INTRODUCTION

The testis is an endocrine-responsive tissue that requires a number of important cell-cell interactions for the maintenance and control of cellular growth and differentiation. The Sertoli cells form the seminiferous tubules and provide the cytoarchitectural support and microenvironment required for the developing spermatogenic cells. Peritubular myoid cells surround the tubule and are separated from the basal surface of the Sertoli cells by a complex extracellular matrix. In the interstitium of the testis are numerous cell types including the Leydig cells that are responsible for the production of androgens. Previous investigation of testicular cell-cell interactions have primarily utilized Leydig, peritubular, Sertoli, and germinal cells. Other cell types such as the lymphatic endothelium, testicular macrophages, lymphocytes, and stromal cells will likely be involved in local cell-cell interactions, but these require further elucidation. Interactions between Leydig, peritubular, Sertoli, and germinal cells will be the focus of the current review.

The types of interactions that occur between cells are numerous and have previously been categorized into environmental, nutritional and regulatory interactions. Structural interactions mediated by extracellular matrix components or cell adhesion molecules are examples of an environmental interaction. The delivery of an essential metabolite or nutritional substance between cells is an example of a nutritional interaction. The production and subsequent action of a paracrine or autocrine agent to elicit a signal transduction event to influence cellular functions on a molecular level is an example of a regulatory interaction. Although environmental and nutritional interactions are critical for normal cellular physiology, regulatory interactions are needed to alter and maintain cellular function and differentiation. The current review will...
focus on regulatory interactions and the specific factors involved. The interactions reviewed will also be discussed in reference to the endocrine regulation of testis function.

**SPECIFIC INTERACTIONS**

*Sertoli Cell–Germinal Cell Interactions*

In 1865, Enrico Sertoli described specialized branched cells within the tubules which were later determined to be essential for both germinal cell development and spermatogenesis. Sertoli cells are columnar epithelial cells which extend from the basal to apical surface of the seminiferous tubule. Germinal cells at all stages of development require the physical support provided by the Sertoli cells and these environmental interactions between Sertoli cells and germinal cells are essential for testis function. Tight junctions are formed between Sertoli cells to prevent the passage of macromolecules from the interstitial space into the lumen of the tubule. Sertoli cells, therefore, provide energy metabolites, such as lactate and pyruvate, to support germinal cell metabolism and binding proteins such as transferrin and ceruloplasmin to transport iron and copper to germinal cells. Although these environmental and nutritional interactions are essential for the maintenance of spermatogenesis, regulatory interactions are required to control cell function on a molecular level.

Investigation of the potential presence of regulatory interactions directed at germinal cells initially utilized co-culture experiments. The presence of Sertoli cells has been shown to increase germinal cell RNA and DNA synthesis, induce the appearance of germinal cell surface antigens and maintain spermatogenic cell glutathione synthesis. Consideration of potential regulatory agents that act on germinal cells have focused on several growth factors produced by the Sertoli cell. Insulin-like growth factor-I (IGF-I) is a somewhat ubiquitous factor that is produced by Sertoli cells. IGF-I receptors have been localized on spermatogenic cells, indicating that IGF-I may act on these cells. The epidermal growth factor-like substance transforming growth factor-alpha (TGF-α) is also produced by Sertoli cells. Germinal cells do not appear to express the EGF receptor; therefore, the paracrine function of TGF-α is unclear. The growth inhibitor transforming growth factor-beta (TGF-β) is also produced by Sertoli cells; however, the actions of TGF-β on germinal cells remain to be elucidated. Another apparent Sertoli cell product is interleukin-1 (IL-1), a cytokine which may stimulate germinal cell growth. Although a number of potential paracrine factors are produced by Sertoli cells (TABLE 1), the direct actions of these factors on germinal cells remain to be established.

Investigation of regulatory interactions directed at Sertoli cells have also utilized co-culture of germinal and Sertoli cells. Spermatogenic cells, particularly pachytene spermatocytes and early stage round spermatids, can influence Sertoli cell function. Germinal cell conditioned medium has been shown to stimulate phosphorylation of specific proteins and gamma-glutamyl transpeptidase activity in Sertoli cells. Conditioned medium also can increase ABP production, increase transferrin gene expression and production, decrease estradiol synthesis, and decrease RNA synthesis. Preliminary characterization of the active components in germinal cell conditioned medium has determined that the activity is heat and trypsin sensitive. Partial purification of the activity has demonstrated that a fraction containing three polypeptides between 10 and 30 kDa has the ability to influence Sertoli cells. Further pur-
### Table 1. Potential Regulatory Agents Involved in Testicular Cell-Cell Interactions

<table>
<thead>
<tr>
<th>Potential Paracrine Factor</th>
<th>Proposed Site Production</th>
<th>Proposed Site Action</th>
<th>Proposed Actions/Function</th>
<th>Site Synthesis/Secretions</th>
<th>Characterization Actions</th>
<th>Physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sertoli Cell-Germinal Cell Interactions</td>
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<tr>
<td>IGF-1</td>
<td>Sertoli</td>
<td>Germinal</td>
<td>? Growth/Metabolism</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>TGF-β</td>
<td>Sertoli</td>
<td>Germinal</td>
<td>? Growth/Differentiation</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>IL-1</td>
<td>Sertoli</td>
<td>Germinal</td>
<td>? Growth</td>
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<td>IGF-2</td>
<td>Sertoli</td>
<td>Germinal</td>
<td>? Metabolism/Growth</td>
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<tr>
<td>SGF/SCSGF</td>
<td>Sertoli</td>
<td>GC/SC</td>
<td>? Growth</td>
<td>X</td>
<td>X</td>
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<tr>
<td>NGF</td>
<td>Germinal</td>
<td>Sertoli</td>
<td>? Growth</td>
<td>X</td>
<td>X</td>
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<td>Peritubular Cell-Sertoli Cell Interactions</td>
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<tr>
<td>PModS</td>
<td>Peritubular</td>
<td>Sertoli</td>
<td>Differentiation</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>TGF-α</td>
<td>PC/SC</td>
<td>PC/?SC</td>
<td>Growth</td>
<td>X</td>
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<tr>
<td>TGF-β</td>
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<td>PC/SC</td>
<td>Growth/Differentiation</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Sertoli Cell-Leydig Cell</td>
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<tr>
<td>Androgen</td>
<td>Leydig</td>
<td>Sertoli</td>
<td>Differentiation</td>
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<td>POMC Peptides</td>
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<td>β-END, MSH, ACTH</td>
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<td>Stimulatory Factor (?)</td>
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<td>Leydig</td>
<td>Alter FSH Action</td>
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<tr>
<td>Inhibitory Factor (?)</td>
<td>Sertoli</td>
<td>Leydig</td>
<td>Steroidogenesis</td>
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<td>X</td>
<td>X</td>
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<td>LHRH-like Factor</td>
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<td>Leydig</td>
<td>Steroidogenesis</td>
<td>X</td>
<td>X</td>
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<td>Leydig</td>
<td>Steroidogenesis</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
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<td>SC/LC</td>
<td>Leydig</td>
<td>Steroidogenesis</td>
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<td></td>
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<tr>
<td>TGF-α</td>
<td>SC/?LC</td>
<td>LC/SC</td>
<td>Growth/Steroidogenesis</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>TGF-β</td>
<td>SC/?LC</td>
<td>LC/SC</td>
<td>Growth/Steroidogenesis</td>
<td>X</td>
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<td>X</td>
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<tr>
<td>Inhibin</td>
<td>SC/LC</td>
<td>LC/?SC</td>
<td>Steroidogenesis</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Leydig Cell-Peritubular Cell</td>
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<tr>
<td>Androgen</td>
<td>Leydig</td>
<td>Peritubular</td>
<td>Differentiation</td>
<td>X</td>
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<td>X</td>
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</table>

*a Potential paracrine factors in the testis with their proposed site of production and action designated, as well as proposed action/function. (?) denotes a postulated cell type or function with negligible data currently available. The inset box designates with an (X) experimental investigation of the site of synthesis, cellular secretion, biochemical characterization, action(s) and physiological relevance for the various paracrine factors with an open box indicating the lack of available information or experimental analysis.

Identification and characterization of the active components is needed to identify or define the unique properties of the paracrine factor(s) and to determine if these factors are actively secreted by the germinal cells or are released as a consequence of poor cell viability and cell lysis. A growth factor that may potentially be involved in germinal cell–Sertoli cell interactions is nerve growth factor (NGF). NGF immunoreactivity and β-NGF gene expression have been observed in spermatocytes and early spermatids. NGF receptor gene expression has been identified in Sertoli cells under an-
drogen regulation.\textsuperscript{21} Although this indicates that NGF may be a paracrine factor for these cells, further investigation is necessary to identify its exact function.

The potential paracrine control of Sertoli and spermatogenic cell function (Table 1) can be influenced by the endocrine system. Follicle-stimulating hormone acts through receptors on the Sertoli cell to effect cellular differentiation and function. Therefore, FSH may indirectly effect germinal cell development by altering the interactions between the Sertoli and germinal cell populations.\textsuperscript{22}

\textit{Peritubular Cell-Sertoli Cell Interactions}

The mesenchymal (i.e., stromal)-derived peritubular-myoid cells contribute to the exterior wall of the seminiferous tubule and are separated from the epithelial-like Sertoli cells by an extracellular matrix. While peritubular cells are important in maintaining the structural integrity of the seminiferous tubule, they also appear to produce paracrine factors that may be important in regulatory interactions in the tubule. Initial observations with co-cultures of peritubular and Sertoli cells indicated that the presence of peritubular cells can alter Sertoli cell morphology and enhance Sertoli cell function, including the production of transferrin and ABP.\textsuperscript{23,24}

Potential regulatory agents that may mediate interactions between Sertoli and peritubular cells include growth factors. Both Sertoli and peritubular cells produce TGF-\(\alpha\) and TGF-\(\beta\).\textsuperscript{12,13,24} Functional EGF receptors are expressed on peritubular cells and possibly Sertoli cells.\textsuperscript{12,26} TGF-\(\alpha\)/EGF can stimulate peritubular cell but not mid-pubertal Sertoli cell growth.\textsuperscript{12} While EGF has been shown to influence Sertoli cell function, little effect is seen in highly purified preparations of Sertoli cells.\textsuperscript{12} Further molecular analysis of EGF receptor localization and TGF-\(\alpha\) action are necessary to elucidate the local interactions mediated by the TGF-\(\alpha\). TGF-\(\beta\) does not appear to have dramatic effects on Sertoli cell growth or function; however, TGF-\(\beta\) can inhibit TGF-\(\alpha\)-induced peritubular cell proliferation. Peritubular cell differentiation may also be induced by TGF-\(\beta\), as indicated by increased production of specific proteins such as matrix components and antiproteases.\textsuperscript{13,28} TGF-\(\beta\) also promotes peritubular cell chemotaxis and colony formation.\textsuperscript{13} Therefore, morphogenesis of the seminiferous tubule may be dependent on this factor. Both Sertoli and peritubular cells produce insulin-like growth factor I (IGF-I). IGF-I can stimulate growth of peritubular and immature Sertoli cells and may also stimulate Sertoli cell function, however, high levels of IGF-I are present in the interstitial fluid available to both cell types.

Potential paracrine factors that primarily influence cellular differentiation are also important in mediating peritubular-Sertoli cell interactions. Peritubular cells have been shown to produce a nonmitogenic factor which modulates Sertoli cell function, termed PModS.\textsuperscript{29,30} The apparent production of PModS is under androgen control, thus allowing for an indirect mechanism for gonadotropin/androgen action in the testis.\textsuperscript{29,30} PModS has been purified into two biologically related forms, PModS(A) and PModS(B), and purified PModS enhances a number of Sertoli cell functions, including transferrin production.\textsuperscript{31} PModS can stimulate many Sertoli cell functions to a greater extent than any known regulatory agent, including FSH.\textsuperscript{31,32} Interestingly, peritubular cell-Sertoli cell interactions provide an example of a mesenchymal (i.e., stromal)-epithelial cell interaction. PModS may provide an example of a mesenchymal inducer substance that can influence the differentiation of the adjacent epithelial cell. It is postulated that PModS may have an important role in the induction and maintenance of Sertoli
cell differentiation. Further molecular characterization of PModS and analysis of in vivo actions of PModS is required.

Leydig Cell–Sertoli Cell Interactions

Regulatory interactions between Leydig cells and Sertoli cells were among the first cell-cell interactions investigated in the testis. The identification of androgen production by Leydig cells and the ability of androgens to maintain the process of spermatogenesis prompted considerable research on the actions of androgens on Sertoli cells. Sertoli cells express the androgen receptor gene; however, the effects of androgens on Sertoli cell function in vitro are absent or less than those obtained with FSH. Since peritubular cells enhance the actions of androgens on Sertoli cells, highly purified preparations of Sertoli cells should be used to investigate the actions of androgens. The role direct actions of androgen have on Sertoli cells remains to be elucidated.

In addition to androgens, a number of peptides and proteins have been localized to Leydig cells. Several peptides derived from proopiomelanocortin (POMC) appear to be produced by Leydig cells, including beta-endorphin (βEND), alpha-melanocyte stimulating hormone (α-MSH), and adrenocorticotropic hormone (ACTH) and it has been speculated that these are involved in the regulation of Sertoli cell function. Endorphin receptors have been localized on Sertoli cells, and βEND can inhibit the actions of FSH. Further work is required to determine the physiological significance of Leydig cell derived peptides and their role in mediating regulatory interactions between Leydig cells and Sertoli cells.

The ability of Sertoli cells or seminiferous tubules to influence Leydig cell function also has been the object of intense investigation. Damage to the seminiferous tubule by cytotoxic agents, vitamin A deficiency, fetal irradiation or cryptorchidism alters Leydig cell morphology. These morphologic studies imply that a regulatory interaction may exist between Sertoli cells and Leydig cells. Observations have been extended with co-culture of the two cell types or co-culture of Leydig cells with specific stages of the seminiferous epithelium. When studies were performed using Sertoli cell-conditioned media both increases and decreases in Leydig cell steroidogenesis were demonstrated. An interesting study which utilized spent media from seminiferous tubules isolated from both normal and cryptorchid testis revealed that under normal conditions predominately inhibitory activity was present while cryptorchidism induced the appearance of stimulatory activity. The active components apparently secreted by Sertoli cells that influence Leydig cell steroidogenesis remain to be purified and characterized.

Several Sertoli cell secretory products have also been identified that can potentially mediate regulatory interactions between Sertoli cells and Leydig cells (TABLE 1). Estrogen, has an inhibitory influence on Leydig cell steroidogenesis and is produced by Sertoli cells. Leydig cells, however, also produce high levels of estrogen which could act in an autocrine manner on the cell. Inhibin, a peptide hormone produced by Sertoli cells and its related protein, activin, both can influence Leydig cell steroidogenesis. Leydig cells, however, have also been shown to produce both inhibin and activin. Several growth factors produced by Sertoli cells that can influence Leydig cells include; IGF-I, TGF-α, and TGF-β. The potential production of these growth factors by Leydig cells, however, questions the importance of paracrine interactions with these growth factors. Additional regulatory agents apparently produced by Sertoli cells that can influence Leydig cell function include an interleukin-1 type
molecule\textsuperscript{49} and an LHRH-like substance.\textsuperscript{50} The Sertoli cell appears to produce a number of regulatory agents that can influence Leydig cells (TABLE 1) and an investigation of their physiological importance is now required.

The majority of Sertoli cell products that may influence Leydig cell function have been identified through the ability to effect Leydig cell steroidogenesis. Although the production of androgen by Leydig cells and subsequent actions of androgen on the seminiferous tubule is essential for the maintenance of testis function, the physiological requirement to modulate steroidogenesis in the adult must be critically evaluated. The concentration of androgen present in the adult testis is significantly higher than the concentration needed to maintain germinal cell development.\textsuperscript{51,52} Although an active regulation of Leydig cell androgen production may be needed during embryonic and pubertal development, local transient alteration in androgen production in the adult may not have major effects on Sertoli cell or germinal cell development. In particular, stimulation of androgen production would not appear to be important for testis function in the adult testis. Factors produced by Leydig cells, other than androgens, however, may need to be actively regulated.

\textit{Leydig Cell–Peritubular Cell Interactions}

Growth factors are potential paracrine factors that may mediate interactions between Leydig cells and peritubular cells. Leydig cells respond to several growth factors shown to be produced by peritubular cells including IGF-I, TGF-\(\alpha\) and TGF-\(\beta\). Although IGF-I, TGF-\(\alpha\) and TGF-\(\beta\) have been shown to be produced by peritubular cells, the production of these growth factors in the interstitium has not been thoroughly assessed; therefore, the significance of growth factor–mediated paracrine interactions is unclear.

The primary regulatory agent produced by Leydig cells that has been shown to influence peritubular cells is androgen. Androgen receptors are present in high numbers in peritubular cells and peritubular cell differentiation appears to be dependent on the presence of androgen.\textsuperscript{53} Androgens increase the apparent production of the paracrine factor PModS by peritubular cells which subsequently can influence Sertoli cell function.\textsuperscript{29} These potential indirect actions of androgens on the Sertoli cells through peritubular cells are speculated to be important for the control and maintenance of spermatogenesis.\textsuperscript{1} The indirect role of androgen action may be that luteinizing hormone influences Leydig cells to produce androgen which stimulates PModS secretion from peritubular cells that then acts to influence Sertoli cell functions vital for the maintenance and control of spermatogenesis. The dramatic effects of PModS on Sertoli cell function and differentiation observed in vitro and the lack of major androgen effects on purified Sertoli cell cultures support this postulated interaction. Further investigation of the hormonal regulation of PModS production and the in vivo actions of PModS, however, is required. The potential ability of LH to influence this series of cell-cell interactions provides an example of how the endocrine regulation of testis function may be indirectly mediated through local cell-cell interactions.

\textbf{SUMMARY}

Regulatory interactions have been shown to occur between all the testicular cell types considered. The paracrine factors mediating these interactions generally influ-
ence either cellular growth or differentiation. The regulation of cellular growth is essential in the developing testis and is required for the maintenance of spermatogenesis in the adult testis. The rapid rate of germinal cell proliferation and the continuous but slowed growth of the peritubular cells and Leydig cells requires the presence of specific growth factors in the adult. Therefore, cell-cell interactions have evolved that involve growth factors such as IGF, TGF-α, TGF-β and NGF (Fig. 1). Other growth factors such as FGF or less characterized components like the seminiferous growth factor (SGF) also may be involved in the paracrine regulation of testis cell growth. An alternate cellular parameter to cell growth to consider is the regulation of cellular function and differentiation. A number of endocrine agents and locally produced paracrine factors have been shown to control and maintain testis cell function and differentiation (Fig. 1). Cell-cell interactions mediated by factors such as androgens, POMC peptides, and PModS are all primarily directed at the regulation of cellular differentiation. Therefore, the agents which mediate cell-cell interactions in the testis can generally be categorized into factors that regulate cell growth or those which influence cellular differentiation. The specific cell-cell interactions identified (Fig. 1) will likely be the first of a large number of cellular interactions yet to be investigated. Although a number of potentially important cell-cell interactions have been identified, future research will require the elucidation of the in vivo physiological significance of these interactions.

The existence of different cell types and potential cell-cell interactions in a tissue implies that the actions of an endocrine agent on a tissue will not simply involve a single hormone and single cell. The endocrine regulation of testis function will have effects on cell-cell interactions and be affected by local cell-cell interactions. The ability of LH to influence Leydig cell androgen production promotes a cascade of interactions mediated through several cell types to maintain the process of spermatogenesis. FSH actions on Sertoli cells also promote cell-cell interactions that influence germinal cell development, peritubular myoid cell differentiation and Leydig cell function. There-
fore, elucidation of the endocrine regulation of testis function requires an understanding of the local cell-cell interactions in the testis.

REFERENCES

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