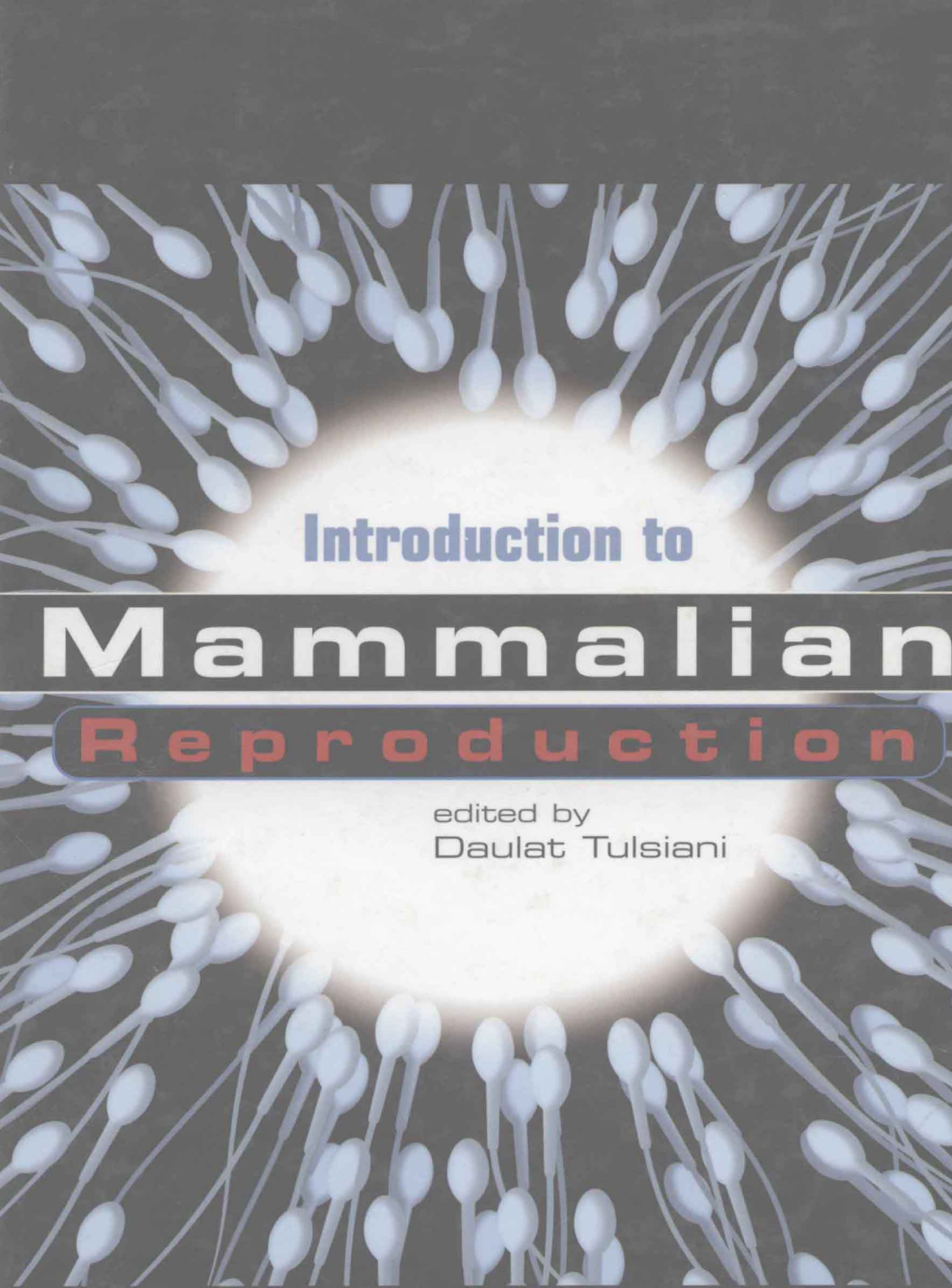
Introduction to
Mammalian
Reproduction
edited by
Daulat Tulsiani

INTRODUCTION TO MAMMALIAN REPRODUCTION

edited by

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PREFACE

One of the goals of reproductive (gamete) biologists is to understand the biochemical processes and molecular mechanisms that regulate the formation and maturation of male and female gametes, and their ultimate union to form a zygote, a cell with somatic chromosome numbers. Development of the zygote begins immediately after sperm and egg haploid pronuclei come together, pooling their chromosomes to form a single diploid nucleus with the parental genes. The major difference between the reproductive and non-reproductive processes is that many events including interaction of the opposite gametes are species specific, and the knowledge gained in a given species may be applicable only in a few closely related species. Thus, the progress in understanding many aspects of gamete biology have been painfully slow. Despite slow advancement, many fascinating discoveries have been made. Recent successes of *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI) techniques are noteworthy, and have helped many couples experience joy of parenthood. The assisted reproductive procedures are now being routinely used to increase the numbers of farm animals and endangered species. Many of these advances, in conjunction with recent successes in the cloning of laboratory and farm animals, were some of the factors behind my decision to undertake the task of organizing this book on mammalian reproduction.

So far as I know, there is no other book that systematically describes the formation and maturation of male and female gametes, and factors that regulate their union during the fertilization process, activation and implantation of fertilized egg, manipulation of the gametes for assisted reproduction, and environmental toxicants. That such book was needed became apparent to me when teaching a course on reproduction to the graduate and medical students at the Vanderbilt School of Medicine. Every attempt has been made to include a wide spectrum of topics (chapters) on morphological and physiological aspects of male and female gametes. These chapters are contributed by investigators currently engaged in "cutting-edge" research in the area of reproductive biology. Needless to say, I am very grateful to all the contributors, whose expertise, willingness to contribute, and hard work have made this book possible. My sincere hope is that the book will succeed in giving pertinent information to most of its readers, which are likely to include undergraduate, graduate and medical students, and perhaps their mentors. If my attempts generate a reasonable interest and stimulate a few young minds to expect exciting possibilities in the area of gamete biology, this book has fulfilled its purpose.

Daulat R.P. Tulsiani, Ph.D.

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I was born in Village Gucherow of the District of Karachi in former British India and moved with my family to the independent India during the partition in 1947. I must acknowledge the help of many kind souls who assisted my refugee family to get settled in India. I am grateful to all my teachers in India for their continuous help and encouragement.

My first encounter in the U.S.A. came when I joined the laboratory of Professor Raul Carubelli in the Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, in 1968 as a postdoctoral fellow. I greatly benefited from the advice and encouragement of my mentor and his colleagues during my young years in Oklahoma.

I joined the research team of Professor and Chairman Oscar Touster at Vanderbilt University, Nashville, in 1972. The university provided a rich academic environment for my professional growth. It is not possible to list the names of all the colleagues, research fellows, and students who have been a continuing inspiration during my stay at Vanderbilt. However, I must acknowledge the following colleagues for their collaborations and discussions throughout my tenure in the area of reproductive biology: Drs. Marjorie D. Skudlarek, Marie-Claire Orgebin-Crist, Benjamin J. Danzo, and Michael K. Holland. I am grateful to Professor Stephen S. Entman, Chairman of the Department of Obstetrics & Gynecology, and Vanderbilt University for providing me with the space and facilities for editing this book.

My sincere thanks to the contributors who graciously sent their assigned chapters in a timely manner. Many chapters needed very little editing; however, there were some that needed extensive editing and formatting. I learned more by editing these chapters than from any other text book or research article.

I am deeply indebted to Loreita Little and Lynne Black for editorial assistance, and to Lynne Black for final preparation of the chapters for the camera-ready format. Without this assistance, the publication of this book would have been considerably delayed. Finally, I am grateful to Joanne Tracy, Editor of Biosciences, at the Kluwer Academic Publishers, for her faith in this project. The research in my laboratory is supported in part by research grants HD25869 and HD34041 from the National Institute of Child Health and Human Development.

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Chapter 1

MAMMALIAN TESTES: STRUCTURE AND FUNCTION

Neelakanta Ravindranath, Luis Dettin, and Martin Dym
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INTRODUCTION

The male reproductive system consists of the primary sex organs, the two testes and a set of accessory sexual structures. The adult mammalian testis performs two important functions, spermatogenesis and male sex hormone production. It is an organ structurally designed to produce the haploid male gametes from diploid postnatal germ-line stem cells, i.e. type A spermatogonia. The process of morphological and functional differentiation of type A spermatogonia into the haploid male gamete, the spermatozoon, is termed spermatogenesis. In addition, the testis elaborates a steroid hormone, testosterone, that is responsible for maintaining the spermatogenic process as well as the secondary male sexual characteristics. Furthermore, testosterone is important for several different functions in various organ systems including the maintainance of muscle mass and bone density. The process of testosterone formation from its precursor, cholesterol, is termed steroidogenesis. In this chapter, we will discuss how the structure and form of the testis contributes to the processes of spermatogenesis and steroidogenesis.

MORPHOLOGY OF THE ADULT TESTIS

Each testis is covered with a thick fibrous capsule, the tunica albuginea. The thick infolding of the tunica albuginea at the posterior margin of the testis forms the mediastinum of the testis. Connective tissue septae originate from the mediastinum and pass into the interior of the testis, and subdivide it into several lobules. Within these lobules lie the convoluted folds of the seminiferous tubule. The space surrounding the seminiferous folds is occupied by the interstitial tissue. The seminiferous tubules form coiled

loops that terminate at both ends into the rete testes located within the mediastinum. Spermatozoa and testicular fluid produced within the seminiferous tubule pass through the rete testes into the ductuli efferentes and epididymis.

Histologically, the adult testis can be divided into two compartments, a seminiferous tubular compartment and an interstitial compartment (Fig. 1). The tubular compartment consists of an outer layer (s) of peritubular myoid cells and an inner layer of seminiferous epithelium separated by an intermediate layer of acellular matrix or basement membrane. The interstititial compartment consists of Leydig cells, immune cells (macrophages and lymphocytes), and fibroblasts. In addition, it also contains blood and lymph vessels, nerves, and loose connective tissue. The tubular and interstitial compartments of the testis perform the defined functions of spermatogenesis and steroidogenesis, respectively.

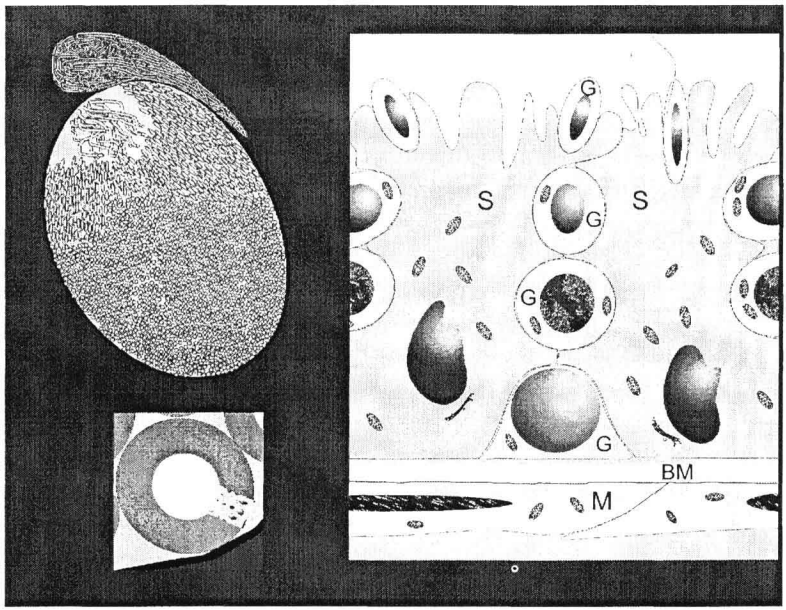

Figure 1. Schematic representation of a mammalian testis (top left), a cross section of a seminiferous tubule (bottom left), and the seminiferous epithelium (right) showing myoid cells (M), the basement membrane (BM), Sertoli cells (S), and germ cells (G).

SEMINIFEROUS EPITHELIUM AND SPERMATOGENESIS

The seminiferous epithelium rests on the acellular basement membrane and contains two types of cells, Sertoli cells and germ cells (Fig. 1). At the

time of birth, the seminiferous epithelium consists of Sertoli cells and only one type of germ cell, i.e., the gonocyte, located in the central part of the seminiferous cord. Gonocytes migrate to the basement membrane during the early postnatal period and are now called type A spermatogonia. Type A spermatogonia could be called 'male germ-line stem cells' as they renew themselves and also differentiate into spermatozoa (1). During the process of differentiation into spermatozoa, the type A spermatogonia undergo several mitotic divisions to yield type B spermatogonia. Type B spermatogonia mitotically divide to yield primary spermatocytes. Primary spermatocyte through two successive meiotic divisions form haploid spermatids. The haploid spermatids morphologically differentiate into spermatozoa. Thus, in the adult testis, the seminiferous epithelium consists of various germ cell types with the stem cells resting on the basement membrane and more differentiated germ cell types arranged progressively towards the lumen. The germ cells at different stages of differentiation are in close anatomical and functional contact with the Sertoli cells. However, tight junctional complexes between adjoining Sertoli cells compartmentalize the seminiferous epithelium into a basal compartment and an adluminal compartment (2). The junctional complexes separate young germ cells, i.e. spermatogonia and the preleptotene spermatocytes, from later spermatocytes, spermatids, and spermatozoa. In addition, they form the morphological basis of blood-testis barrier. This barrier creates a unique microenvironment in the adluminal compartment. The germ cells in the basal compartment communicate with the neighbouring Sertoli cells, the basement membrane, the peritubular myoid cells, and the blood and lymphatic vessels. More advanced germ cells in the adluminal compartment derive substances in blood or lymph through the Sertoli cell (3). Thus, Sertoli cells interact with all types of germ cells via desmosomes and gap junctions (4, 5). In addition, Sertoli cells develop ectoplasmic specializations (actin-rich filaments sandwiched between plasma membrane and endoplasmic reticulum) and tubulobulbar complexes with spermatids (6). The development and degradation of these structural complexes between neighbouring Sertoli cells at the base of the seminiferous epithelium and between elongating spermatids and the Sertoli cell at the apical end of the seminiferous epithelium has been correlated with the movement of spermatocytes from basal to adluminal compartment and the release of sperm to the lumen, respectively (6).

Sertoli Cell

Generally, the Sertoli cells exhibit an infolded nuclear envelope with pores, a homogeneous nucleoplasm, and a single tripartite nucleolus (Fig. 2). Within the cytoplasm of Sertoli cells, a large Golgi apparatus, numerous mitochondria, lysosomes, multivesicular bodies, lipid droplets, and residual

bodies have been described. Sertoli cells present a profuse network of both rough and smooth endoplasmic reticulum suggesting their capability for both protein and steroid synthesis and secretion. Sertoli cells lack secretory granules, large vacuoles, and exocytotic vesicles (7, 8). Lack of these structures indicate that the synthesized proteins may be transferred to the plasma membrane where they are either secreted after cleavage or remain membrane-bound for interaction with the corresponding receptor on germ cell types. A classic example of the growth factor that is expressed by Sertoli cells in both membrane-bound and secretory form is stem cell factor (9). The corresponding receptor, c-kit, is expressed on the surface of type A spermatogonia (10, 11). This concept appears to be true as Sertoli cells have been shown to extend cytoplasmic processes (conical at the base, sheet-like in the middle, and tapered apical towards the lumen) that interact with the plasma membranes of spermatogonia, spermatocytes, spermatids, and spermatozoa (12). The shape of the Sertoli cells in 3-dimension

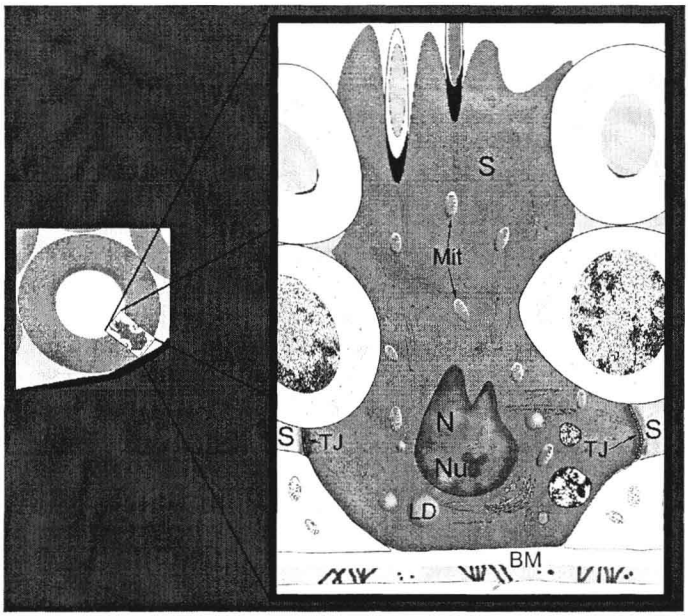

Figure 2. A schematic representation of a Sertoli cell. Morphological details of the Sertoli cell is shown in the magnified image of the portion of the seminiferous tubule from the drawing on the left. Note that the Sertoli cell (S) is placed perpendicular to the basement membrane (BM). It possesses an infolded nucleus (N) with cytoplasm containing numerous mitochondria (Mit) and lipid droplets (LD). A tripartite nucleolus (Nu) is apparent within the nucleus. A tight junction (TJ) between adjoining Sertoli cells is also shown.

changes continuously to accommodate the developing and differentiating germ cells and their mobilization from the base to the lumen (13). Apart from the above mentioned structures, Sertoli cells possess elaborate

cytoskeleton consisting of microtubules and filaments that may be involved in transport of spermatids through the seminiferous epithelium (6).

Germ Cells

In the seminiferous epithelium of the adult testis where spermatogenesis is progressing actively, germ cell types begining with the most primitive germ cell, i.e., type A spermatogonia, to the most differentiated type, i.e., spermatozoa, are observed. The intermediary cell types during this differentiation pathway are type B spermatogonia, preleptotene spermatocytes, spermatocytes in different phases prior to meiotic division (leptotene, zygotene, and pachytene), secondary spermatocytes, and spermatids (round and elongating). A schematic representation of the stages of differentiation of type A spermatogonia into spermatozoa is shown in Fig. 3.

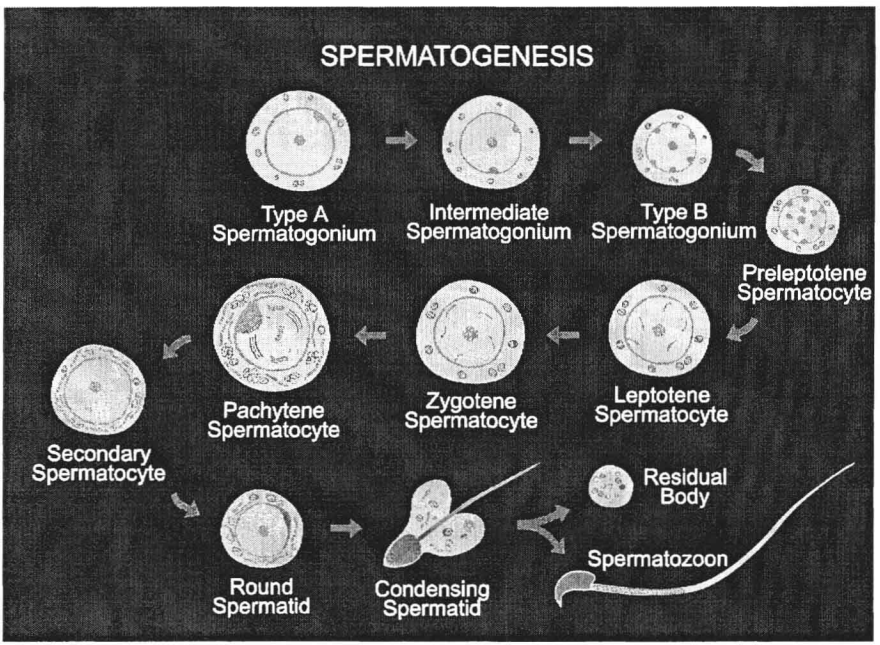

Figure 3. A schematic representation of the process of spermatogenesis. Type A spermatogonia that are present at the base of the seminiferous epithelium undergo a series of mitotic divisions to yield intermediate and type B spermatogonia. Further mitotic divisions of type B result in the formation of preleptotene spermatocytes. The preleptotene spermatocytes through leptotene, zygotene, and pachytene stages undergo the first meiotic division. The resultant secondary spermatocytes proceed through the second meiotic division to yield round spermatids. Round spermatids morphologically differentiate into spermatozoa.