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INVITED REVIEW

Sperm Biology

Peroxisome proliferator-activated receptor gamma signaling in human sperm physiology

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Peroxisome proliferator-activated receptor gamma (*PPARγ*) is a member of the *PPARs*, which are transcription factors of the steroid receptor superfamily. *PPARγ* acts as an important molecule for regulating energy homeostasis, modulates the hypothalamic-pituitary-gonadal (HPG) axis, and is reciprocally regulated by HPG. In the human, *PPARγ* protein is highly expressed in ejaculated spermatozoa, implying a possible role of *PPARγ* signaling in regulating sperm energy dissipation. *PPARγ* protein is also expressed in Sertoli cells and germ cells (spermatocytes). Its activation can be induced during capacitation and the acrosome reaction. This mini-review will focus on how *PPARγ* signaling may affect fertility and sperm quality and the potential reversibility of these adverse effects.

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INTRODUCTION

Peroxisome proliferator-activated receptor gamma (*PPARγ*) was originally named for its ability to induce hepatic peroxisome proliferation in mice in response to xenobiotic stimuli.[1] It belongs to the nuclear hormone receptor superfamily of ligand-activated transcription factors. The *PPAR* family consists of three primary subtypes, *PPARγ*, *PPARβ/δ* and *PPARγ*, which are encoded by separate genes.[2] These receptors play a central role in the physiological processes that have an impact on lipid homeostasis, inflammation, adipogenesis, reproduction, wound healing, and carcinogenesis.[3–5] *PPARγ* is also implicated in a wide variety of cellular functions and regulates the expression of gene networks required for cell proliferation, differentiation, morphogenesis and metabolic homeostasis. It is possible to hypothesize that *PPARγ* potentially activates lipogenic genes and adipocyte differentiation.[6–8] *PPARγ* is highly expressed in adipose tissue[9] and it is necessary for adipocyte differentiation and transformation of many nonadipogenic cell lines into adipocyte-like cells. *PPARγ* is also an important transcriptional regulator that modulates cellular glucose and lipid metabolism.[10] Intensive studies and compelling evidence have demonstrated that *PPARγ* is a link between energy metabolism and reproduction, as in male infertility because of obesity, which is frequently associated with insulin resistance.[11]

Thorough studies have demonstrated a close link between energy status and reproductive functions.[12] In mice, loss of the *PPARγ* gene in oocytes and granulosa cells results in impaired fertility.[13] Moreover, Aquila *et al*. have demonstrated that human spermatozoa express *PPARγ* protein and investigated its functions.[14] Recently, repetition of thorough studies have indicated that sperm cells express various receptor types,[15,16] and also produce their ligands, suggesting that an autocrine short loop may modulate sperm cell's function independently by systemic regulation.[17,18] Nevertheless, it is necessary for spermatozoa to regulate their metabolism to affect the changes in signaling pathways encountered during their life. However, the mechanisms underlying the signaling events associated with the change in sperm energy metabolism are, to date, poorly understood.

Here, we will briefly review the mechanisms of sperm physiology, determining whether *PPARγ* signaling affects sperm capacitation and the possible targets of therapy of male infertility. *PPARγ* agonists may be used in artificial insemination or other biotechnologies, including cryopreservation.

EXPRESSION AND PUTATIVE ROLES OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA SIGNALING IN THE REPRODUCTIVE TISSUES

Hypothalamic-pituitary-gonadal axis

Early studies elucidated that adenoma cells can suppress the proliferation of pituitary cells,[19] and the administration of thiazolidinediones (TZDs) inhibits the development of pituitary adenomas in mice and man. Furthermore, in the pituitary gland of mice, the expression of *PPARγ* is reduced by 54% after 24 h of food restriction.[20] In the hypothalamus, *PPARγ* regulates a variety of molecules involved in energy homeostasis,[20] mainly playing a role in temperature regulation through its natural ligand 15-deoxy-delta12, 14-prostaglandin J2 (PGJ2), which is secreted into cerebrospinal fluid.[21] It is still unclear

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whether the effect of *PPARγ* on reproductive function is mediated by this signal pathway. Some pituitary tumors secrete hormones such as prolactin (*PRL*) and growth hormone (*GH*). In most of PRL- and *GH*-secreting pituitary tumors, these hormones control tumor growth or induce tumor shrinkage.[22] Moreover, pituitary *PPARγ* is abundantly expressed in human *PRL*-, *GH*-secreting, and nonfunctioning pituitary tumors.[19] Conditional knockout of *PPARγ* in pituitary gonadotrophs causes an increase in luteinizing hormone levels in female mice, a decrease in follicle-stimulating hormone (*FSH*) in male mice, and a fertility defect in knockout mice characterized by reduced litter size.[23] Moreover, it has been reported that *PPARγ* functions are regulated by *FSH* through mitogen-activated protein kinase (*MAPK*) signaling pathways (**Figure 1**).[24] Thus, it is suggested that *PPARγ* signaling participates in the regulation of pituitary hormones.

In the testis, the *PPARs* are expressed in both somatic and germ cells.[25] *PPARα* and *PPARβ* are widely expressed in the interstitial Leydig cells and the seminiferous tubule cells (Sertoli and germ cells),[26] whereas, *PPARγ* is believed to be restricted to Sertoli cells.[27] Sertoli cells are the first cells to differentiate recognizably in the undifferentiated fetal gonad, an event, which enables seminiferous cord formation, prevention of germ-cell entry into meiosis, differentiation, and function of Leydig cells.[28] During puberty, Sertoli cells also play vital roles in supporting spermatogenesis. Without the physical and metabolic support of Sertoli cells, germ-cell differentiation, meiosis and transformation into spermatozoa would not occur.[29] Moreover, Thomas *et al*. have recently detected *PPARγ* mRNA in the germ cells (spermatocytes).[30] It may be that *PPARγ* signaling regulates the pattern of expression of key lipid and glucose metabolic genes in the Sertoli cells.

Spermatogenesis

Spermatogenesis is the successful transformation of round spermatids into the complex structure of the spermatozoon (**Figure 2a**).[31] However, the physiological demands of reproduction are energetically costly and mating behavior and physiological responses are inhibited when fuel reserves or food intake is limited. Indeed, inadequate metabolic fuel utilization is the common factor of nutritional infertility.[32] Of the sources of stored energy that can be tapped for fuel

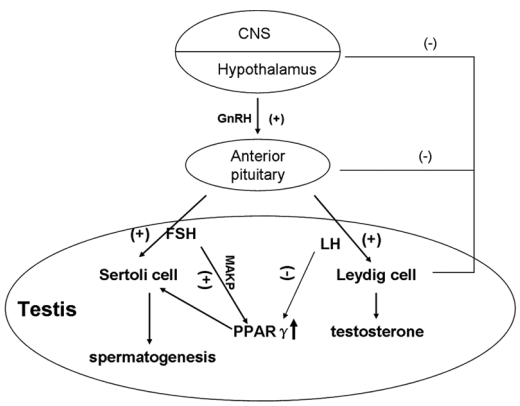

Figure 1: *PPARγ* functions in hypothalamic-pituitary-gonadal axis. Pulsatile GnRH production signals gonadotroph cells in the anterior pituitary to produce *FSH* and *LH* that then act on the testis to regulate spermatogenic potential. *FSH* up-regulates the expression of *PPARγ* through *MAPK* signaling pathways while *LH* inhibits the function of *PPARγ* via various pathways. High expression of testosterone suppresses the secretion of *LH* by negative feedback, providing a relatively persistent high-expression of *PPARγ*. *PPARγ*: peroxisome proliferator-activated receptor gamma; *FSH*: follicle-stimulating hormone; *LH*: luteinizing hormone; *MAPK*: mitogen-activated protein kinase.

reproductive energy requirements, the largest depot is white adipose tissue (WAT), which is primarily composed of white adipocytes that store lipid fuels as triacylglycerols.[33–35] Epididymal WAT (EWAT) is necessary for normal spermatogenesis and could produce a locally acting factor responsible for maintaining spermatogenesis since a decrease in EWAT causes a disturbance in spermatogenesis.[34,36] However, removal of comparable amounts of WAT from other sites (inguinal) shows no effect, disproving the idea that the effect is due to a decreased energy supply or the need for some minimal amount of fat.[37] It has been suggested that it might be due to the presence of a local, but currently unidentified, growth or nutritive factor from EWAT that promotes spermatogenesis. *PPARγ*, known as one of the master regulators in adipogenesis, is also developmentally expressed both in differentiating germ and Sertoli cells,[27,30] where it is involved in regulating the patterns of expression of key lipid metabolic genes in Sertoli cells.[30] It is also indicated that *PPARγ* signaling plays an important role in spermatogenesis.[30]

Mechanisms of peroxisome proliferator-activated receptor gamma signaling in spermatogenesis

The *PPARs* form obligate heterodimers with the retinoid X receptors (*RXRs*) to produce functional transcription factors that are involved in transactivation of several key genes during energy homeostasis and cellular differentiation (**Figure 2b**).[30,38–40] TZDs, the synthetic ligands of *PPARs*, have been demonstrated to modify *PPAR*-mediated transcriptional activation of a number of key genes involved in energy homeostasis.[41] Furthermore, Thomas *et al*. have demonstrated that *PPAR* and *RXR* transcripts encoding members of the *PPAR* and *RXR* nuclear receptor family reach maximum levels of expression in the germ cells during the early meiotic stages of spermatogenesis.[30] *PPARγ* levels peak at a slightly later stage of spermatogenesis in leptotene/zygotene spermatocytes, concomitant with increased levels of *RXRβ* and *RXRγ* expression. *PPARγ*/*RXRγ* heterodimeric transcription factor complexes, the predominant transcripts expressed in mature Sertoli cells, up-regulate lipid metabolic target genes in Sertoli cells, providing them with enough energy to support spermatogenesis. Infertility occurs if there is an interruption of the spermatogenic program.[42,43] In addition, male fertility can be compromised by inactivation of genes involved in lipid metabolism.[44] In summary, except for its role in spermatogenesis, *PPARγ* participates in fertilization by supporting energy provision.

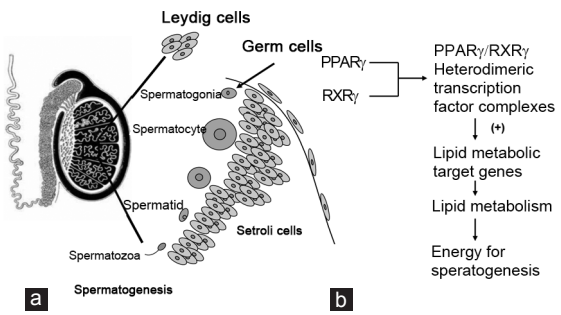

Figure 2: Expression patterns of PPARg shows in the testis. (**a**) In the testis, *PPARγ* protein is detected at high expression in Sertoli cells and weak expression in spermatocytes. The names of cells expressing *PPARγ* are underlined. (**b**) *PPARγ* forms obligate heterodimers with *RXRγ* for regulation of lipid metabolic target genes, providing energy for spermatogenesis. *PPARγ*: peroxisome proliferator-activated receptor gamma; *RXRγ*: retinoid X receptor gamma.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA ACTION IN FERTILIZATION

Roles of peroxisome proliferator-activated receptor gamma in fertilization

Fertilization is a complex program of biochemical changes that spermatozoa undergo in the female reproductive tract. Once capacitated, the spermatozoon can bind to the zona pellucida of the oocyte and undergo the acrosome reaction (AR), a process that enables sperm penetration and fertilization of the oocyte.[45] Some intracellular changes, including an increase in cholesterol efflux, a rise in membrane fluidity, an increase in intracellular Ca^{2+} concentration,[46,47] and actin polymerization,[48,49] have been considered to be the acceptable markers of capacitation. *PPARγ* agonist was able to elevate the functional maturation of sperm by evaluating its action on capacitation.[50] Recent research has demonstrated that *PPARγ* is expressed by ejaculated spermatozoa of humans and pigs, improving their motility, capacitation, AR, survival and metabolism.[14,50]

Roles of peroxisome proliferator-activated receptor gamma in infertility

Infertility is the inability to conceive after 12 months of regular, unprotected intercourse,[51] which is a problem of public health importance in China and many other developing nations because of its high prevalence and its serious social implications for affected couples and families. Epidemiological studies have confirmed that infertility affects approximately 5% of newly married couples in Shanghai, China. Under infertility treatment, about 60% of couples subsequently have a higher chance of having children than the untreated.[52] Recently, reports have asserted that sperm concentrations have been identified a potential decline over the past several decades, which may result in the decline in male fertility; however, the causes and extent of declining sperm quality and fertility remain unknown in most cases. Beyond the growing burden of disease, male infertility, associated with a high cost of care, generates significant psychosocial and marital stress. In addition, paternal health cues can be passed to the next generation, with male age associated with an increase in autistic spectrum disorders[53] and environmental exposures associated with increases in incidences of childhood diseases.[54,55] Likewise, there is now evidence that paternal infertility may be transferred to the offspring, including metabolic diseases.[56] As a result, several factors relating to general health and well-being, such as diet,[57] exercise,[58] obesity,[59] and psychological stress,[60,61] have been extensively studied for their effects on male reproductive potential. Special attention has been paid to the connections between obesity and sperm function. It is of great importance to find out the causes of declining sperm quality and fertility, which adversely affect human reproduction. It is a matter of great concern triggering large-scale studies into its causes and possibilities for prevention.

There is now emerging evidence that male obesity has a negative impact on male reproductive potential not only by reducing sperm quality, but in particular by altering the physical and molecular structure of testicular germ cells and ultimately mature spermatozoa.[59] Meanwhile, hyperinsulinemia and hyperglycemia are common in obese individuals and are constant confounding factors in many rodent studies of male obesity.[62–64] Apart from these, the fuel sensors glucose, insulin[65,66] and leptin[67–69] are known to be directly involved in the regulation of fertility at each level of the hypothalamic-pituitary-gonadal (HPG) axis.[70] The discovery of the *PPAR* family of transcription factors has revealed a link between lipid or glucose availability and long-term metabolic adaptation.[70] Historically, the roles of *PPARγ* have been associated with preadipocyte expansion and differentiation.[71] *PPARγ* mainly plays key roles in the regulation of cellular lipid metabolism, redox status and organelle differentiation in adipose tissue and other organs such as the prostate.[72,73] Therefore, it remains plausible that *PPARγ* participates in the regulation of male reproductive function, by reducing sperm motility and inducing male infertility.

Roles of peroxisome proliferator-activated receptor gamma in sperm capacitation and sperm metabolism

Sperm capacitation is an intricate program in which a myriad of events take place with the result that spermatozoa can penetrate and fertilize the oocyte. The bioenergetics of sperm capacitation is poorly understood despite its fundamental role in sustaining the biochemical and molecular events occurring during gamete activation. Adenosine triphosphate is synthesized by spermatozoa through either aerobic or anaerobic metabolic pathways. Santoro *et al*. demonstrated that in the majority of spermatozoa, *PPARγ* was expressed in the apical region of the head, in the subacrosomial region and prevalently in the midpiece, while the signaling was almost absent from the tail. However, in capacitated spermatozoa, the location of the receptor mirrors that observed in uncapacitated sperm cells.[50] It has been confirmed that PGJ2, an agonist of *PPARγ*, increases the viability of spermatozoa, whereas all these events are reduced by the irreversible *PPARγ* antagonist GW9662,[50] confirming the involvement of *PPARγ* in sperm viability. Meanwhile, *PPARγ* antagonist GW was able to attenuate the functional maturation of spermatozoa by evaluating its action on capacitation which has been correlated with functional and biochemical changes in sperm cells, including cholesterol efflux and tyrosine phosphorylation of sperm proteins.[50] Hence, it is reasonable to believe that *PPARγ* participates in capacitation by glucose metabolism or other metabolic pathways, and increases the motility of capacitated spermatozoa.

Glucose metabolism is a critical pathway that can produce sufficient energy for the sustenance of life. Given the beneficial effects of *PPARγ* ligands in therapies aimed at lowering glucose levels in type 2 diabetes, a role for *PPARγ* in glucose metabolism has been explored.[74,75] The effect of glucose on the fertilizing ability of spermatozoa appears to be mediated by the pentose phosphate pathway (*PPP*).[76] Metabolism of G-6-P through the *PPP* yields much more nicotinamide adenine dinucleotide hydrogenase (*NADPH*) than glycolysis and TCA cycle, and *NADPH* acts as a hydrogen donor in many chemical reactions *in vivo*.[77] *G6PDH* is a key rate-limiting enzyme in this metabolic pathway and has been shown to be functional in human spermatozoa.[78] *PPARγ* is able to modulate in a dose-dependent way the activity of *G6PDH* in spermatozoa.[50] Meanwhile, *PPARγ* has the potential to increase peripheral tissue sensitivity to insulin, thereby improving insulin resistance. Insulin resistance appears to negatively affect the sperm quantity and quality.[79] Moreover, insulin is a known mediator and modulator of the HPG axis, contributing to the regulation of male reproductive potential and overall wellbeing.[79,80] Its disruption of the HPG axis can render patients hypogonadal. It has been shown that hyperinsulinemia is associated with increased seminal insulin concentrations, which may negatively impact male reproductive function in obesity.[80]

CROSS-TALK BETWEEN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA AND SIGNALING TRANSDUCTION PATHWAYS

Peroxisome proliferator-activated receptor gamma and the PI3K signal transduction pathway

Once an insulin receptor substrate (*IRS*) combine with its catalytic subunits, the *IRS* catalyzes the phosphorylation of membrane

phosphatidylinositol (*PI*). *PI3K*, which has been shown to be active in human spermatozoa,[81] is important in a wide variety of cellular processes in which *PI3K* activation leads to production of 3'-phosphoinositide second messengers, such as PI- 3,4,5-trisphosphate, which activate a variety of downstream cell survival signals.[82] Accumulation of PI 3,4,5-trisphosphate in the membrane recruits a number of signaling proteins containing pleckstrin homology domains, including *AKT* and *PDK1*.[82,83] On recruitment, *AKT* becomes phosphorylated and activated by a series of enzymes, kinases and transcription factors downstream, and yields a variety of biological functions, including intracellular trafficking, organization of the cytoskeleton, cell growth and transformation, and prevention of apoptosis.[84,85] Interestingly, *AKT* is able to stimulate the metabolism of glucose through activation of AS160, the substrate of *AKT*, and promotes transposition of *GLUT4* and absorption of glucose into muscle cells. *PPARγ* activation has been reported to regulate components of the *PI3K* signaling cascade in various cell types,[86] enhancing the sensitivity of insulin. Elevation of *Glut4* and *PPAR* gene expression in parallel with glucose uptake has been confirmed by *in vitro* glucose uptake activity.[87] There is evidence that increasing doses of *PPARγ* agonists increase *Akt1/Akt2/Akt3* significantly, whereupon. *AKT*, the major downstream gene of *PI3K* signal transducer, is fully activated.[50]

Peroxisome proliferator-activated receptor gamma and the leptin signal transduction pathway

In addition to its role in metabolic control, leptin has pivotal roles in reproduction[88] and neuroendocrine signaling.[89,90] Various pieces of evidence have pointed to a direct role of leptin in the control of male reproduction.[91–94] In particular, *ob/ob* male mice (lacking functional leptin) or *db/db* male mice (lacking functional leptin receptor) are infertile and fail to undergo normal sexual maturation.[95] In human, leptin is expressed in the seminiferous tubules[96,97] and in seminal plasma[98,99] while the leptin receptor is found in the interstitium, primarily in the Leydig cells.[97] Worthy of note, Camiña *et al*. first proposed that human leptin is present in seminal fluid, with at least two charge variants and no binding proteins, the most likely source being either the seminal vesicles or prostate.[99] Hence, it is reasonable to speculate that leptin has a direct (paracrine, autocrine or both) effect on epithelial cells of the male accessory genital glands, and on the spermatozoa via sperm leptin receptors.[100] OBR, a single membrane-spanning glycoprotein, belonging to the class I cytokine receptor superfamily, shares sequence homologies for interaction with Janus kinase (*JAK*) as well as *STATs*.[101] Nonetheless, *PPARγ*, whose promoter region is rich in multiple Stat5 DNA binding consensus sequences, is downstream of the *JAK/STAT* signaling pathway,[102] suggesting that expression of this gene is regulated by the *JAK/STAT* pathway.

Experimental studies have shown that leptin treatment results in a significant increase in cholesterol efflux from and protein tyrosine phosphorylation of pig spermatozoa, stimulates pig sperm acrosin activity,[103] two events associated with capacitation.[104–106] Compelling evidence suggests that leptin has a direct inhibitory effect on rosiglitazone-induced adipocyte differentiation and *PPARγ* expression, in which *ERK1/2 MAPK* and *JAK/STAT1* signaling pathways are involved.[107,108] Several studies have supported a relationship between increased leptin production and regulation of reproductive function. Indeed, leptin plays a critical role at every level of the HPG axis in males. Most obese male mice become insensitive to increased endogenous leptin production and develop functional leptin resistance.[109,110] This deregulation of leptin signaling might result in abnormal endocrine and reproductive functions with altered leptin dynamics, and may contribute to male infertility in different ways, leading to hypogonadism.[111] Therefore, *PPARγ* agonists may enhance the sensitivity of insulin, acting as a potential therapy for hypogonadism.

SUMMARY

Peroxisome proliferator-activated receptor gamma may play a key role in linking lipid metabolism and reproduction in general. Energy from glucose and fat metabolism mediated by *PPARγ* signaling is required for sperm physiology, affecting male fertility. These recent experiments raise several questions. One question concerns *PPARγ* agonist activation of related metabolic pathways. Owing to the role of *PPARγ* in sperm capacitation, the use of its agonists may be considered a strategy in artificial insemination or other biotechnologies. Another question is whether the positive effects of *PPARγ* agonists are due to a direct effect on the testis or a positive effect on glucose homeostasis. Further experiments are needed to increase our knowledge of the way in which *PPARγ* signaling maintains sperm viability.

AUTHOR CONTRIBUTIONS

LL drafted the manuscript. HX and JCC participated in the design of the study and helped draft the manuscript. CZ, YHZ, MMC and YQ helped draft the manuscript. MJ conceived of the study, and participated in its design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

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