DOI: 10.2436/20.1501.02.4

Endocrinologia molecular
(Jaume Reventós, ed.)

Treballs de la SCB. Vol. 56 (2005) 33-46

PARACRINE REGULATION OF SPERMATOGENESIS: THE VIRTUE OF DIALOGUE

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RESUM

Molt aviat en la història de la humanitat, la gent ja sabia que hi havia una relació entre l'existència dels testicles i algunes funcions importants. L'accessibilitat d'aquest òrgan tenia, indubtablement, quelcom a veure amb això. Des del període neolític, la castració ha estat utilitzada per a domesticar o engreixar alguns animals i per a castigar els homes que cometien adulteri. La idea de relacionar el testicle amb una funció endocrina i l'experimentació en aquesta àrea tingué lloc el 1849, amb els treballs de Berthold, que varen consistir a trasplantar els testicles d'un pollet normal a un pollet castrat, i va mostrar a continuació que la cresta i els caràcters sexuals secundaris del darrer animal es mantenien després d'aquest transplantament. El 1889, a París, els experiments de Brown-Séquard, que consistien en l'autoinjecció d'extractes testiculars, varen ser igualment crucials per a establir el concepte de *hormona*, a pesar de la ridiculització a la qual sovint, fins encara avui en dia, són sotmesos. Émile Zola, per escriure el seu llibre titulat *Le docteur Pascal*, de les sèries Rougon-Macquart, es va inspirar en aquest treball. En aquest llibre, el doctor s'injecta a si mateix extractes de testicle de conill i proclama el recobrament de la vigoria, incloent-hi la sexual. Nogensmenys, la paradoxa referent al testicle persisteix, mentre que se'n parla molt sense saber realment encara avui com funciona.

Paraules clau: espermatogènesi, cèl·lula de Sertoli, cèl·lula germinal, paracrinologia.

SUMMARY

Very early in the history of humanity, people discovered that there was a relationship between the existence of testicles and certain important functions. Undoubtedly, the accessibility of this organ had something to do with this. Since the Neolithic Period, castration has been used as a means of domesticating and fattening certain animals and of punishing men for adultery. The idea of an endocrinological function and of experimentation in this area first arose in 1849 with the work of Berthold, who transplanted testes from a normal cockerel into a castrated cockerel and showed that the cockscomb and secondary sexual characteristics of the animal

were maintained after this transplantation. In 1889, in Paris, the experiments of Brown-Séquard, involving the self-injection of testicular extracts, were also crucial in establishing the concept of a hormone, despite the derision with which such experiments would be regarded today. Emile Zola's book in the Rougon-Macquart series, *Le docteur Pascal*, was inspired by that study. In the book, the doctor injected himself with extracts of rabbit testis and claimed to have recovered all his vitality, including his sexual vigor. However, the paradox concerning the testis remains, as we regularly continue to discuss it without really understanding how it functions.

Keywords: testis, spermatogenesis, Sertoli cell, germ cells, paracrinology.

MALE STERILITY, AN ABNORMALITY OF POORLY UNDERSTOOD ORIGIN

The large degree of ignorance on how the testis functions has diverse consequences, some of which may be damaging, since the cause of male sterility is frequently unknown. In half of all cases, we cannot establish the origin of this sterility or sub-fertility. This situation has resulted in a lack of available treatments and has presented major difficulties in the development of male contraception. Another consequence of this ignorance is that we are currently reduced to more or less futile speculation about the reasons for the deterioration in sperm quality reported at various sites around the world and in the Parisian region, in particular (Jégou *et al.*, 1999). Furthermore, although everyone is aware of the serious problems posed by sexually transmitted diseases at the levels of the individual, the society and the planet, we still know nothing about how the viruses gets into the testes.

There are many possible reasons for this extremely large gulf between knowledge about the testis and knowledge about other physiopathological situations. There are certainly cultural blocks. For instance: why are we more interested in female fertility than in male fertility? And, why does social pressure more often lead to the woman being identified as the one responsible for infertility in the absence of formal investigations?

Considerable progress in the control of fertility in other mammals has been made in the zootechnical domain, where initial advances have been made in work with female domesticated animals. These findings have been transferred to clinical practice and have been applied to women, though obviously, they could not be applied to men. Another aspect that may account for the lack of progress in the field of male fertility is the complexity of the testis. It is much easier to control ovulation in women than to control the daily production of 100 to 200 million spermatozoa in men.

AN INTIMATE "CONVERSATION" BETWEEN CELLS

We will now take a journey to the centre of the testis to unravel a secret: the intimacy of the "conversation" between cells in this organ.

On a testis section (see figure 1) seen under a scanning electron microscope, we can easily recognize the seminiferous tubules, in the center of which the spermatozoa are released. We can also see the material between these tubules, known as the "interstitial tissue," that contains blood vessels and Leydig cells, which produce androgens, and macrophages. The testis can certainly be divided into two compartments—the seminiferous tubules and the interstitial tissue—but these compartments are not independent. In particular, we cannot say, as was once asserted in works on this subject, that the seminiferous tubules are responsible for testicular exocrine function and that the interstitial tissue is responsible for endocrine function. Indeed, it

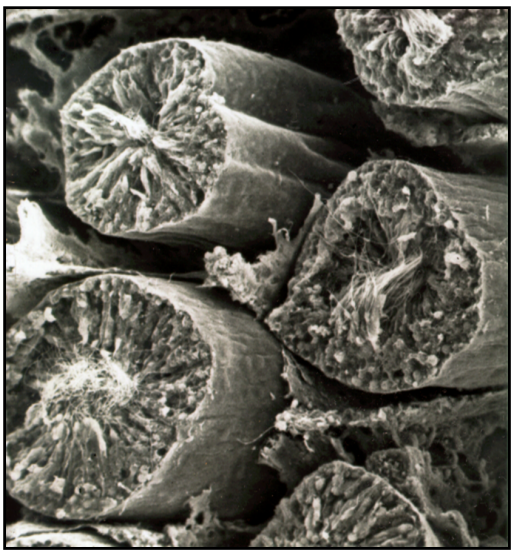

FIGURE 1. Testicular architecture in the rat.

has been clear for at least fifteen years that the Sertoli cells in the seminiferous tubules produce hormones such as inhibin and activin. The seminiferous tubules, therefore, also possess extremely important endocrine functions.

The two key elements are spermatozoa and testosterone. There is certainly no need to explain why spermatozoa are so important, and without testosterone there would be no spermatozoa. Testosterone is also responsible for the maintenance of secondary sexual characteristics, control over the skeletal muscle mass and sexual behavior in many species.

NUMEROUS COMMUNICATION FACTORS

The following is a brief explanation of the role of hormonal control in testicular function. The pituitary gland produces two hormones: luteinising hormone (LH) and follicle-stimulating hormone (FSH). LH induces the production of testosterone and androgens in Leydig cells, whereas FSH acts upon Sertoli cells and regulates certain functions essential to spermatogenesis. In return, testosterone exerts a negative control over the hypothalamus and pituitary gland. The Sertoli cells produce certain hormones, including inhibin, which have a peripheral effect. However, we do not know how the 500 to 1000 million testicular cells co-ordinate their activities to produce 100 to 200 million spermatozoa every day in men. We understand the broad framework of sperm production regulation, but we still know little about the details of the fine regulation of this process within the organ itself.

Our journey to the center of this organ moves us towards the infinitely small, in terms of scale and in terms of the distance between the cells producing a factor and those responding to it. This corresponds to the transition from endocrinology to paracrinology that was described by José Saez and his collaborators (Lejeune *et al.*, 1992; see figure 4).

Endocrinology deals with the concept of a "hormone" in the traditional sense of the word. This involves the production by an organ of a molecule, which is then transported elsewhere in the bloodstream and, in most cases, acts from a distance upon another organ.

In paracrinology, the distances involved are much smaller, with one type of cell in a given organ producing a factor that acts upon a neighboring cell of a different type. In most cases, this recipient cell is also capable of regulating intercellular dialogue.

From paracrinology, we can move on to notions of an even greater intimacy between cells: the notions of autocrinology and intracrinology. Autocrinology corresponds to the communication between two cells of the same type, such as two Leydig cells, for example. These two cells produce a factor (e.g., testosterone), which acts upon cells of the same type. In intracrinology, the distance is even shorter because the factor is not even secreted; rather, it acts upon the cell that produced it (see figure 2). For the sake of simplic-

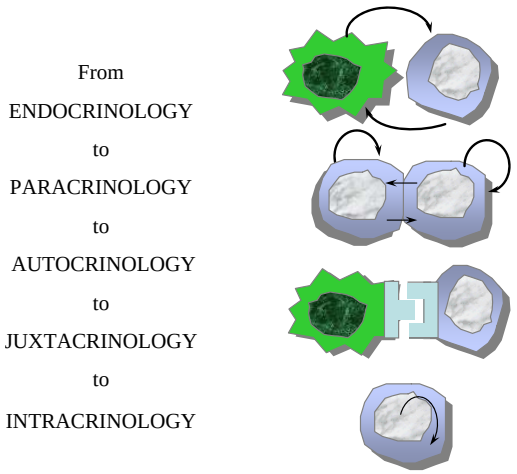

FIGURE 2. From endocrinology to intracrinology.

FIGURE 3. A "double image" of Salvador Dali.

ity, we will use the single term "paracrinology" to refer to paracrinology, autocrinology and intracrinology.

Within the scientific community, there are those who would contest the notion that the intimate regulation within the organ itself has any real importance when compared to genetic programming, the veritable "biological clock" of cells of different types. To those who express skepticism about the crucial importance of cell to cell interaction in the control of testicular function, I would like to answer them with a metaphor, as represented in a painting by S. Dalí, in which the observer can discern the image of a face looking forwards and also see a dog looking to the right (see figure 3). Those who cannot see the point of paracrine regulation are like those who can only see the face in S. Dalí's painting, without being able to see the image of the dog. In 1938, S. Dalí said that seeing the *double image* (the dog and the face) was going beyond simple appearances: once the observer sees it, it provides the key to a third image, and then a fourth, and so on. He said that the only intellectual limitation is the "paranoid" limitation, otherwise known as imagination. It would seem that for a scientist, this metaphor has great significance.

Testosterone was the first paracrine factor to be identified. Between the seminiferous tubules and the interstitial tissue, which contains the Leydig cells, there is a space from which it is possible to sample lymph and blood. This ability allowed the identification of testosterone as the first paracrine factor. This distance is not found in the dialogue between the cells composing the seminiferous tubules. Testosterone does not seem to act directly upon germ cells. Instead, it acts upon the peritubular and Sertoli cells, which in turn, emit certain signals that regulate spermatogenetic functions (Jégou *et al.*, 1999; Sharpe, 1994).

Testosterone may act directly on Leydig cells and, of course, it may be exported from the testicle via nearby blood vessels. The seminiferous tubules, in exchange, send a certain number of signals to the interstitial cells. Some of these signals have been previously identified and reviewed in the last fifteen years (Lejeune *et al.*, 1992, 1996; Jégou, 1992, 1993) (see figure 4). Here, we will only deal with inhibin and activin from this list. However, the existence of this list demonstrates the existence of a dialogue between the tubules and the interstitial tissue.

We will now look at the dialogue that takes place within the seminiferous tubules, as this is the central interest of my research team.

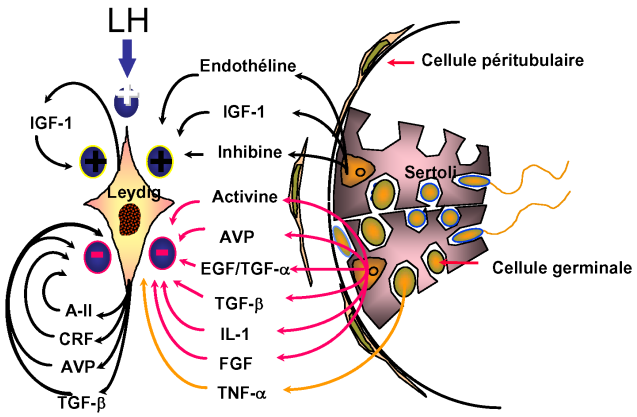

FIGURE 4. Endocrine and paracrine regulation of Leydig cell function.

Let's consider a single tubule (see figure 1). The seminiferous epithelium lining the tubule and defining the limits of the tubule lumen is extremely unusual, and it is unique in its complexity. Don Fawcett, the greatest anatomist of the first half of the 20th century, was among those who introduced the techniques of electron microscopy into cell biology. He said that this epithelium was the most complex to be found anywhere in the body (Fawcett, 1975). It remodels itself continually, displays cell division and differentiation, and is the only site of meiosis in men. There is only one problem in this structure: the distance between the neighboring cells is minimal or even nonexistent. Cell contact is, therefore, maximal ("juxtacrinology"; see figure 2) and as intimate as it can be, to the extent that it was only with the advent of electron microscopy that the debate (which had lasted a whole century) about the existence or absence of separation between Sertoli cells finally came to an end (Jégou *et al.*, 1992).

In 1865, E. Sertoli identified the Sertoli cell as a self-contained entity (Sertoli, 1865). This conclusion was immediately contradicted by others who claimed that the Sertoli cell formed a syncytium with the germ cells. The intercellular boundaries were definitively delimited thanks to electron microscopy, which showed Sertoli and his supporters to have been correct (Jégou *et al.*, 1992).

The obvious problem is how to study interactions when there is no distance between cells. How can we understand any interactions without disrupting them? Here, we diverge from the views of Claude Bernard, who established the criteria for biological experimentation in his *Introduction à l'étude de la médecine experimentale* in 1865 and for whom it was essential to prove the existence of a phenomenon. When we address a problem, such as the communication between cells within a seminiferous tubule, it is often impossible to determine whether what we observe *in vitro* corresponds to the true situation *in vivo*. Experimentation is becoming increasingly automated, but, paradoxically, the consequence of this is that the experimenter is becoming increasingly important. This is due to the fact that the interpretation of the complex network of cell-cell interactions requires the integration of data obtained from different approaches: *in vivo*, *in vitro*, *in situ*, *ex vivo*, etc.

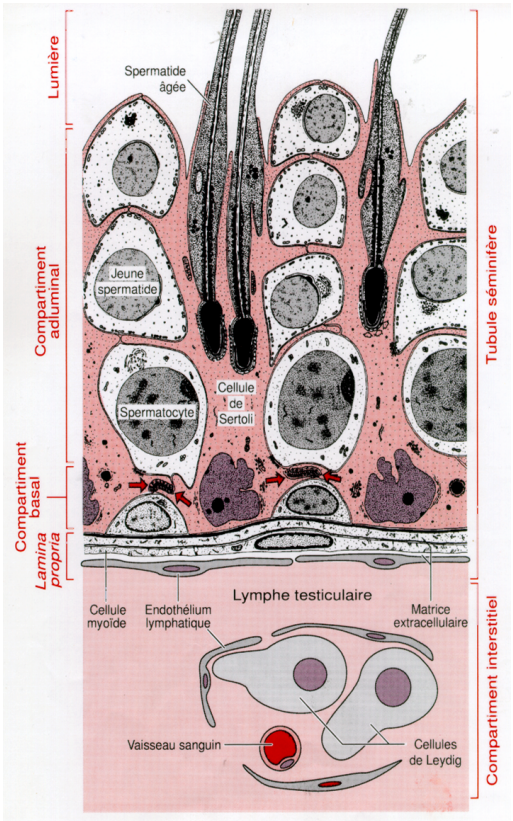

FIGURE 5. Schematic representation of a section of a seminiferous tubule (adapted by Jégou, Med/Sciences. *La cellule de Sertoli, Actualité du Médecine Sciences*. Original figure by Russell *et al.*, 1990).

THE SOCIETY OF TESTICULAR CELLS: PARTICIPANTS IN CLOSELY COORDINATED ACTIONS

Before going any further, certain familiar notions concerning the participants in this small society of gonad cells need to be briefly recalled. They are represented in figure 5.

Andropause (an analogy of menopause and defined as gamete exhaustion) does not really exist, due to the continuous self-renewal of spermatogenetic stem cells in the testis. The spermatogonia differentiate by means of a series of mitoses and then, at a given moment, they generate the primary spermocytes. These cells then enter meiotic prophase, undergoing the two successive divisions of meiosis to generate the haploid cells: the spermatids. Each spermatid is then transformed into a spermatozoon.

Spermatogenesis can be broken down into three steps—the mitotic phase, the meiotic phase, and the metamorphic phase known as spermiogenesis.

Is there a difference between spermatids and spermatozoa? In the recent past, some practitioners of the assisted reproductive technologies have claimed that spermatids and spermatozoa are more or less the same thing, although this has not yet been demonstrated. We disagree, and, given our current lack of knowledge on this point, it seems to me very risky to use spermatids for microinjection into ovocytes in medically assisted procreation. We often warn doctors to pay attention, because the gulf between science and technoscience is widening, and this may generate serious problems in the future (e.g., cloning). As pointed out in the introduction, research into this poorly understood domain can guarantee that the transfer of technology into clinical practice still remains an adventure, in the best sense of the word, without becoming adventurism (Jégou, 1995).

Each of the vertical columns in figure 6 corresponds to an association between cells that may be encountered in testis sections at a particular location in the tubule (Clermont, 1972). As the tubules are wound up within the testis, one transverse section may differ considerably from another. The reason for these associations, which are fixed for a given species, is that the stem cells involved in spermatogenesis do not multiply continually at a given site in the tube. Instead, in the rat they multiply every twelve days. The inter-generation time, therefore, corresponds to exactly twelve days: the interval between two stem cell divisions. Extraordinarily, spermatogenesis is coordinated both transversely and longitudinally within the tubule (associ-

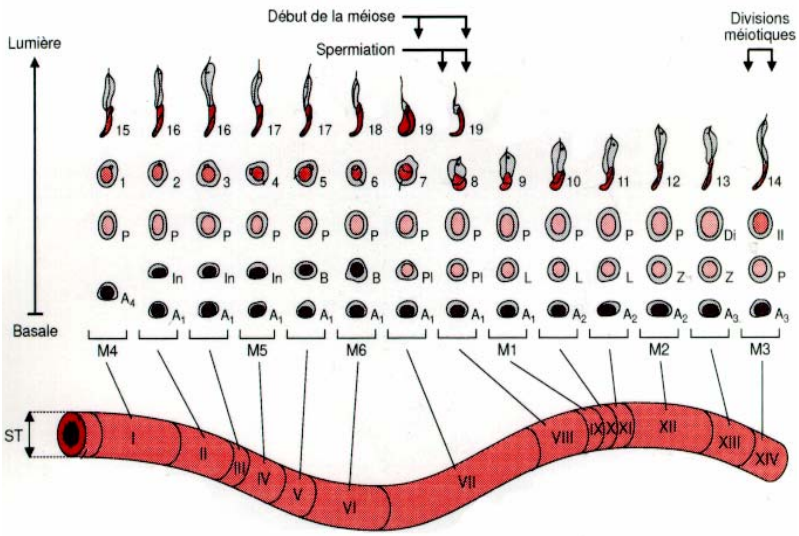

FIGURE 6. "Cycle et onde de l'épithélium séminifère chez le rat" (SHARPE, R. M. (1994). In: KNOBIL, E.; NEIL, J. D. [ed.]. *The Physiology of Reproduction.* New York: Raven Press, p. 1363-1434.)

ation 1 preceding association 2, which itself precedes association 3, and so on).

How can we explain this coordination? We know nothing about the nature of the factors that generate a transverse signal initiating the division of a stem cell in spermatogenesis and then generating a signal that stops the division.

One possible explanation is that all the germ cells—from the spermatogonia to the most mature spermatids—are connected by intracytoplasmic bridges (Russell, 1980), resulting in clonal germinal development. Theoretically, sixteen spermatogonia are capable of generating more than 4,000 mature spermatids (Huckins, 1965). However, in reality there is considerable wastage. Humans have the lowest spermatogenetic yields among mammals because, during the various mitoses and meioses, "quality control" mechanisms eliminate 70% of germ cells by means of apoptosis. This longitudinal interconnection between cells in the tubules thus facilitates the passage of developmental signals, but it also provides instructions for cellular suicide. Here again, it seems that regulation of the spermatogenetic process and of the germinal genome involves more than just the biological clock or cellular genetics.

THE SERTOLI CELL: ORCHESTRATOR OF THE TESTICULAR MICROENVIRONMENT

There is another possible explanation for germinal coordination: along the entire length of the tubule, we know that there are open junctions between Sertoli cells. These cells can, therefore, communicate with each other along the entire length of the tubule. If we place a microelectrode at a certain point in the tubule, we can pass a current through the tubule and rapidly measure the distance covered by this signal, clearly demonstrating that communication does indeed occur between Sertoli cells. There is also another type of coordination based on a dialogue between Sertoli cells and germ cells. Each type of association between cells shown in this diagram imprints

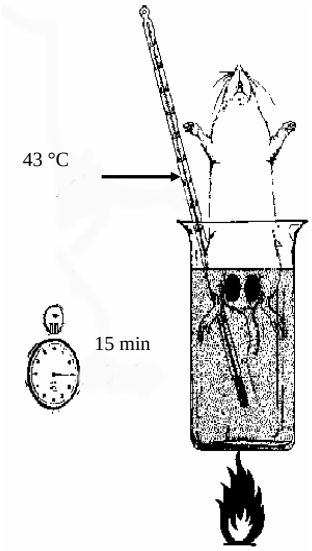

FIGURE 7. Schematic representation of the exposure of rat testes to a moderate temperature.

a certain physical and biological message on the Sertoli cells. The Sertoli cells at a given site in the tubule are not exactly in phase with the neighboring Sertoli cells. They are synchronized, but they do not function in exactly the same way. This enables the Sertoli cells to orchestrate tubule functions. Sertoli cells carry out essential functions, including protecting the testis against infection and harmful molecules via the blood-testis barrier. This barrier is often compared to the blood-brain barrier because, at the start of the last century, the first histologists found that dyes injected into animals penetrated most organs but did not enter the brain or the interior of the seminiferous tubules. This lack of penetration to the brain is accounted for by the existence of junctions between the endothelial cells of the blood vessels. Similar junctions exist between Sertoli cells in the testis, which perform the same function (Ploen and Setchell, 1992). These tight junctions prevent the passage of dyes and other experimental markers. The Sertoli cells create an absolutely unique microenvironment in the organism, allowing meiosis and post-meiotic maturation to occur. From the earthworm to humans, these tight junctions between Sertoli cells isolate the meiotic cells. Thus, meiosis seems to need such security, and the Sertoli cells seem to produce all the substances required for the development of meiotic germ cells.

Sertoli cells are thought to produce several hundred thousand proteins, a minority of which have already been identified. These proteins include proliferation factors, differentiation factors, binding proteins, transport proteins, proteases, protease inhibitors, matrix components, antioxidant agents protecting germ cells, junctional constituents and other membrane components (Jégou, 1992, 1993; Jégou *et al*., 2002; Griswold, 1993).

THE BASIS OF CELLULAR COORDINATION

We have carried out a series of experiments over the years that will now be discussed. Our investigations into the synchronization of the seminiferous epithelium cycle have been carried out over a period of fifteen years and will be briefly summarized here.

What do we mean by the synchronization of the seminiferous epithelium cycle? The transverse and longitudinal synchronization within the tubules has already been mentioned. Researchers have long been intrigued by the fact that in rats, and in many other species, the spermatozoa detach from the seminiferous tubules (in a process known as spermiation) at the moment when the stem cells for spermatogenesis enter the first mitotic division. We need to understand how this system is synchronized so that the youngest cells start to divide at the precise moment that the oldest cells leave.

This story began in Melbourne in the 80s in the laboratory of DM de Kretser and has since continued in Rennes. Developments occurred in New York with W. Bardin and in Finland

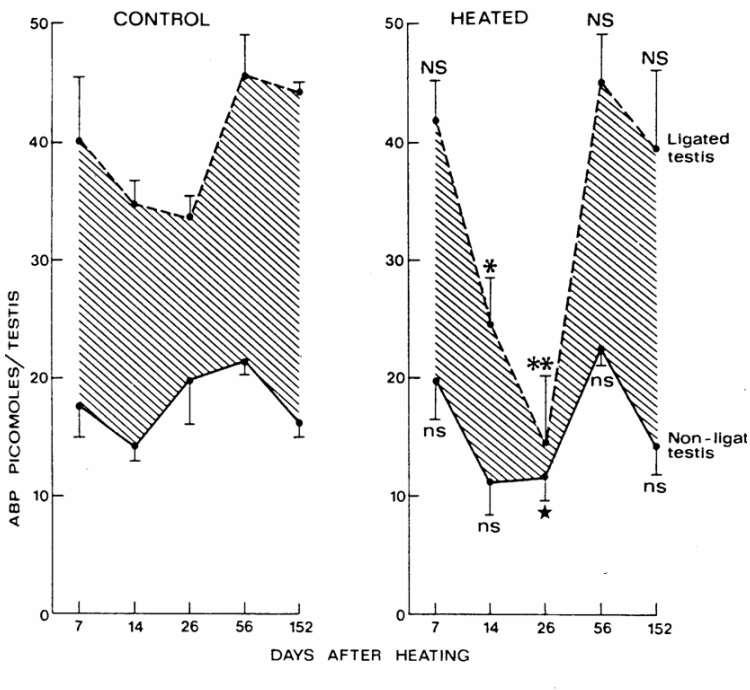

FIGURE 8. Effect of heating on ABP production. NS: Normal production; *: $P < 0.05$; **: $P < 0.001$.

with M. Parvinen and, in its latest developments, in the framework of a collaboration between our laboratory and José Saez in Lyon (Cudicini *et al.*, 1997).

The distances between cells are minute or non-existent in the tubules, and currently, we only have rudimentary tools to study the cellular interactions in the testis. We can isolate testicular cells and culture them, but by doing so, we destroy the delicate interactions between these cells. So, to reconstitute cellular intersections, we need to reconstitute the testis—a real paradox. Therefore, we need to develop different, but highly complementary approaches to tackle this difficult question.

We and other international groups have developed systems for the culture of Sertoli cells *in vitro*. We have also developed another system for the isolation of germ cells. However, as soon as they have been isolated, these cells die. This clearly demonstrates that the influence of Sertoli cells is essential for the development of the germ cell lineage. We have also managed, in a limited manner, to co-culture Sertoli and germ cells. We carry out immunocytochemical staining *in situ*, with markers for tubules. In collaboration with our colleagues led by M. Parvinen in Finland, we have also developed a system for the short-term culture of seminiferous tubules in the presence of tritiated thymidine, with and without various factors thought to affect germ cell proliferation (Dorval-Coiffec *et al.*, 2005; Syed *et al.*, 1995). I will now present an example of a subtractive approach: moderate heating of the testes in anaesthetized rats (see figure 7). The testes were heated to 43 °C for 15 minutes. Previous studies showed that this treatment specifically destroyed the primary spermacytes present (Steinberger and Dixon, 1959). The various

TABLE 1. *Maturation-depletion of the germ cell line following exposure of the rat testis to 43 °C for 15 minutes, from 7 to 56 days post-exposure. N: Normal appearance; ↓: decreased; ↓↓: moderate decrease; ↓↓↓: very important decrease*

Stages of germ cell development	*Control*	*7 days*	*14 days*	*26 days*	*56 days*
Spermatogonia	N	N	N	N	N
Leptotene-zygotene spermatocytes	N	N	N	N	N
Pachytene spermatocytes	N	↓↓	↓↓	N	N
Early spermatids	N	↓↓	↓↓	N	N
Late spermatids	N	↓	↓	↓↓↓	N

categories of germ cells, from spermatogonia to mature spermatids—the oldest and most differentiated cells—are shown in Table 1. The "N" indicates normality in the controls. Seven days after the exposure of the testes to moderate heating, we saw a "window" in the creation of primary spermatocytes and young spermatids, despite the pool of mature spermatids being only slightly reduced in size. Fourteen days later, this window was displaced with the cells repopulating the seminiferous tubules. It should be noted that 26 days after this very short treatment, the only population of germ cells lacking in the seminiferous tubules was that of the mature spermatids. Everything returned to normal within 56 days of the heat treatment. This demonstrated an absence of intrinsic suffering as a result of this treatment.

In parallel, we followed Sertoli cell activity. We did this by determining the levels of a very specific marker for Sertoli cells: androgen-binding protein (ABP; see figure 8). This figure shows the specific production of ABP in normal seminiferous tubules in a normal testis, throughout the duration of the experiment. In animals exposed to heat, there was a break in the curve corresponding to the absence of ABP at day 26. ABP levels then gradually began to recover, reaching normal levels when the germ cell complement of the seminiferous tubules was completely restored. Thus, at 26 days, when the tubule lacked only mature spermatids, Sertoli cell function was completely disrupted. It was easy to draw a conclusion from this manipulation: *in vivo,* the elongated spermatids positively controlled the normal physiology of the testis and the functioning of Sertoli cells, in particular (Jégou, 1991; Jégou *et al*., 1984).

MECHANISMS OF INTERCELLULAR COORDINATION

It is always rewarding for a researcher to be the first to describe a phenomenon. However, the reward is even greater for researchers demonstrating the mechanisms underlying the observed biological effect. This raises questions about the mechanisms by which mature spermatids exert their controlling effects on Sertoli cells. We have formulated a number of hypotheses. Mature spermatids may produce factors secreted into the intercellular space (that is several microns wide), which interact with Sertoli cells. This seems unlikely, because, as already mentioned, the chromatin of a spermatozoon or of a mature spermatid is so compact that transcription and translation rates are likely to be low. The membrane contacts may be involved since the number of contacts with Sertoli cells decreases in mature spermatids. However, this cannot be studied experimentally as we cannot isolate mature spermatids without destroying them. The effects of mature spermatids on Sertoli cells may also be due to physical constraints. During spermiogenesis (the process generating spermatozoa from spermatids), the cytoplasm of the Sertoli cell remains connected to that of the

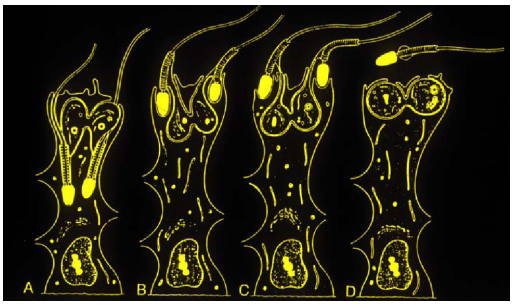

FIGURE 9. Diagrammatic representation of successive stages in sperm release (FAWCETT, D. V. (1973). In: SEGAL, J. S. [*et al.*] [ed.]. *The Regulation of Mammalian Reproduction.* Springfield: III, Charles C. Thomas.)

germ cells throughout the extraordinary deformation of these cells. Physical constraints leading to changes in the cytoskeletal architecture of the Sertoli cells have consequences for transcription activity, because the cytoskeleton is anchored to the nuclear envelope.

Unfortunately, this third hypothesis is also impossible to test experimentally. These experimental impossibilities have led us to focus on a fourth possibility that can be tested. Researchers are imaginative, it is true, but nature often imposes constraints on their choices. It is possible that material is transferred from the spermatids to the Sertoli cells. In figure 9, we can see a spermatid at a stage when it is strongly anchored in the Sertoli cell cytoplasm. The spermatid gradually disengages from the Sertoli cell during the last stages of its maturation. In the final stage, in which it becomes a spermatozoon, 90% of the spermatid's cytoplasm is jettisoned, becoming a "residual body" that is rapidly taken up by phagocytosis and digested by the Sertoli cell. We can isolate both Sertoli cells and these residual bodies. We are also interested in this aspect, and it is with this, at the start of the last century, that morphologists, who pushed concepts so far forward, defined in their work all the concepts on which the current notion of paracrinology is based. Perhaps the most important of these morphologists was a German, Roosen-Runge, who fled anti-Semitic persecution in Hamburg to seek refuge in the United States, where he carried out remarkable scientific work. In 1952, he published an article in which he suggested that since the only extraordinary event in Sertoli cells, following the departure of the spermatozoa, was the phagocytosis of residual bodies, this process might be involved in the entrance of stem cells into the process of spermatogenesis, as these two phenomena occured at the same time (Roosen-Runge, 1952, 1962).

Our favorite experimental model for culture and co-culture is the rat. However, we sometimes use human material, when available. We have used Sertoli cells from rats of different ages, which we cultured with or without peritubular cells (the cells bordering the tubules). We then added (or did not add) residual bodies. We collected the culture media and determined various Sertoli cell markers, including interleukin 1. We have observed that none of the previously discussed Sertoli cell parameters (e.g., ABP and inhibin) were affected by residual bodies. In contrast, major changes were observed in interleukin 1 (IL-1) levels. Why should we be interested in IL-1? A few years ago, a foreign scientist came to our laboratory, Dr. V. Syed, who had just discovered that the testes produced large amounts of IL-1, a cytokine implicated in inflammatory phenomena. At first, we could not fit this discovery into a specific physiological context.We showed that it was the Sertoli cells that produced this cytokine. In the immune system, IL-1 is produced primarily by monocytes or macrophages. (Monocytes are the circulating form of this particular cell type of the haemoatopoietic lineage.) The macrophage displays low levels of IL-1 gene transcription and translation and is said to be quiescent. The function of the macrophage is phagocytosis, leading to the destruction of bacteria and serving as the first line of defense in infections. This analogy between the phagocytic activ-

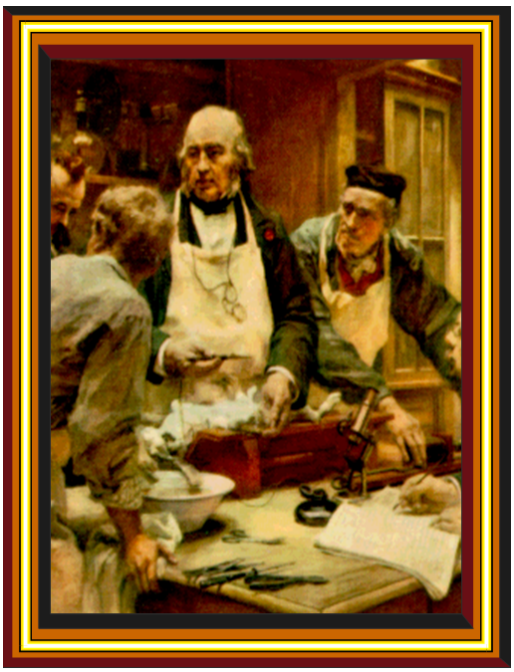

FIGURE 10. "Les théories ne sont que des vérités partielles et provisoires qui nous sont nécessaires, comme des degrés sur lesquels nous nous reposons, pour avancer dans l'investigation." Claude Bernard, *Introduction à l'étude de la médecine expérimentale,* 1865.

ity of macrophages and the phagocytosis of residual bodies by Sertoli cells, as well as the observation of IL-1 production by both Sertoli cells and macrophages led us to investigate whether this molecule was activated in Sertoli cells following *in vitro* exposure to various molecules.

THE ACTIVATORS OF SERTOLI CELLS

First, we investigated classical macrophage activators: silica granules, latex beads, zymosan (yeast extract), and lipopolysaccharides (constituents of the gram-negative bacterial cell wall). All these activators of macrophages proved to be powerful activators of IL-1 production in Sertoli cells (Gérard *et al.*, 1992).

We then wanted to identify the physiological activators of Sertoli cells. We considered the residual bodies, because these bodies are taken up by phagocytosis in Sertoli cells, just as bacteria are taken up by macrophages. Our results showed that the residual bodies did indeed activate IL-1 production in Sertoli cells.

However, all these experiments were carried out *in vitro*, so it is difficult to affirm that they reflect reality. A study that had recently been carried out in Sweden showed that the production of IL-1 *in situ* increased following the release of spermatozoa (Soder *et al.*, 2000). We are, therefore, taking this possibility very seriously.

The next question to be tackled was the role played by this IL-1. A group had just shown that IL-1 stimulated the incorporation of tritiated thymidine into the DNA of mitotic and meiotic germ cells (Soder *et al.*, 2000).

This suggests that when spermatozoa detach from the tubule, phagocytosis of the residual bodies induces the production of IL-1, which is mitogenic and which may trigger the replication of spermatogenic stem cells and the entry into meiosis of spermatocytes.

Nonetheless, we know that in the immune system and other systems, IL-1 often acts by stimulating the production of many other cytokines, including interleukin 6 – IL-6). This inflammatory molecule is also produced by macrophages. We investigated whether Sertoli cells produced IL-6, and we showed that this was the case (Syed *et al.*, 1993).

REALITIES AND COINCIDENCES IN THE ESTABLISHMENT OF A THEORY

If we follow IL-1 production by Sertoli cells in co-culture after the addition of residual bodies and then the production of IL-6, there is a time lag of about ten hours in IL-6 production. This time lag suggests that IL-1 production probably induces IL-6 production in the testis. We checked this coin-

FIGURE 11. "The story I wanted to tell you has ended, but this is another story." Fyodor Dostoevsky.

cidence (according to Claude Bernard, the greatest danger in biology is coincidence!) by adding antibodies that specifically neutralized Il-1 to the medium.

We found that that abolishing IL-1 activity blocked Il-6 production. We have shown *in situ* that little IL-6 is produced in the presence of very small quantities of IL-1. We have also shown that IL-1 production peaks at the time of spermatozoon release, with IL-6 production occurring only once this peak has been reached (Syed *et al*., 1995).

We have also demonstrated that IL-6 inhibits meiotic DNA replication. This suggests that the release of residual bodies during spermiation triggers the production of IL-1, which triggers the production of IL-6 via an autocrine mechanism. According to this model, IL-1 triggers the entry of cells into meiosis or mitosis, with IL-6 having the opposite effect, to limit the action time (Syed *et al*., 1995).

CONCLUSION

This long history of research demonstrates the importance of using different methodological approaches.

However, we should not forget the teachings of Claude Bernard who said that theories are only partial and temporary truths, which enable us to advance in our investigations (see figure 10). In this context, Claude Bernard referred to "philosophical doubt." If this message has been understood, then the last figure in this review in homage to José Saez will make sense. It is inspired by a contemporary of Claude Bernard, Fyodor Dostoevsky, who wrote, "The story I wanted to tell you has ended, but this is another story." (See figure 11.)

Thank you so much, José, for your friendship and the trust you have expressed in our work.

This article deals with a domain in which José Saez was a pioneer.

It was written in homage to this outstanding researcher whose intellectual rigor and scientific advice have been a source of inspiration and support to us.

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