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Cell-to-cell cross talk in the testis

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Introduction

The testis consists of the seminiferous tubules as well as interstitial cells, specifically Leydig cells. The seminiferous tubules include germ cells as well as somatic cells such as Sertoli cells. Other somatic cells, the peritubular (myoid) cells, surround the tubules. Spermatogenesis in mammals is characterized by intense, continuous proliferative activity with a complex pattern of mitotic divisions in different types of spermatogonia, culminating in meiotic cleavage of primary spermatocytes to produce noncycling haploid cells (spermatids) [79]. Development of male germ cells in the seminiferous tubules is dependent on the action of pituitary gonadotropins and on androgens secreted by Leydig cells [112].

The concept that mesenchymal cells or their products are required for expression of functions and morphology of adjacent epithelial cells during organogenesis was proposed by Clermont [12]. Numerous investigators have validated this concept [3, 110]. Increasing evidence indicates that apart from gonadotropins and other hormones, cell-to-cell interactions are important in control of testicular function. Increasing numbers of growth factors and cytokines have been implicated in interactions between cells and localized to the testis, where they are produced by different cell types at different phases of testicular development [3, 56, 95, 101, 103, 106, 110]. However, the physiological roles of many of these factors in the testis are still unknown. Investigation of these complex cellular and molecular interactions is very important to understanding the causes of spermatogenic dysfunction. The findings that emerge are likely to have important implications for treatment of male infertility.

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Structural relationships of Sertoli cells and germ cells

Anatomical relationships between Sertoli cells and germ cells were first described by Enrico Sertoli [97]. Morphological studies have given rise to two very important concepts (Fig. 1). Firstly, the tubules are separated into two compartments by tight junctional complexes between adjacent Sertoli cells [1, 19, 30, 36, 67, 87, 93]. The array of junctional complexes is completed at puberty, resulting in a seminiferous tubule barrier that separates the adluminal compartment from the basal compartment of the tubule. Throughout spermatogenesis, developing germ cells migrate from the basal lamina of the seminiferous epithelium to the adluminal compartment [16, 99]. All spermatogonial divisions take place in the basal compartment, while zygotene spermatocytes complete meiosis in the adluminal compartment. Finally mature spermatozoa are released into the tubular lumen during spermiation. Serum macromolecules are effectively excluded from the inner or adluminal compartment, and the extracellular milieu in this compartment is composed of secretions from Sertoli cells and germ cells. The second key morphologically derived concept is that the highly organized, precisely timed sequences of germ cell development involve interaction of groups of germ cells with Sertoli cells [54].

Specialized junctions between Sertoli and germ cells such as ectoplasmic specializations [35, 89], tubulobulbar complex [90, 91], desmosomes [20], and gap junctions [62, 77, 94] are continuously disassembled and reassembled to facilitate the migration of developing germ cells. Disruption and reassembly of Sertoli-germ cell junctions are likely to require the active participation of proteases, protease inhibitors, junctional complex components, signaling molecules, growth factors,

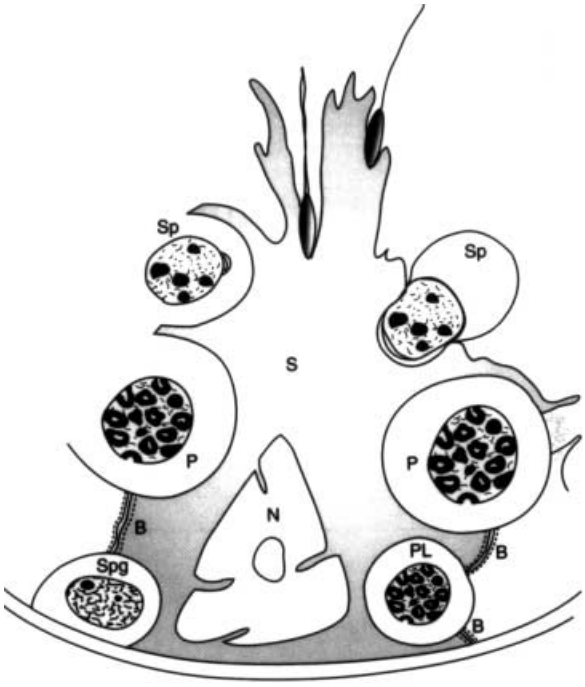

Fig. 1 Structure of the seminiferous tubules *S* Sertoli cells, *Spg* spermatogonia, *PL* preleptotene spermatocyte, *P* pachytene spermatocyte, *Sp* spermatid, *B* barrier (tight junctional complexes between adjacent Sertoli cells), *N* nucleus

and cell adhesion molecules. An intimate association of Sertoli cells and germ cells in vivo is demonstrated by the elaborate branches of Sertoli cell cytoplasm that interdigitate with differentiating germ cells and by the tubulobulbar complexes that extend from developing germ cells deep into the cytoplasm of Sertoli cells [88, 90]. Fawcett identified 1000Å vesicles opening at the surfaces of both Sertoli cells and germ cells that may transport secretory proteins [21]. Junctional structures also have been demonstrated in cocultures of Sertoli cells and pachytene spermatocytes [132] that might allow passage of small molecules between cells [133].

Cell–cell recognition and binding

As noted above, morphological studies of the testis have emphasized an intimate physical relationship between Sertoli cells and differentiating germ cells in vivo [22, 92], contributing to speculation that regulatory influences on spermatogenesis may be mediated by direct contact between Sertoli cells and germ cells [23, 64]. In keeping with such regulation, evidence for specific recognition and adhesion between Sertoli cells and germ cells has come from several in vitro studies [17, 117]. Germ cells added to cultures attached preferentially to Sertoli cells but not to peritubular cells or cell-free culture plates. These attached germ cells are capable of extended viability and some degree of differentiation. Neither somatic cells from the testis other than Sertoli cells nor culture medium conditioned by Sertoli cells can substitute for actual attachment of germ cells to Sertoli cells [117]. In addition, germ-Sertoli cell interaction in vitro is specifically related to the differentiative stage of germ cells [132]; the interaction is shown by middle-to-late pachytene spermatocytes but not by round spermatids. In addition, germ cell adhesion to Sertoli cells is specific, temperature-dependent, and requires a viable Sertoli cell but not necessarily a viable germ cell [17]. Fixation of Sertoli cells with paraformaldehyde effectively inhibits germ-Sertoli cell binding, while fixed spermatogenic cells still are able to bind to native Sertoli cells [17]. These data imply involvement of Sertoli cell-specific constituents in seminiferous cell adhesion events.

Multiple germ cell and Sertoli cell surface molecules evidently are involved in regulation of cellular adhesion within the seminiferous epithelium. Newton et al. identified a Sertoli cell surface polypeptide with an apparent marker of 55 kDa that is involved in spermatogenic-Sertoli cell adhesion [68]. D'Agostino demonstrated components with apparent molecular weights of 78,000 and 51,000 as potential participants in adhesion between Sertoli cells and pachytene spermatocytes [13]. However, which molecules, identified or as yet unidentified, are most important for germ-Sertoli cell adhesion is unknown.

Sertoli cell functions in spermatogenesis

Sertoli cells synthesize specific products that are necessary for germ cell survival. Together, these products form a unique environment in the adluminal compartment that is essential for meiosis and spermiogenesis [34, 98]. Some of these products are testis-specific [11, 52, 126]; other factors are homologous to serum proteins [128]. Representative secreted proteins are listed in Table 1.

Glycoproteins secreted by the Sertoli cells can be placed in several categories according to their known biochemical properties:

1. Transport or bioactive proteins. These are secreted in relatively high abundance and include androgen-binding protein [113] and the metal ion transport proteins such as transferrin and ceruloplasmin [41, 113]. Sertoli cells produce transferrin, considered part of a proposed iron shuttle system that efficiently transports iron between the tight junction complexes to developing germ cells [114, 115]. Analysis of a line of hypotransferrinemic mice demonstrated that transferrin synthesis by the Sertoli cells is essential for normal spermatogenesis [6]. Other transport proteins include sulfated glycoprotein (SGP)-1 and SGP-2, which function as carriers of lipids [53, 75, 100].
2. Proteases and protease inhibitors. These inhibitors, such as plasminogen activator, are important in tissue remodeling processes that occur during spermiation

Table 1 Secreted proteins and peptides from Sertoli cells and its regulation by germ cells. Sertoli cells were cocultured with germ cells (pachytene spermatocytes or round spermatids) or spent media from cultured germ cells. *ABP* androgen binding protein, *SGP* sulfated glycoprotein, *bFGF* basic fibroblast growth factor, *TGF* transforming growth factor, *SGF* seminiferous growth factor, *IL-1* interleukin-1, + stimulation, – inhibition, *NE* no effect

Sertoli cell products	Pachytene spermatocytes		Early spermatids	
	Cells	Culture media	Cells	Culture media
Transport or bioactive proteins				
ABP	+	+	+	+
Transferrin	+	+	+	+
ceruloplasmin		+		+
SGP-1		+		
SGP-2		+		+, –
α2 macroglobulin				
γ-GTP	+	+		
Protease and protease inhibitors				
Plasminogen activator				
Cyclic protein 2				
Cystatin C				
Extracellular matrix components				
Collagen type IV				
Laminin				
Growth factors, cytokines, and related factors				
Antimüllerian inhibiting substance				
Activin				
Inhibin	+	+	+	
Insulin growth factors				
bFGF				
TGFα				
TGFβ				
SGF				
IL-1	NE		NE	
Meiosis-inducing substance	NE		NE	
Estradiol	–	–	–	–
Energy metabolites				
Lactate				
Pyruvate				
Others				
Testins		+		+
Nitric oxide		NE		+

and movement of preleptotene spermatocytes into the adluminal compartment [25]. Cyclic protein 2 is also involved in sperm release [129].

3. Extracellular matrix components. Extracellular matrix components (collagen type IV, laminin, and unique proteoglycans) are among the glycoproteins that form the basement membrane between Sertoli cells and peritubular cells [24, 25, 107].
4. Growth factors, cytokines, and hormones. Glycoproteins function as growth factors. These include müllerian inhibiting substance, meiosis-inducing substance [53, 75, 100], activins, inhibin [108], insulin-like growth factor [116, 117], transforming growth factors α (TGF-α) and β (TGF-β) [3, 63, 76, 102, 106], basic fibroblast growth factor (bFGF), interleukin (IL)-1, and seminiferous growth factor (SGF). Estrogen produced by Sertoli cells may be important in adult endocrine regulation or developing seminiferous tubules [61].
5. Energy metabolites. Sertoli cells can secrete lactate and pyruvate, metabolites required by germ cells [32, 34, 46, 47, 48, 65] because germ cells cannot use glucose as an energy source.

Finally, although the factors involved remain unknown, intimate germ-Sertoli cell contact is necessary for prolonged germ cell survival and function [13, 74, 117]. Apoptosis of germ cells is inhibited by contact with Sertoli cells [26].

Regulation of Sertoli cell function by germ cells and other testicular cells

Several reports have suggested that Sertoli cell function varies depending upon the developmental stage of immediately adjacent germ cells [78, 86, 121]. These cyclic variations are generally considered to reflect paracrine interactions between Sertoli cells and various germ cell constituents representing each stage of spermatogenesis [53, 85, 129].

A variety of observational and experimental approaches have been used to explore regulation of Sertoli cell function by germ cells [44, 106]. Morphological analysis showed that Sertoli cells assume two distinct different shapes as they interact with germ cells during the cycle of the seminiferous epithelium [94, 123, 127]. One might hypothesize that a specific Sertoli cell shape would be elicited only during a limited portion of the cycle of seminiferous epithelium and thus only by specific stages of germ cells. Since methods have been developed to fractionate germ cells to collect fractions rich in spermatocytes and round spermatids, the germ cell

stages responsible for the observed stimulation of Sertoli cells can be determined.

Representative effects of germ cells on Sertoli cell function are shown in Table 1. Protein and steroid secretion by Sertoli cells can be either stimulated by germ cells (in the case of androgen biding protein, transferrin, SGP-1, SGP-2, γ-GTP, inhibin, and testin) or inhibited, in the case of estradiol [8, 9, 45, 55, 57, 70, 72]. These influences are conveyed by factors secreted by spermatocytes and spermatids; thus, to an extent, germ cells specify the milieu in which they develop. Spermatids stimulate nitric oxide production by Sertoli cells, while spermatocytes have an inhibitory effect [28]. Stage-dependent cyclic variations in the level of Sertoli cell mRNAs for transferrin and SGP-2 [66] and for preproenkephalin [27] indicate transcriptional regulation of synthesis of these proteins. In addition, intact germ cells and germ cell-conditioned medium stimulate phosphorylation of some Sertoli cell proteins [43]. Germ cell plasma membranes and soluble factors derived from germ cell cytoplasm stimulate the activity of adenylate cyclase activity in Sertoli cell plasma membranes [125].

Many investigators have sought to identify the regulatory molecules secreted by germ cells that influence Sertoli cell function. While germ cells have been shown to synthesize a wide variety of intracellular [31, 38] and membrane-associated proteins [64], the molecules that regulate Sertoli cell function have not been characterized completely. So far, bFGF derived from pachytene spermatocytes can stimulate Sertoli cell transferrin expression [37]. Transferrin synthesis by Sertoli cells is reduced in the absence of germ cells and can be restored by spermatocytes or bFGF [33, 63]. Onoda and Djakiew have characterized a 29-kDa protein isolated from round spermatids that, like bFGF, stimulates transferrin synthesis in Sertoli cells [71, 72]. Additionally, germ cells have been shown to produce nerve growth factor (NGF) [69, 73] and interferon-α and -γ [15]. Secreted by round spermatids, NGF acts as a potent mitogen [69, 73].

Given the mechanism of action of these paracrine factors, rat germ cells and Sertoli cells may interact via the phosphatidylinositol pathway [124], but this has not yet been confirmed because numerous compounds are involved [29]. Thus, mechanisms of these paracrine effects are not completely understood.

In addition to interactions between germ cells and Sertoli cells, other cell types are probably involved in testicular intercellular communication. Hoeben found that the mixture of cytokines present in media conditioned by activated human peripheral blood mononuclear cells was more potent in stimulating Sertoli cell transferrin secretion than any known single factor; IL-1 and IL-6 may be responsible for some of this effect, but other cytokines are probably involved as well [40]. In addition to the effects of cytokines derived from blood cells, P-Mod-S secreted by peritubular cells can stimulate inhibin secretion by Sertoli cells [108].

Leydig cell products and their local role

Leydig cells produce a number of peptides and proteins and steroids with demonstrated or putative paracrine activity (Table 2). Some of these have inhibitory or stimulatory effects on tubular function. Testosterone is an important paracrine factor in the testis and one of the few substances clearly demonstrated to act as a local regulator of spermatogenesis in animals and humans. The precise targets of testosterone within the tubules are also well known. Androgen receptors have been detected in round and elongating spermatids [122], peritubular, and Sertoli cells [96, 119]; testosterone elicits a number of biological responses in the latter two cell types including direct stimulation of Sertoli cell function by testosterone [120].

Regulation of Leydig cell protein synthesis by tubule cells

Several reports have suggested that Leydig cell morphology and function are controlled locally by cells of the seminiferous tubules. When spermatogenesis was disrupted by irradiation [80], vitamin A deficiency [81], treatment with hydroxyurea, antiandrogen implantation [2], or experimentally induced cryptorchidism [51,

Table 2 The effect of Leydig cell-secreted proteins on Sertoli and germ cell function. *CRF* corticotropin-releasing factor, *SPARC* secreted protein, acidic and rich in cysteine, *TGF* transforming growth factor, IGF insulin-like growth factor, *PDGF* platelet-derived growth factor, *LIF* leukemia inhibitory factor, + stimulation, − inhibition

	Sertoli cell function	Germ cell function
Testosterone	+	+
Pro-opiomelanocortin		
Prodynorphin		
Proenkephalin		
Oxytocin		
Vasopressin		
Renin-angiotensin		
Activin		+
Inhibin		−
Gastrin		
CRF		
SPARC		
bFGF	+	+
β-Endorphin		
Interferon	+	−
Estradiol		
TGFα		
TGFβ		−
Oncostatin M	+	
IGF-1	+	+
Endothelin-1		
PDGF		
IL-1	+	+
IL-6		−
LIF	+	

Table 3 The effect of Sertoli cell products on Leydig cell functions. *IGF* insulin growth factor, *FGF* fibroblast growth factor, *TGF* transforming growth factor, + stimulation, – inhibition

Sertoli cell product	Effect
Activin	–
Inhibin	+
IGF-1	+
IGF-2	+
FGF	+
TGF-α	+, –
TGF-β	–
Estradiol	–

82, 83], Leydig cell hypertrophy and hyperplasia were observed. Bergh [4, 5] demonstrated cyclic changes in the size and volume of the smooth endoplasmic reticulum (SER) of Leydig cells depending on the stage of the seminiferous epithelium of neighboring tubules. Hedger [39] found that disruption of spermatogenesis predictably affects the number, morphology, and function of Leydig cells in vivo. Sharpe reported that Leydig cell regeneration after ethane dimethanesulfonate (EDS) varied with the number of germ cells in neighboring tubules [105]; Leydig cell regeneration was most prominent adjacent to the most damaged tubules, where the fewest germ cells were observed, leading to the speculation that germ cells exert negative control on Leydig cell proliferation via Sertoli cells [105]. Based on these observations, a paracrine mitogenic factor produced by seminiferous tubules has been postulated [18, 84, 104].

Sertoli cells factors that may regulate Leydig cells are listed in Table 3. These have either stimulatory or inhibitory effects on the production of testosterone in Leydig cells [10, 42, 50, 58, 59, 60, 111]. Effects of estradiol secreted by Sertoli cells on Leydig cell activity are probably restricted to the fetal and neonatal periods, when estradiol is known to be involved in negative control of Leydig cell proliferation and perhaps differentiation [7].

Coculture of Leydig and Sertoli cells stimulated Leydig cell DNA synthesis and proliferation but decreased hCG-stimulated testosterone formation and LH/hCG receptor levels [130]. Moreover, proliferation of Leydig cells was associated with a decrease in testosterone production cocultured with Sertoli cells when the temperature was increased from 33 °C to 37 °C [131]. Therefore, temperature modulates the effect of Sertoli cells on Leydig cell function and proliferation.

Neuropeptide Y (NPY) mRNA expression by Leydig cells was stimulated by media from cultured Sertoli cells and particularly enhanced by media from Sertoli cells exposed to follicle-stimulating hormone (FSH) [49]. The Sertoli cell-secreted product mediating these changes in Leydig cells has not yet been conclusively determined, although the responsible Sertoli cell factor is clearly regulated by FSH.

Perspectives

In recent years, several techniques have been developed that permit investigation of molecular events in cell-to-cell interactions, and a number of autocrine, paracrine, and endocrine factors have been reported. However, many questions remain concerning physiology and pathophysiology of testicular function and development. Clarification of these complex mechanisms will prove helpful in identifying causes of spermatogenic dysfunction. Ultimately, we should define these interactions at the molecular level in order to make reliable diagnostic and therapeutic application possible.

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