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Male Reproductive Function and Semen

Themes and Trends in Physiology, Biochemistry and Investigative Andrology

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Preface

To present a coherent and meaningful survey of scientific research endeavour in an area that has expanded as fast as physiology and biochemistry of reproduction in the male is no mean task these days. No less prodigious than the growth of knowledge of male reproductive function has been the rate at which the outpouring of publications on this subject has continued since the appearance of 'The Biochemistry of Semen and of the Male Reproductive Tract' in 1964. Since cyclopaedic treatment of this vast literature did not appeal to us, we have made no attempt either to rehash the material contained in that book or to enlarge the bibliography beyond the nearly 3500 references included in the present treatise. At the same time, whilst writing, we felt strongly that to advance, it is necessary to understand the past, and for this reason we have not hesitated to refer (especially in the introductory chapter) to a number of those fundamental early discoveries in which today's knowledge is deeply and firmly rooted. As regards progress since 1964, rather than attempt to tackle the research area as a whole, we have preferred to deal foremost with current trends and concepts, emphasizing the importance of topics that were the object of intensive study around the time of our writing, and in particular, we have tried to provide examples illustrating how the progress in male reproductive physiology and biochemistry is contributing, on a steadily mounting scale, to the development of investigative and diagnostic andrology. We have also assumed, correctly we hope, that a goodly proportion of our readers will take a direct interest in selected parts only, and not the whole of the book, and hence we did not hesitate to permit ourselves a certain amount of deliberate repetition and overlap between some of the chapters.

Because the comparative approach applied to biology of male reproduction proved to be very fruitful in the interpretation of various types of spermatogenic failure, hormonal disarray and malfunction of male accessory organs, and in view of the experience which we ourselves have gained over some 30 years, whilst studying and teaching comparative aspects of reproductive physiology and biochemistry at the University of Cambridge, we have described a certain number of findings made in this area of human and animal investigations. Reference has therefore, been made, sometimes rather briefly, to animal species as far apart as farm animals, laboratory rodents, insectivores, marsupials, birds, insects, echinoderms, and especially one large cephalopod mollusc, the North-Pacific Giant Octopus of the Puget Sound. This creature's impressively long spermatophores have been the study object of happy collaboration with Drs. Arthur Martin and John Thiersch at the University of Washington, Seattle, for several memorable years. We hope that by choosing the comparative approach we shall have succeeded in extending the readership of the present book, so as to attract not only our scientific colleagues and clinicians, but also environmentalists, sociologists, historians of medicine, and perhaps even those members of the public who evince nowadays a keen interest in matters relating to male fertility and sterility, often in conjunction with problems such as industrial health, population growth and adolescent sexual development.

In a large measure, much that has been achieved lately is due to highly specialized new techniques and refinement of older methods, and this prompted us to give in two chapters (II and III) certain general methodological guidelines for the inspection of male reproductive organs, and the collection, quality appraisal and preservation of human and animal semen.

Four chapters (IV-VII) are devoted, respectively, to exocrine and endocrine testicular function leading to the formation of testicular semen; the role of the epididymis in sperm maturation and storage, and the formation of epididymal semen; the physiology of the vas deferens and some of the hazards of vasectomy; and the secretory processes in the prostate, seminal vesicle, Cowper's gland and other male accessory organs, leading to the formation of seminal plasma.

A notable trend in modern research on semen has been the shift of emphasis from studies on intact spermatozoa to attempts at defining the structure, chemical make-up and function of separated sperm organelles, such as the plasma membrane, acrosome, nucleus, mitochondrial sheath and the axoneme of the flagellum. This is reflected in the treatment which we have accorded in two chapters (VIII and IX) to the biochemistry of spermatozoa and the seminal plasma, in which we have also dealt with the application of chemical methods of semen analysis to andrological problems. Finally, in the last chapter (X) we have outlined the adverse pharmacological effects upon male reproductive function of certain chemosterilants, anti-androgens, and antispermatogenic and spermicidal substances.

The task of writing the book has occupied a great deal of our working time for about 4 years. The initial work was undertaken with the support of the Agricultural Research Council and the Leverhulme Trust in Great Britain; during the last 3 years its continuation has depended entirely on the magnanimous support of the National Institutes of Health and the Lalor Foundation, in the United States of America. We owe an immense debt of gratitude to our many American friends and colleagues, but most of all to Drs. C. Lalor Burdick, Kevin Catt, Philip Corfman, Maria Dufau and Richard Sherins, who together with their families, have made our stay in Bethesda a memory that we shall always treasure. We thank Dr. Roy Jones for his valuable cooperation and Mrs. Carmen Frankl for the preparation of figures; to Mrs. Jennifer Constable goes out our joint gratitude for the excellent work done in the typing and assembling of the manuscript, and especially of the extensive bibliography. We also wish to put on record the assistance received from the staff of Springer-Verlag, and to acknowledge with thanks the permission granted by various individuals and publishing houses to reproduce material from certain journals, namely our colleagues Drs. Winston Anderson (Washington DC), David Brooks (Adelaide, Australia), Hector Dott and David Cran (Cambridge, England) and Don Fawcett (Boston, Mass), and the following publishers: The Royal Society of London, Biochemical Journal, Fertility and Sterility, Journal de Microscopie, Journal of Endocrinology, Journal of Reproduction and Fertility, S. Karger Publishing House, Messrs Methuen & Co. (Associated Book Publishers) and Nature Macmillan Journals.

November 15, 1980

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CHAPTER I

Male Reproductive Function and the Composition of Semen: General Considerations

> "Now, when a man is unable to beget children by his wife, although his virility is unimpaired, he is said in common parlance to have a cold nature. To my mind, however, it would be more apt to say that no living animalcules will be found in the seed of such a man, or that, should any living animalcules be found in it, they are too weakly to survive long enough in the womb."
>
> Antoni van Leeuwenhoek 1685

The modern era of the physiology of reproduction in the male is commonly, and rightly so, accepted as having been ushered in by Leeuwenhoek's sensational letter to the Royal Society, dated November 1677, reporting the first-ever demonstration in semen of motile spermatozoa. From another of his famous communications, cited above and submitted 8 years later, it is evident that Leeuwenhoek intuitively associated the existence of spermatozoa with male fertilizing ability, by recognizing that even though a man may be keenly interested in the opposite sex, this alone is not enough to guarantee the birth of offspring. He anticipated, moreover, yet another basic concept of male reproductive biology, by proclaiming that for the act of procreation to be fulfilled, the spermatozoa, as well as being motile, must also be sufficiently energetic to survive in the female tract for a certain period, presumably to attain their full potential. The precise duration of that critical time-span he was, of course, unable to back up with experimental evidence.

A long time was to pass before spermatogenesis was discovered in all its intricacy and became generally recognized as the prerequisite for the formation of spermatozoa; how this came about is fully and lucidly recapitulated by Roosen-Runge (1977). It took a great deal more patient scientific effort before it became clear that the differentiation of the fetal gonad into a testis depends on several determinants, of which the H-Y antigen is a major one. Although it was clear by 1970 that the undifferentiated gonad of a mammalian fetus with an XX sex chromosomal complement is destined to become an ovary, and the XY complement is necessary if the gonad is to develop into a testis, it was still uncertain at that time how the Y chromosome directs the fetal gonad to become a testis. During the succeeding years convincing evidence accumulated to support the concept that the male sex chromosome acts on testicular organogenesis through a Y-linked gene locus which mediates the production of the H-Y antigen (Ohno 1979; Wachtel et al. 1975; Wachtel 1980). Even now the idea that the H-Y antigen is the principal evocator of testicular function still has to account for clinical observations on patients with various problems of sexual differentiation in whom the expression or nonexpression of the H-Y antigen does not seem to tally with the presence or absence of testicular development (Nagai et al. 1980; Winters et al. 1979d). Another important sex determinant is the Müllerian tract-inhibiting factor ('anti-müllerian hormone') of Jost (1947), which enables the genital tract to acquire male characteristics (Josso et al. 1980; Jost et al. 1977). Only within the recent past has due recognition been accorded to certain other factors controlling testicular function, such as the vascular and nervous supply network of the gonad and scrotum, the blood-testis barrier, testicular thermoregulation, and the mechanism of fluid secretion and entry of substances into the seminiferous tubules (Fawcett 1979; Setchell 1978, 1980; Setchell et al. 1980).

Another area in which progress has been spectacular concerns the endocrine function of the testis and the ways in which the gametogenic and hormonal activity of the male gonad proceed side by side in a manner that ensures a coordinated formation of both parts of semen, that is, the spermatozoa and the seminal plasma. Our early knowledge concerning the influence the testis exerts on the accessory organs, and thereby on the formation of seminal plasma, stems from John Hunter's famous experiments (1786) on the postcastrate regression of the prostate gland and seminal vesicles. Over a century later conclusive evidence was provided that the involutional changes can be reversed by parenterally administered male sex hormone; the latter was applied at first as a rather crude testicular extract and subsequently in its pure form, following the successful chemical isolation of testosterone (David et al. 1935). It was largely as a direct result of this achievement that it became possible at last to quantitate the action of testosterone upon the growth and secretory activity of the male accessory organs of reproduction and hence on the production of seminal plasma.

Technical modifications and improvements in quantitative analysis of testosterone soon followed, enabling better insight into the mechanism whereby testosterone promotes the growth and secretory activity of male accessory organs. Another important development has been the discovery of other major testosterone-dependent functions, such as promotion of the male phenotype during embryogenesis, initiation and maintenance of spermatogenesis in the pubescent male, and control of gonadotrophin secretion by the hypothalamus-pituitary system, among others. A direct outcome of these advances in male reproductive physiology was the formulation of new concepts concerning the pathogenesis of certain endocrine disorders, such as the syndrome of androgen resistance in male pseudohermaphrodites and the demonstration that male infertility is frequently the result of a combination of hitherto unrecognized genetic, phenotypic, endocrine and enzymatic defects. Reviews giving comprehensive coverage of this rapidly widening research area are available (Griffin and Wilson 1980; Steinberger and Steinberger 1980).

1. Functional Dualism of the Testis

The beginnings: conceptual formulation of testicular function

Brown-Séquard (1889) was the first to formulate the idea of functional duality of the testis, when he wrote:

"Je considère les glandes spermatiques, ainsi que les autres principales glandes (foi, reins, etc.), comme douées, en outre de leur puissance sécrétoire, d'une influence spéciale sur le sang, à la manière des glandes sans sécrétion extérieure, comme la rate, la thyroïde, etc. Conduit par cette idée, j'ai déjà fait des expériences avec le sang revenant des testicules."

In these sentences Brown-Séquard (then 72 years old) was alluding to experiments in which he had injected himself with blood and juices pressed out of animal testicles, to overcome a feeling of physical and mental fatigue. Leaving aside judgement on the efficacy of such treatment on lassitude, its more important impact was that the concept of testicular dual function was taken up by others, and in particular Regaud (1899), who suggested, tentatively at first, that the interstitial or Leydig cells act primarily as a source of nutrients for the seminiferous epithelium, but in addition they are also representative agents "de cette sécrétion interne du testicule si nettement mise en évidence par Brown-Séquard." A subsequent, more precise formulation (Regaud and Policard 1901) of the dual role of the testis was expressed thus:

"Nous croyons pouvoir conclure que la fonction sécrétoire des cellules interstitielles s'établit bien avant la fonction spermatogénétique (testicule impubère), et qu'elle persiste lors même que la fonction spermatogénétique ne s'est jamais établie (testicule ectopique).
Il y a donc une indépendance relative, anatomique et fontionelle, entre les cellules interstitielles et les tubes séminifères; et il est permis de rattacher à une sécrétion interne particulière, depuis longtemps soupçonnée, les phénomènes sécrétoires dont les cellules interstitielles sont le siège."

Soon thereafter Bouin and Ancel (1903) quite boldly expressed their conviction that the interstitial tissue is endowed with an internal secretory activity:

"La glande interstitielle nous apparaît donc comme un organ qui élabore probablement des matériaux pour la glande séminale, et qui, par sa sécrétion interne, tient sous sa dépendance l'ardeur génitale et le déterminisme des caractères sexuels secondaires."

The notion that male genital ardour depends in the first place on the testes dates back a long time, and has had among its scientific proponents keen observers such as John Hunter (1786, 1792) and Berthold (1849).

Exocrine and endocrine activity

The groundwork on testicular function gradually clarified the dual mechanism whereby the testes control semen production. Testicular exocrine activity associated with the seminiferous tubules, tubuli recti, rete testis, and efferent ducts was decisively proved to result in the formation of testicular semen (a suspension of spermatozoa in testicular plasma) which is ultimately released into the epididymis. On the other hand, testicular endocrine activity residing in the interstitial tissue was shown to be responsible for the synthesis of several steroid hormones, first and foremost testosterone, which is carried from the testes to the target tissues mainly in the bloodstream but partly also via the lymphatic system and, to a minor extent, the testicular semen. On further study it also became apparent that much of the testosterone released by the testes is carried to the target organs not in the free form but bound to a special type of transport protein.

Testosterone, as we now know, influences a wide range of biological functions, and hardly any organ in the male body can be said to be unaffected by it. In the testis itself it plays a pivotal role in the initiation and maintenance of spermatogenesis. In the pituitary gland, testosterone coordinates the process of gonadotrophin formation and release. Its anabolic effects manifest themselves in the response of various organs, including musculature (maintenance of a positive nitrogen balance), kidney, liver, skin and hair follicles (Chap. IV). Each end-organ reacts to the androgenic stimulus in a specific way. The reason for this behaviour is that upon arrival at the target cells the androgen becomes bound to organ-specific high-affinity protein receptors, which facilitate interaction with the chromatin of the cells and thus enable the hormone to induce its specific action in the target organ. In relation to semen formation, the most outstanding feature is the stimulatory influence which testosterone exerts on the secretory function of the male accessory glands. The end-result of the modulating influence of androgen receptors in the various accessory organs is the stimulation by androgens of cellular growth, metabolism and secretory activity, a process which in its entirety leads to the appearance of organ-specific secretory products in the seminal plasma.

2. Male Reproductive Tract: Main Characteristics

Representative accessory organs in mammals

On emerging from the testis, testicular semen in the form of a thin suspension of as yet immotile and infertile spermatozoa enters the epididymis, and having completed their passage and maturation in the epididymal duct, mammalian spermatozoa pass into the vas deferens. In the course of the ejaculatory process they encounter secretory fluids produced by several accessory organs of reproduction located along the male tract. At the time of semen emission these secretions blend into seminal plasma, which is the native element for the spermatozoa at this stage. The accessory

Table 1. Species differences in volume and sperm density of ejaculated semen

Species	Volume of single ejaculate: Normal variations (ml)	Volume of single ejaculate: Average value (ml)	Sperm density in semen: Normal variations (sperm/μl)	Sperm density in semen: Average value (sperm/μl)
Ass	10-80	40	100 000- 600 000	300 000
Bat		0.05	5 000 000- 8 000 000	6 000 000
Blue Fox	1.5-2.5	2	100 000- 230 000	150 000
Boar	150-500	250	25 000- 300 000	100 000
Buffalo	0.5-4.5	2.5	200 000- 800 000	600 000
Bull	2-10	4	300 000- 2 000 000	1 000 000
Camel	4-12	8	100 000- 700 000	400 000
Cat	0.02-0.12	0.03	100 000- 2 600 000	1 700 000
Cock	0.2-1.5	0.8	50 000- 6 000 000	3 500 000
Dog	2-15	9	60 000- 300 000	300 000
Drake	0.34-0.45	0.4	7 000 000-11 000 000	9 500 000
Fox	0.2-4	1.5	30 000- 250 000	70 000
Goat	0.2-2.5	1	1 000 000- 5 000 000	3 000 000
Goose	0.4-1.3		400 000- 1 500 000	
Guinea Pig	0.4-0.8		5 000- 17 000	10 000
Man	2-6	3	50 000- 150 000	80 000
Pigeon	0.002-0.04	0.016	800 000- 3 800 000	2 000 000
Rabbit	0.4-6	1	50 000- 350 000	150 000
Ram	0.7-2	1	2 000 000- 5 000 000	3 000 000
Red Deer	2-20	4	100 000- 1 300 000	200 000
Stallion	30-300	70	30 000- 800 000	120 000
Turkey	0.2-0.8	0.3		7 000 000