining of the branched cells surrounding germ cells. His drawings indicated that the united branched cells were protecting or extending a hand to the germ cells (Fig. 1.4).

Even without the privilege of seeing Sertoli's notebook, we can imagine that it contained numerous pages of drawings and descriptions, many of which he used as the basis for writing the following narrative, as translated by Setchell from Sertoli's original 1865 paper [10]:

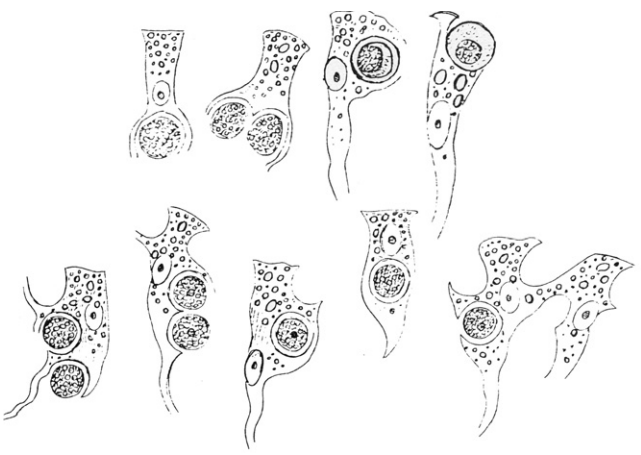

FIGURE 1.4 Drawings taken from the original paper (Figs. II a–d and III a–e) of Sertoli [15].

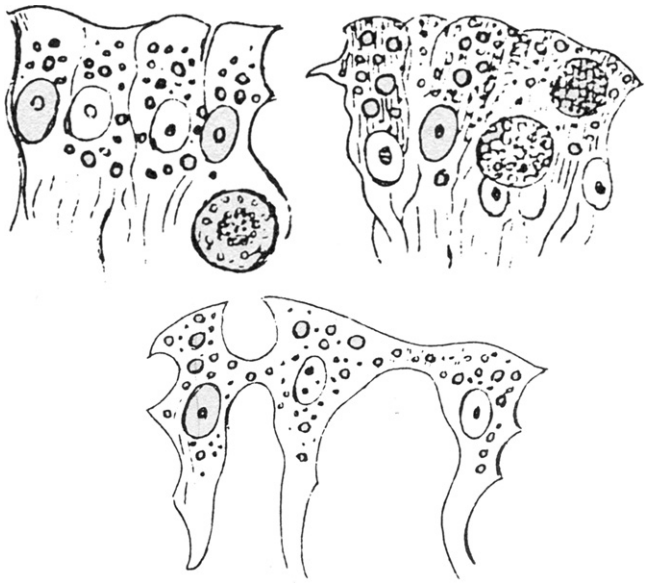

FIGURE 1.5 Drawings taken from the original paper (Figs. IV and V a–b) of Sertoli [15].

> IV. Finally, some special cells, which I saw in moderate number in the preparations and which, to my knowledge, have not previously been observed and described by anyone. They appear in the form of irregularly cylindrical or conical cells, with indistinct margins, provided with a nucleus, always containing a nucleolus. Their contents comprise fine droplets of fat in a substance that is reasonably transparent because it is homogeneous. These cells almost always have quite transparent extensions, in the interior of which fine droplets of fat can frequently be seen. They have an irregularly shaped body from which often protrude one or more extensions, and two extremities of which the upper is usually large and bounded by a well-marked margin that sometimes appears double (Fig. I a,c,d). Lower down the cell often is contracted somewhat, formed like a sort of collar (Fig. I a,d, Fig. III b). The other, luminal extremity, becomes narrower and forms an extension, which often ends abruptly in a rounded off tip with delicate outlines (Fig. III c,d). Often, the tip is torn and it is not possible to determine how it ends normally. I have observed that other cells bifurcate and send out secondary extensions (Fig. I b,d).

Such detailed descriptions by Sertoli are amazing, considering the crude equipment and conditions that he worked under at the time. To put his work in perspective, it is helpful to consider the time frame of events in microscopy and histology in the 19th century that is shown in Table 1.1.

Thus, by the time Sertoli published his first paper in 1865, most of the accepted standard methods of histology were lacking: fixation of tissue, embedding, sectioning with microtomes, and routine histological stains. In the year that Sertoli published his last manuscript as a professor, 1886 [19], major breakthroughs in

TABLE 1.1 Significant Scientific Discoveries Surrounding the Period of Sertoli

1839	Theodor Schwann presented his famous "cell theory."
1839	The 3- × 1-inch glass microscope slide was established as a standard by the Microscopical Society of London.
1840	The first commercial glass coverslips were used.
1841	Kölliker reported that spermatozoa arose from cells within the testis.
1850	Leydig's article on interstitial cells is published.
1850s	Clark's alcohol-acetic solution and Müller's solution for fixation were revealed.
1855	Water immersion lens first displayed at the Paris Exposition.
1858	Carmine stain first used by Joseph Gerlach.
1859	Butlerov discovered formalin.
1860s	Wilhelm Waldeyer first used aniline dyes, one called *Paris blue*.
1863	Waldeyer was first to stain histological sections with an extract that became *hematoxylin*.
1864	Fromman first demonstrated the use of silver for the identification of axons.
1865	Sertoli's paper on "branched cells" of the testis is published.
1869	Theodor Klebs first used melted paraffin to support a tissue block while sectioning.
1870s	First standard microscopical slides used for teaching.
1873	Ernst Abbe published his work on the theory of the microscope, which explained the difference between magnification and resolution. His formula was used to calculate resolution.
1873	Ernst Leitz microscope is introduced with a revolving mount (turret) for five objectives.
1879	Carl Zeiss Jena produced its first oil immersion objective in 1880, designed by Ernst Abbe.
1879	Walther Flemming discovered mitosis.
1880	August Kohler determined the optimum spacing for the light source and the condenser, which would produce sharper images; thus, was born *Kohler illumination*.
1880s	First electrical illumination, rather than sunlight, is reflected off a substage mirror.
1885	The first sliding microtome was developed.
1886	The first rotary microtome was developed.
1886	The substage condenser was developed.
1886	Ernst Abbe introduces the apochromatic objective lens, bringing the red, yellow, and blue colors into one focus, and requiring numerous lens elements.
1886	Ehrlich invented a stable solution of hematoxylin with a long shelf life.
1886	Benda introduced iron-hematoxylin techniques.
1893	Blum tested formalin as a fixative, and Carl Weigert introduced it as routine preservative for tissues.
1894	Zenker's fixative became available.
1896	"Sudan" stains for lipids introduced by Daddi.
1897	Bouin's fixative became available.

microscopic technique and instrumentation were just coming on the scene, such as Kohler illumination and Ernst Abbe's apochromatic lenses, which became available just before Sertoli retired. Despite these handicaps in technology, Sertoli worked with whatever was available and carried the observations to the limits of current microscopic resolution. Thus, we must conclude that Sertoli was an exceptional and determined student, with a keen skill for observation and capable of having original thoughts that others of his day were not giving consideration.

VI. COMPLETING THE MANUSCRIPT

Before Sertoli completed the first draft of his manuscript, he probably had numerous discussions with his adviser, Professor Oehl. Professor Eusebio Oehl had just founded the first Institute of Physiology at the University of Pavia in 1861. His research was not focused on the testis and, at the time that he mentored Sertoli and Golgi, he was studying extracted human saliva and salivary ducts. Thus, we must assume that Sertoli's focus on the testis may not have been top priority for his major adviser. On the other hand, in the late 1800s, every observation under the microscope was something new and it is not inconceivable to think that the adviser would recommend that each new student study a different organ. Regardless of the reason why he studied the testis, Sertoli was making major discoveries that would contradict the published literature and this must surely have made his professor either very excited or very worried.

The last figure in Sertoli's manuscript was a low magnification of a seminiferous tubule cross section showing germ cells and even spermatozoa associated with the branched cells that contained lipid droplets (Fig. 1.6). He had a great desire to observe the borders of the branched cell, and in his final experiment he tried something new [10]: "… in some sections treated with a weak solution of ammonia, I have seen that quite definite dark borders, limit the basal extremity of the branched cells …." After these remarks, he showed restraint by pointing out the limits of his observations.

He ended the paper with his conclusions from numerous observations. One of the most important of which was his conclusion that "it is not likely that these cells produce the spermatozoa …"[10], for which he gave three arguments:

> (1) Spermatozoa have been observed in the extension of only a very few cells. (2) The spermatozoa are found only in the extensions, where it would be possible for them to have

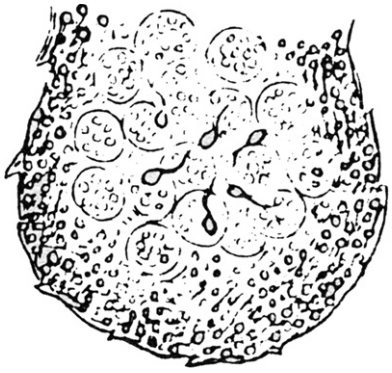

FIGURE 1.6 Drawing taken from the original paper (Fig. VI) of Sertoli [15].

> entered accidentally, and I have never seen spermatozoa inside the cells. (3) The formation of the spermatozoa would be consistent neither with the form of the branched cells, which are different from the seminiferous cells, the real progenitors of the spermatozoa, nor with their constant position inside the tubule, nor their tendency to enclose the seminiferous cells among their branches, nor finally their communication with one another through the extensions.

Yet, in the manner of an honorable scientist, he said he could not categorically deny the possibility, but that he did not think the branched cells produced spermatozoa. Nevertheless, with the insight of a keen scientist, Sertoli ended the paper with a suggestion that the "function of the branched cells is linked to the formation of spermatozoa." This comment along with that on page 1 of the manuscript regarding "mother cells" indicates that Sertoli was indeed the first to suggest that the "branched cell" served as a "cellule madri" or "mother cell" or "sustentacular cell."

It is likely that Sertoli made significant progress in writing the manuscript during 1864. The entire process of manuscript preparation, rewrite, submission, review, resubmission, and printing sometimes takes nearly 1 year nowadays, so it is reasonable to picture the same process taking more than 1 year in the 1800s. However, the time from submission to publication may have been considerably shorter than today, because every observation in the 1800s was publishable and the number of capable reviewers for any subject was limited. Therefore, it is likely that Sertoli completed his major observations and writing in late 1863 or early 1864 and submitted the manuscript for publication prior to graduation in 1865.

There has been no mention of why Sertoli was the single author on the paper, even though it is reasonable to assume that his research professor approved of the work in his laboratory. Until about 1950, it was common to see single-author papers or papers with just two authors. This may reflect the fact that much of the research performed during the late 1800s and early 1900s did not require collaboration and involved the use of simple tools of investigation. Research efforts back then required a tremendous personal endeavor. Furthermore, until the late 20th century, the evaluation of professorships did not depend on counting the total number of publications.

The first manuscript is always very special. A young scientist will dream of the day when his or her own name appears beneath the title of a manuscript. Sertoli may have written the title several times, but the published title had a very specific focus: "Dell' esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano," or as interpreted: "On the existence of special branched cells in the seminiferous tubules of the human testis." He restricted the study to just one cell type, although he had observed all of the germ cells, had noted what is now called stages of the seminiferous epithelium [20], and even recognized the beginnings of a "wave" of spermatogenesis.

VII. AFTER GRADUATION

Sertoli graduated in 1865 and traveled to the University of Vienna to study with Ernst Wilhelm von Brücke, a physiologist [3]. In 1866 he returned home briefly to defend his country in the war between Prussia and Austria. After the war was over, he stayed in the army for a while before returning to science. In 1867 he went to work in the laboratory of Ernst Felix Immanuel Hoppe-Seyler in Tübingen (not yet part of Germany). Throughout his career, Sertoli's research studies focused on many different organ systems other than male reproduction. At different times, his studies included the following: the lymphatic system, lungs, coccygeal gland, nutrition, kidney, tactile hairs, and smooth muscle [12].

After a series of lectures at the Politecnico in Milan in 1870, he was given a professorship at the Advanced Royal School of Veterinary Medicine in Milan [3]. From 1871 to 1880, Sertoli was professor and chair of anatomy and physiology, and then from 1880 to 1907, he was chair of physiology. It was reported by Negrini [3] that in 1900 at the Anatomy Congress in Pavia, the works of numerous eminent Italian and foreign scientists were presented on a series of microscopes, and one microscope held the label "Cells of Sertoli." By this date, others were also referring to the branched cells in association with Sertoli's name [21, 22].

In the fall of 1906, a year before Sertoli retired as professor, we can easily imagine that Golgi's name