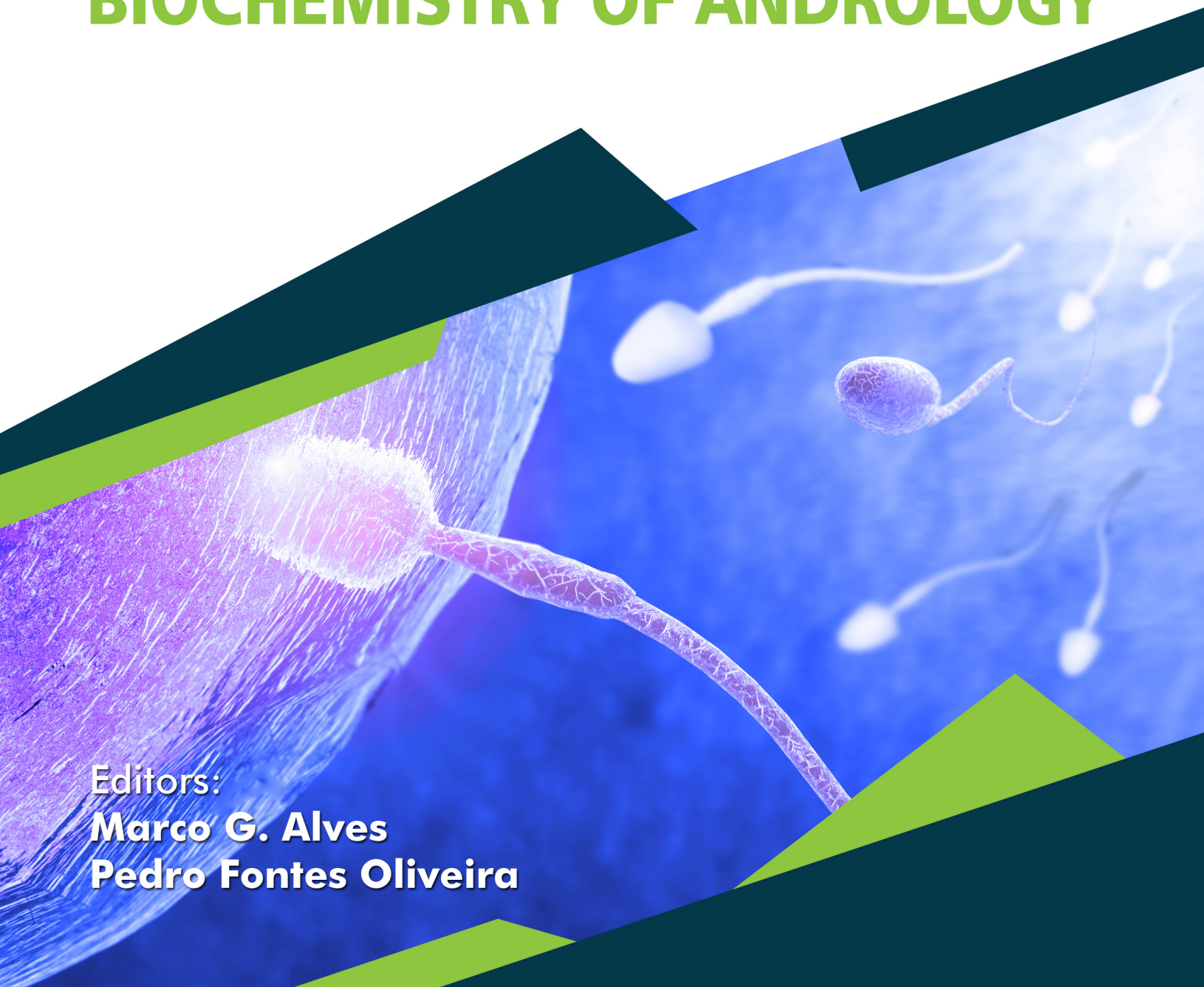
eISBN: 978-1-68108-500-5
ISBN: 978-1-68108-501-2

eISBN: 2543-1617
ISBN: 2543-1609

ANDROLOGY: CURRENT AND FUTURE DEVELOPMENTS

VOLUME 1

BIOCHEMISTRY OF ANDROLOGY



Editors:
Marco G. Alves
Pedro Fontes Oliveira

Bentham e Books

Andrology: Current and Future Developments

(Volume 1)

(Biochemistry of Andrology)

Editors

Marco G. Alves
Health Sciences Research Centre (CICS-UBI), Faculty of Health Sciences, University of Beira Interior, Covilha, Portugal

&

Pedro F. Oliveira
Department of Microscopy, Institute of Biomedical Sciences Abel Salazar UMIB - Unit for Multidisciplinary Research in Biomedicine I3S - Institute for Innovation and Health Research, University of Porto, Porto, Portugal

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Volume # 1

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eISSN (Online): 2543-1617

ISSN (Print): 2543-1609

eISBN (Online): 978-1-68108-500-5

ISBN (Print): 978-1-68108-501-2

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PREFACE

Andrology is the discipline that corresponds to gynecology for men, but while the latter is a well-established discipline, the former is still an emerging field. A simple definition of andrology will include the study of all subjects that may affect the male reproductive system. In the last years, a striking large number of topics has emerged in this field and there is an increasing amount of information that may be useful for graduate, post-graduate and even well-established researchers. Herein, we summarize the available information so far in this area, presenting a review and a critical analysis of the available data on the most relevant subjects with interest to andrology, with emphasis on the biochemistry of the processes. This e-book is divided into 14 chapters, all coordinated by Dr. Marco G. Alves and Prof. Pedro F. Oliveira.

The first chapter is a brief introduction to the historical evolution of andrology and how biochemistry has emerged as a partner, contributing to the emerging relevance of this discipline. The second chapter describes general considerations on testis physiology, testicular anatomy and functional organization, as well as its embryonic development. The third chapter discusses the basic aspects of testicular cells physiology and function. The fourth chapter presents the basic aspects of spermatogenesis, a key event for species propagation, from a biochemical perspective. It is mainly focused on the mechanisms responsible for postnatal testis development but it also presents an overview on the complex events that control the spermatogenic cycle. Many of those changes are under the action and function of male-associated hormones that trigger signaling pathways thus, the fifth chapter is dedicated to those issues. The endocrine regulation of sexual maturation and sperm formation is still a matter of great debate and with an enormous interest. In the sixth chapter, the transition period between childhood and adulthood is discussed, particularly the biochemical changes that control pivotal events responsible for the sexual maturation of the individuals. There is also an overview on puberty-associated disorders, pinpointing the clinical features that should be taken into consideration and the deleterious signals that may occur until sexual maturation is achieved. In the seventh chapter, the biochemical events occurring in the epididymis that end-up in sperm maturation, are discussed. It also discussed the structural organization of epididymal epithelial cells and secretory proteins and their involvement on the spermatozoa modifications that occur during the process of maturation. The eighth chapter is dedicated to the formation and biochemical properties of seminal plasma and male accessory glands. Those changes are essential for spermatozoa to acquire fertility capacity. In the ninth chapter, the functional and physiological aspects of spermatozoa, as well as its epigenome are presented, which may have an enormous implication to the success of the pregnancy and latter to the offspring health. The male gamete is very dynamic and has to move, capacitate, migrate through the female tract, bind to the egg membrane and fuse to the oocyte, resulting in a viable embryo. The tenth chapter is dedicated to the sequential modifications and the molecular mechanisms that occur during the journey of spermatozoa through the female reproductive tract since they have a pivotal role in couple's fertility success and offspring health. Those events may be compromised by several factors that compromise male reproductive health. Congenital disorders, such as hypospadias, undescended testis, testicular atrophy and testicular cancer have increased among young males and even erectile dysfunction and sexually transmitted diseases are still problems that compromise male reproductive health. These issues are discussed in chapter eleven. The twelfth chapter discusses how the pandemic incidence of metabolic diseases is contributing for the worldwide decline in both, sperm quality and male reproductive health. The biochemical changes induced by lifestyle factors and nutrition in the testis are on spotlight to unveil the mechanisms by which metabolic diseases affect nativity rates and the offspring. There is an

intense debate whether worldwide sperm quality is decreasing and the factors that may be responsible for that. Environmental contaminants have arisen as main contributors to the decline on sperm quality. Thus, the molecular mechanisms by which environmental cues alter male reproductive health remain a matter of great interest and are discussed in the thirteenth chapter. The last chapter is dedicated to the biochemical changes in the reproductive function of the aging male. Later parenting is very frequent in modern societies. Nevertheless, the quality and the altered patterns of epigenetics/gene expression in aging sperm remain to be disclosed. Thus, the biochemical changes that occur in testis and sperm and that go along with aging will be on the spotlight for the next decades.

Nowadays, there is a huge investment in reproductive healthcare that is mainly applied in assisted reproductive technologies. However, the long-term effects of these treatments and the causes for male infertility are not cautioned. Overall, this book discusses all the major topics of interest for andrology and mainly presents a focus on the biochemistry of andrology without avoiding the debate on the clinical relevance of the discussed topics. This is a fast growing discipline and thus, there is a great need to educate and prepare students, scientists and physicians for the novel challenges. As scientists working in the field, we felt that most books focused on Andrology lack a strong biochemical view on the topics. As biochemists working in the field for more than a decade, we gathered our team and prepared a book that discusses a large spectrum of topics with high relevance for andrologists all over the world. This book will be valuable for all those working on andrology that aim to understand the magnificent biochemical control of the male reproductive health. Our team had great pleasure preparing this book and we are sure that it will be very useful.

Marco G. Alves
Health Sciences Research Centre (CICS-UBI),
Faculty of Health Sciences, University of Beira Interior, Covilha,
Portugal

Pedro F. Oliveira
Department of Microscopy, Institute of Biomedical Sciences Abel Salazar,
UMIB - Unit for Multidisciplinary Research in Biomedicine,
I3S - Institute for Innovation and Health Research,
University of Porto, Porto,
Portugal

List of Contributors

Ana D. Martins Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

Ana M. Cardoso University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Health Sciences Research Center, Covilhã, Portugal

Bernardo C. Rodrigues University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Health Sciences Research Center, Covilhã, Portugal
Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

Bruno P. Moreira University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Health Sciences Research Center, Covilhã, Portugal
Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

João P. Monteiro Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

Luís Rato University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Health Sciences Research Center, Covilhã, Portugal

Marco G. Alves University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Health Sciences Research Center, Covilhã, Portugal
University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, Porto, Portugal

Mário Sousa University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, Porto, Portugal

Maria J. Meneses New University of Lisbon, 1150-082, ProRegeM PhD Program, CEDOC – Chronic Diseases Research Center and NOVA Medical School, Lisboa, Portugal
Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

Pedro F. Oliveira Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal
University of Porto, Rua Alfredo Allen, 4200-135, i3S – Institute for Innovation and health Research, Porto, Portugal

Raquel L. Bernardino Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

Susana P. Almeida Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal

Tânia R. Dias University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Health Sciences Research Center, Covilhã, Portugal
Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Department of Microscopy, Laboratory of Cell Biology, Porto, Portugal
Faculty of Pharmacy, University of Porto, Rua do Campo Alegre, 4150-755, LAQV/REQUIMTE — Laboratory of Bromatology and Hydrology, Porto, Portugal

CHAPTER 1

Introduction

Marco G. Alves[1,*] and **Pedro F. Oliveira**[2,3,*]

[1] *Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[2] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[3] *i3S – Instituto de Investigação e Inovação em Saúde, University of Porto, Rua Alfredo Allen, 4200-135, Porto, Portugal*

Abstract: Andrology has emerged since the 1950's, when gynecologists started to consistently refer to this word. However, in 1891, there was already an editorial in JAMA suggesting that andrology could evolve to become an important discipline. It was proposed that, as gynecology is a discipline that is focused on the study of genito-urinary female system, andrology could emerge as the discipline focused on the genito-urinary system of males. For many years, this issue was disregarded and there was a long period until the first societies of andrology appeared and establish it in a definitive way. This historical affirmation of andrology as a discipline will be briefly presented, together with a critical view on some aspects that are still a matter of controversy. Reproductive science is a growing discipline that needs economic support from health care systems, institutions responsible for funding research, and training centers. There was never a greater need for trained and well-prepared scientists and physicians to study human reproductive health. Most countries, developed and developing, are witnessing unprecedented rates of people seeking for assisted reproductive technologies. Decreased sperm quality and male reproductive complications are factors that unquestionably contribute to the observed decline in nativity rates. On the other hand, even though females have various contraceptive methods available, men are still limited. This could be improved if more knowledge on sperm formation, maturation and overall testicular physiology arises. In this introductory chapter, we will discuss some challenges for the upcoming years in the field of Andrology.

Keywords: Andrology, Male reproductive tract, Male fertility, Male reproductive health.

[*] **Corresponding authors Marco G. Alves & Pedro F. Oliveira:** Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal; Tel/Fax: +351967245248; E-mails: alvesmarc@gmail.com, pfobox@gmail.com

In 1891, a JAMA editorial referred to andrology as a possible specialty with difficulties in being established, since it was causing some controversy among the genito-urinary surgeons [1]. Nevertheless, it is usually considered that andrology was firstly used with authority by the gynecologist Dr. Harald Siebke from the University of Bonn in 1957 [2]. Although there was great controversy among some physicians, the first step to establish andrology as a valid specialty was taken when the Congress of American Physicians and Surgeons formed the section of Andrology. This decision was welcomed by many researchers and physicians. Andrology established itself as a growing field of research with potential to rapidly develop into a mandatory discipline to evaluate men's quality of life and health. After this, it developed within the field of dermatovenereology and gained great interest from several important researchers. Validation also came from the German Society for the Study of Fertility and Sterility that acknowledged andrology as part of its activities in 1958. Though, it was only in 1970 that an international committee of andrology was founded in Barcelona. Later, several other associations of andrology were formed, including the Nordic Association of Andrology (1973), the American Association of Andrology (1974), the German Society of Andrology (1975), and the American Society of Andrology (1976). This ended up with the formation of the International Society of Andrology in 1981. The need for a high level of formation highlighted that the training centers should be grouped and thus, in 1992, the European Academy of Andrology was formed to gather and establish the guidelines for the training in andrology at a European level. Then, all the most relevant societies involved in reproductive medicine recognized and gave attention to this specialty, including the European Society of Human Reproduction and Embryology (ESHRE). This resulted in a rapid development of the discipline in the last three decades [3]. Nevertheless, even today, there are few formal board-certified training programs in andrology. There is a parallelism between andrology and gynecology taking in consideration that the latter is dedicated to the study of the genito-urinary system of females, and the former of males. This is of particular relevance because male's fertility and the study of their reproductive system has always been overlooked when compared with females, in such a way that the term "diseases of women" is used by several physicians to summarize their specialty, while the same is rare in the case of men. Even today, the field of andrology is not widely recognized by non-experts as a clinical discipline or a research field of great interest like others such as gynecology or urology. Thus, public consciousness of the existence and relevance of this discipline is also mandatory. In summary, andrology is a young interdisciplinary specialty that deals with the male, particularly with the physiology and pathophysiology of male reproductive functions and fertility. We may go further and state that the main focus of andrology is to provide a diagnosis and treatment to males with fertility disturbances.

Andrology evolved as a branch of science that deals with male reproduction and its disorders, including erectile dysfunction, infertility and sexual development. In 1969, the first journal "Andrologie" appeared and gave visibility to this emerging field of research. A few years later, the "Andrologia" journal further contributed to the internationalization of the topic. Initially, the works published were mostly focused on the analysis of the ejaculate, particularly sperm morphology. The clinicians, veterinarians and biochemists started to publish important information on the characterization of sperm and the molecular mechanisms responsible for male fertility. Limited analytical methods, at the time, hampered the initial findings, but enormous progress was made in the first years of those journals. With the advent of molecular biology techniques, omics technologies and hormonal knowledge, andrology entered in a new era of findings. We never had so much information and ways to study testicular physiology, hormonal network, sperm physiology, testicular disorders, and the genetics of the individuals, as we have nowadays. It is also important to highlight that andrology emerged as a discipline that is forced to cooperate with others, including urology, dermatology or endocrinology and thus, it relies on a multidisciplinary work. In addition, concerning the fertility of couples and the treatment of childless couples, it is pivotal that andrologists and gynecologists cooperate and work together to solve the problems beyond the use of assisted reproductive technologies. The diagnosis and therapies of couples would greatly benefit from that. Family planning is also another important matter that benefits from the joint work of both specialties. Nowadays, there are several physicians engaged in andrology and thus, universities and research groups focused on this discipline have highly increased in the last decades. In addition, there is a high number of scientists, besides medical doctors, such as biochemists, veterinaries or biologists that focus their research interest in andrology. Training of highly qualified people is still a major need, as well as support from funding agencies to explore new methods of examination and fundamental research in this field. Basic scientists have also greatly contributed to the exponential growth of this discipline, particularly those with strong formation on biochemistry, biology, pharmacology, genetics and molecular biology. This multidisciplinary approach has allowed a rapid advancement in the understanding of the physiology and biochemical events involved in male reproduction, from the hormonal regulation to the genetic mechanisms responsible for those processes [4, 5]. Nevertheless, the translational gap between basic science and clinical practice still hampers some effective developments that could be useful to improve male reproductive health. We are witnessing an unprecedented need for scientists working on reproductive science. In fact, most developed countries present high rates of induced abortion. Notably, the oral contraceptive method was introduced in 1960, and some authors alerted to the fact that the fundamental biochemical research that served as the basis for this

CHAPTER 2

Testis Physiology

Bruno P. Moreira[1,2], João P. Monteiro[2] and **Maria J. Meneses[2,3,*]**

[1] *Health Sciences Research Center, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal*

[2] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[3] *ProRegeM PhD Program, CEDOC – Chronic Diseases Research Center and NOVA Medical School, New University of Lisbon, 1150-082 Lisboa, Portugal*

Abstract: In multicellular organisms, and particularly in mammals, both gonadal and germ cell development are essential for the transmission of genetic information to the next generations. The testes are paired ovoid organs located inside the scrotum but outside the abdominal cavity. They have two major functions: spermatogenesis and steroidogenesis. The former corresponds to the production of male gametes, spermatozoa; the latter, to the production of hormones that will influence spermatogenesis and consequently male reproductive function and health. The male and female reproductive organs have the same precursor tissues. Initially, the embryo has a bipotential gonad which may have a testicular or ovarian fate. Accordingly, Müllerian ducts form the uterus and fallopian tubes in females and Wolffian ducts form the epididymis, *vas deferens*, and ejaculatory duct in males. On the other hand, male sex determination is triggered by sex-determining region Y (*SRY*), which is located on the Y chromosome and works as a master regulator, initiating *SOX9* expression. The latter causes urogenital development, a highly complex process, through a complex cascade of transcription factors and signaling events. These will promote testis differentiation and ultimately the production of hormones that will lead to male development and testicular function during adulthood. In this chapter, we will provide a brief overview of the testicular anatomy and functional organization, as well as its embryonic development.

Keywords: Genital ridge, Interstitial compartment, Leydig cells, Male reproductive tract, Peritubular myoid cells, Prenatal development, Pre-Sertoli cells, Sex-determining region Y, Sex differentiation, Testis cords, Tubular compartment.

[*] **Corresponding author Maria J. Meneses:** CEDOC - Chronic Diseases Research Center, Rua Câmara Pestana, nº 6, 6A, 1150-082 Lisboa, Portugal; Tel: +351 218803101; Fax:+351 218851920; E-mail: mariajoaocmo@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

INTRODUCTION

Testes are the primary organs of the male reproductive system. They are central in the production of sperm and responsible for the synthesis of male sex hormones, fundamental to the normal development of male internal and external genitalia. The secondary organs of the male reproductive system support the testes in these tasks. Accessory glands produce secretions that constitute the semen. Other accessory structures support and nourish the developing germ cells. In addition, a network of ducts is responsible for storing and the transport of sperm to the female reproductive tract, where fertilization occurs.

Sexual and asexual reproduction are the two basic processes through which organisms reproduce. In the first, a male and a female are needed and each one of them equally contributes to the formation of the new individual. The egg and the spermatozoon will form a zygote, which contains genetic information from both progenitors. However, and unlike other processes of embryonic development, sex determination is a poorly conserved event among the different species, ranging from being controlled by environmental factors to being genetically determined. In mammals, sex determination is genetically determined at the time of conception, with the formation of either an XX embryo or an XY embryo. This process depends on the chromosome acquired from the father, X or Y chromosome, since the one acquired from the mother is always an X chromosome. Male and female reproductive systems are quite different and thus, evolve independently although sharing a common origin. Bipotential gonad can differentiate into testes or ovaries, depending on the stimuli received. In the male, the presence of the testis determining factor shifts the bipotential gonad into a testicular fate, leading to the development of the testes. This complex process is tightly controlled and involves the action of several different signaling molecules and transcription factors. In this chapter, we present the most relevant aspects of the male reproductive system anatomy. We also discuss the development and differentiation of the testes, from the early stages at the time of conception until they are fully developed.

ANATOMY OF THE MALE REPRODUCTIVE TRACT

The reproductive system is not essential for the survival of the individual; it is, however, required for the survival of the species. It is through the reproductive system that new individuals are born; the species are constantly repopulated and the genetic code is transmitted over generations. In humans, the sexual reproduction is the method used, which has several advantages, namely at the level of variability induced by the combination of progenitors genes. This variability ensures the evolution of the species throughout time.

The reproductive system has some unique features. Unlike any other body systems, it is not fully functional at the time of birth, and it requires the action of sex hormones around the time of puberty to be fully active and ready to perform its purpose. In addition, the gender differences between the male and female reproductive system are clearly observed, a fact that does not occur in the other body systems [1].

Structure of the Male Reproductive System

The male reproductive system has different structures that can be divided in primary and secondary sex organs. In males, the primary sex organs, also known as gonads, are the testes. They are responsible for the production of spermatozoa and the secretion of sex hormones. The secretion of sex hormones is then responsible for the development of secondary sex organs. Surrounding the testes is the scrotum, an outpouching of the abdominal wall that protects the testes. The secondary sex organs are structures responsible for the nourishment and storage or transport of the spermatozoa to the exterior or into the female reproductive tract. One of the organs responsible for this transport is the penis. The penis is the male organ used in sexual intercourse and can be divided into three structures: the root linked to the abdominal wall, the body of the penis that corresponds to the major portion of this organ and the glans, also referred as the head of the penis [2]. There are other secondary sex organs, such as the epididymis, *vas deferens*, ejaculatory ducts and urethra responsible for the storage, maturation and transport of the spermatozoa and others responsible for the secretion of fluids that are part of the ejaculate, such as seminal vesicles, prostate gland and bulbourethral glands (Fig. **2.1**). The sex hormones are also responsible for the development of the secondary sex characteristics, that appear during puberty, such as body hair, deep voice and development of the Adam's apple (see Chapter 6) [3].

Testes

The testes are the male gonads, paired ovoid organs that are responsible for the production of spermatozoa and sex hormones. They are suspended in the scrotum by the spermatic cords. Each one is about 4-5 cm long and 2.5 cm in diameter and weighs between 14-18 g in humans [4]. Both testes are covered by two tunics. The outer tunica is the tunica vaginalis and their visceral layer covers the surface of each testis, except where the testis attaches to the epididymis and spermatic cord. This tunica is a thin closed peritoneal sac that has origin on the peritoneum during the descent of the testes. The parietal layer of the tunica vaginalis covers more tissue than the previous one, extending superiorly onto the distal part of the spermatic cord. The separation between the visceral and parietal layers is filled with fluid, allowing the movement of the testis in the scrotum [1, 3, 5].

CHAPTER 3

Basic Aspects of Testicular Cells: Physiology and Function

João P. Monteiro*

Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal

Abstract: Within the testes there is a considerable histological diversity, reflected in a significant variety of different circumscribed environments and cells. As is always the case regarding structures slowly forged by evolution, this translates into meaningful differences in the physiology and function for each cell type. Leydig cells are essentially known for their steroidogenic potential; Sertoli cells are known for their local support to germ cells, and peritubular myoid cells are rapidly transcending a simple structural role. They are all known to actively determine and contribute to spermatogenesis in some way. Moreover, the physiological interplay between these types of cells is known to functionally impact male fertility. However, the specific physiological mechanisms by which each cell type governs spermatozoa production are not fully accounted for, and pathways underlying the cooperative action of these cells in the process are far from being clarified. Increased knowledge regarding the function and interaction of these cells could potentially lead to important breakthroughs within the contexts of testes disease, infertility and contraception.

Keywords: Blood-testis barrier, Cell differentiation, Energetic metabolism, Hormonal regulation, Leydig cells, Paracrine regulation, Peritubular myoid cells, Sertoli cells, Spermatogenesis, Steroidogenesis.

INTRODUCTION

The continuous production of competent spermatozoa by sexually mature males is a complex, highly regulated multistep process implying an intricate interaction between different cooperating cell types present in the testes. Spermatogenesis is regulated by a large number of endocrine and testicular paracrine/autocrine factors. Classically, the process is perceived as being dependent on interactions between somatic and germ cells, involving the coordinated action of three main

* **Corresponding author João P. Monteiro:** Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal; Tel: +351 220 428 000; E-mail: jpspmonteiro@yahoo.com

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cell lineages: supporting cells, steroidogenic cells, and germ cells. Therefore, in the testes, there is a need for a provision of differentiated cells types, and the high rate of cell turnover and differentiation displayed these organs surely is associated with that.

Sertoli cells are viewed as supporting cells in the process of spermatogenesis (they are even called "nurse cells" by some researchers). They are morphological and physiologically very distinct from all other cells present in the testes, and their importance for spermatogenesis ranges from mere physical support, to immunoprotection and nutritional support of developing germ cells. Leydig cells are mostly recognized as being steroidogenic cells. Therefore, they are known to play an important role in the hormonal regulation of the spermatogenic process and, furthermore, in ensuring an adequate definition and sustenance of male secondary sexual characteristics. Peritubular myoid cells have been relatively overlooked regarding their role in spermatogenesis. Other than just a structural role in the establishment of the basement membrane, a more active role in the process has been lately disclosed, involving relevant paracrine function. These three cell types, and their respective importance and involvement in the spermatogenic process, will be the subject of this chapter, and the relative location and organization of each cell type within the testes can be perceived in Fig. (**3.1**). Aspects related to their functional relationships and interactions that are meaningful for competent spermatozoa production will be discussed. Peritubular myoid cells, through the establishment of the basement membrane, preclude direct physical proximity between Leydig and Sertoli cells. However, despite actual mechanical constrictions, these cells were all shown to interact with each other locally, through paracrine mechanisms, and these interaction mechanisms have substantiated many justified studies.

LEYDIG CELLS: STRUCTURE AND FUNCTION

Leydig cells were first characterized in the 1850's by Franz Leydig, from whom they got their name [1]. Leydig cells, located within the interstitial compartment of the testes, are arguably the most relevant cell type involved in endocrine function of the testes, being the main cells responsible for the synthesis and secretion of hormones, namely testosterone in male adults [2]. This important role of Leydig cells implies that the normal function of these cells is crucial for the reproductive activity of males. Leydig cells produce testosterone under the control of luteinizing hormone, which is produced by gonadotropic cells in the anterior pituitary gland [3] and binds to G protein-coupled receptors, activating adenylyl cyclase and therefore increasing cAMP formation [4]. Testosterone was reported to regulate a multiplicity of genes (mostly repressed) in the testes [5].

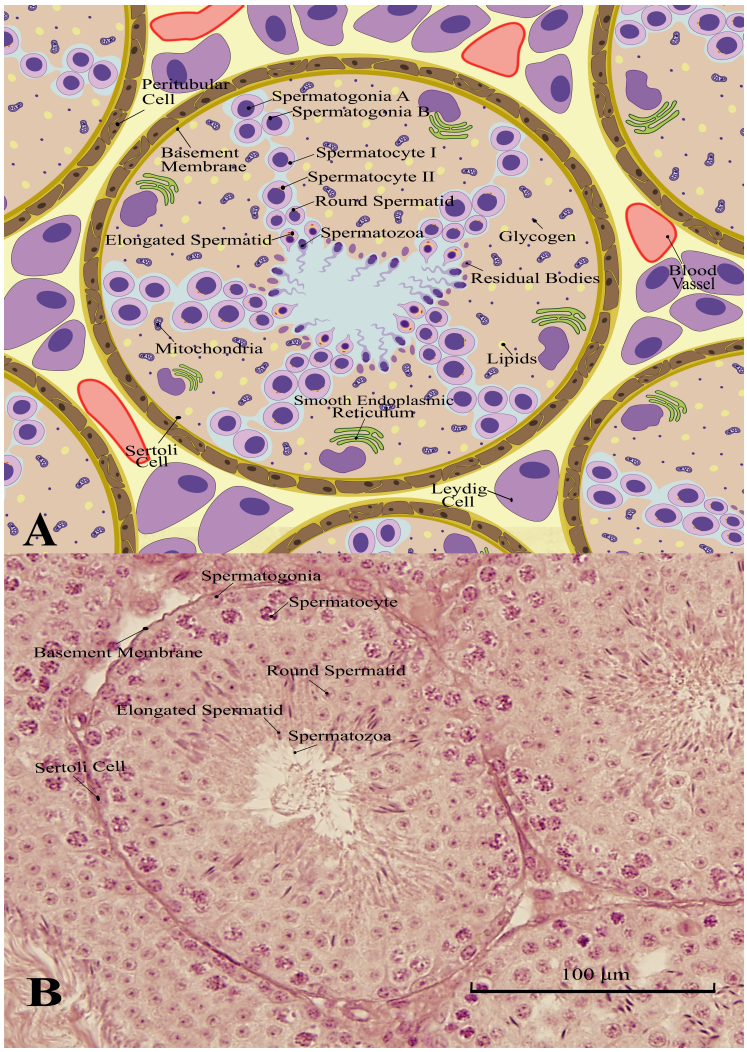

Fig. (3.1). (A) Schematic representation of a transversal cut of mouse (*Mus musculus*) seminiferous tubules, exposing the location of Leydig cells (outside the tubules), peritubular myoid cells (disposed around the tubules, in the basement membrane), and Sertoli cells (inside the basement membrane, in close contact with germ cells, at different maturation stages). **(B)** Actual microscopy image of the transversal cut shown in A), where the same cells type elements are also visible.

Leydig cells have, therefore, an important role in the definition and maintenance of the secondary sexual features defining male characteristics, intermediating testes development, maintenance of spermatogenesis and general male fertility [6, 7]. Some of the processes by which testosterone impacts spermatogenesis include the sustenance of spermatogonia population, preservation of a functional blood-

CHAPTER 4

Basic Aspects of Spermatogenesis

Bernardo C. Rodrigues[1,2,*] and **Mário Sousa**[2,3]

[1] *Health Sciences Research Center, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal*

[2] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[3] *Centre for Reproductive Genetics Prof. Alberto Barros (CGR), Av. Bessa. 4100-012 Porto, Portugal*

Abstract: In what male reproduction is concerned, postnatal testis development comprises five sequencial stages: neonatal, infantile, juvenile, peripubertal and late pubertal. Each of these stages is associated with several regulation factors that may directly or indirectly affect them. After full testis differentiation, the individual is ready to commit to spermatogenesis. Spermatogenesis is a highly complex process that aims to produce spermatozoa through three consecutive steps (mitosis, meiosis and spermiogenesis) that culminate in spermiation. All these checkpoints have an intrinsic relationship with the cycle of the seminiferous epithelium, which allows their deeper understanding and integration. Given all the participants involved in spermatogenic cycle, it can be easily realized that there is a need for a controlled environment that maintains its correct development. This is achieved through the interconnected role of hormonal and paracrine/autocrine regulation factors. Each of them target a specific variety of somatic and germ cells, balancing their response in accordance with testis needs. Attention has also been given to the factors that control the genetic environment. In fact, male fertility is associated with a unique and indispensable set of genes, which are naturally influenced by several protein families with transcriptional and/or translational approaches. With this information in mind, the present chapter aims to discuss the most relevant research on the mechanisms involved in the basis of spermatogenesis.

Keywords: Autocrine Regulation, Cycle of the Seminiferous Epithelium, Hormonal Regulation, Male Fertility, Meiosis, Mitosis, Paracrine Regulation, Phases of Spermatogenesis, Postnatal Testis, Regulation of Spermatogenesis, Spermatogenesis, Spermiation, Spermiogenesis, Testis Development, Transcriptional Regulation, Translational Regulation.

[*] **Corresponding author Bernardo C. Rodrigues:** Av. Infante D. Henrique 6200-506 Covilhã, Portugal; Tel: +351 275 329 002; Fax:+351 275 329 099; E-mail: bernardorodrigues.biomedical@gmail.com

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INTRODUCTION

Male fertility is a highly complex concept that involves an evolutionary process, initiating in the embryo and finishing in the adult individual. There are countless moments in which an impairment can occur, so it is pivotal to understand and describe in detail all the hallmarks that contribute to offspring's maintenance.

Mammalian species do not have a fully differentiated and mature sexual organ at birth. In addition, postnatal testis development involves the proliferation and differentiation of somatic and germ cells through five sequential periods: neonatal, infantile, juvenile, peripubertal and late pubertal. Each of these stages is associated with several regulation factors that may directly or indirectly affect them. Any disturbance in these regulators, as seen in some animal models, results in sexual disruption and, in most cases, infertility.

After full differentiation of the testis, male mammals are ready to develop one of the most important biological processes of cellular transformation: spermatogenesis. This event starts at puberty and continues throughout the entire humans' life, with a well-defined goal: the production of spermatozoa, one of the most specialized and capacitated haploid cells. This mechanism involves three consecutive fully characterized steps. Firstly, mitosis, which is associated with the proliferation and differentiation of spermatogonia. Then, meiosis, which represents the reduction of the number of chromosomes (from diploid to haploid). Finally, spermiogenesis, that transforms round spermatids into highly structured spermatozoa. It also must be highlighted the vital role of spermiation, which allows spermatozoa to be released into the lumen of the seminiferous tubule. All these periods have an intrinsic relationship with the cycle of the seminiferous epithelium, which allows their deeper understanding and integration.

With all the participants involved in the spermatogenic cycle, it can be realized that there is a need for a controlled environment that maintain not only the correct growth and differentiation of germ cells, but also the proliferation and function of the somatic ones. This happens through the interconnected role of hormonal and paracrine/autocrine regulation factors. Concerning the hormonal control, the participation of the hypothalamus and pituitary is unquestionable. The hypothalamic secretion of gonadotropin-releasing hormone stimulates the gonadotroph cells in the anterior pituitary that consequently secrete two gonadotrophins, luteinizing hormone and follicle-stimulating hormone. These, latter then, act directly on the testis to stimulate somatic cell function. In the paracrine/autocrine pathway, local metabolites, growth factors and cytokines have been shown to complement the mechanisms under hormonal control. Each of

factors target a specific variety of somatic and germ cells, balancing their response in accordance with testis' needs.

Researchers have been giving attention not only to the hormonal and paracrine/autocrine regulation of spermatogenesis, but also to the factors that control the genetic environment. This is because male fertility is associated with a unique set of genes, known as chauvinist genes, which are stimulated by germ cells and encode a set of proteins that are essential for the correct interplay seen in the seminiferous epithelium. The process of gene expression is naturally influenced by several protein families with transcriptional and/or translational approaches.

The present chapter aims to discuss the most relevant findings concerning the mechanisms involved in the basis of spermatogenesis, focusing not only in the regulation of postnatal testis development, but also in the complex and structured mechanisms that occur throughout the spermatogenic cycle.

FACTORS THAT REGULATE POSTNATAL TESTIS DEVELOPMENT

At birth, human testis consist of sex cords with interstitial tissue in between, enclosed in a capsule. The cords, which give rise to the seminiferous tubules where the spermatozoa are formed, contain gonocytes, precursors of the germ cells, and the undifferentiated cells, precursors of the Sertoli cells. The interstitial tissue contains fetal-type Leydig cells.

During postnatal testis development, five time periods are recognized in male mammals. These stages include the neonatal, infantile, juvenile, peripubertal and late pubertal periods [1]. In rat, the neonatal period (postnatal days, PNDs 0–7) is the time of transition from fetal gonocytes to mitotically active spermatogonia, high rate of Sertoli cells mitosis and maturation and replacement of fetal Leydig cells by progenitor Leydig cells. The infantile period (PNDs 8–20) is the time when Sertoli cells cease to divide, the tubules start to segregate into stages and spermatocyte development occurs. The juvenile period (PNDs 21–32) is characterized by maintenance of the first wave of spermatogenesis to round spermatids and dramatic increase in tubular diameter. During the peripubertal period (PNDs 33–55) there is active spermiogenesis, as tubular diameter continues to expand and spermatids develop. During the late pubertal period (PNDs 56–70) there is continued growth of the testis and morphological features, consistent with the appearance of a normal adult testis [2]. Along this process, there are innumerous regulators identified as pivotal to the correct development of postnatal testis. The following information synthetize some of that elements.

CHAPTER 5

Hormonal Control of Male Reproductive Function

Maria J. Meneses[1,2] and **Ana D. Martins**[1,*]

[1] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, 4050-313 Porto, Portugal*

[2] *ProRegeM PhD Program, CEDOC – Chronic Diseases Research Center and NOVA Medical School, New University of Lisbon, 1150-082 Lisboa, Portugal*

Abstract: Hormones are key regulators of the reproductive system. These signaling molecules are transported in the blood stream to reach target organs in order to regulate physiologic processes and their function. The most relevant hormones for male reproductive system are those involved in the hypothalamus-pituitary-gonads axis. Through several stimuli, anterior pituitary produces luteinizing hormone and follicle-stimulating hormone that act on testicular cells modulating both steroidogenesis and spermatogenesis. In fact, steroidogenesis, namely the production of testosterone, is crucial for the normal occurrence of spermatogenesis and for feedback actions to the pituitary and hypothalamus. However, spermatogenesis and Sertoli cells are also important to the regulation of this axis through the production of activin and inhibin B that, along with testosterone, also transmit feedback to the brain. Interestingly, in the last years, new intervenient have appeared in the regulation of male reproductive function with the discovery that adipose tissue is an endocrine organ and thus also produces hormones that may be important for this process. Along with the latter, gut hormones, which are related with the nutrient homeostasis, also modulate the function of testicular cells. In some cases, this interaction was only found due to metabolic disorders, like hyper- or hypothyroidism, obesity or diabetes mellitus. Herein, we propose to discuss the action and function of these hormones that interact with male reproductive system.

Keywords: Adipokines, Androgens, Estrogens, Follicle stimulating hormone, Ghrelin, Glucagon like peptide-1, Gonadotropin-releasing hormone, Hormonal control, Hormones, Luteinizing hormone, Obestatin, Resistin, Testosterone, Thyroid hormones, 5α-dihydrotestosterone.

INTRODUCTION

Reproduction is a key process for the survival of a specie thus being subjected to a

* **Corresponding author Ana D. Martins:** Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS), Rua de Jorge Viterbo Ferreira, Nº 228, 4050-313 Porto, Portugal; Tel: +351 220 428 000; E-mail: anacdmartins@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

tight control. The main players in this regulation are hormones which act as dynamic signaling molecules that modulate several events such as gene transcription and translation, directly influencing the reproductive phenotype. The most known hormones are those who belong to the hypothalamic-pituitary gonadal (HPG) axis. In males, the latter is based on the interaction between the hypothalamus, pituitary and the testes [1]. In brief, gonadotropin releasing hormone (GnRH) is synthesized by the hypothalamus, which will stimulate the pituitary to produce gonadotropins: the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH). LH binds to membrane receptors on Leydig cells and stimulates testosterone production while FSH binds to membrane receptors on Sertoli cells, stimulating among other events, the production/secretion of 17β-estradiol, activin and inhibin B [2]. The 17β-estradiol acts on Leydig cells in order to inhibit the production of testosterone [3]; and while, activin produce a positive feedback [4] and inhibin B and 17β-estradiol produces a negative feedback on pituitary [4 - 7].

Over the last few years, other intervenient in the control of male reproductive function have been unveiled. In fact, male fertility is also dependent on overall metabolic and energetic homeostasis [8]. Although testicular metabolism was overlooked during several decades, the adequate overall body metabolic functioning and particularly in testicular cells is crucial for the normal occurrence of spermatogenesis. As nowadays this is a hot research field, other hormones associated with all body metabolic regulation were found to influence male reproductive function [9]. In fact, insulin, which is linked with glucose homeostasis is now known as an important regulator of spermatogenesis [10] (see Chapter 12). Similarly, gut hormones have been described as important regulators of male reproductive potential, besides its well-known role in controlling feeding/fasting status [11]. Moreover, as adipose tissue was recognized as an endocrine organ, the hormones that it produces, the adipokines, were also reported to interact with several other body systems, namely the reproductive system. Thyroid hormones (THs) are also known to influence the reproductive function of males [12]. In fact, they are so important for the modulation of male reproductive potential, that any fluctuation in thyroid hormone levels, either hypo- or hyperthyroidism may lead to impaired spermatogenesis. During this chapter, we will discuss the most relevant hormones and pathways that interact with male reproductive system.

THE HYPOTHALAMIC-PITUITARY-GONADAL (HPG) AXIS

The HPG axis is formed by the hypothalamus, pituitary, and gonads (testes in the male) (Fig. **5.1**). This axis is the key hormonal control system that modulates spermatogenesis and all the events occurring on the male reproductive tract. The

hypothalamus synthesizes and releases neuro-hormones, namely GnRH [1]. The mammalian GnRH is a peptide hormone synthesized and released from GnRH-producing neurons within the arcuate nucleus of the hypothalamus [13]. Still, this secretion is not uniform during life, and changes happen during sexual development [14] (see Chapter 6). GnRH-producing neurons have an intrinsic pulse-generating ability leading to a pulsatile release of GnRH [15]. This pulsatile frequency and the concentration of GnRH influences the subsequent release of LH and FSH [16] having a crucial role in maintaining a normal steroidogenesis and gametogenesis. After its secretion, GnRH enters to the hypothalamic-pituitary portal system and bind to GnRH cell membrane receptors (GnRHR) on gonadotropic cells. These cells are located in the adenohypophysis, which represents 80% of the pituitary gland. The GnRHR is a transmembrane protein and its levels are regulated by GnRH. In fact, GnRHR levels increase when endogenous GnRH is increased. However, a continuous exposure to GnRH leads to desensitization causing a GnRHR downregulation [17]. When GnRHR is activated, it triggers several signal transduction pathways that lead to the release of the gonadotropins LH and FSH [18].

As GnRH, these gonadotropins are released in a pulsatile manner. However, while slower GnRH pulses lead to FSH synthesis, faster pulses favor LH synthesis and release [19]. An increase in the amplitude of LH pulses marks the beginning of puberty and the reactivation of reproductive axis, stimulating the secretion of gonadal sex steroid hormones. As puberty progresses, testosterone starts to control GnRH release, maintaining the frequency of LH pulses [20]. The major androgen produced in the testes is testosterone, a key regulator of spermatogenesis. This hormone is produced by Leydig cells in response to LH and targets Sertoli cells (Fig. **5.1**) that have androgens receptors (ARs) located in the nucleus and cytoplasm [21 - 23]. The other pituitary hormone secreted in response to GnRH is FSH that binds to G-protein coupled receptors, exclusively located in Sertoli cells [2] (Fig. **5.1**).

The activation of FSH receptor results in an increase of cyclic AMP signaling pathway, leading to increased levels of phosphorylated protein kinase B (PKB-P) through a phosphatidylinositol 3-kinase (PI3K)-dependent mechanism [24] and to the production and secretion of inhibin B by Sertoli cells. Inhibin B is a member of the transforming growth factor β family of proteins and its co-receptor betaglycan bind to the activin type II receptor, blocking its association with activin. Therefore, it selectively inhibits FSH biosynthesis and secretion through a negative feedback controlled by GnRH [25] without affecting LH secretion [26] (Fig. **5.1**). Inhibin is a dimer composed by an α subunit and either a βA- or a βB-subunit. In contrast, activin, a dimer composed of two identical inhibin β subunits, plays a positive feedback to the anterior pituitary and stimulates FSH release [27,

CHAPTER 6

Male Puberty: A Triggered Biochemical Event towards Sexual Maturation

Ana D. Martins and **João P. Monteiro***

Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, 4050-313 Porto, Portugal

Abstract: The finding of a concise definition for puberty has proved to be a difficult task for the scientific community and it remains an intense matter of discussion. Nowadays, there is consensus that puberty is a dynamic process influenced by many factors. In this chapter, we will discuss some of the most relevant biochemical markers of puberty and briefly emphasize their relevance in the development and onset of puberty. We will expose the neuroendocrine control that lies behind this very complex hormonally-dependent process. In addition, since puberty is definitely not experienced in the same way by all individuals, we will also discuss genetic, metabolic and nutritional factors as key modulators for the control of puberty onset. The final section of this chapter is dedicated to a brief overview on puberty-associated disorders, pinpointing the clinical features that should be taken into consideration and the deleterious signals that may occur until sexual maturation is achieved.

Keywords: Anti-müllerian hormone, GABA, Glutamate, Growth Hormone, Insulin Growth Factor Binding Protein-3, Insulin Growth Factor-1, Kisspeptin, Leptin, Melatonin, Tanner Stages.

INTRODUCTION

Over time, the establishment of a definitive definition for puberty by the scientific community has proved to be a real challenge, consistently wrapped in interesting debate. If one takes a look into the definition present in dictionaries, it always points to something along the lines of: “the period in people's lives when they develop from a child into an adult, because of changes in their body that make them able to have children”. This definition, requires significant further clarification and is rather simple but is globally accepted by both law and scientific communities. In a more detailed definition, we can define puberty as a period in which we attain secondary sexual characteristics and develop a repro-

* **Corresponding author João P. Monteiro:** Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal; Tel: +351 220 428 000; E-mail: jpspmonteiro@yahoo.com

ductive capacity. Yet, as a very complex event, puberty is not a single and immediate episode since it requires a whole set of processes, not only physical but also psychological that occur in human body during a specific life phase. There are many physical manifestations of puberty in males, including the increase of testis; development of pubic, axillary and facial hair; accelerated body growth; increased muscle strength, among others. In addition, psychological changes may also occur to an extent that is dependent on the individual. Recent studies provided compelling evidence that puberty onset is not only the product of spontaneous body changes but also of hormonal and biochemical-mediated events. The entire environment surrounding the child, from the place of birth, to their nutritional status and their genetic heritage, are all suggested to influence not only puberty but particularly its onset. Physical changes can be observed as a result of differentiation and growth, which in the human case may be more visible during the last phase of growth. In fact, puberty is characterized by a cascade of morphological, physiological, psychosocial, cognitive, emotional and behavioral adjustments associated with the increased gonadal and, in some cases, adrenal activity. The beginning of puberty and its duration is determined by two main physiological processes: the gonadarche and the adrenarche. The gonadarche involves the maturation and enlargement of the gonads, along with increased secretion of sex steroids and, consequently, the initiation of spermatogenesis. The adrenarche encloses the maturation of the adrenal cortex and consequent increase in the secretion of adrenal androgens. The physical manifestations of adrenarche, known as pubarche, embrace the appearance of pubic and axillary hair, acne and adult apocrine odor. Interestingly, the absence of adrenarche does not inhibit fertility, neither influences the timing of gonadarche. In sum, puberty comprises many biological changes, some of which are physically observed while others are discrete and can only be biochemically understood.

BIOLOGY OF PUBERTY

Growth and physical maturation during puberty enclose a wide range of cellular and somatic changes. Evaluation of growth is traditionally based on the assessment of height, but changes in body proportions and composition are also important elements to be taken into consideration. Puberty is characterized by increased growth rate and the appearance of somatic sexual differences. The beginning of puberty is expected to take place at the biological age of 13 in boys [1]. The testis of pre-pubertal boys display a very active and marked hormonal profile [2 - 4]. Testis growth is already visible during childhood, and they increase their volume between the 9 to 13 years until they reach the adult volume [5, 6], mainly due to changes in the seminiferous tubules, during the gonadarche. Leydig cells in the testis of pre-pubertal boys are relatively scarce, although recognizable [7 - 9]. In the beginning of puberty, the number of germ cells exponentially

increases, leading to a rise in the seminiferous tubule diameter and testicular growth. However, the seminiferous tubule length and Sertoli cell numbers remain relatively constant [4, 10]. At the beginning of puberty, Sertoli cells form the blood-testis barrier, the secretion of seminiferous fluid starts, and the lumen is formed in the seminiferous tubules, resulting in increased diameter [11]. Germ cell population raises, as they are first disposed around the base of Sertoli cells, and then move towards the center of the tubules, according to their maturation stage. FSH was shown to be at least in part responsible for the population of Sertoli cells in rats in a post-natal phase [12, 13]. In addition, FSH receptors increase in Sertoli cells and maximal FSH sensitivity is achieved, along with a very high metabolic activity [14] associated with the many functions of these cells, including aromatase activity and secretion of estrogen and anti-müllerian hormone (AMH). The first indicator of pubertal maturation of seminiferous tubules occurs at the peak of spermatogonial multiplication, and involves the entrance of spermatocytes in meiosis [3]. For a period of 2-4 years, meiotic spermatocytes/spermatids are continuously produced and degraded, gradually progressing in the maturation course while reducing germ cell degradation, leading to an increase in spermatogenic ratios [4], and ultimately to accomplished fertility [4]. The increase in testicular testosterone is due to an early activation of Leydig cell precursors, which respond to LH stimulation [15 - 17]. During pre-puberty, the increase of pulsatile LH levels activates the steroidogenically competent Leydig cell precursors first. At this point, immature Leydig cells and their precursors are abundant [4]. The rise in testosterone concentration in the testis precedes the pronounced growth of seminiferous tubules and the development of secondary sexual characteristics [15, 16]. Therefore, it promotes the increase of seminiferous tubule diameter and testicular volume, as well as spermatogenic development [3].

The five stages defined by Tanner and colleagues [18] for the development of secondary sexual characteristics are nowadays most frequently used to define physical development in developing individuals. Tanner stages go from stage 1 (pre-pubertal) to stage 5 (post-pubertal), and their definition takes into account different physical parameters, such as the growth of pubic hair, genital development and height spurt in boys. Tanner stage 1 represents a pre-pubertal stage and stage 5 is attained at complete development, while stages 2-4 are intermediate stages of development. Tanner stage 3 is reached at mid-puberty and is related with the surge in gonadal hormones (testosterone). Tanner stages are a good external indicator physical sexual development, although they are far from capturing all features related to and leading to biological maturity [19].

CHAPTER 7

Biochemical Events Occurring in the Epididymis

Tânia R. Dias[1,2,3,*] and **Raquel L. Bernardino**[2]

[1] *Health Sciences Research Center, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal*

[2] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[3] *LAQV/REQUIMTE — Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Rua do Campo Alegre, 4150-755, Porto, Portugal*

Abstract: The epididymis is a long convoluted organ of the male reproductive tract. Its functionality has been overlooked for many years, but it is currently accepted that it has a preponderant role in spermatozoa post-testicular maturation. The epididymis presents a high secretory activity. Several proteins can be released in bulk through apical blebs or can be associated with epididymosomes, which fuse with sperm plasma membrane becoming integral proteins. The interaction of epididymal proteins with spermatozoa is a very important factor in the regulation of spermatozoa maturation along their passage through the different epididymal regions. A mature sperm cell recovered from the epididymal caudal region should present the ability to move (activated motility) and to fertilize the oocyte. The atmosphere created within the epididymal lumen is very dynamic, since the epididymal fluid composition is relatively different between the tubule compartments. The blood-epididymal barrier (BEB) created by junctions between principal cells of the epididymal epithelium is not only responsible for the control of epididymal luminal fluid composition, but also acts as a defense mechanism to protect spermatozoa from the immune system, harmful xenobiotics and oxidative stress. While the caput and corpus of epididymis are mainly involved in sperm maturation, the caudal region is the site of mature sperm storage in a quiescent and protected state. In this chapter, we discuss the biochemical events occurring during the transit of spermatozoa through the epididymis. We will focus on the involvement and structural organization of epididymal epithelial cells and secretory proteins on spermatozoa modifications during their maturational process.

Keywords: Androgens, Apical blebs, Blood-epididymal barrier, Coating proteins, Epididymal epithelial cells, Epididymal secreted proteins, Epididymis, Epididymosomes, Fertilizing ability, Integral membrane proteins, Luminal micro-

[*] **Corresponding author Tânia R. Dias:** Avenida Infante D. Henrique 6200-506 Covilhã, Portugal; Tel: +351 275 329 002; Fax: +351 275 329 099; E-mail: taniairdias@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

environment, Motility acquisition, Sperm protection, Sperm maturation, Sperm storage.

INTRODUCTION

Spermatozoa development goes far beyond their complex process of production in the seminiferous tubules. Their post-testicular progress involves ultrastructural and macromolecular modifications during their course to reach the site of fertilization in the oviduct [1]. These alterations result from sequential, temporally controlled interactions between male reproductive tract secretions and the transiting male gamete [2]. After being shed from the testes, spermatozoa follow their pathway through the rete testis and efferent ducts, reaching the epididymis. The epididymis is a long convoluted tubule that is structurally organized into three principal regions: the caput (head), the corpus (body) and the distal cauda epididymis (tail) [3]. The epididymal epithelium is constituted by a diverse set of cell types: principal, narrow, apical, clear, basal, halo and surrounding peritubular myoid cells [4, 5]. Each cell type has a particular function and compartmentalization throughout the epididymal duct. Though, all cellular types contribute to the establishment and regulation of the epididymal luminal environment, which is crucial for the attainment of spermatozoa peculiar morphological and functional characteristics [1, 4]. Among the wide range of epididymal functions, we can highlight the following: (1) sperm concentration, to facilitate ejaculation; (2) functional maturation, to acquire motility and fertilizing ability; (3) storage in a quiescent viable state until ejaculation; (4) removal of degenerating sperm and (5) protection of spermatozoa [6].

From the rete testis to the end of the epididymis, spermatozoa are bathed in a continuously and progressively changing medium of fluid proteins and other chemical components [7]. The epididymal fluid composition is highly regulated by a selective structure known as blood-epididymal barrier (BEB). The formation of this barrier is a critical factor for sperm maturation, since it enables the development of the proper epididymal luminal environment for spermatozoa maturational process. Besides, the epididymis has a great proteinaceous secretory activity [8]. In fact, it releases a wide range of proteins that directly influence the composition of the epididymal epithelium (*e.g.* pH, osmolality), but also contributes for sperm protection, since it modulates oxidative stress (OS) [9]. The maturational process of spermatozoa includes many changes in sperm physiological properties, such as the acquisition of forward motility, the ability to recognize and bind to the zona pellucida, and the capacity to fuse with the plasma membrane of an oocyte [10]. During epididymal transit, spermatozoa acquire new proteins, some of which are coating proteins that can be removed by washing with isotonic or hypertonic solutions, while others are assimilated by sperm plasma membrane as integral membrane proteins. These latter are incorporated in

spermatozoa membranes by small vesicles called epididymosomes [11]. However, the underlying mechanisms are not yet fully understood.

The mature spermatozoa are finally stored in a quiescent state in the cauda epididymis [12]. Since they can be stored for several days until ejaculation, the characteristic microenvironment of this epididymal section should also be controlled. Particularly, there is a need to protect spermatozoa from OS, since they are highly susceptible to reactive oxygen species (ROS) damage [13]. Moreover, at this stage, it is essential to remove degenerating spermatozoa, since they can damage the viable quiescent cells [14]. This chapter is focused on the major sequential modifications of spermatozoa during their transit through the epididymis, highlighting the role of epididymal secretome and the biochemical modifications occurring from the immotile testicular spermatozoa to the quiescent, but actively motile, spermatozoa at the end of the epididymal duct.

STRUCTURE AND FUNCTION OF THE EPIDIDYMIS

The epididymis is an accessory organ derived from the Wolffian duct, anatomically connected to the testes, which at birth essentially consist of mesenchymal tissue [15]. During prenatal development, cell proliferation in Wolffian duct is dependent on the presence of androgens and mesenchymal factors, whereas postnatal development is influenced by lumicrine factors, such as androgens, growth factors and several enzymes secreted by the testes [16]. The epididymal postnatal development consists of three major stages: an undifferentiated period, a period of differentiation and a last period of expansion or proliferation [15]. In the first period, from birth to early infancy, the proximal regions of epididymis begin to coil, while the cauda coiling still uncomplete. Moreover, this accessory organ undergoes extensive remodeling and duct elongation/convolution until puberty, where it becomes fully differentiated [15, 17]. In the second period, from infancy to puberty, the epididymal epithelium differentiates into specific cells: principal, apical, basal, clear and narrow cells. During puberty, the epithelial cell differentiation is completed, since there is a high rate of cell division and epididymal expansion. In the third period, there is a continued growth of the epididymis and spermatozoa appear in the lumen of the duct [15]. The adult epididymis consists in a several meters-long contorted duct that, if uncoiled, measures about 5.5 meters in humans [3], 1 meter in mice [18], 3 meters in rat [19] and up to 80 meters in horses [20]. Human epididymis is quite different from that of other mammalians. The macroscopic aspect of the human epididymis allows the anatomically division of the duct, which includes the proximal region (caput), corpus and distal cauda (Fig. **7.1**). This segmentation is also based on structural and functional parameters of each region. In most mammals, each region of the epididymis is structured into lobules that are

CHAPTER 8

Formation and Biochemistry of Seminal Plasma and Male Accessory Fluids

Raquel L. Bernardino*

Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal

Abstract: An appropriate microenvironment in each segment of the male reproductive tract is crucial for the successful maturation and motility of sperm and thereby for male fertility. Spermatozoa are produced in the testes and transported to the epididymis along with the seminiferous tubular fluid. The epididymis contains an epididymal milieu that maintains the optimal conditions necessary for sperm maturation and storage. The composition of the luminal fluid is gradually changed along the epididymal duct due to absorption and secretion processes. The main changes in epididymal fluid and other fluids produced by accessory glands will be reflected in the ionic content, osmolality, pH and spermatocrit. Sperm motility is a good predictor of human male fertility that is controlled by some parameters such as bicarbonate and calcium concentrations, which constantly fluctuate throughout the reproductive ducts. The spermatozoa leaving the epididymis along with the epididymal fluid will join the secretions from the prostate and seminal vesicles, thus forming the seminal plasma. More attention should be paid to male reproductive tract fluids, namely its ionic composition and pH in order to unravel the causes of idiopathic infertility, which represents an elevated percentage of infertile men.

Keywords: Bicarbonate, Calcium, Epididymal fluid, Epididymis, Ions, Ionic transporters, pH, Seminal plasma, Seminal vesicles, Seminiferous tubular fluid, Sperm capacitation and motility, Spermatozoa, Prostate.

INTRODUCTION

The male reproductive system is very complex and highly sophisticated. Spermatozoa are generated in the testes and undergo a maturational process while traveling through the long epididymal tubule, until they reach the cauda epididymis. The sperms are continually exposed to a specialized luminal fluid microenvironment. The composition of the fluid that bathes spermatozoa is

* **Corresponding author Raquel L. Bernardino:** Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal; Tel: +351 938626516; Fax: +351 220 428 000; E-mail: raquellbernardino@gmail.com

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unique in all ducts of the male reproductive tract and is rather different from blood plasma and interstitial fluid [1]. The composition of the testicular fluid and epididymal fluid is variable, and major changes from one region to the next may be detected. The processes underlying the formation of seminal fluid are essential for male reproduction, since the biochemical composition of this fluid is critical for sperm function. Seminal fluid contributes to the process of sperm capacitation and fertilization, controlling pH, nourishing spermatozoa and creating a proper environment for immunological protection [2]. The motility of spermatozoa is also severely affected by the composition of seminal plasma and is, in fact, favored by a controlled pH in the seminal fluid [3].

Failure to maintain pH homeostasis along the male reproductive tract may impair the production and maturation of spermatozoa, therefore causing subfertility or infertility [4, 5]. The importance of understanding the functional relevance of the luminal fluids of the male reproductive tract relies in the fact that up to 40% of infertile men present idiopathic infertility, which may be a reflection of disorders in sperm maturation and storage [6]. This chapter will discuss some of the key components of the testicular fluid, epididymal fluid and seminal plasma and briefly explain how these very specific microenvironments are created.

THE EPIDIDYMAL MILIEU

The epididymis is an organ consisting of a highly convoluted duct system supposed to cover a total length of about 20 meters in man. The whole duct is strictly dependent upon testicular products for the maintenance of its structure as well as secretory, reabsorptive, biosynthetic and metabolic activity [7, 8]. The epididymal lumen has usually the most complex fluid found in any exocrine gland. This may be due to the continuous changes in its composition, such as the presence of some substances in unusually high concentrations for unknown reasons, or the presence of others, not found in any other body fluids [9]. The epididymal fluid compostition is largely influenced by the end-product of the exocrine activity of the testes, testicular fluid or testicular plasma. Besides being dependent on the presence of androgens [10], the epididymis also relies on the presence of luminal fluid factors obtained from the testes and the epididymis itself. Some studies described that without testicular luminal fluid factors, many cells within the initial segment of epididymis would undergo apoptosis [11, 12]. Therefore, factors originated from testes are responsible for the preservation of the integrity and survival of cells within epididymis. The testicular fluid that goes to epididymis is mainly composed by the seminiferous tubular fluid (STF). There is a layer of spindle-shaped cells that fills the space among the external and internal lamella of the seminiferous tubules surrounding tissue, to which the tubules owe their contractibility [13]. STF was thought to be a mixture of primary fluid, most

likely secreted by Sertoli cells [7]. However, the seminiferous epithelium secretes a fluid that is later modified when enters the rete testis, by alterations in potassium, bicarbonate, sodium, chloride, proteins and steroid concentrations [14]. For instance, STF is characterized by being potassium-rich, and is later altered to a sodium chloride-rich when entering the rete testis [7]. Thereby, the epithelium of rete testis is active in the regulation of the luminal fluid microenvironment, and not as much in controlling the volume of fluid produced in the STF that goes to the epididymis [7]. Nevertheless, the role of the rete testis in the regulation of luminal fluid environment has been overlooked. The testicular fluid undergoes a sequence of changes before it entries the epididymis, and along its passage through the seminiferous tubules, rete testis and, lastly, the efferent ducts. Therefore, the "testicular semen" is a suspension of spermatozoa released by the germinal epithelium into a combination of fluids originated from different compartments of the testes and referred to as testicular fluid [15]. The epididymal lumen has a complex fluid that is controlled by the blood-epididymal barrier (BEB), which maintains a specialized luminal fluid, providing a suitable environment for sperm maturation and survival [16]. Luminal fluid microenvironment is important in the processes of sperm maturation and storage. The epididymal epithelium is composed by very active cells in intermediary metabolism that generate products of energy metabolism and reactive oxygen species [6, 16]. The epididymal lumen is rich in inorganic ions and small organic molecules, which create an environment that is hyperosmotic when compared to serum [17]. Perturbations in the microenvironment that surrounds the spermatozoa, such as alterations in the luminal pH or extreme temperatures, can affect maturation and development [4].

LUMINAL FLUID COMPOSITION: RELEVANCE OF BICARBONATE

The luminal fluid surrounding the spermatozoa displays peculiarities between each region of male reproductive tract. The production process begins inside the testes where spermatozoa are released into the lumen of the seminiferous tubule. Inside the seminiferous tubules the spermatozoa are bathed in STF, which is mainly produced and released by Sertoli cells [18]. The establishment of the ionic composition of the STF implies the net movement of water, K^+ secretion and Cl^-, Na^+ and HCO^-_3 reabsorption, which contribute for luminal acidification [18]. Substantial differences in ionic composition of STF have been reported comparatively to blood plasma, particularly concerning the concentration of K^+, Na^+, Cl^- and HCO^-_3. The reported composition of the STF differs according to the method of collection [19 - 22]. The study of male reproductive tract fluids is very complex, with micropuncture, which has originally been used to study renal physiology, being the most common method. The major problem of micropuncture analysis is the available quantity of sample, since it obtains very

CHAPTER 9

Functional and Biochemical Aspects of Spermatozoa

Tânia R. Dias[1,2,3,*]

[1] *Health Sciences Research Center, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal*

[2] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[3] *LAQV/REQUIMTE — Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Rua do Campo Alegre, 4150-755, Porto, Portugal*

Abstract: The spermatozoon is a highly specialized cell that is formed through a complex cellular program of differentiation during spermatogenesis. It has a unique structure and chromatin that reflects its vital function. Morphologically it comprises a head, a midpiece and a tail. The sperm DNA is confined to the nucleus of the head and it has a characteristic protamine-based chromatin that makes it the most condensed eukaryotic DNA. This super compaction of sperm chromatin enhances the protection of DNA from damage since this cell type do not possess robust repair mechanisms. The midpiece is considered the "source of power" of spermatozoa, since it contains many mitochondria, which are responsible for the energy production required for motility. The tail, also known as flagellum, is crucial for spermatozoa movement and transit until they reach the female gamete. The morphological integrity of spermatozoa is of extreme importance for their responsiveness to testicular and epididymal factors involved in maturation. One of the main features that spermatozoa acquire during the maturational process is their motility capacity. This characteristic is not only dependent on the communication of spermatozoa with their surroundings, but also on sperm intrinsic factors, such as adenosine triphosphate (ATP) and specific membrane and secretory proteins. In recent years, sperm epigenome has been a matter of debate among researchers. Its importance is related to its impact on the embryo fate and offspring development. This chapter will discuss the significance of spermatozoa exclusive structure and function for human reproduction and the preservation of generations.

Keywords: Activated motility, Assisted Reproductive Technologies, DNA damage, Epigenetics, Fertilization, Histone, Hyperactivated motility, Protamine, Offspring, Sperm Chromatin, Sperm DNA, Spermatozoa morphology.

[*] **Corresponding author Tânia R. Dias:** Avenida Infante D. Henrique 6200-506 Covilhã, Portugal; Tel: +351 275 329 002; Fax: +351 275 329 099; E-mail: taniairdias@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

INTRODUCTION

Spermatozoa are the male gametes shed from the seminiferous tubules after suffering a myriad of changes during the process of spermatogenesis (see Chapter 4). This cell type is essential for human fertilization and reproduction. To accomplish this concrete but fundamental goal, it is necessary that only one spermatozoon delivers its genetic material to the female egg, activates its development, and generates a new individual. The specialized structural features of mammalian spermatozoa reflect their unique functions. While the acrosome contains the essential enzymes to allow the spermatozoon to penetrate the egg and achieve fertilization, the midpiece contains the energy sources and the flagellum the machinery to generate motility [1]. Moreover, the nucleus contains the paternal genome, which is organized in 23 haploid chromosomes with a high degree of condensation. At fertilization, this set of chromosomes combines with the haploid set of the female gamete to form a diploid cell. The chromatin structure of the spermatozoon presents three main features that allow its distinction from the one of somatic cells: (1) the large majority of DNA is packaged by protamines instead of histones; (2) only 5-15% of the histone-bounded DNA is not replaced by protamines; and (3) the DNA is attached to the sperm nuclear matrix at matrix attachment regions (MARs) at medium intervals of roughly 50 kb throughout the genome [2]. Since protamines have about half the size of a typical histone [3], they contribute to the 6-fold condensation of sperm chromatin relative to somatic cells' chromatin. This specific organization presumably increases the protection of DNA during spermatozoa transit from the male to the oocyte prior to fertilization. In addition to the structural integrity of spermatozoa, its maturation and motility acquisition are also crucial factors for a successful natural reproduction. The attainment of motility by spermatozoa, is only possible in proper and controlled environmental conditions during their transit throughout the male and female reproductive tracts (see Chapter **10**). The motile pattern of spermatozoa is vital for the sperm to reach the egg. Firstly, spermatozoa motility is activated during their transit through the epididymis although this capability is only necessary after ejaculation. Then, spermatozoa are hyperactivated in the female tract, so they can achieve their fertilizing ability. In fact, if a spermatozoon lacks motility or presents an altered motile pattern, it will not be able to fertilize the oocyte, unless technological intervention is made.

Nevertheless, sperm chromatin and DNA are always susceptible to damage, which constitutes a risk factor for the development of embryo abnormalities. In fact, exposure to physical agents or chemicals, including therapeutic drugs and environmental toxicants, can affect the integrity of sperm chromatin, inducing structural, genetic and/or epigenetic alterations. Anomalies on sperm chromatin

structure such as poor chromatin packaging and/or DNA damage may have an impact on male fertilizing ability. Epigenetics englobes the study of gene expression alterations that occur in the absence of changes in DNA sequence and that are fairly stable across the life of an individual [4]. Histone retention and modification, protamine incorporation into the chromatin, DNA methylation, and spermatozoa RNA transcripts appear to play important roles in the epigenetic state of mature spermatozoa. In fact, the histone-bound chromatin identifies genes that are important for embryonic development. The mechanisms by which such damage is triggered are still largely unresolved and the susceptibility of each individual will depend on their genetic background, lifestyle and exposure to various insults. Depending on the nature of the chemicals, they may directly target the DNA, induce oxidative stress, or modify the epigenetic elements. All these topics will be addressed in the present chapter, highlighting the importance of spermatozoa unique architecture for human reproduction and evolution.

SPERM ULTRASTRUCTURE AND CHROMATIN

Morphology of Human Spermatozoa

Spermatozoa present a unique and complex morphology. In general, they comprise a head, a midpiece and a tail region (Fig. **9.1**), commonly known as flagellum [5]. The spermatozoon is smaller than most cells in the body, but its size does not reflect its fundamental function of generating a new human being. The size, shape of the head, length and relative amount of the different components of the flagellum is species-specific [1]. The head of human seminal spermatozoa is pear-shaped, with a median length of 4.4 μm and width of about 3 μm [6]. The nucleus occupies most of the sperm head area and contains a haploid set of condensed, genetically inactive chromosomes [7]. The apical half of the nucleus is covered by the acrosome (Fig. **9.1**), which represents about 48% of the sperm head surface [6]. This acrosomal cap is a membrane-enclosed cytoplasmic vesicle originating from the Golgi apparatus during sperm formation [8]. It contains several polysaccharides (*e.g.* galactose, mannose, fructose, and hexosamine) [9] and hydrolytic enzymes with a preponderant role in the penetration of the sperm into the egg membranes [5]. The part of the nucleus that is not overlaid by the acrosome cap constitutes the postacrosomal region [10]. The sperm head also contains a small amount of cytoplasm and several cytoskeletal structures, including the dense perinuclear layer that is made of basic proteins (*e.g.* calicin and cylicin) associated with calmodulin and actin filaments [11]. On the base of the sperm head there is a small structure called connecting piece (or neck) that connects the head to the midpiece. The connecting piece harbors the proximal centriole and the empty vault. The proximal centriole is composed of nine microtubule triplets and has a vital role on the orchestration of cell division

CHAPTER 10

Biochemistry Behind the Journey of Spermatozoa Through the Female Reproductive Tract

Luís Rato*

Health Sciences Research Centre, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal

Abstract: Sperm released in the lumen of seminiferous tubules are functionally immature. These cells acquire maturation during their passage through the epididymis. The epididymis exhibits an extraordinary structure showing different segments with distinct luminal composition, which act as a whole for the gradual differentiation of sperm. This organ is permanently targeted by neuronal and hormonal factors, in particular by androgens. The study of the biochemical mechanisms that mediate sperm maturation has been a matter of intense debate. The current advances on the knowledge of sperm physiology was possible due to studies conducted in laboratory and domestic species, whereas in humans these processes remain to be fully explored. This is a subject with high relevance in the field of reproductive biology since defects in these events may end-up in infertility. Maturation of sperm begins in the epididymis, but does not end in this organ, since after ejaculation sperms are still unable to fertilize the oocyte. After being deposited in the reproductive female tract, sperm undergo a number of structural and biochemical changes to become "capacitated". Non-capacitated sperm cannot interact with eggs *In vivo* as their failure to hyperactivate motility precludes ascent to the site of fertilization. Sperm and egg must interact so that gamete fusion and the introduction of paternal information into the egg occurs, and the program of development must be activated. Male gametes are the "vehicle" by which the genetic information is passed from fathers to the offspring. In this context, there is increasing evidence that parental lifestyle and the environment influence phenotypes of the next generation. Sperm epigenome has huge implications for the success of male fertility, fertilization, pregnancy and in the transmission of undesirable information to the next generations. In this chapter we will discuss these topics from a biochemical point of view by exploring the mechanisms that govern the most relevant processes.

Keywords: Acrosome reaction, Capacitation, Epididymis, Fertilization, Glycosylation, Male Reproductive tract, Phosphorylation, Post-translational modifications, Sperm-egg interactions, Sperm Epigenome, Sperm proteins.

* **Corresponding author Luís Rato:** Health Sciences Research Centre, Av. Infante D. Henrique, 6201-506, Covilhã, Portugal; Tel: +351 275 329 002; Fax: +351 275 329 099; E-mail: luis.pedro.rato@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

INTRODUCTION

During spermiation, sperm are released in the lumen of the seminiferous tubules morphologically mature, however the proteome of spermatozoa with testicular source does not allow for itself that the spermatozoa is functionally competent. In the later stages of spermatogenesis, sperm are devoid of transcriptional, as well as translational activity. To become mature, sperm require two post-testicular maturation steps; the first occurs in the epididymis and is known as epididymal maturation. During their passage through the epididymis, sperm are exposed to a unique environment, since these cells remain "isolated" due to the blood-epididymal barrier. In the epididymal lumen, sperms are bathed in fluid produced by the cells of the epididymal epithelium, which results from an extraordinary absorptive and secretory activity. At this site there is a constant exchange of proteins between protein epididymal epithelium and sperm where the epididymosomes were reported as main "actors" in this process. At the same time, sperms suffer several modifications known as post-translational modifications (PTMs), namely phosphorylation and glycosylation. Acquisition of motility is one of the significant changes mediated by protein phosphorylation, which begins in the epididymal environment. Sperm motility is regulated by the phosphorylation profile in the several parts of the flagellum and axoneme. This process is coordinated by protein kinases and phosphatases.

The second step of sperm maturation occurs within the female tract after ejaculation in an event known as capacitation. Capacitation is essential for fertilization since sperms are prepared to undergo an exocytosis process known as acrosome reaction, as well as changes in the sperm motility known as hyperactivated movement. At the molecular level, capacitation is associated with molecular changes such as loss of cholesterol from sperm plasma membrane, changes in intracellular ion concentrations, namely HCO_3^- and Ca^{2+}, hyperpolarization of sperm plasma membrane, increased activity of protein kinase A (PKA), and protein tyrosine (Tyr) phosphorylation. All these points will be discussed in detail below. These events have been independently studied and the information regarding how they interconnect to regulate sperm motility and to prepare sperm to undergo the acrosome reaction is still poorly explained. Mammalian fertilization comprises sperm migration through the reproductive female tract, where it undergoes biochemical and morphological alterations and sperm-egg interactions in the oviduct as well. Fertilization occurs through a series of coordinated steps, which ends with the fusion of the two gametes to produce a genetically distinct individual. In this regard, there is an increasing awareness that the molecular changes that occur in the genome, called epigenetic alterations, not only influence the success of male fertility but also fertilization and even pregnancy. Furthermore, the most recent evidence demonstrated that the genetic

information transmitted through male gametes has enormous implications in the subsequent generations, particularly due to the transmission of undesired information that predispose the offspring to metabolic diseases. In this chapter we will discuss, from the biochemical point of view, all steps that sperm experiment from the moment that enters in the epididymal duct till fusion with oocyte.

SPERM EJACULATION

After being released in the lumen of seminiferous tubules, sperms proceed through the rete testis, efferent ducts and begin their journey towards the epididymis which acts as a site for sperm storage until ejaculation. Ejaculation involves a series of events triggered by central nervous system activation of the sympathetic nervous system that creates an emission containing the semen and the secretions from seminal vesicles, prostate, and bulbourethral glands [1, 2]. This process can be divided into two distinct phases: emission and expulsion. The emission phase involves the distal epididymis, vas deferens, seminal vesicles, prostate gland, prostatic urethra, and the bladder neck. The initial step in emission starts with closure of the bladder neck due to sympathetic innervation of the base of the bladder. After bladder neck closure, secretion of fluid from the prostate (10% of total ejaculate volume) provides zinc ions, citric acid and prostate specific antigen (PSA), which facilitates the liquefaction of semen after ejaculation. Subsequently, seminal vesicles contribute to most of the volume (75–80% of total volume) of the ejaculate presenting a rich concentration in HCO_3^- (alkalinization), prostaglandins, fructose, ascorbic acid, and seminogelin I and II. A minor contribution also includes excretion of fluid from bulbourethral glands. The expulsion phase follows the emission and consists of discharge of the semen from the urethra through the coordinated actions of the bladder neck, urethra, and pelvic striated muscles in a process mediated by somatic nervous system [2].

Beyond the neural control, ejaculation is also target of neurocrine regulation. Indeed, androgens can affect the ejaculation process, thus affecting sexual behavior in men because acts both on central and peripheral nervous system, where many areas of which are connected to ejaculatory reflex [3]. Evidences from animal models demonstrated that testosterone (T) regulates the central serotonin pathway. Treatment with T decreased the serotonin release and its metabolite, 5-hydroxyindoleacetic acid by the hypothalamus, thus suggesting that high levels of T may induce premature ejaculation, which is associated with impaired serotonin pathway [4]. At peripheral level T regulates the activity of nitric oxide (NO), as well the expression and activity of phosphodiesterase-5 (PDE-5), an important system involved in the penile erection and in the contractility of genital male tract during emission phase [5]. The initial part of the

CHAPTER 11

Testicular Cancer, Erectile Dysfunction and Male Reproductive Health

Luís Rato*

Health Sciences Research Centre, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal

Abstract: In recent years, there has been growing awareness that male reproductive performance has declined. Numerous studies have focused on various aspects of adverse trends in male reproductive health. There is a wealth of data that congenital disorders, such as hypospadias, undescended testis, testicular atrophy and testicular cancer have increased among young males. Testicular cancer, in particular germ cells tumors, are the most common malignancy among young males accounting more than 10 cases per 100000 men per year in Europe. Both genetic predisposition and environmental contaminants probably contribute for its etiology. Testicular germ cell tumors arise from malignant transformation of testicular germ cells in a multistep process where several aberrant modifications occur in genes involved in proliferation/survival and differentiation. Individuals with testicular germ cell tumors present a high survival rate but during treatments they are exposed to radio- and/or chemotherapy that may induce permanent damages in male fertility. In this context, it is essential to decipher the molecular mechanisms underlying testicular-related cancers. However, other pathologies have also contributed to the decline of male reproductive health, and particularly affect male sexual behavior. Inadequate penile erection, commonly termed as erectile dysfunction (ED) mostly occurs in men older than 40 years. It is quite common in developed countries and compelling evidences have linked the development of ED to diabetes mellitus, hypertension, hyperlipidemia, metabolic syndrome and depression. In fact, it has been shown that certain environmental and factors related to daily life, such as smoking, obesity, and limited or an absence of physical exercise may also be key predictors of ED. Physicians have looked with particular concern to these issues, but also for the sexually transmitted diseases (STDs). Some STDs are resolved without treatment, but others have chronic lifelong infections. In this chapter those topics will be discussed from a biochemical point of view and the pathways that regulate the most relevant processes.

Keywords: *Carcinoma in situ*, Erectile dysfunction, Libido, Phosphodiesterase inhibitors, Testicular cancer, Testicular germ cell tumors, Sexual behavior, Sexual desire, Sexually transmitted diseases.

* **Corresponding author Luís Rato:** Health Sciences Research Centre (CICS-UBI), Av. Infante D. Henrique, 6201-506, Covilhã, Portugal; Tel: +351 275 329 002; Fax:+351 275 329 099; E-mail: luis.pedro.rato@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

INTRODUCTION

For many years, reproductive health of males has been overlooked. This has occurred because reproductive problems such as infertility have been suggested to have only origin in females. For instance, for many years, women's reproductive capacity has been a target for contraceptive development, which illustrates how the health systems define "priorities" around women's and not men's health needs. However, in the last two-three decades a decline in male reproductive health has been observed, mostly due to the decline in spermatozoa quality. The prevalence of infertility in couples in reproductive age is extremely high and in many developed countries affects one in seven couples, with the "male factor" accounting for the 50% of the cases. Male reproductive health is extremely vulnerable to adverse effects that arise from lifestyle factors. Though there is not yet a direct association, compelling evidence suggested that all these external insults have contributed to several male reproductive disorders that have a common fetal origin, triggered by testicular maldevelopment [1]. This has been termed by "testicular dysgenesis syndrome" (TDS) [1]. Amongst the several reproductive anomalies that may arise from TDS, testicular cancer is one of the most relevant problems since it mostly affects young males. In fact, testicular cancer is the most common malignancy among young men. Most of these cases are treated with radio- and/or chemotherapy, which lead to severe effects for male fertility (most of them permanent). It is therefore imperative to unravel the molecular mechanisms by which testicular-related cancers establish and progress.

On the other hand, the current lifestyle, based on sedentarism and work stress has led to psychological and emotional distress that have also compromised male reproductive health by disturbing male reproductive behavior. All these factors can affect the endocrine system and the molecular pathways that govern it, therefore affecting male sexual behavior (namely libido and erectile function). Physicians have looked with particular concern to these issues but also for the problems caused by pathogens that can be acquired and transmitted through sexual activity. Sexually transmitted diseases (STDs) have accounted to the most common infection diseases. The alarming number of individuals getting STDs has reached pandemic proportions [2]. Most of STDs may be asymptomatic and therefore people may be unaware that have it or are at high risk to be infected. Some of the STDs are resolved without treatment, but others are chronic lifelong infections. In this chapter, those topics will be discussed from a biochemical point of view, with particular focus on the pathways that regulate these processes.

MOLECULAR MECHANISMS INVOLVED IN TESTICULAR CANCER

Testicular cancer is rare accounting to only 1–1.5% of male cancers. It mostly

affects young males during their third or fourth decade of life [3, 4]. The World Health Organization (WHO) has classified testicular tumors in three main categories: testicular germ cell tumors (TGCTs) accounting for the most common form of testicular cancer; cord stromal tumors, and miscellaneous germ cell/sex cord stromal tumors [2]. Since TGCTs account for 90-95% of all testicular neoplasia and are a heterogeneous group of neoplasms with diverse histopathology and clinical behavior, we will focus in this type of cancer. TGCTs may occur throughout men's life and are divided into three main types: type I which consists of benign mature teratomas or malignant yolk sac tumors which occur in children usually before four years of age and always become apparent before puberty [5 - 7]; in the type II, TGCTs can be sub-divided into two main types: classic seminoma and nonseminomas, both derived from a common precursor cells, called carcinoma in situ (CIS) or intratubular germ-cell neoplasia. CIS cells are large atypical germ cells that are found between the thickened basement membrane and the Sertoli cell layer within the seminiferous tubules; the third type of TGCTs are known as spermatocytic seminomas and usually occur in men older than 50 years. These benign tumors exhibit slow growth and share some genetic markers with type B spermatogonia [5, 6, 8]. Contrastingly, there has been a marked increase in the incidence of type II germ cell tumors in developed countries in the last 50 years [5, 6, 8]. Type II TGCTs mainly affects men in their reproductive age (20s to 40s years of age). These tumors are associated with a pre-invasive lesion or undifferentiated intratubular germ cell neoplasia. Interestingly the incidence of TGCTs shows geographic and ethnic differences, with Caucasian people being mostly affected in developed countries [8 - 12]. Even within this group, it has been observed marked differences between populations, as evidenced by two North European countries, Denmark and Finland. The rapid increase in the incidence of TGCTs cannot be only explained by genetic predisposition, indicating the possible involvement of environmental or lifestyle factors in its etiology. This is supported by the fact that increased incidence of TGCTs has occurred at the same time with the tendency of other reproductive disorders associated with TDS, such as impaired testicular descent, genital malformations, and male subfertility [13, 14]. Based on these facts, it has been proposed that TGCTs may arise from congenital disorders. For instance, it has been generally accepted that cryptorchidism is one of the best known risk factors for testicular cancer [15], but other components of TDS, such as hypospadias, also arise as good candidates. Interestingly, all these risk factors are directly linked to prenatal or perinatal period of development. TGCTs development is a multistep process that, in its principle, follows the general multiple-hit hypothesis of malignant transformation where accumulation of several aberrations affecting groups of genes involved in proliferation/survival and differentiation/cell repair, at subsequent stages of life, are required for the

CHAPTER 12

Metabolic Disorders and Male Reproductive Health

Maria J. Meneses[1,2,*]

[1] *ProRegeM PhD Programme, CEDOC – Chronic Diseases Research Center and NOVA Medical School, New University of Lisbon, 1150-082 Lisboa, Portugal*

[2] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Unit for Multidisciplinary Research in Biomedicine (UMIB), University of Porto, 4050-313 Porto, Portugal*

Abstract: Metabolic disorders represent a major public health burden nowadays. From these metabolic disorders, obesity and diabetes *mellitus* (DM) may be considered the most significant ones. Obesity is characterized by an excess of body fat, where body mass index (BMI) is used for its classification. When an individual has a BMI between 25 and 30 kg/m^2 is considered overweight, while a BMI over 30 kg/m^2 classifies an individual as obese. This excessive fat is very harmful and may even reduce life expectancy. On the other hand, DM encompasses a cluster of disorders characterized by chronic hyperglycemia that are a result of defects in insulin action, insulin secretion, or both. The exponential increase of these metabolic disorders is, in part, due to erroneous dietary habits that lead to an inadequate intake of essential nutrients. Moreover, while the prevalence of metabolic diseases increases, the fertility trends decrease, illustrating an association that may, or may not, be direct. In fact, there is an increasing number of children, adolescent and men in reproductive age suffering from metabolic disorders. It is well known that the occurrence of a normal spermatogenesis is dependent on the metabolic cooperation established between testicular cells, particularly concerning glucose metabolism and insulin signaling. Therefore, it is crucial to unveil these mechanisms in individuals with metabolic disorders, how they are affected by the disease and how they change the fertility of males. In recent years, several studies have provided new information concerning alterations induced by metabolic disorders in male reproductive health. In addition, it was highlighted that testicular cells possess several mechanisms that react to hormonal fluctuations to counteract hyper- and hypoglycemic events. In this chapter, we will discuss the effects of DM and obesity in the regulation of testicular insulin signaling and glucose metabolism as well as the importance of an adequate diet and how these events are implicated in the reproductive health of males.

[*] **Corresponding author Maria J. Meneses:** CEDOC - Chronic Diseases Research Center, Rua Câmara Pestana, nº 6, 6A, 1150-082 Lisboa, Portugal; Tel: +351 218803101; Fax:+351 218851920; E-mail: mariajoaocmo@gmail.com

Marco G. Alves and Pedro F. Oliveira (Eds.)

Keywords: Antioxidants, Diabetes *mellitus*, Fertility, Glucose, Insulin, Micronutrients, Nutrition, Obesity, Oxidative Stress, Sperm, Vitamins.

INTRODUCTION

In the last years, the world is fronting an emergency in public health, due to the radical increase of metabolic disorders [1], as obesity or diabetes *mellitus* (DM) [2]. Obesity is a metabolic disorder characterized by an excessive body fat accumulation and is measured in part through the body mass index (BMI) [3]. When an individual has a BMI above 25 kg/m^2 but lower than 30 kg/m^2, the individual is considered overweight. However, when BMI surpasses 30 kg/m^2 the individual is considered obese [3]. Obesity may lead to several co-morbidities, being DM one of those [4]. DM is part of a group of metabolic disorders characterized by chronic hyperglycemia. The latter may be due to defects in insulin action, insulin secretion or even both [5]. In this group of diseases, the most representative ones are type 1 DM and type 2 DM. The first one, also known as insulin dependent DM, is an autoimmune disease where T lymphocytes react against insulin-producing pancreatic beta cells [6]. However, the vast majority of diabetic individuals have type 2 DM [5]. It embraces individuals who have insulin resistance and increasing levels of insulin deficiency. Nevertheless, unlike type 1 DM, the key triggering factor of type 2 DM appears to be wrong dietary habits rather than genetic reasons, though the latter can also play a critical role in the appearance of the disease [7]. The deleterious effects of these metabolic disorders is denoted by a widespread list of comorbidities and social problems. Concurrent with a drastic increase in the prevalence of metabolic disorders, we have also been witnessing to a dramatic decline in fertility rates. In fact, research dedicated to fertility trends has been encouraged by reports showing that semen quality has been declining in European populations from a few decades ago until now [8, 9]. Furthermore, these two trends appear to be interrelated for several reasons. One of the possible explanations is the enlarged frequency of men on reproductive age suffering from metabolic disorders [10]. As discussed in the previous chapters, the metabolism of testicular cells is crucial for a normal spermatogenesis. Besides, glucose, insulin and lipids have a key role in the control of the metabolic cooperation established between Sertoli and developing germ cells [11, 12]. Thus, the dysregulation promoted by either obesity or DM in the testicular metabolic cooperation might be a key factor to the decrease in fertility rates observed in countries with high incidence of metabolic diseases. Nowadays, there is evidence from several studies performed either *in vitro* or *in vivo*, that the presence of metabolic disorders or some of their main features have a profound impact on the reproductive potential of males [11, 12]. Moreover, in the last years, several studies have been performed to unveil the mechanisms by which metabolic diseases alter the reproductive potential of males. Therefore, in this we will

discuss the possible mechanisms involved in the connection between metabolic disorders, sperm quality and thus (in)fertility. We will also discuss in what way dietary components can act as regulators of testicular metabolism and thus, be useful to counteract the undesirable effects of metabolic disorders.

OXIDATIVE STRESS AND ITS EFFECTS ON MALE REPRODUCTION

Reactive oxygen species (ROS) are byproducts of oxygen metabolism and energy production [13]. This broad term includes both oxygen radicals and certain non-radical oxidizing agents that can be easily converted into radicals, namely superoxide anion radical ($O_{2}\cdot^{-}$), singlet oxygen ($^{1}O_2$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical ($\cdot OH$). Free radicals are defined as any species that contain one or more unpaired electrons [14]. ROS are normally produced in cells, playing important roles in cell signaling [15] and being capable of damaging cellular components, namely lipids, proteins and even DNA [16] (Fig. **12.1**). Concerning male reproductive potential, ROS play a crucial role in sperm function as second messengers, namely in capacitation, acrosome reaction [17], and sperm-oocyte fusion when they are produced in small quantities [18]. However, when the antioxidant capacity of the cells is insufficient to neutralize ROS, either by an increase of ROS or by a decrease in antioxidant defenses, oxidative stress (OS) occurs [19] (Fig. **12.1**). OS may then impair sperm cells through damages in plasma membrane and DNA integrity affecting sperm motility and viability, sperm-oocyte fusion, development of embryo and even maintenance of pregnancy [20]. One of the main reasons for spermatozoa being so susceptible to OS is the high percentage of polyunsaturated fatty acids (PUFA) that they contain [21]. The attack to the latter by free radicals leads to the formation of lipid radicals. These combine with oxygen, the universal electron receptor, generating a lipid peroxyl radical [22]. Then, lipid radicals are formed because lipid peroxyl radical extracts hydrogen atoms from adjacent lipids in order to stabilize as a hydroperoxide [23]. In consequence of lipid peroxidation, lipid aldehydes (such as 4-hydroxynonenal) are formed and bind to mitochondrial proteins, stimulating the generation of even more free radicals [22]. This further enhances lipid peroxidation in a vicious cycle that leads spermatozoa to apoptosis. However, and due to the structure of spermatozoa, the nucleases activated in the midpiece, either in the cytoplasm or in mitochondria, cannot enter the nuclear compartment. H_2O_2 is the only product of apoptosis that can pass from the midpiece to the sperm head and consequently damage the DNA. Thus, this is the main reason why the great percentage of DNA damage in spermatozoa has oxidative origin [24].

CHAPTER 13

Environmental Cues and Sperm Quality

Ana M. Cardoso and **Luís Rato***

Health Sciences Research Centre, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal

Abstract: The increase in the occurrence of defective spermatogenesis and other important fertility issues in males evidenced over the past few decades have prompted the research on the possible contribution of environmental factors to this adverse trend. Environmental contaminants may act through different molecular targets in male reproductive system, being able to disrupt the functioning of reproductive axis and, consequently, testicular physiology and metabolism. In addition, endocrine disruptors and environmental compounds that favor adipogenesis, namely obesogens, are also related to the imbalance of tightly regulated metabolic processes and to a host of other adverse reproductive outcomes. Such effects may result from an exposure during gestation, prepubertal age or adulthood, emphasizing the importance of different environmental impacts throughout the life course. Environmental contaminants may also promote disturbances in the metabolic performance of the following generations, through epigenetic modifications passed by male gametes. As society increasingly introduces new potentially toxic substances into daily life, unveiling the molecular pathways by which environmental contaminants induce toxicity that may end-up in epigenetic modifications is imperative. Otherwise, a transgenerational susceptibility to metabolic diseases may be favored. Herein, we discuss the suggested molecular targets and potential mechanisms for environmental contaminants action and the subsequent effects of exposure during different life stages of the male. We also present an up-to-date overview about the impact of endocrine disruptors and obesogens on male reproductive health, as well as the epigenetic modifications induced by these environmental cues.

Keywords: Environmental contaminants, Endocrine disruptors, Epigenetic modifications, Glucose metabolism, Lipid metabolism, Male fertility, Molecular toxicology, Obesogens, Spermatogenesis, Sertoli cells, Sperm quality, Transgenerational effects.

INTRODUCTION

Nearly 50% of infertility cases, affecting millions of couples worldwide, are

* **Corresponding author Luís Rato:** Centro de Investigação em Ciências da Saúde (CICS-UBI), Av. Infante D. Henrique, 6201-506, Covilhã, Portugal; Tel: +351 275 329 002; Fax: +351 275 329 099; E-mail: luis.pedro.rato@gmail.com

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exclusively attributed to the male factor [1]. As in these cases treatment is not usually directed at a specific identified cause, it becomes difficult to establish an accurate diagnosis for the observed anomalies in male reproductive health. Moreover, men apparently normal may suffer abnormalities in the quality of spermatozoa [2]. Treatment is usually associated with assisted reproductive technologies, which are applied to increase the chances of conception. Indeed, a negative correlation has been suggested in this context: while life expectancy increases, the quality of spermatozoa substantially declines, thus becoming a matter of great concern specially in most developed societies [3]. Although the etiology of this adverse trend remains a subject of great debate, there has been a consensual awareness regarding the contribution of external factors, such as lifestyle habits and its associated factors, as is the case of permanent exposure to environmental contaminants. These contaminants can be found virtually everywhere, with exposures throughout lifespan, from gestation to adulthood, emerging as an important health issue. Male reproductive function is highly susceptible to the effects of environmental contaminants, such as pesticides, plasticizers, heavy metals, food additives and others [4]. Most of these environmental contaminants exhibit lipophilic characteristics and can mimic naturally occurring hormones acting as endocrine disruptors (EDCs). EDCs are defined as exogenous agents that interfere with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body, which are responsible for the maintenance of homeostasis and the regulation of developmental processes [5]. Considering reproductive function, most of the effects are exerted through disturbance of estrogens-, anti-estrogens-, androgens- and anti-androgens-mediated processes [6, 7]. The majority of these substances act by interfering with the receptors of endogenous hormones and impairing the usual gene transcription response [8]. However, some EDCs are also capable of modifying hormone bioavailability by interfering with its secretion and transport or by disrupting the enzymatic pathways involved in hormone synthesis and metabolism [9, 10]. EDCs are a heterogeneous group of substances and several harmful effects were already associated to their exposure in healthy individuals. This is extremely important concerning male reproduction, which is highly dependent on endocrine regulation, specially reproductive events (such as steroidogenesis and spermatogenesis) that are dependent on Leydig and Sertoli cells, respectively [11]. However, the molecular mechanisms by which the environmental cues affect male reproductive health are not entirely dissected yet. Besides, the adverse effects of an exposure to these compounds was mainly studied at an occupational context and not applied to the general population [12], making people less concerned about their effects. Information regarding human data is scarce. Furthermore, humans are exposed to at least hundreds of environmental chemicals of which dozens are classified as EDCs. One limitation

of epidemiological studies is that they only evaluate human exposure to a single environmental contaminant, or at best to a set of isomers or congeners within a family of EDCs. Understanding the potential human health risks requires the study of the complex mixtures to which we are permanently exposed. In this context, animal studies have suggested that environmental cues play a significant role in spermatozoa quality and thus, in male fertility [13 - 15].

Spermatogenesis is an exclusive function of mature testes, since it begins during puberty and continues spanning through the entire reproductive life [16]. This event is extremely complex and its function becomes easily vulnerable to the effects of environmental contaminants [17]. However, it is important to highlight that the effects of insults from these environmental contaminants occurring at early ages, even gestational, may be manifested only at adulthood [18]. In this chapter, the impact of environmental contaminants on male reproductive health and testis physiology will be discussed from a biochemical point of view. It will also explore the putative effects of environmental contaminants on sperm parameters and the subsequent consequences to overall male fertility potential.

MOLECULAR TARGETS OF ENVIRONMENTAL CONTAMINANTS

The impact of environmental compounds on male fertility has been under discussion for more than 50 years, when Ratcliffe [19] associated the dramatic decline in the population of certain bird species to a persistent pesticide exposure. Pesticides, heavy metals, chemicals in plastics, phytoestrogens and other environmental chemicals have been described since then to act like "uncontrolled medicines" for humans [20], being capable of affecting their health. Originally it was thought that environmental contaminants, in particular those acting as EDCs, exerted its effects through nuclear hormone receptors, including estrogen receptors, androgen receptors, progesterone receptors, thyroid receptors and others [21]. However, today it is known that those mechanisms are much broader than was initially postulated. The group of molecules classified as EDCs is very heterogeneous and includes industrially produced chemicals, such as polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins, bisphenol A (BPA), phthalates, pesticides (methoxychlor, chlorpyrifos, dichlorodiphenyltrchloroethane (DDT)), fungicides and pharmaceutical drugs. Likewise, natural chemicals found in human and animal diets (e.g. phytoestrogens, such as genistein) can also act as EDCs. Individuals are mainly exposed to these contaminants through ingestion, inhalation and/or dermal absorption. EDCs such as dioxins, PCBs, PBBs and pesticides often contain a phenolic moiety that is thought to mimic natural steroid hormones and enable EDCs to interact with steroid hormones receptors as agonists or antagonists. Furthermore, the majority of these compounds are lipophilic and this is

CHAPTER 14

Biochemical Changes in the Reproductive Function of the Aging Male

Susana P. Almeida[1] and **Luís Rato**[2,*]

[1] *Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar and Unit for Multidisciplinary Research in Biomedicine, University of Porto, Rua de Jorge Viterbo Ferreira, 4050-313, Porto, Portugal*

[2] *Health Sciences Research Center, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001, Covilhã, Portugal*

Abstract: Late-onset hypogonadism (LOH) is a situation where a middle-aged or older man has low serum testosterone in conjunction with diffuse symptoms resembling those of genuine male hypogonadism. Testosterone replacement therapy has become a popular choice for the treatment of LOH. Aging is a process that includes irreversible changes because of a large variety of endogenous and environmental factors. Paternal aging also causes genetic and epigenetic changes in spermatozoa that damage male reproductive functions through adverse effects on sperm quality and count, as well as on sexual organs and also on the hypothalamic-pituitary testicular axis. If on one hand, hormone production, spermatogenesis, and testes undergo changes as a man ages, on the other hand, the offspring of older fathers show high prevalence of genetic abnormalities, childhood cancers, and several disorders. Information on the impact of age on male fertility is of growing importance, therefore, further studies should investigate the onset of changes in the reproductive function and its effects on aging men. The aim of this chapter is to briefly discuss the effects of aging on the male reproductive system and function.

Keywords: Aging, Epigenetics, Infertility, Late-onset hypogonadism, Male fertility, Oxidative stress, Paternal age, Replacement therapy, Semen parameters, Spermatogenesis, Spermatozoa, Testes, Testosterone.

INTRODUCTION

Aging of males exerts effects on reproductive organs and tissues and such changes seem to evolve progressively without a well-marked threshold of age. Among those alterations are the testicular changes that are most often accompanied by variations on the levels of reproductive hormones [1, 2]. In

* **Corresponding author Luís Rato:** Health Sciences Research Centre (CICS-UBI), Av. Infante D. Henrique, 6201-506, Covilhã, Portugal; Tel: +351 275 329 002; Fax: +351 275 329 099; E-mail: luis.pedro.rato@gmail.com

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menopausal women, the cessation of ovarian function is due to the inevitable decline and final exhaustion of the oocyte pool, paralleled by increase in the levels of follicle-stimulating hormone (FSH). However, in males, spermatogenesis continues throughout life [2]. As they age, men experience a gradual and progressive decline in reproductive function when compared to women. Also, in contrast to menopause, which is universal and a well characterized timed process related with absolute gonadal failure, this condition in men is characterized by insidious beginning and slow progression [3]. This variability is a trait of male reproductive system aging and of the general male aging process. As reported in several recent studies, androgens play an important role within the development and maintenance of male reproductive and sexual functions. Diminished levels of circulating androgens lead to innate abnormalities of the male reproductive tract. Indeed, testosterone levels decrease as age advances. The symptoms caused by this decline can be regarded as a normal part of aging. At more advanced age, this is reported to be associated with reduced fertility, sexual disorders, decreased muscle formation and bone mineralization, disturbances of metabolism, and psychological feature dysfunction. Moreover, low testosterone levels are also related to many chronic diseases, and symptomatic patients could benefit from testosterone treatment [4] though this remains a matter of intense debate. Though the biochemical changes induced by aging in the male reproductive tract remain largely unknown, we will briefly discuss the mechanisms known so far.

IMPACT OF AGING ON TESTICULAR ANATOMY AND PHYSIOLOGY

Generally, as men grow older, they retain their fertility though some changes may occur. Indeed, they develop certain physiological changes affecting the endocrine system and testicular function (Fig. **14.1**). Notwithstanding individual variations, alterations in testicular morphology are one of the several effects of aging on the reproductive system of males. Testicular function declines with advancing age, but this reduction has a magnitude similar as that of other body organs [5 - 7]. Still, the decreased efficiency in testicular function has been the subject of a great number of studies, which evidenced the relationship between testicular function and age [8 - 12]. During aging there is a thickening of the basal membrane of the seminiferous tubules, accompanied with a reduction in both the height of the seminiferous epithelium and in the vascularization of the testes [13, 14], which are associated with testicular hernia-like protrusions [15]. Sertoli cells and germ cells represent up to 90% of the testicular volume, while Leydig cells contribute to less than 1%. Aging leads to a reduction in the number of Sertoli cells and Leydig cells [2, 16] which could results in a decrease of testicular volume [1]. In fact, a negative association between increasing age and reduction in testicular volume for men over 80 years was already established [17]. In general, the observed mean testicular volume between 20 and 30 years of age is 16.5 cm^3 and

the maximal testis volume is observed at 25 years of age, after which there is a slight but significant decline to a mean volume of 14 cm^3 between 80 and 90 years of age [18]. Compared to the age group 18-40 years, men aged over 75 years have a 31% smaller mean testicular volume. This variation in the testes volume is related with higher average serum levels of gonadotropins and lower serum free testosterone [1]. Age-related increment in gonadotropins is mostly due to primary testicular failure. The aforementioned decrease in Leydig cells number is reflected in the observation that older men display a diminished secretory capacity compared with younger men when testes are stimulated with human chorionic gonadotropin or via pulsatile GnRH [19]. In fact, the testicular volume decrease in older men showed strong direct correlation with serum levels of inhibin B and inhibin B/FSH ratio, and indirect correlation with FSH [1] and on LH levels [1, 6]. Serum gonadotropins levels increase due to feedback mechanisms that cause increased secretion of gonadotropins [20, 21]. In fact, testicular androgen metabolism increases between the 11^{th} and 40^{th} year of age and progressively decreases between the age of 40 and 90 [8, 9].

ENDOCRINE DYSFUNCTION IN THE AGING MALE

Aging is related to important alterations in the control of the hormonal axis that regulate male fertility [22]. The hypothalamic-pituitary-testicular (HPT) axis is the key regulator of the male reproductive function and controls the synthesis of sex hormones and the formation and maturation of male germ cells. As referred in previous chapters, it is constituted by three major elements, including the hypothalamus, anterior pituitary, and the testes. In this axis, gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus reaches the anterior pituitary gland via the hypophyseal portal system and stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), by the gonadotropic cells, into the bloodstream. In broad terms, LH induces the production of testosterone by the Leydig cells, while FSH stimulates Sertoli cells to secrete androgen-binding protein (ABP) and inhibin and plays a vital role in spermatogenesis [23].

Independent from primary, events that affect the hypothalamic-pituitary axis have a major outcome on the reproductive tract and the reproductive potential of the aging male. Aging causes a decrease in the secretion of GnRH, which in turn leads to smaller LH pulses. In older men, there is an impairment on the response of gonadotropic cells to exogenous GnRH, a decrease of LH pulse size and/or reduction of LH bioactivity [23, 24]. Moreover, it has been reported that serum FSH levels rise more pronouncedly in men after 40 years of age, reflecting a progressive tenacity of the gonadotropic support to the germinal epithelium [25 - 27]. This increase in FSH is concurrent with age-dependent alterations in

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Marco G. Alves

Marco G. Alves was conferred a PhD in biochemistry, branch of bioenergetics at the University of Coimbra, Portugal. After his PhD, he started to work on Andrology and endocrinology with a biochemical approach. His main lines of research are focused on reproductive biology, obesity, diabetes, metabolism, metabolic modulation and cellular metabolic profiles. He is particularly focused on the understanding of the molecular and biochemical mechanisms that control male fertility. He has more than 100 publications in the last years (2009-2017) in leading peer-review journals including some invited reviews. These publications are mostly focused on biochemistry of andrology. He has been awarded with several fellowships and prizes and is the Principal investigator and team member of several ended and ongoing funded projects. Marco G. Alves is an active member of several national and international societies. He has served as a peer-reviewer for more than 70 indexed journals and has been invited as an evaluator/expert for several national and international agencies.

Pedro Fontes Oliveira

Pedro F. Oliveira was conferred his degree in biochemistry, in 1996, at the Faculty of Sciences of the University of Porto. In the same year, he began his scientific activities as a young researcher at the Institute for Biomedical Sciences Abel Salazar (ICBAS) from the University of Porto. In 2004, he was conferred a PhD degree in biomedical sciences at ICBAS - University of Porto, during which he was awarded various fellowships and prizes. In 2005, as a Post-Doctoral researcher, he shifted his research area and started working on the ionic and metabolic regulation of the male reproductive tract. His work was pioneer in the characterization of several membrane transporters that control the ionic regulation in sertoli cells, as well as their metabolic features. In this context, in 2009, he was hired by the University of Beira Interior to develop endocrinology and reproduction group. During the period of 2009-2015, he established a strong research team with high international recognition. Translational biomedical research has been the focus of his team in recent years. For that reason, he was invited to join the medical schools of the University of Porto as a principal researcher and professor. He currently co-leads a research team focused on reproductive biology with several postdoctoral researchers, PhD and MSc students, who contributed to the more than 100 publications in the international and national peer-reviewed journals, books and book chapters. Pedro F. Oliveira is an active member of several national and international societies, and has been invited as an expert for the European Commission and other international research agencies.