THE NATURE OF SOMATIC CELL INTERACTIONS IN THE SEMINIFEROUS TUBULE

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1. INTRODUCTION

It is generally accepted that the hormonal control of mammalian spermatogenesis is mediated by direct actions of hormones on testicular somatic cells, and that the optimal expression of programs for germinal cell development is dependent upon the microenvironment provided by adjacent somatic cells (Fig. 1) (for reviews, see 16, 17).

In this presentation, we shall focus upon the nature of interactions between Sertoli cells and peritubular cells, with emphasis on observations from our laboratory on the properties of these cells from the rat testes in monoculture and in co-culture. Before becoming totally immersed in this rather specialized topic, however, it may be useful to consider some of the recent developments in male reproductive biology which have led to current views of the hormonal control of spermatogenesis, and to describe in general terms the basis for the growing importance attributed to the role of testicular somatic cells in the formation and maintenance of a unique local environment in the seminiferous tubule.

It has long been known that germinal cell development is dependent upon the maintenance of an appropriate hormonal milieu. Yet, germ cells do not appear to respond directly to FSH, LH or to androgens. These hormones, which are required to restore spermatogenesis in hypophysectomized animals, elicit their overall effects by modulating the functions of testicular somatic cells. The hormonally-stimulated somatic cells interact at several levels with adjacent germinal cells, providing in some manner all that is required for the orderly progression of spermatogonia to spermatozoa. Within this framework, much remains to be defined and delineated:

1) The easiest and most straightforward task consists simply of cataloguing the list of functional activities of Sertoli cells and peritubular cells which can be directly modulated by various hormones. Advances in our knowledge in this area are progressing rapidly, along with a better comprehension of hormone actions.
speculate upon the biological relevance of these interactions in establishing the unique microchemical and cytoarchitectural arrangements within the seminiferous tubule required for germinal cells to develop. Finally, we shall attempt to relate these observations to the general field of mesenchymal cell-epithelial cell interactions.

II. PROPERTIES OF PERITUBULAR CELLS

A. Overview

In the intact seminiferous tubule, spermatogonia and a relatively small percentage of the basal surfaces of Sertoli cells rest upon a basal lamina which separates these cells from peritubular myoid cells in the boundary tissue. The tubule wall in rat testis is comprised of layers of cells and cell-free matrix, commencing with the basal lamina or basement membrane adjacent to the seminiferous epithelium; a relatively clear zone containing a reticulum of collagen fibrils; a thin basal lamina-like structure which provides an inner coating for the continuous layer of peritubular myoid cells; another clear zone; and finally an outer layer of flattened endothelial cells that line the peritubular lymphatic sinusoids (11). Structural and ultrastructural characteristic of peritubular myoid cells have been well described (5, 6, 11, 13, 30, 31, 42). The development of peritubular myoid cells in vivo is androgen-dependent (5), and peritubular cells have been shown to contain androgen receptors (47, 62).

The peritubular myoid cell layer provides an incomplete barrier to the passage of molecules from the interstitial space into the basal compartment of the seminiferous tubule (11). Some peritubular cells are in close apposition, joined by tight junctional complexes which exclude the passage of small molecules such as lanthanum nitrate. However, sufficiently large intercellular clefts exist between other peritubular myoid cells at various stages of the cycle of the seminiferous epithelium to permit the penetration of lanthanum past the boundary tissue. In these apparently randomly located regions of the tubule, which are estimated to represent about 15% of the length of the tubule, lanthanum injected intravenously has been visualized in the space between the peritubular myoid cell layer and the germinal cells (11). Data reviewed permit the conclusion that peritubular cells form an initial barrier which partially excludes the passage of molecules past the tubule wall. These properties are insufficient to account for those of the seminiferous tubule barrier (often inappropriately called the “blood-testis” barrier).

Other functions of the peritubular cells are not clear, but they are thought to play a role in tubular contractions (22).
### B. Properties of Peritubular Cells Maintained In Culture

Many of the structural characteristics of peritubular cells (5, 11) are retained in preparations in culture (Table 1 and Fig. 2) (34, 57, 58, 59, 61).

![Image](https://via.placeholder.com/150)

**Fig 2.** Transmission electron micrographs of peritubular cells isolated from testes of 20-day-old rats and maintained in culture for 10 days (panel A) or 6 days (panel B) in modified Eagle's minimal essential medium containing 10% calf serum. Panel A: 4000x; Panel B: 16000x. For details, see the original publications (57, 59).
Peritubular cells readily spread to form monolayers or multilayers when plated on various substrata. However, the addition of serum is required for efficient plating, subculture and growth. No chemically defined-serum-free

<table>
<thead>
<tr>
<th>Cell Type in Culture</th>
<th>Levels of ECM Components in Medium During 48 Hour Collection Period Starting on Day 5</th>
<th>(ng/mg DNA)</th>
<th>Collagen I</th>
<th>Collagen IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritubular Cells</td>
<td>432 ± 68</td>
<td>254 ± 37</td>
<td>350 ± 54</td>
<td></td>
</tr>
<tr>
<td>Sertoli Cells</td>
<td>0</td>
<td>0</td>
<td>322 ± 52</td>
<td></td>
</tr>
<tr>
<td>Peritubular and Sertoli</td>
<td>182 ± 34</td>
<td>96 ± 15</td>
<td>197 ± 30</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as the mean ± SEM for 3 separate experiments, each of which was analyzed in triplicate (n = 9). For details of the competitive ELISA assays, and other information, consult the original publication (55).

has thus far been found which can support these functions of peritubular cells in monolulture. After cells have grown to confluence, they can survive for several weeks in serum-free medium. Under these conditions, peritubular cells have the capacity to synthesize fibronectin (61); types I and IV collagen (55); and proteoglycans containing chondroitin sulfate (51) (Table 2). The gel electrophoretic patterns of other proteins synthesized by peritubular cells in culture and released into the medium have been described (24, 61). The presence of specific protein(s) synthesized by peritubular cells which modulate the functional activities of Sertoli cells has recently been detected in conditioned medium (52). This is discussed below in the section on peritubular cell — Sertoli cell interactions.

### III. PROPERTIES OF SERTOLI CELLS

#### A. Overview

The structure and functions of these cells have been exhaustively reviewed (12, 16, 17, 35, 36, 39, 40, 41, 46, 63). A three-dimensional reconstruction of a rat stage IV Sertoli cell has recently been achieved, allowing an increased appreciation of the extraordinary complexity and diversity of interactions of Sertoli cells with all classes of cells in the seminiferous tubule (44, 64, 66). It is abundantly clear from information reviewed that Sertoli cells form the basic architecture and organization of the seminiferous tubule, including the generation and maintenance of the testis barrier separating the adluminal from the basal compartments (12). The barrier, which functions to maintain the unique chemical composition of fluids in the lumen of the seminiferous tubule and in the rete testis (49, 63), also serves to provide an immunologically privileged environment for germinal cells in the adluminal compartment (38).

#### B. Properties of Sertoli Cells Maintained in Culture

As in the case of peritubular cells, many of the in vivo structural characteristics of Sertoli cells (12) are retained in cells cultured in chemically defined medium (Table 1, and Fig. 3) (56, 59). The normal cytoarchitecture is better maintained in Sertoli cells cultured on an extracellular matrix substratum than in cells plated on an uncoated plastic substratum (60). Evidence of maturation of Sertoli cells in culture has been presented (56).

Patterns of proteins synthesized by Sertoli cell-enriched preparations have been described (9, 24, 65, 67). The gel electrophoretic profile of proteins secreted by Sertoli cells is clearly different from patterns of proteins secreted by peritubular cells (24, 61). For example, Sertoli cells in culture do not synthesize Type I collagen or fibronectin, but peritubular cells do (61) (Table 2). Sertoli cells secrete transferrin (53), ceruloplasmin (53a), and a host of other proteins (Table 3) which are not synthesized by peritubular cells.

The list of components known to be synthesized by Sertoli cells is rapidly growing (Table 3). Most of this information has been obtained by investigating the secretory products of Sertoli cell-enriched preparations in culture. Additional information which strengthens interpretations of the physiological relevance of in vitro observations has been obtained with experiments on seminiferous tubules in organ culture, and with analyses of testes depleted of germ cells by irradiation in utero (for reviews see, 36, 63). In the cases of those proteins isolated from testes or rete testis fluid to which antibodies have been generated, immunocytological examination of testes has demonstrated the presence in Sertoli cells of androgen binding protein (ABP) (1); clusterin (60a); dimeric acidic glycoprotein (20); transferrin (20) and plasminogen activator (Tung et al., in preparation). However, none of these proteins is necessarily unique to Sertoli cells (17).

### Table 2. Levels of Soluble Extracellular Matrix (ECM) Components Produced by Peritubular Cells or Sertoli Cell Maintained in Serum-Free MEM in Monoculture or Co-Culture

<table>
<thead>
<tr>
<th>Cell Type in Culture</th>
<th>Fibronectin</th>
<th>Collagen I</th>
<th>Collagen IV</th>
</tr>
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<tbody>
<tr>
<td>Peritubular Cells</td>
<td>432 ± 68</td>
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</tbody>
</table>

For details, see references 16, 17, 20, 34, 35, 36, 39, 40, 46 and 50.

### Table 3. Products Synthesized and Released by Primary Cultures of Sertoli Cell-Enriched Preparations

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Collagen IV</th>
<th>Proteoglycans</th>
<th>Clusterin</th>
<th>Dimeric Acid Glycoprotein</th>
<th>Others (&quot;Cyclic&quot; Proteins; &quot;Lactalbumin&quot;, etc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen Binding Protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Mullerian Hormone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Transferrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ceruloplasmin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasminogen Activator</td>
<td></td>
<td></td>
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</tbody>
</table>

Spermiogenesis: 17-β Estradiol

Carbohydrates: Inositol, Lactate, Pyruvate
IV. INTERACTIONS OF SERTOLI CELLS WITH PERITUBULAR CELLS

A. Extracellular Matrix Formation and Deposition

Sertoli cell-enriched aggregates plated on top of peritubular cells spread to form a monolayer. Sertoli cells reaggregate, and then form mounds, nodules, and eventually complex protrusions as adjacent nodules merge (58). Aggregates are surrounded by ribbons of peritubular cells, separated by a limiting membrane and collagen fibrils (Figure 4B). The structure formed in vitro in the co-cultured system resembles the histologic appearance of seminiferous tubules in situ (Figure 4A). Basal lamina formation is evident (Figure 4B). In contrast, peritubular cells and Sertoli cells in monoculture spread to form relatively uniform layers, devoid of mounds, nodules or protrusions (25, 58, 61). Sertoli cells and peritubular cells in co-culture can survive in serum-free MEM for months, whereas neither cell type can do so in monoculture (58). ABP production by Sertoli cells is enhanced and sustained by the presence of peritubular cells in co-culture (23, 58).

These remarkable changes in properties of each cell type when placed in co-culture provided a strong incentive to investigate the nature of the apparent cooperativity. We first explored the influences of cell interactions on the formation and deposition of extracellular matrix components (Table 2). Sertoli cells, which did not produce Type I collagen during the first week of culture, stimulated peritubular cells to synthesize more soluble Type I collagen during co-culture. In addition, co-culture resulted in a greater deposition in extracellular fibrils of Type I collagen. Type IV collagen was synthesized by each population of cells; fibronectin was synthesized by peritubular cells but not by Sertoli cells; and laminin was detectable in Sertoli cells but not in peritubular cells. Deposition of all ECM components investigated was more pronounced in co-cultures of Sertoli cells and peritubular cells than in monocultures of either cell type (Table 2; 55). We interpret data presented to indicate...
that the two cell types in co-culture act cooperatively to form the basal lamina-like structure observed in vitro (Figure 4B). We propose that the same processes may occur in vivo.

B. Evidence for the Existence of Paracrine Factors

1. P Mod-S

Conditioned medium obtained from peritubular cells in culture stimulates Sertoli cell to increase the formation and release of ABP (52) (Table 4). As indicated previously, ABP production by Sertoli cells is augmented when peritubular cells are present during co-culture (23, 58). We interpret data shown to indicate that a protein, designated "P Mod-S", is released by peritubular cells (P), and this factor modulates functions of Sertoli cells (S). Addition of P Mod-S stimulates the formation of transferrin to as great an extent as it augments the synthesis of ABP by Sertoli cells (Table 4). Addition of a mixture of FSH, insulin, retinol and testosterone (FIRT) is required to elicit maximal stimulation of transferrin formation by Sertoli cells (S4a). The same mixture increased the synthesis of ABP and transferrin to an extent comparable to that observed in Sertoli cells cultured in the presence of P Mod-S, but it should be noted that P Mod-S was more stimulatory than FSH, insulin, retinol or testosterone alone (Table 4). The formation of plasminogen activator (PA) is also greatly stimulated by addition of FIRT, in confirmation of observations previously reported on the effects of FSH on PA synthesis by Sertoli cells in culture (26, 27, 28). P Mod-S addition did not enhance PA levels in the medium. Instead, the formation of PA by Sertoli cells cultured in the presence of P Mod-S appeared to be reduced slightly (Table 4). In contrast, the synthesis of transferrin by peritubular cells than in monocultures of either cell type (Table 2, 55). We interpret data presented to indicate

| Table 4: Effects of P Mod-S on Levels of Androgen Binding Protein (ABP), Transferrin and Plasminogen Activator (PA) Produced by Primary Cultures of Rat Sertoli Cells |
|---|---|---|---|
| Additions to Basal Medium | Levels of Protein Released into Medium By Sertoli Cells in Culture |
| | (ng/µg DNA/24h) | (ng/µg DNA/24h) | (units/µg DNA/24h) |
| Control | 4.5 ± 0.5 | 58 ± 10 | 60 ± 50 |
| FIRT* | 16.0 ± 4.0 | 180 ± 20 | 210 ± 20 |
| P MOD-S** | 14.5 ± 3.5 | 200 ± 15 | 35 ± 5.0 |

*FIRT represents follicle-stimulating hormone (FSH, NIH-S-16, 100 ng/ml); insulin (5 µg/ml); retinol (0.35 µM); and testosterone (1 µM) in serum-free MEM.

**P Mod-S is the partially purified 50-100 kDa fraction prepared from 200X-concentrated serum-free conditioned medium obtained from secondary cultures of peritubular cells. The concentration of P Mod-S added to the Sertoli cell culture medium was 1.5 µg protein/ml. All values are given as the mean ±S.D. for n = 9. For details consult the original publication (52).
Mechanisms involved are unknown, but it may be concluded that P Mod-S influences Sertoli cell functions in a manner which is not identical to the actions of any of the components present in the FIRT mixture. Experiments are in progress to isolate P Mod-S and to determine its site(s) of action (52). Thus far, our observations indicate that P Mod-S is a protein having an apparent molecular mass of 70 KDa, and that its addition increases the incorporation of $^{38}$S-methionine into several proteins released into the medium by Sertoli cells. In primary cultures of peritubular cells maintained in the presence of androgens (testosterone or dihydrotestosterone), levels of P Mod-S activity secreted are greater than those detected when peritubular cells are maintained in MEM alone or in MEM containing estrogens (52). We have also recently observed that the stimulation by androgens of ABP production by populations of Sertoli cells nearly devoid of contaminating peritubular cells is less than that obtained in Sertoli cell-enriched preparations containing peritubular cells. Reconstitution experiments have shown that androgen stimulation of ABP production by Sertoli cells is enhanced by the presence of peritubular cells (52a).

We interpreted these data to indicate that androgens stimulate peritubular cells (stromal or mesenchymal cells) to synthesize P Mod-S, which then modulates the functions of adjacent Sertoli cell (epithelial cells). In this manner, P Mod-S may serve as a local mediator of androgen actions on Sertoli cells. The actions postulated are independent of the direct effects of testosterone on Sertoli cells (32, 33, 46).

2. Speculations on Other Paracrine Factors Produced by Testicular Cells

The existence of other paracrine factors has been suggested by several lines of evidence. Since Sertoli cells alter the functions of peritubular cells in coculture (58), we postulate the existence of a putative S Mod-P, a component secreted by Sertoli cells which modulates the activities of peritubular cells. The influences of LH on the formation of interstitial fluid of the testis (50), and the influences of hormone on the properties of capillary distribution and permeability in the testis during gonadal maturation (25) suggest the presence of factors produced by testicular cells under hormonal regulation which may affect the functions of vascular endothelial cells. The presence of androgen receptors in testicular endothelial cells has recently been reported (37), suggesting the possibility that these cells may directly respond to androgens. Alternatively, peritubular cells could secrete a factor (P-Mod-V) which alters the properties of vascular endothelial cells. Several investigators have reported that FSH modulates the synthesis of androgens by Leydig cells (for reviews see 16, 45). These data suggest the possibility that Sertoli cells (the only cells known to respond directly to FSH) can produce S-Mod-L, a factor which modulates the properties of Leydig cells. Finally, several types of interactions between Sertoli cells and germinal cells have been reported (for review, see 39). Not only do Sertoli cells secrete products which affect the development of germ cells, but the germinal cells association pattern can modulate the functions of Sertoli cells. For example, basal rates of formation of plasminogen activator by Sertoli cells are over 10-fold greater in cells in stages VII and VIII of the cycle of the seminiferous epithelium (19, 29).

![Diagram](image)

**Fig. 5.** Hormonal control of spermatogenesis: 1984 model. Solid arrows represent known actions or effects, while dotted arrows indicate postulated actions. In the germinal cell column, «A» represents spermatagonia, «c» represents spermatocytes, «T» represents round and elongating spermatids, and «Z» represents spermatocytes released into lumen. «P-Mod-S» designates the protein(s) secreted by peritubular cells which modulate the activities of Sertoli cells (52). «S-Mod-P» and «S-Mod-L» designate hypothetical protein(s) secreted by Sertoli cells which modulate the activities of peritubular cells (58), and Leydig cells (45), respectively. «P-Mod-V» designates the hypothetical protein(s) secreted by peritubular cells which modulate the activities of vascular endothelial cells. For details, see the text and (17).

The likelihood emerges that factors may be produced by many types of cells in the seminiferous tubule which exert local control, and that these paracrine or autocrine components may play an integral role in establishing the nature of the microenvironment required for spermatogenesis. The 1978 version of the hormonal control of spermatogenesis presented earlier (Figure 1) is therefore accordingly modified to reflect these new concepts (Figure 5). Somatic cells of the seminiferous tubule are regulated directly by systemic hormones (primar-
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AKNOWLEDGMENTS

Work reported from this laboratory was supported by a grant from the Canadian Medical Research Council, and Michael Skinner was supported by an MRC postdoctoral Fellowship. We express our gratitude to Krystyna Burdzy, Lyn Dean and Kathy Karmally for expert technical assistance, and to Donna McCabe and Fern Teodoro for typing the manuscript.

REFERENCES

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