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Emerging Role for Mammalian Target of Rapamycin in Male Fertility

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Abstract

Male fertility is modulated by environmental, endocrine, paracrine, and metabolic cues. Mammalian target of rapamycin (mTOR) coordinates many cellular events in response to those signals. Here, we discuss how the mTOR pathway integrates and mediates signals throughout the male reproductive system, acting as a central player in the control of spermatogenesis.

mTOR and Male Fertility: From Concept to Clinical Evidence

mTOR is a highly conserved multifunctional serine/threonine protein kinase that regulates protein synthesis, cellular growth, proliferation, motility and survival, as well as autophagy. It associates with different proteins such as Raptor and Rictor, to form two complexes, mTORC1 and mTORC2, respectively. These two complexes display different sensitivity to the inhibitor rapamycin; with mTORC1 being acutely inhibited by rapamycin, whereas mTORC2 inhibition requires prolonged treatment and is cell/tissue dependent. mTORC1 responds to multiple signals to control protein and lipid synthesis, autophagy, among other processes, while mTORC2 regulates cytoskeletal function and insulin signaling (reviewed in [1]). Thus, mTOR orchestrates and integrates signals that control fundamental processes of cellular physiology. Despite the extensive work already done on mTOR signaling, its functions and how they are coordinated remain elusive. However, rapamycin has shown potential to extend lifespan in mammals, and treat cancer and age-related diseases. mTOR inhibitors are widely used as immunosuppressants and anticancer agents but unfortunately they come with numerous negative side effects including a phenotype of compromised fertility in men.

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A role for mTOR in male reproductive physiology was originally proposed when male patients treated with sirolimus (rapamycin analog) displayed reversible infertility. Those patients presented lower testosterone production by Leydig cells (which was later shown to also occur *in vitro*), resulting in increased systemic follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels [via upregulation of the hypothalamus–pituitary–gonad (HPG) axis], and reduced sperm counts [2]. Withdrawal of sirolimus treatment improved sperm parameters and sex hormone levels [2]. These first case reports suggested that the use of rapamycin analogs affected the HPG axis, although the exact mechanisms remain unclear.

mTOR, Testis Physiology and Sertoli Cell (SC) Function

Recent studies highlighted that mTOR is involved in critical mechanisms in testis physiology. mTOR and its associated partner proteins are expressed in both germ (particularly in spermatogonia) and somatic (Sertoli and Leydig) cells within the testes, with mTORC1 and mTORC2 being differentially present in all these cellular types [3–6]. In spermatogonia, *in vitro* exposure to rapamycin results in decreased proliferation through blockage of the mTOR/p70s6k pathway, suggesting a role for mTORC1 in the maintenance of the germinal cell line pool [6]. *In vivo* studies have shown that inhibition of mTORC1 by rapamycin leads to atrophy and vacuolization of seminiferous tubules, and is associated with reduced sperm production, further suggesting a role for mTORC1 in spermatogenesis [6]. mTORC1-deficient mice, due to the constitutive deletion of Raptor (*Raptor fl/fl* KspCre* mice), present spermatozoa with decreased motility, suggesting that mTORC1, besides controlling glandular size and seminal vesicle fluid composition, also regulates sperm physiology during passage through the epididymis [5].

The somatic SCs are known as nurse cells because they sustain germ cell development during spermatogenesis. Adjacent SCs also establish the Sertoli/blood–testis barrier (BTB). SCs are under strict hormonal and paracrine control and integrate most of the signals and pathways that control spermatogenesis (reviewed in [7]). These cells express mTORC1 and mTORC2. mTORC1 is involved in FSH-mediated proliferation of *in vitro* cultured immature SCs, via the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signal transduction pathway, which is known to promote cell proliferation [8]. mTORC1 also regulates the redox balance of *in vitro* cultured human SCs, and modulates the nutritional support that these cells provide to developing germ cells [9]. In addition, mTORC2 is involved in the control of BTB dynamics during spermatogenesis. *In vitro* studies have shown that mTORC1 and mTORC2 have opposing effects on BTB dynamics, with rpS6 (a pivotal downstream effector of mTORC1) promoting its opening by making the BTB leaky, whereas Rictor promotes BTB integrity by making the BTB tighter [4,10]. Juvenile mice with conditional knockout of mTOR (*MTOR^{fllox/flox}; Amhr2^{cre/+}*) in SCs showed impaired organization of the seminiferous epithelium and loss of SC polarity, with a redistribution of BTB gap junction protein-1 (GJA1). This results in increased germ cell apoptosis, decreased sperm quality, and infertility [11]. Thus, specific inactivation of the mTOR pathways in SCs blocks their capacity to sustain spermatogenesis. Moreover, deregulated activation of mTORC1 in SCs of mice with conditional deletion of the *Lkb1* gene (*Lkb1^{cko}*) results in germ cell loss and a phenotype of SC only tubules in adult mice. mTOR signaling is negatively regulated by liver kinase (LK) B1, and upregulation of mTORC1 activity caused

by loss of LKB1 leads to an increase in SC proliferation, with remarkable defects in SC polarity and localization of junctional complexes in young mice (with these latter effects being suggested as side effects of excessive SC proliferation) [12]. Short-term *in vivo* postnatal administration of rapamycin does not affect SC numbers, arrangement, or morphology in prepubertal mice [3], although the functional characteristics of these cells have not been evaluated. Hence, further studies are needed to evaluate the role of mTOR in testis physiology and how it controls SC function, in particular, cell proliferation and differentiation.

mTOR and Transcriptional and Translational Control of Spermatogenesis

The expression of several key genes during spermatogenesis is tightly regulated to allow germ cell differentiation versus maintenance. Retinoic acid (RA) regulates several such genes and is required for spermatogonial differentiation and entry into meiosis. A recent report demonstrates that RA stimulates the PI3/AKT/mTOR signaling pathway. *In vivo* inhibition of mTORC1 by rapamycin blocks the RA-dependent gene expression and stimulation of kinase pathways; both essential for germ cell development, resulting in accumulation of undifferentiated spermatogonia. These findings illustrate that mTORC1 activation is required for spermatogonia differentiation prior to meiotic initiation and reveals a crucial *in vivo* mechanism by which mTORC1 may control male fertility [3].

Activation of mTOR stimulates protein synthesis by causing the release of the translational repressor eukaryotic translation initiation factor (eIF) 4E-binding protein (4E-BP)1 from eIF4E, and recruitment of eIF4G. External cues (such as temperature and growth factors) known to modulate mTOR activity and expression, have distinct effects on meiotic and haploid cells [13]. eIF4G and poly(A)-binding protein (PABP)1 are more abundant in postmeiotic cells, while the expression of mTOR and 4E-BP1 decreases during spermatogenesis [13]. In addition, *in vivo* labeling shows that pachytene spermatocytes display higher rates of protein synthesis (a process partially mediated by mTOR) than haploid spermatids. Formation of the eIF4F complex is also enhanced by mTOR in pachytene spermatocytes, but not in round spermatids. Interestingly, translational regulation is differentially dependent on the mTOR pathway in spermatocytes and spermatids, suggesting that the translation initiation complex assembles during the late stages of germ cell differentiation [6]. This illustrates that mTOR may mediate the specific somatic–germ cell interactions that occur during those stages. Treatment of highly pure and viable spermatocytes with rapamycin suppresses the activity of mTOR and phosphorylates p70S6 kinase, but not that of phosphorylated 4E-BP1, and modulates expression of spermatocyte-specific miRNA [14]. These data suggest that mTOR also mediates the tight regulation that occurs at each stage. The targets of mTOR (p70S6K, rpS6, and 4E-BP1) are also downregulated with age [6], suggesting an association of the mTOR pathway with aging-related decline in male fertility; a hypothesis that warrants further investigation.

Deletion of mitochondrial elongation factor (mtEF)4, in mice results in dysfunction in oxidative phosphorylation in the testis, and male infertility [15]. Deletion of mtEF4 accelerates mitochondrial translation of unstable proteins. This is compensated by mTOR activation in somatic tissues to increase and match cytoplasmic to mitochondrial translation.

Because the mTOR signaling is downregulated in spermatogenic cells, accelerated mitochondrial translation thus leads to cell cycle arrest and apoptosis [15]. This work provides evidence of the involvement of mTOR in mitochondrial translation and how this may compromise male fertility.

Concluding Remarks

Compelling evidence suggests that both mTORC1 and mTORC2 play key roles in the transcriptional and translational control of genes during spermatogenesis; the post-translational mechanisms responsible for sperm maturation; the BTB dynamics that allow preleptotene spermatocytes entering the adluminal compartment to prepare for meiosis; and the nutritional support of spermatogenesis (summarized in Figure 1). These crucial events are under metabolic and endocrine control and determine the reproductive potential of males. Both mTORC1 and mTORC2 have been implicated in several testicular functions and in the control of male fertility [2–5]. However, additional studies are needed to define the specific roles for each complex in the male reproductive system. In addition, as disruption of mTORC2 requires prolonged exposure to rapamycin, this creates a therapeutic window through which mTORC1 inhibition can be specifically targeted. The development of highly specific inhibitors that may target or improve male fertility relies on the thorough understanding of the molecular mechanisms underlying the differential sensitivity of cells and tissues of the male reproductive system to mTORC1 and mTORC2 inhibition. This will unleash potential therapies for male fertility or development of male contraceptives based on manipulating mTOR signaling pathways.

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Figure 1. Schematic Illustration Showing the Known Effects of mTOR Inhibition or Absence on Testicular Cells and Spermatogenesis

The mTOR pathway is associated with cell-specific effects throughout the seminiferous tubules and in spermatozoa. mTOR inhibition/absence in Leydig cells decreases testosterone production, while in SCs, it increases vacuolization and decreases proliferation. In addition, mTOR regulates SC redox balance and metabolic activity, which has implications in the nutritional support of spermatogenesis. The Sertoli/BTB dynamics and permeability are also under strict control by mTOR. During spermatogenesis, mTOR also mediates the transcriptional and translational control of genes, particularly in germ cells. mTOR inhibition/absence results in decreased differentiation, decreased miRNAs expression and higher apoptosis levels in germ cells. Notably, mTOR inhibition/absence results in decreased sperm production and motility. Though the detailed molecular mechanisms by which mTOR integrates and mediates these effects remain largely unknown, it appears that this signaling pathway is emerging as a master rheostat of male fertility. Both mTORC1 and mTORC2 have been implicated in the control of male fertility but their separate contribution or interaction throughout the male reproductive tracts remains a matter of debate and an attractive field of research for years to come. Abbreviations: BTB, blood–testis barrier; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; SC, Sertoli cell.