Peritubular Myoid Cells in Testis

Eric E Nilsson and Michael K Skinner, Washington State University, Pullman, WA, United States

This is an update of Ryan P. Thompson, Eric E. Nilsson, Michael K. Skinner, Peritubular Myoid Cells in Testis, Editor(s): Michael K. Skinner, Encyclopedia of Reproduction (Second Edition), Academic Press, 2018, Pages 42–46, ISBN 9780128151457, https://doi.org/10.1016/B978-0-12-801238-3.64361-1.

Abstract

Peritubular myoid cells are cells located along the basal surface (outside wall) of the seminiferous tubules. They are responsible for the seminiferous tubules holding their shape and have contractile ability which promotes the transport of immotile sperm. There are notable differences between humans and rodents, with the number of layers of peritubular cells varying and the size of the extracellular matrix differing. Even after sexual maturity peritubular myoid cells continue the process of dividing. For this reason, growth regulation is needed even after reaching adulthood. Peritubular cell–Sertoli cell interactions are vital to the testis and are responsible for many of the components produced in the testis. TGF-β (transforming growth factor-beta) is an agent used by these cells to help drive the maintenance of spermatogenesis. Peritubular myoid cells have been found to regulate spermatogonial stem cells and Sertoli cell function. Leydig cell and peritubular myoid cell interactions are required for Sertoli cell development and function, indicating that peritubular myoid cells are vital to testis biology and function.

Key Points

- Peritubular Myoid Cells in Testis.
- Peritubular myoid cells serve structural functions, including through their contractile activity.
- They produce factors that regulate both somatic and germ cell behaviors in the testis.
- Peritubular myoid cells retain proliferative activity into adulthood.

Introduction

In men of good health, there are layers of flattened cells and extracellular matrix (ECM) proteins that make up the wall of the seminiferous tubules. The cells located along this wall are peritubular myoid cells, which have smooth muscle-like properties (Mayerhofer, 2013). Peritubular myoid cells fall under the classification of a mesenchymal/stromal cell type. They reside immediately exterior to the extracellular matrix (ECM) that is at the basal surface of the Sertoli cells in seminiferous tubules (Skinner, 1989). Peritubular cells have been found in all mammalian species looked at thus far, although there are differences in the numbers of layers of peritubular cells (Skinner and Griswold, 2005).

Peritubular myoid cells possess the ability to contract and use this feature to transport immotile sperm along the seminiferous tubules. Peritubular cells express growth factor receptors that respond to endothelins (ET), vasopressin, angiotensin II, and platelet-derived growth factor beta (PDGFβ), in turn leading to contractility of the seminiferous tubule (Skinner, 2005; Rossi *et al.*, 2002). Adrenomedullin stimulates receptors to inhibit contractility (Rossi *et al.*, 2000). Human testicular peritubular cells produce prostaglandin E2 (PGE2), and also have PGE2 receptors. Stimulation of PGE2 receptors induces human testicular peritubular cells to increase expression of smooth muscle protein, which are involved in contraction, and glial cell-derived neurotrophic factor (GDNF) involved in supporting spermatogenesis (Mayerhofer, 2020). Human testicular peritubular cells manufacture extracellular matrix components such as the proteoglycan decorin, thought to influence paracrine signaling between testicular cells. Peritubular cells, along with extracellular matrix proteins, form the walls of the seminiferous tubules. There are peritubular macrophages which are

interdigitated with peritubular myoid cells. These were only recognized relatively recently and shown to also be important in seminiferous tubule formation and function (DeFalco *et al.*, 2015; Heinrich and DeFalco, 2020).

There are distinct differences in peritubular cells between humans and rodents. In a human, there are several layers of stretched out, thin, spindle-shaped peritubular cells and extracellular matrix (ECM) proteins that form the wall of the seminiferous tubule. However, in a laboratory rodent this same wall is constructed of a single peritubular cell layer with a minimal extracellular matrix. No matter the model, mature peritubular cells express markers of smooth muscle-like cells. This expression of smooth muscle actin is induced around the age of sexual maturity in primates, primarily by androgens. Androgens are thought to play a critical role in the differentiation process that occurs in peritubular cells. After expression of smooth muscle actin is evoked, removing the androgen stimulation appears to have no effect (Mayerhofer, 2013). Peritubular myoid cells (PMCs) also provide structural support to seminiferous tubules. It is worth noting that the seminiferous tubular wall compartment in men who are subfertile or infertile with impaired spermatogenesis is often abnormal. Testicular tubular fibrosis, which is easily diagnosed by thickened deposits of extracellular matrix, is known as a clear sign of male infertility. Testicular tubular fibrosis and morphological alterations to peritubular cells, such as hypertrophy, likely suggest that particular capacities of peritubular cells, such as hormonal and contractile functions, are modified. **Figs. 1** and **2**. Observations clearly illustrate the differences between a healthy layer of peritubular cells in comparison to that in an infertile man (Mayerhofer, 2013).

Peritubular cells continue to divide throughout life and therefore need growth regulation even after adulthood (Skinner, 1989). Early in embryonic development during the time of testis determination, peritubular cells originate from mesenchymal cells within the mesonephros next to the developing gonad. Mesonephric cells travel into the gonad to form precursor peritubular cells that are part of the outer layer of what will become the seminiferous tubules, along with Sertoli cell and primordial germ cell aggregates (Skinner, 2005).

Sertoli Cell–Peritubular Cell Interactions

Previous studies suggest both peritubular cells and Sertoli cells produce Transforming growth factor α (TGF-alpha), which is capable of acting as an EGF (epidermal growth factor)-like substance to trigger peritubular cell growth. The assumption is made that TGF-α has an important role in controlling testis cell growth and mediates a peritubular cell–Sertoli cell interaction. Conversely, transforming growth factor-β (TGF- β) is a growth factor that can act as an inhibitor in these interactions. TGF- β is a family of gene products that promote cell differentiation and inhibit cell division, in particular in TGF-α responsive cells. TGF-β originates from peritubular cells as well as Sertoli cells. TGF-β was found to have no major effects on Sertoli cell function or growth. However, TGF-β blocked the ability of TGF-α to promote peritubular cell proliferation. TGF-β was also found to induce peritubular cells to form colonies in cell culture and heighten the production of extracellular matrix components. As a result, TGF-β is thought to likely play a part as a chemotactic agent for peritubular cells and impact seminiferous tubule morphogenesis (Skinner, 1989; Konrad *et al.*, 2000).

Peritubular cells express activin, a product that can act on Sertoli cells to promote prepubertal Sertoli cell proliferation. Peritubular cells express heregulins, also known as neu differentiation factors (NDF), such as NDFα and NDFβ, which act through receptors located on Sertoli cells (Skinner, 2005). In addition to these factors, peritubular cells express leukemia inhibitory factor (LIF), which has the ability to act on early-stage spermatogenic cells and likely also Sertoli cells (Piquet-Pellorce *et al.*, 2000). Several other potential paracrine factors produced by peritubular cells may influence Sertoli cell function (**Table 1**). The connection between Sertoli cells and peritubular cells and their ability to regulate one another was discovered as a result of androgens not being able to promote peritubular cell differentiation on their own, rather, the presence of gonadotrophin-stimulated Sertoli cells was necessary as well. A combination of Leydig cell and peritubular cell interactions with Sertoli cells have been noted as being essential to Sertoli cell development and function (Skinner, 2005).

Studies have shown that both Sertoli cells and peritubular myoid cells express androgen receptors (Norton *et al.*, 1994). It was found that the effect of androgens on Sertoli cells was greater in Sertoli cell aggregates in co-cultures containing peritubular cells as opposed to Sertoli cell preparations lacking peritubular cells. Therefore, it's postulated that the stimulatory effects of peritubular cells on functions of Sertoli cells in co-culture, specifically to sustain the production of androgen binding protein (ABP), are affected at least moderately by component(s) manufactured by peritubular cells and secreted into the medium. Peritubular cells accumulate androgens and react to testosterone *in vivo* in addition to in organ culture. Peritubular cells lack the ability to synthesize androgen binding protein (Skinner and Fritz, 1985; Thompson *et al.*, 1995). Peritubular cells in culture, when stimulated by androgens, secrete substances that maintain elevated rates of androgen binding protein production by Sertoli cells. It is important to note that co-culture of Sertoli cells with peritubular cells assists in maintaining the production of androgen binding protein (ABP) with or without the presence of follicle stimulating hormone (FSH).

After Sertoli cell ablation in an adult mouse testis, a single peritubular myoid cell layer with a standard appearance was found to encompass the majority of seminiferous tubules up to 1 year later. After 1 year, there was loss of integrity in the connective tissue of either the peritubular or interstitial regions. These findings indicate that seminiferous tubule remodeling occurs over a long period of time. It is postulated that the remodeling process incorporates several different cell types such as peritubular myoid and interstitial cells. Although the presence of the basement membrane is able to preserve peritubular myoid cell fate for many months following Sertoli cell ablation, the Sertoli cells are vital to maintaining routine peritubular myoid cell function. It is apparent that peritubular myoid cells regulate immune access to the seminiferous epithelium. It

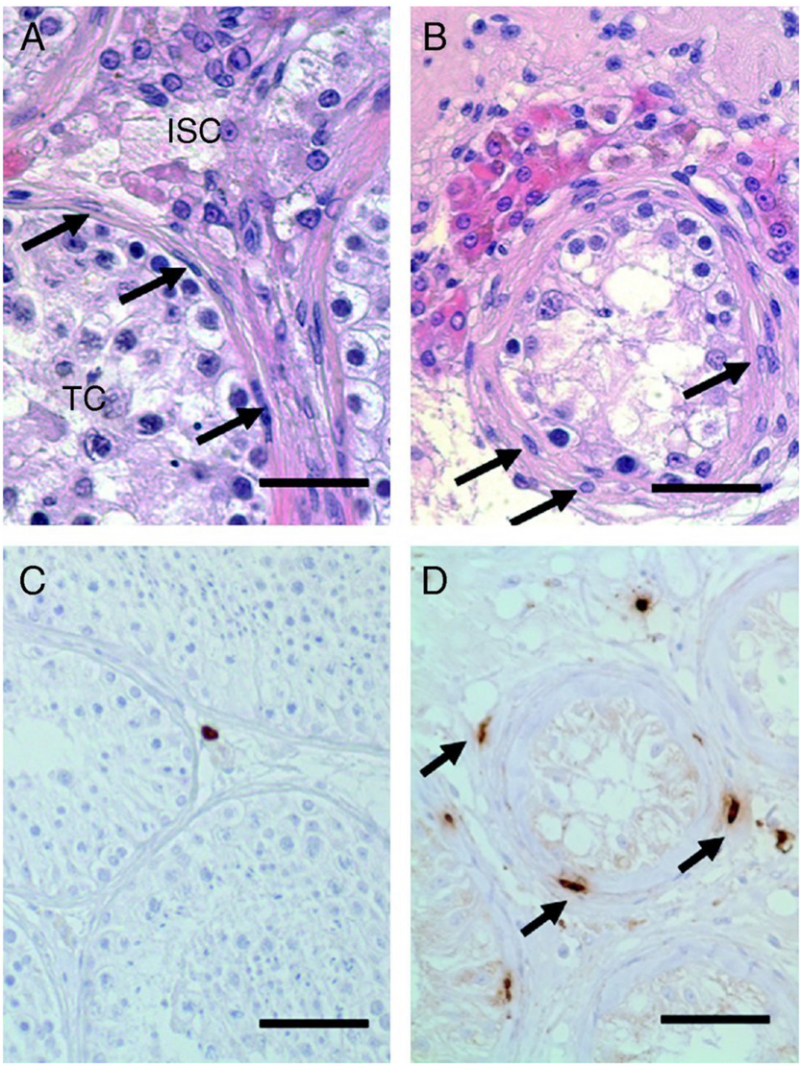

Fig. 1 Testicular morphology and composition of the tubular wall in the adult human testis in health (normal spermatogenesis; A and C) and disease (infertility B and D). (A) The tubular wall (*arrows*) is normally an inconspicuous compartment, in which only few nuclei of peritubular cells are visible in hematoxylin and eosin (H&E)-stained sections. Note the close proximity of the tubular wall to the tubular compartment (TC) containing Sertoli cells and germ cells and to the interstitial compartment (ISC) with Leydig cells. Bar: ~30 μm. (B) In samples from infertility patients, the tubular wall is often enlarged, deposits of ECM are seen, and both elongated and round peritubular cell nuclei (*arrows*) are seen in the thickened wall. H&E stain. Bar ~30 μm. (C and D) Immunohistochemical identification of mast cells containing tryptase, in a normal testis (C) and in a testicular biopsy of an infertility patient (D). Note that in the section of the normal testis, only one mast cell is present in the interstitial compartment (C). In contrast, in the section from the infertility patient, several mast cells are seen and accumulate in the wall of seminiferous tubules (*arrows* in D) Bar ~60 μm. From Mayerhofer, A., 2013. Human testicular peritubular cells: More than meets the eye. Reproduction 145, R107–16.

was noted that where macrophage invasion of the tubule transpired, peritubular myoid cell morphology was changed, however, it is not known whether this was responsible for causing the macrophage invasion or just a repercussion (Rebourcet *et al.*, 2014).

Studies in rodents have revealed that when Sertoli cells were not present the peritubular myoid cells were not able to form a functional barrier to biotin. This result points towards Sertoli cells being able to support the barrier properties of peritubular myoid cells. When Sertoli cells are not present, the peritubular myoid cells and underlying extracellular matrix appear to be adequate for keeping macrophages out of most tubules. Therefore, it is thought that peritubular myoid cells mediate the immune privilege of the seminiferous tubules (Rebourcet *et al.*, 2014).

During fetal and neonatal life Sertoli cells are crucial to maintaining seminiferous tubular structure and the preservation of peritubular myoid cell phenotype. However, as an animal enters puberty, Sertoli cells are not necessary to retain seminiferous tubule structure as well as peritubular myoid cell differentiated function, since at this stage the basement membrane is capable of

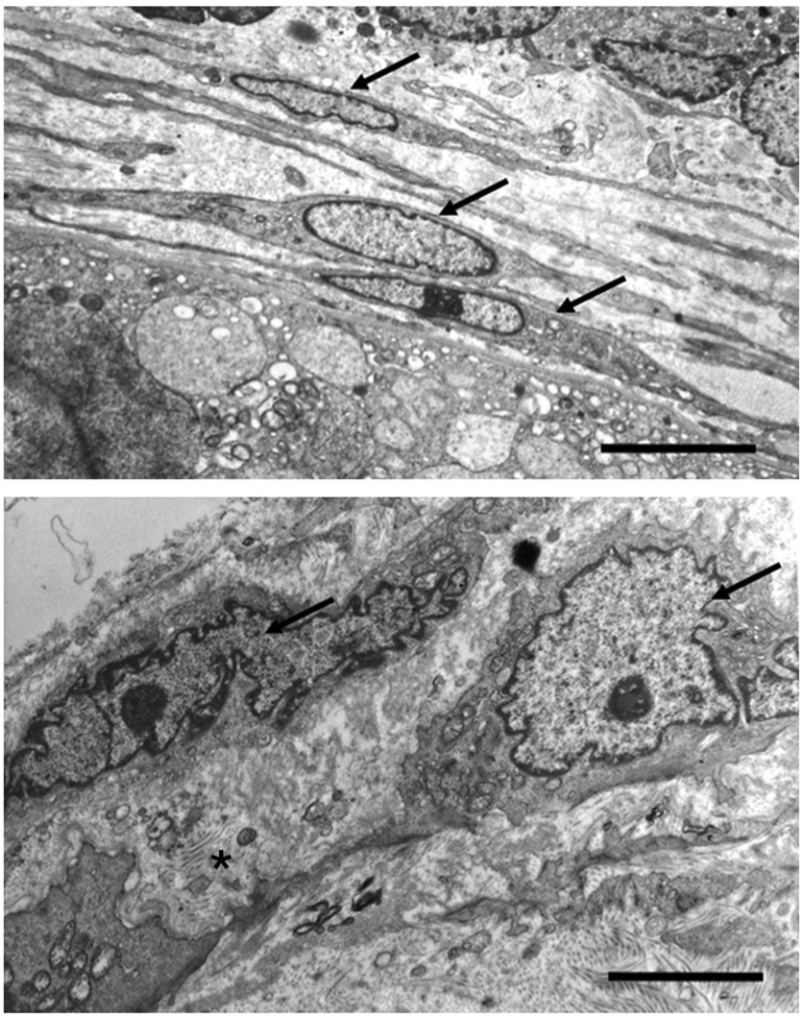

Fig. 2 Electron microscopy of human peritubular cells in the wall of seminiferous tubules. (Top) In the healthy adult, several layers of thin, peritubular cells (*arrows* at nuclei) and ECM form the wall of seminiferous tubules. Bar: ~3 μm. (Bottom) The morphology of the tubular wall of an infertility patient reveals cellular hypertrophy (*arrow* at nucleus) and increased ECM (*asterisk*). Bar: ~4 μm. These micrographs are taken with permission from **Fig. 2** of our previous paper (Schell *et al.*, 2010). Copyright 2010, reproduced with permission from The Endocrine Society. From Mayerhofer, A., 2013. Human testicular peritubular cells: More than meets the eye. Reproduction 145, R107–16.

supporting these functions. Previous developmental studies have indicated that Sertoli cells are fundamental at all ages to the upkeep of all germ cell populations, including spermatogonia (Rebourcet *et al.*, 2014).

Signaling Pathways

Peritubular cells generate the paracrine factor known as PModS that is responsible for mediating mesenchymal–epithelial interactions and regulating Sertoli cell functions necessary for the process of spermatogenesis. PModS has been observed to stimulate cGMP levels in Sertoli cells and sustain heightened cGMP levels in culture for as long as 5 days. There are two different isoforms of PModS that have been isolated, PModS (A) and PModS (B), although it appears there is little difference between the two and they share the same apparent biological activities. PModS (A) is a processed form of PModS (B). PModS has the ability to affect tyrosine phosphorylation of Sertoli cell proteins, and blockage of tyrosine phosphorylation puts an end to the activity of PModS on Sertoli cells. The gonadotrophin follicle stimulating hormone (FSH) and the testicular paracrine factor PModS promote actions affiliated with a more differentiated state of the Sertoli cell, such as the secretion of androgen-binding protein and transferrin. PModS has been shown to raise Sertoli cell cGMP levels, however, cGMP does not directly influence the actions of PModS. Signaling mechanisms such as cAMP levels, calcium uptake, and phosphatidyl inisitol hydrolysis, were not affected by the paracrine factor PModS. Findings suggest that PModS potentially shapes an important phosphorylation event. PModS has been observed to have an impact on the tyrosine

Table 1 Major Sertoli cell and peritubular cell paracrine regulatory products

Potential paracrine factors	*Site production*	*Site action*	*Action/proposed function*
PModS	Peritubular	Sertoli	Paracrine regulatory agent
TGF-α	Peritubular	Sertoli	Growth stimulation EGF-like
TGF-β	Both	Both	Growth inhibition
TGF-I	Both	Both	Maintenance cell growth/differentiation
NDFα/NDFβ	Peritubular	Sertoli	Increase differentiation
LIF	Peritubular	Gonia/Sertoli	Growth stimulation
Activin	Peritubular	Sertoli	Growth stimulation
NT3	Sertoli	Peritubular	Embryonic chemotactic factor
bFGF	Sertoli	Peritubular	Growth stimulation

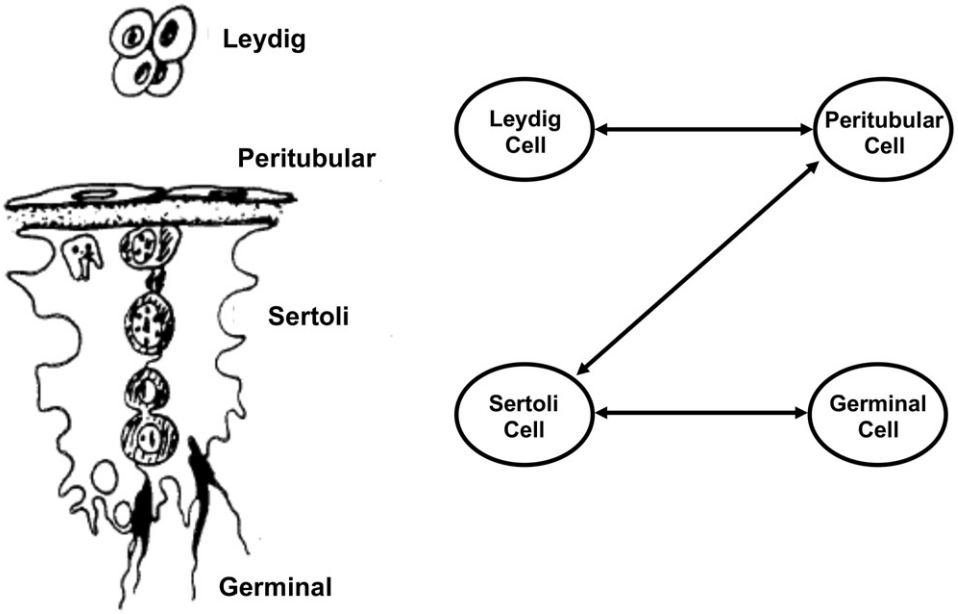

Fig. 3 Diagram of testis cell associations and the interactions between cells. From Skinner, M.K., 1989. Peritubular myoid cell–Sertoli cell interactions which regulate testis function and growth. Perspectives in Andrology, 175–182.

phosphorylation of a Sertoli cell protein(s) following a 72-h treatment. It is not yet determined whether these tyrosine phosphorylations are correlated with the increased differentiation of the cell and/or influence the actions of PModS (Norton *et al.*, 1994).

Androgen Receptors

Peritubular cells react to androgens by heightening the production of PModS (Anthony and Skinner, 1989). Mice with a targeted disruption of the androgen receptor gene (*Ar*) in peritubular myoid cells exhibit a continuous loss of spermatogonia. This finding led researchers to suggest that peritubular myoid cells depend on testosterone (T) action to generate factors influencing the spermatogonial stem cell in its niche. The addition of testosterone to adult mouse peritubular myoid cells in vitro was found to result in considerable spikes in the amount of glial cell line-derived neurotrophic factor (GDNF) mRNA and protein. The findings revealed that testosterone has the ability to control the production of glial cell line-derived neurotrophic factor by peritubular myoid cells *in vitro* and support the hypothesis that peritubular myoid cells have a major effect on the microenvironment of the niche and spermatogonial stem cell maintenance (Chen *et al.*, 2014; Potter and DeFalco, 2017).

Lgr4 Role in the Maintenance of Spermatogenesis

A study in mice found that Leucine-rich repeat G-protein-coupled receptor 4 (Lgr4) is selectively expressed in peritubular myoid cells. Knockout of Lgr4 leads to germ cells going into arrest at meiosis I, followed by apoptosis. The levels of androgen receptor, alpha-smooth actin and extracellular matrix proteins were substantially lowered in Lgr4 mutant ($Lgr4^{1}-$) mice. Sertoli cell nucleus

position as well as functional protein expression differed in Lgr4$^{1}-$ mice. Wnt/β-catenin signaling was disabled in the peritubular myoid cells of Lgr4$^{1}-$ mice. After Wnt/β-catenin was reactivated through breeding with Apc$^{min/+}$ mice or by Gsk3β inhibitor treatment, the deficiency of Lgr4 was incompletely rescued. These findings, as a whole, indicate that peritubular myoid cell Lgr4 signaling by means of Wnt/β-catenin is critical to spermatogenesis (Qian *et al.*, 2013).

Connection to Male Fertility

Spermatogonial stem cells (SSCs) are essential for lifelong spermatogenesis. In men, human testicular peritubular myoid cells (HTPCs) are thought to partly responsible for regulating the overall testicular microenvironment through the actions of secreted proteins. Similar to observations from studies of mice, there is evidence that human peritubular myoid cells also play a critical role in SSC niches in men. Human peritubular cells have been shown to supply several neurotrophic factors, such as glial cell line derived neurotrophic factor (GDNF). GDNF is essential for the renewal of SSCs throughout species. GDNF is a factor that comes from both peritubular cells and Sertoli cells. Due to the proximity of peritubular cells to spermatogonial stem cells, peritubular cells, by the way of GDNF, regulate spermatogonial stem cells in a paracrine manner (Chen *et al.*, 2016).

Conclusions

Peritubular cells of the seminiferous tubule wall are important to the spermatogonial stem cells (SSC) niche in the human testis. Peritubular cells are also thought to be a part of the general regulation of testicular functions by producing elements which affect Sertoli cells and Leydig cells (Wen *et al.*, 2016; Parekh *et al.*, 2019). **Fig. 3** indicates the relationship between three of the major cell types found in the testis (Skinner, 1989). In summary, peritubular myoid cells have a critical function for testis biology and it is the cellular interactions of all cell types that is required.

References

Anthony, C.T., Skinner, M.K., 1989. Cytochemical and biochemical characterization of testicular peritubular myoid cells. Biology of Reproduction 40, 811–823.

Chen, L.Y., Brown, P.R., Willis, W.B., Eddy, E.M., 2014. Peritubular myoid cells participate in male mouse spermatogonial stem cell maintenance. Endocrinology 155, 4964–4974.

Chen, L.-Y., Willis, W.D., Eddy, E.M., 2016. Targeting the Gdnf Gene in peritubular myoid cells disrupts undifferentiated spermatogonial cell development. Proceedings of the National Academy of Sciences of the United States of America 113 (7), 1829–1834. 16.

DeFalco, T., Potter, S.J., Williams, A.V., *et al.*, 2015. Macrophages contribute to the spermatogonial niche in the adult testis. Cell Reports 12 (7), 1107–1119.

Heinrich, A., DeFalco, T., 2020. Essential roles of interstitial cells in testicular development and function. Andrology 8 (4), 903–914.

Konrad, L., Albrecht, M., Renneberg, H., Aumüller, G., 2000. Transforming growth factor-beta2 mediates mesenchymal-epithelial interactions of testicular somatic cells. Endocrinology 141 (10), 3679–3686.

Mayerhofer, A., 2013. Human testicular peritubular cells: More than meets the eye. Reproduction 145, R107–R116.

Mayerhofer, A., 2020. Peritubular cells of the human testis: Prostaglandin E2 and more. Andrology 8 (4), 898–902.

Norton, J.N., Vigne, J.L., Skinner, M.K., 1994. Regulation of Sertoli cell differentiation by the testicular paracrine factor PModS: Analysis of common signal transduction pathways. Endocrinology 134, 149–157.

Parekh, P.A., Garcia, T.X., Hofmann, M.-C., 2019. Regulation of GDNF expression in Sertoli cells. Reproduction 157 (3), R95–R107.

Piquet-Pellorce, C., Dorval-Coiffec, I., Pham, M.D., Jegou, B., 2000. Leukemia inhibitory factor expression and regulation within the testis. Endocrinology 141, 1136–1141.

Potter, S.J., DeFalco, T., 2017. Role of the testis interstitial compartment in spermatogonial stem cell function. Reproduction 153 (4), R151–R162.

Qian, Y., Liu, S., Guan, Y., *et al.*, 2013. Lgr4-mediated Wnt/beta-catenin signaling in peritubular myoid cells is essential for spermatogenesis. Development 140, 1751–1761.

Rebourcet, D., O'shaughnessy, P.J., Monteiro, A., *et al.*, 2014. Sertoli cells maintain Leydig cell number and peritubular myoid cell activity in the adult mouse testis. PLoS One 9, e105687.

Rossi, F., Guerrini, L., Pasimeni, G., *et al.*, 2000. Testicular peritubular myoid cells are a target for adrenomedullin. Archives of Andrology 44 (2), 103–107.

Rossi, F., Ferraresi, A., Romagni, P., Silvestroni, L., Santiemma, V., 2002. Angiotensin II stimulates contraction and growth of testicular peritubular myoid cells in vitro. Endocrinology 143 (8), 3096–3104.

Schell, C., Albrecht, M., Spillner, S., *et al.*, 2010. 15-Deoxy-delta 12-14-prostaglandin-J2 induces hypertrophy and loss of contractility in human testicular peritubular cells: Implications for human male fertility. Endocrinology 151, 1257–1268. doi:10.1210/en.2009-1325.

Skinner, M.K., 1989. Peritubular myoid cell–Sertoli cell interactions which regulate testis function and growth. Perspectives in Andrology. 175–182.

Skinner, M.K., 2005. Sertoli cell-somatic cell interactions. In: Griswold, M.D., Skinner, M.K. (Eds.), Sertoli Cell Biology. San Diego, CA: Elsevier-Academic Press.

Skinner, M.K., Fritz, I.B., 1985. Androgen stimulation of Sertoli cell function is enhanced by peritubular cells. Molecular and Cellular Endocrinology 40, 115–122.

Skinner, M.K., Griswold, M.D., 2005. Sertoli Cell Biology. San Diego, CA: Elsevier-Academic Press.

Thompson, E.W., Blackshaw, A.W., Raychoudhury, S.S., 1995. Secreted products and extracellular matrix from testicular peritubular myoid cells influence androgen-binding protein secretion by Sertoli cells in culture. Journal of Andrology 16 (1), 28–35.

Wen, Q., Wang, Y., Tang, J., Cheng, C.Y., Liu, Y.X., 2016. Sertoli cell Wt1 regulates peritubular Myoid cell and fetal Leydig cell differentiation during fetal testis development. PLoS One 11, e0167920.