The central role of Sertoli cells in spermatogenesis

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Sertoli cells are the somatic cells of the testis that are essential for testis formation and spermatogenesis. Sertoli cells facilitate the progression of germ cells to spermatozoa via direct contact and by controlling the environment milieu within the seminiferous tubules. The regulation of spermatogenesis by FSH and testosterone occurs by the action of these hormones on the Sertoli cells. While the action of testosterone is necessary for spermatogenesis, the action of FSH minimally serves to promote spermatogenic output by increasing the number of Sertoli cells.

THE CELLULAR ASSOCIATIONS in the mammalian testis have been a subject of discussion since the production of quality microscopes was coupled with inquiring minds. The possiblity of a functional relationship between the 'branched cells' and the production of spermatozoa was first described by Enrico Sertoli when he was a new university graduate at the age of 23.[1]

> ''....I do not think that the branched cells I described are destined to produce spermatozoa, although it is not possible to deny it categorically, but I think that the function of the branched cells is linked to the formation of spermatozoa.''
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> Enrico Sertoli, 1865 from a translation by B.P. Setchell[1]

Since 1865, scientists have been pursuing the question of how the functions of the branched cells are linked to the formation of spermatozoa. Research on the descriptive morphology and endocrine physiology has given rise to an important and fundamental principle, i.e. successful spermatogenesis in higher vertebrates requires the functions of Sertoli cells. The determination of exactly which properties or functions of Sertoli cells are crucial in spermatogenesis has been a major research goal. Sertoli cells can influence testis formation in the embryo and spermatogenesis in the adult by regulating the immediate environment of the developing germ cells. Sertoli cells are arranged such that they can maintain physical contact with germ cells; they can cordon off germ cell populations into environmentally distinct compartments of the seminiferous epithelium; and they can regulate the biochemical surroundings of the germ cells. It is important to note that the regulation of the environment of the seminiferous tubules is likely a joint responsibility of the Sertoli cells and the resident germ cells.

Requirement for Sertoli cells in testis development and spermatogenesis

The relationship between the germinal cells and the Sertoli cells is obligatory in testis development as well as in spermatogenesis (for a review see ref. 2). In the initial formation of the embryonic testis, Sertoli cells sequester the germ cells (gonocytes) inside of newly formed seminiferous tubules and inhibit the proclivity of these cells to enter into meiosis. Unsequestered gonocytes destined for the ovary enter into meiotic prophase shortly after organ formation. This process of testis formation requires the expression of specific genes on the Y-chromosome. Following testis formation Sertoli cells and germ cells undergo rapid proliferation. Puberty generally involves the cessation of mitosis of Sertoli cells, the formation of tight junctions between adjacent Sertoli cells and the progression of germ cells through meiosis and differentiation into spermatozoa.

In addition to the role in testis formation, Sertoli cells provide critical factors necessary for the successful progression of germ cells into spermatozoa. These critical factors may be in the form of physical support, junctional complexes or barriers, or they may be biochemical stimulation in the form of growth

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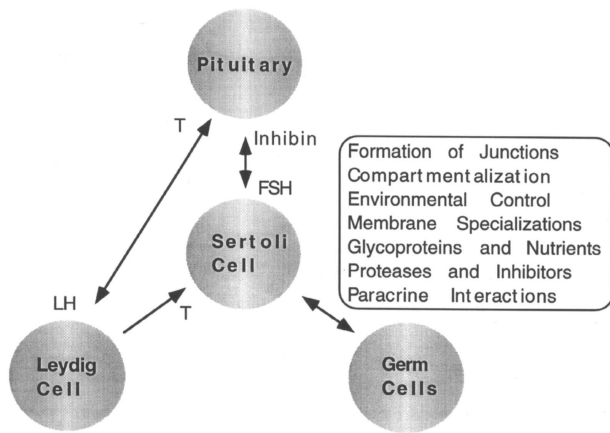
Figure 1. Diagram illustrating the central role of the Sertoli cell in spermatogenesis. The relationships between the Sertoli cells and the pituitary gland, the Leydig cells and the germ cells are shown. In the boxed area is a list of the ways that Sertoli cells interact with the germ cells. The pituitary secretes follicle stimulating hormone (FSH) and luteinizing hormone (LH) and is regulated in turn by feedback of testosterone (T) and inhibin from the Leydig and Sertoli cells.

factors or nutrients (Figure 1). There are a number of excellent reviews dealing with this topic.[3–13] There is abundant experimental evidence that the persistence of the tight junctional complexes and the two-compartment system in the testis is required for sper-

matogenesis. Sertoli cells make specific products that are necessary for germ cell survival and those products combine to form a unique and essential environment in the adluminal compartment. Some of the functions of Sertoli cells may not be absolute requirements for spermatogenesis but may influence the efficiency of the process.

Experimentation over the last decade has revealed that Sertoli cells make and secrete a number of proteins that form the molecular basis for Sertoli–germ cell interactions.[6,7,14] The glycoproteins secreted by the Sertoli cells can be placed in several categories based on their known biochemical properties. The first category includes the transport or bioprotective proteins that are secreted in relative high abundance and include metal ion transport proteins such as transferrin and ceruloplasmin. The second category of secreted proteins includes proteases and protease inhibitors, which allegedly are important in tissue remodeling processes that occur during spermiation and movement of preleptotene spermatocytes into the adluminal compartment. The third category of Sertoli cell secretions includes the glycoproteins that form the basement membrane between the Sertoli cells and the peritubular cells. Finally, the Sertoli cells secrete a class of regulatory glycoproteins that can be made in very low abundance and still carry out their biochemical roles.
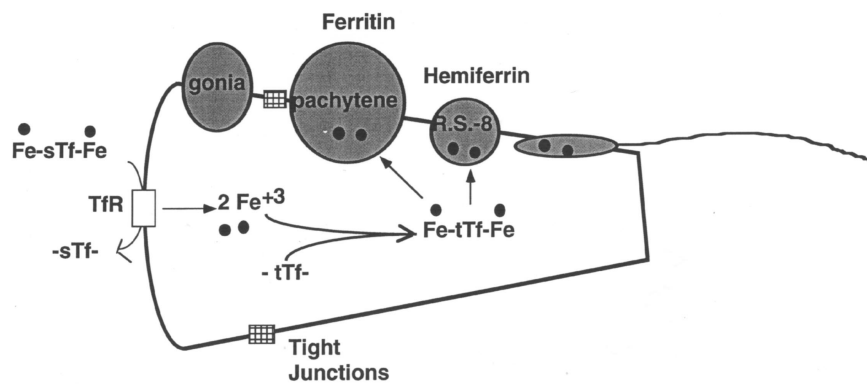

Figure 2. Diagram illustrating the components of the iron transport system in the testis. The need for the transport system arises because of the tight junctions between adjacent Sertoli cells. Ferric ions are delivered to the basal aspect of the Sertoli cells via diferric serum transferrin (Fe-sTf-Fe). The serotransferrin interacts with transferrin receptors (TfR) on the Sertoli cells. Two ferric ions are delivered and internalized per transferrin molecule. The ferric ions are put on newly synthesized testicular transferrin (Fe-tTf-Fe), which is secreted adluminal to the junctions where iron is delivered to the germ cells and stored as ferritin. Round spermatids from the rat contain a truncated transferrin molecule termed hemiferrin, but its role in this process is unknown. (gonia, spermatogonia; pachytene, pachytene spermatocytes; R.S.-8, step 8 round spermatids.)

These glycoproteins function as growth factors or paracrine factors and include products such as Müllerian inhibiting substance (MIS), *c kit* ligand and inhibin. In addition, Sertoli cells may secrete bioactive peptides such as prodynorphin and nutrients or metabolic intermediates.

The requirement for the glycoproteins in the process of spermatogenesis is inferred from the known properties of the proteins. However, *in vivo* evidence for the role of these proteins in spermatogenesis is lacking in most cases. One exception is the role of transferrin, which is an iron transport protein also made in the liver and the brain. Sertoli cells make transferrin as part of a proposed shuttle system that effectively transports iron around the tight junction complexes to the developing germ cells.[15] The proposed model includes basal transferrin receptors on Sertoli cells, movement of iron through the cell, secretion of ferric ions associated with a newly synthesized testicular transferrins and incorporation of iron in the newly synthesized transferrin into ferritin in the developing germ cells (Figure 2). Most aspects of the model have been experimentally verified *in vivo*.

Evidence for the essential nature of Sertoli cells

The notion that Sertoli cells are essential in spermatogenesis originates from three general experimental observations. The first observation is that the condition where testes might contain germ cells but no Sertoli cells has never been observed. In addition, the ability of germ cells in cell culture independent of co-culture with Sertoli cells is very limited. Mammalian spermatogenesis cannot be demonstrated easily in culture, but when claims for success have been published there is a requirement for Sertoli cells (see Brinster and Nagano, this volume). In the amphibian, germinal cell development will occur in culture and some elements of the process are independent of the presence of Sertoli cells.[16] However, the advancement of amphibian spermatocytes through meiosis and some aspects of the maturation of spermatids require the presence of Sertoli cells in the co-culture. A similar reliance on Sertoli cells in the mammalian testis can be postulated.

The second group of experimental observations demonstrating the essential nature of Sertoli cells leads to the conclusion that the function and efficiency of Sertoli cells appear to be limiting to germ cell numbers. While the maximum number of germ cells supported by Sertoli cells varies between species, it is constant within a species. This concept of the limiting nature of Sertoli cells comes from several experiments in which the size of the testis and the spermatogenic output was manipulated by changing the number of Sertoli cells. Orth *et al.* (1988) inhibited the proliferation of Sertoli cells during testicular development and produced a rat with smaller than normal testes.[17] Propylthiouracil was used to inhibit thyroid hormone action during development and this treatment resulted in rats with larger than normal testes.[18] Hemicastration of developing testes in several mammalian species at the appropriate age results in an increased proliferation of Sertoli cells in the remaining testis.[19–24] In each of these animal models the ratio of spermatids to Sertoli cells was relatively constant before and after the treatments. The conclusion is that the number of germ cells appears to be related directly to the number of functional Sertoli cells.

Finally, the third group of experimental observations has led to the conclusion that the endocrine requirement for spermatogenesis in higher vertebrates is a result of the action of reproductive hormones such as FSH and testosterone on Sertoli cells and not on germ cells.

Endocrine regulation of spermatogenesis via Sertoli cells

The primary hormonal controls on spermatogenesis involve the action of FSH and testosterone on Sertoli cells. The actions of FSH on Sertoli cells from prepubertal animals is demonstrated biochemically by increased levels of cAMP, increased protein synthesis and increased estradiol production.[25] However, responses of Sertoli cells from adult mammals to FSH are more difficult to demonstrate and may vary from species to species. In many cases the biochemical activities of FSH in the prepubertal animal appears to be assumed by testosterone in the adult. These findings have led to considerable controversy concerning the role of FSH in male reproduction.[26]

The availability of antibodies to the androgen receptors has led to the demonstration of receptors in Sertoli and peritubular cells (for a review see ref. 27). Evidence for the lack of androgen action directly on germ cells in the testis was obtained originally from genetic studies.[28] In mice, the gene for the androgen receptor is located on the X-chromosome and in

those animals bearing the testicular feminization mutation (*Tfm*) where functional androgen receptors are absent, a testis is formed and germ cell development proceeds only as far as primary spermatocytes. In 1975, Mary Lyons *et al*, by using techniques involving the production of chimeras from *Tfm* and normal mice, generated male mice that sired *Tfm* offspring.[29] The authors of the original article postulated that this outcome was possible only if the germ cells of the chimera were of *Tfm* origin and the somatic cells came from the normal blastula. This experiment provided evidence that the presence of a functional androgen receptor in Sertoli cells but not in germ cells was sufficient for successful spermatogenesis.

Despite the wealth of biological information pertaining to the importance of androgen action in spermatogenesis and detailed molecular information on the androgen receptor, there is little reliable information about the molecular events stimulated by testosterone in Sertoli cells.[30] Results from experiments with cultured Sertoli cells have shown little or no effect of testosterone on the synthesis of specific macromolecules. In addition to regulating the synthesis of key genes, it is possible that testosterone plays a general trophic role in Sertoli cells in the adult testis. The mode of action of testosterone in spermatogenesis remains one of the major enigmas in male reproduction.

The role of FSH in spermatogenesis

As previously stated, the role of FSH in spermatogenesis has been controversial.[26] In the past couple of years some very important experiments have clarified the overall biological role of FSH. First, Handelsman and colleagues administered testosterone alone to gonadotropin releasing hormone (GnRH) deficient mice and showed that this treatment was sufficient for testicular maturation and fertility.[31] GnRH-deficient mutant mice treated with testosterone implants had normal spermatogenesis but reduced testis size and germ cell numbers. In a parallel study, the same group showed that FSH treatment, in addition to testosterone treatment, resulted in GnRH-deficient mice with quantitatively normal spermatogenesis and testes of normal size.[32] The authors treated newborn mouse pups with FSH for 14 days, with or without a single dose of testosterone. At 21 days of age, all of the treated mice received a silastic implant containing testosterone. The exogenous FSH treatment increased testis size by 43%. They concluded from these studies that FSH treatment during the first 2 weeks of life increased Sertoli cell numbers and total sperm production by the mouse testis. Another Australian group showed that prepubertal treatment of normal rats with additional FSH produced larger than normal testes and higher numbers of germ cells.[33] Finally, a FSH β gene deletion by homologous recombination has resulted in a line of mice where the males were fertile but had smaller than normal testes and reduced numbers of germ cells.[34] The FSH null mutants had testes that were about 1/2 normal size and at 6−7 weeks of age the number of epididymal sperm was reduced by 75%. A FSH receptor null mutation (566C → T) has been reported for five male humans.[35] The men showed variable degrees of spermatogenic failure but were not infertile. None of the five men had normal sperm parameters and testicular size was reduced, but two of the men had fathered two children each.

The use of the mice with the null mutations in the GnRH and FSH genes and the discovery of fertile men with inactivating mutations in the FSH receptor gene have shown clearly that FSH is not required for fertility in mice or men. However, these experiments also support the role for FSH in testis size and ultimately in spermatogenic capability. While FSH may not be necessary for fertility, it is very important for species where propagation of an individual depends on competitive numbers of sperm.

Spermatogonial transplants — what have we learned about Sertoli cells?

In 1994 and 1995 Brinster and colleagues described a technique for transplanting germ cells from one mouse to another and from a rat to a mouse by microinjection into the lumen of the seminiferous tubules of recipient animals[36–39] (see Brinster and Nagano, this volume). These were the first successful transplantation experiments in which isolated germ cells from one individual have been shown to seed tubules in another individual and ultimately to continually produce sperm. Spermatogonia in a syngeneic transplant formed cell associations that were characteristic of the normal mouse testis.[40,41] In the initial studies, light and electron microscopy were utilized to show that transplanted spermatogonia injected originally into the lumen of the tubules had moved to the basal compartment of the testis. This movement implies that the adluminal surface of Sertoli cells can recognize spermatogonia and can transport them to

their normal location in the basal compartment. This movement requires the breaking and making of tight junctions between adjacent Sertoli cells. The germ cells that were transplanted in these studies consisted of a mixture of the cell types present in the donor testis and the spermatogonia must have comprised a very small percentage of the injected cells. It follows that the Sertoli cells in the recipient testis must selectively recognize spermatogonial cells. Since rat germ cells transplanted into mouse testes developed into normal rat spermatozoa, it also follows that the information necessary for this developmental process is inherent in the germ cell and the role of the Sertoli cells is to facilitate the progression of this developmental process.

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