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 PPARα, 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.4-6 PPARγ is highly expressed in adipose tissue 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,21 mainly playing a role in temperature regulation through its natural ligand 15-deoxy-delta12, 14-prostaglandin J2 (PGJ2), which is secreted into cerebrospinal fluid.22 It is still unclear
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. Moreover, pituitary PPARγ is abundantly expressed in human PRL-, GH-secreting, and nonfunctioning pituitary tumors. 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. Moreover, it has been reported that PPARγ functions are regulated by FSH through mitogen-activated protein kinase (MAPK) signaling pathways (Figure 1). 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. PPARα and PPARγ are widely expressed in the interstitial Leydig cells and the seminiferous tubule cells (Sertoli and germ cells), whereas, PPARγ is believed to be restricted to Sertoli cells. 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. 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. Moreover, Thomas et al. have recently detected PPARγ mRNA in the germ cells (spermatocytes). 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). 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. Of the sources of stored energy that can be tapped for fuel reproductive energy requirements, the largest depot is white adipose tissue (WAT), which is primarily composed of white adipocytes that store lipid fuels as triacylglycerols. 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. 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. 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 in the epididymal germ and Sertoli cells, where it is involved in regulating the patterns of expression of key lipid metabolic genes in Sertoli cells. It is also indicated that PPARγ signaling plays an important role in spermatogenesis.

**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). 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. 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. 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. In addition, male fertility can be compromised by inactivation of genes involved in lipid metabolism. In summary, except for its role in spermatogenesis, PPARγ participates in fertilization by supporting energy provision.

![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 up-regulates 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.](image1)

![Figure 2: Expression patterns of PPARγ 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.](image2)
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. Some intracellular changes, including an increase in cholesterol efflux, a rise in membrane fluidity, an increase in intracellular Ca²⁺ concentration, and actin polymerization, 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. Recent research has demonstrated that PPARγ is expressed by ejaculated spermatozoa of humans and pigs, improving their motility, capacitation, AR, survival and metabolism.

Roles of peroxisome proliferator-activated receptor gamma in infertility

Infertility is the inability to conceive after 12 months of regular, unprotected intercourse, 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. 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 and environmental exposures associated with increases in incidences of childhood diseases. Likewise, there is now evidence that paternal infertility may be transferred to the offspring, including metabolic diseases.

As a result, several factors relating to general health and well-being, such as diet, exercise, obesity, and psychological stress, 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. Meanwhile, hyperinsulinemia and hyperglycemia are common in obese individuals and are constant confounding factors in many rodent studies of male obesity. Apart from these, the fuel sensors glucose, insulin and leptin are known to be directly involved in the regulation of fertility at each level of the hypothalamic-pituitary-gonadal (HPG) axis. The discovery of the PPAR family of transcription factors has revealed a link between lipid or glucose availability and long-term metabolic adaptation. Historically, the roles of PPARγ have been associated with preadipocyte expansion and differentiation. 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. 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 subacrosomal 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 uncapsulated sperm cells. 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, 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. 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. The effect of glucose on the fertilizing ability of spermatozoa appears to be mediated by the pentose phosphate pathway (PPP). 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. G6PDH is a key rate-limiting enzyme in this metabolic pathway and has been shown to be functional in human spermatozoa. PPARγ is able to modulate in a dose-dependent way the activity of G6PDH in spermatozoa. 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. Moreover, insulin is a known mediator and modulator of the HPG axis, contributing to the regulation of male reproductive potential and overall wellbeing. 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.
phosphatidylinositol (PI). PI3K, which has been shown to be active in human spermatozoa, 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. Accumulation of PI 3,4,5-trisphosphate in the membrane recruits a number of signaling proteins containing pleckstrin homology domains, including AKT and PDK1. 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. 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, 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. 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.

**PPARγ and spermatozoa**

In addition to its role in metabolic control, leptin has pivotal roles in reproduction and neuroendocrine signaling. Various pieces of evidence have pointed to a direct role of leptin in the control of male reproduction. 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. In human, leptin is expressed in the seminiferous tubules and in seminal plasma, while the leptin receptor is found in the interstitium, primarily in the Leydig cells. Worthy of note, Caminha 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. 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. 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. Nonetheless, PPARγ, whose promoter region is rich in multiple Stat5 DNA binding consensus sequences, is downstream of the JAK/STAT signaling pathway, 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, two events associated with capacitation. 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. 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. 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. 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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**REFERENCES**

1. Issermann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990; 347: 645–50.
2. Sørensen HN, Treuter E, Gustafsson JA. Regulation of peroxisome proliferator-activated receptors. Vitam Horm 1998; 54: 121–66.
3. Ehrmann J Jr, Varvatsou N, Collan Y, Kolar Z. Peroxisome proliferator-activated receptors (PPARs) in health and disease. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2002; 146: 11–4.
4. Robinson E, Greive DJ. Significance of peroxisome proliferator-activated receptors in the cardiovascular system in health and disease. Pharmacol Ther 2009; 122: 246–63.
5. Vanneçq J, Cherkauki-Malki M, Andreolletti P, Latruffe N. The human peroxisome in health and disease: the story of an oddity becoming a vital organelle. Biochimie 2014; 98: 4–15.
6. Cho HK, Kong HJ, Nam BH, Kim WJ, Noh JK, et al. Molecular cloning and characterization of olive flounder (Paralichthys olivaceus) peroxisome proliferator-activated receptor gamma. Gen Comp Endocrinol 2009; 163: 251–8.
7. Oku H, Umino T. Molecular characterization of peroxisome proliferator-activated receptors (PPARs) and their expression in the differentiating adipocytes of red sea bream Pogonias major. Comp Biochem Physiol B Biochem Mol Biol 2008; 151: 268–77.
8. Abbott BD. Review of the expression of peroxisome proliferator-activated receptors alpha (PPAR alpha), beta (PPAR beta), and gamma (PPAR gamma) in rodent and human development. Reprod Toxicol 2009; 27: 246–57.
9. Li S, Gui Y, Wang W, Qian X, Zhao Y. PPARγ, an important gene related to lipid metabolism and immunity in Megalobrama amphiblophela: cloning, characterization and transcription analysis by GeNorm. Gene 2013; 512: 321–30.
10. Tsai ML, Chen HY, Tseng MC, Chang RC. Cloning of peroxisome proliferators activated receptors in the cobra (Naja naja) and their expression at different life-cycle stages under cage aquaculture. Gene 2008; 425: 69–78.
11. Froment P, Gizard F, Defever D, Staels B, Dupont J, et al. Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. J Endocrinol 2006; 189: 199–209.
12 Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: a review. Fertil Steril 2002; 77: 433–44.
13 Cui Y, Miyoshi K, Claudio E, Siebenlist UK, Gonzalez FJ, et al. Loss of the peroxisome proliferator-activated receptor gamma (PPARgamma) does not affect mammalian development and propensity for tumor formation but leads to reduced fertility. J Biol Chem 2002; 277: 17830–5.
14 Aquila S, Bonifoglio D, Gentile M, Middea E, Gabriele S, et al. Peroxisome proliferator-activated receptor (PPAR) gamma is expressed by human spermatozoa: its potential role on the sperm physiology. J Cell Physiol 2006; 209: 977–86.
15 Aquila S, Middea E, Catalano S, Marsico S, Lanzino M, et al. Human sperm express a functional androgen receptor: effects on P31KAKT pathway. Hum Reprod 2007; 22: 2594–605.
16 Guido C, Perrotta I, Panza S, Middea E, Avena P, et al. Human sperm physiology: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) influence sperm metabolism and may be involved in the pathophysiology of varicocele-associated male infertility. J Cell Physiol 2011; 226: 3403–12.
17 Aquila S, Gentile M, Middea E, Catalano S, Andò S. Autocrine regulation of insulin secretion in human ejaculated spermatozoa. Endocrinology 2005; 146: 522–7.
18 Aquila S, Gentile M, Middea E, Catalano S, Morelli C, et al. Leptin secretion by human ejaculated spermatozoa. J Clin Endocrinol Metab 2005; 90: 4735–61.
19 Heaney AP, Fernando M, Melmed S. PPAR gamma receptor ligands: novel therapy for pituitary adenomas. J Clin Invest 2003; 111: 1381–8.
20 Wiesner G, Morash BA, Ur E, Wilkinson M. Food restriction regulates adipose-specific cytokines in pituitary gland but not in hypothalamus. J Endocrinol 2004; 180: R1–6.
21 Manna CS, Minke A, Boissé L, Pittman GJ. A novel antiprybic action of 15-deoxy-Delta 12,14-prostaglandin J2 in the rat brain. J Neurosci 2004; 24: 13112–8.
22 Giustina A, Barkan A, Casanueva FF, Cavagnini F, et al. Gonadal development in mammals at the cellular and molecular levels. Int Rev Cytol 2000; 200: 47–99.
23 Krishnamoorthy G, Selvakumar K, Venkataraman P, Elumalai P, Arunakaran J. Inhibition of phosphofructokinase in isolated rat epididymal white adipose tissue. Mol Med 2011; 365: 1465–80.
24 Lampiao F. Variation of semen parameters in healthy medical students due to exam running on reproductive hormones, hypothalamus–pituitary–testis axis, and semen quality: a randomized controlled study. Asian J Androl 2009; 11: 292–7.
25 Lampiao F. Variation of semen parameters in healthy medical students due to exam running on reproductive hormones, hypothalamus–pituitary–testis axis, and semen quality: a randomized controlled study. Asian J Androl 2009; 11: 292–7.
26 Ghanayem BI, Bai R, Kissling GE, Travlos G, Hoffler U. Diet-induced obesity in male mice is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity. Biol Reprod 2013; 88: 964–70.
27 Sale EM, Denton RM. Beta-adrenergic agents increase the phosphorylation of actin cytoskeleton during mammalian sperm capacitation and acrosome reaction. Cell Biol Int 2010; 34: 655–62.
28 Cui Y, Miyoshi K, Claudio E, Siebenlist UK, Gonzalez FJ, et al. Peroxisome proliferator-activated receptor-ß/d in septic shock. J Biol Chem 2002; 277: 17830–5.
29 Floryk D, Kurosaka S, Tanimoto R, Yang G, Goltsov A, et al. Peroxisome proliferator-activated receptor-ß/d in septic shock. J Biol Chem 2002; 277: 17830–5.
30 Dhungana B, Pizzino DE, Concors SA, et al. Capacitation of mouse spermatozoa. II. Protein tyrosine phosphorylation and capacitation are regulated by a cAMP-dependent pathway. Development 1995; 121: 1139–50.
31 Brener E, Rubinstein S, Cohen G, Shternail K, Rivlin J, et al. Remodeling of the acrosomal membrane in mammalian sperm capacitation and acrosome reaction. Biol Reprod 2003; 68: 837–45.
32 Wang L, Chen W, Zhao C, Huo R, Guo XJ, et al. The role of ezrin-associated protein network human sperm capacitation. Asian J Androl 2010; 12: 667–76.
33 Santoro M, Guido C, De Amicis F, Sisci D, Vizza D, et al. Sperm metabolism in pigs: a role for peroxisome proliferator-activated receptor (PPAR). J Exp Biol 2013; 216: 1085–92.
34 D. Brinsden, PM24, T.M. Lishko NE, J. Geresdorff, J. I. Rabinovitch, J. C. Artzt, et al. Protective role in regulating the Ssm, a novel retinoic acid target gene in the mouse testis. Genes Dev 2001; 15: 4619–29.
35 P.M. Safarinejad, A. Martin, B. Safarinejad, C. Safarinejad, et al. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus–pituitary–testis axis, and semen quality: a randomized controlled study. J Endocrinol 2009; 200: 259–71.
36 D. Pal, T. H. H. Pal, C. Pal, et al. Central effects of leptin and ghrelin on the reproductive axis. J Clin Endocrinol Metab 2006; 91: 4364–70.
37 S.A. Aquila, E. Bono, M. Aquila, et al. Peroxisome proliferator-activated receptor gamma (PPARgamma) does not affect mammary gland development and propensity for tumor formation but leads to reduced fertility. J Biol Chem 2002; 277: 17830–5.
38 D. Pal, A. Martin, K. P. G. L. Liu, et al. PPARalpha and pituitary hormone system in the female reproductive tract. Reproduction 2000; 85: 526–9.
39 S.A. Aquila, E. Bono, M. Aquila, et al. Peroxisome proliferator-activated receptor gamma (PPARgamma) does not affect mammary gland development and propensity for tumor formation but leads to reduced fertility. J Biol Chem 2002; 277: 17830–5.
proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 2003; 100: 15712–7.

72 Jiang M, Shappeil SB, Hayward SW. Approaches to understanding the importance of insulin and leptin alongside reduced fertility parameters in a controlled male cohort. *Reprod Domest Anim* 2009; 44: 345–9.

73 Aquila S, Guido C, Middea E, Perrotta I, Bruno R, et al. Human male gamete endocrinology: 1alpha, 25-dihydroxyvitamin D3 (1,25(OH)2D3) regulates different aspects of human sperm biology and metabolism. *Reprod Biol Endocrinol* 2009; 7: 140.

74 Cany A, Frieze EA. Type 2 diabetes, psoriasis and thalassemidiones. *Int J Clin Pract* 2006; 60: 362–3.

75 Savk RS, Miller AR. Investigational PPAR-gamma agonists for the treatment of Type 2 diabetes. *Expert Opin Investig Drugs* 2006; 15: 763–78.

76 Miraglia E, Lussiana C, Viarisio D, Racca C, Cipriani A, et al. The pentose phosphate pathway plays an essential role in supporting human sperm capacitation. *Fertil Steril* 2010; 93: 2437–40.

77 Peña FJ, Rodriguez Martínez H, Tapia JA, Ortega Ferusola C, González Fernández L, et al. Mitochondria in mammalian sperm physiology and pathology: a review. *Reprod Domest Anim* 2009; 44: 345–9.

78 Aquila S, Guido C, Middea E, Perrotta I, Bruno R, et al. Human male gamete endocrinology: 1alpha, 25-dihydroxyvitamin D3 (1,25(OH)2D3) regulates different aspects of human sperm biology and metabolism. *Reprod Biol Endocrinol* 2009; 7: 140.

79 Deppert C, Brannigan RE. Metabolic syndrome and infertility in men. *Best Pract Res Clin Obstet Gynaecol* 2014 Oct 24. pii: S1521-6934(14)00217-X. doi: 10.1016/j.bpobgyn.2014.10.006. [Epub ahead of print].

80 Leisegang K, Bouic PJ, Menkveld R, Henkel RR. Obesity is associated with increased seminal insulin and leptin alongside reduced fertility parameters in a controlled male cohort. *Reprod Biol Endocrinol* 2014; 12: 34.

81 Luconi M, Carloni V, Marra F, Ferruzzi P, Forti G, et al. Increased phosphorylation of AKAP by inhibition of phosphatidylinositol 3-kinase enhances human sperm motility through tail recruitment of protein kinase A. *J Cell Sci* 2004; 117: 1235–46.

82 Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2001; 296: 1655–7.

83 Lawlor MA, Alesi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses. *J Cell Sci* 2001; 114: 2903–10.

84 Ballester J, Fernández-Novell JM, Rutllant J, García-Rocha M, Jesús Palomo M, et al. Evidence for a functional glycogen metabolism in mature mammalian spermatozoa. *Mol Reprod Dev* 2000; 56: 207–19.

85 Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. *Trends Biochem Sci* 1997; 22: 267–72.

86 Bonfiglio D, Gabriele S, Aquila S, Catalano S, Gentile M, et al. Estrogen receptor alpha binds to peroxisome proliferator-activated receptor response element and negatively interferes with peroxisome proliferator-activated receptor gamma signaling in breast cancer cells. *Clin Cancer Res* 2005; 11: 6139–47.

87 Anandharajan R, Pathmanathan S, Shankernarayanan NP, Vishwakarma RA, Balakrishnan A. Upregulation of Glut-4 and PPAR gamma by an isoflavone from Pterocarpus marsupium on L6 myoblasts: a possible mechanism of action. *J Ethnopharmacol* 2005; 97: 253–60.

88 Prolo P, Wong ML, Licinio J. Leptin. In *J Biochem Cell Biol* 1998; 30: 1285–90.

89 Auwers I, Staelens B. Leptin. *Lancet* 1998; 351: 737–42.

90 Ahima RS, Flier JS. Leptin. *Annu Rev Physiol* 2000; 62: 413–37.

91 Tena-Sempere M, Barreiro ML. Leptin in male reproduction: the testis paradigm. *Mol Cell Endocrinol* 2002; 188: 9–13.

92 Caprio M, Fabbriin E, Isidori AM, Aversa A, Fabbri A. Leptin in reproduction. *Trends Endocrinol Metab* 2001; 12: 65–72.

93 Tena-Sempere M, Marina PR, Zhang FP, Pinilla L, González LC, et al. Molecular mechanisms of leptin action in adult rat testis: potential targets for leptin-induced inhibition of steroidogenesis and pattern of leptin receptor messenger ribonuclease expression. *J Endocrinol* 2001; 170: 413–23.

94 White JT, DeSanto CL, Gibbons C, Lardner CK, Panakos A, et al. Insulins, leptin and feeding in a population of Peromyscus leucopus (white-footed mouse) with variable fertility. *Horm Behav* 2014; 66: 169–79.

95 Mouniz K, Lu R, Chehab FF. Leptin treatment rescues the sterility of genetically obese ob/ob males. *Endocrinology* 1997; 138: 1190–3.

96 Soupek S, Armagan A, Serel TA, Hoscan MB, Perk H, et al. Leptin expression in the testicular tissue of fertile and infertile men. *Arch Androl* 2005; 51: 239–46.

97 Ishikawa T, Fujioka H, Ishimura T, Takenaka A, Fujisawa M. Expression of leptin and leptin receptor in the testis of fertile and infertile patients. *Andrologia* 2007; 39: 22–7.

98 Glander HJ, Lammert A, Paasch U, Glasow A, Kratsch J. Leptin exists in tubuli seminiferi and in seminal plasma. *Andrologia* 2002; 34: 227–33.

99 Camifía JL, Mage L, Menendez C, Graña M, García-Devesa J, et al. Evidence of free leptin in human seminal plasma. *Endocrine* 2002; 17: 169–74.

100 Sayed-Ahmed A, Abd-Elmaksoud A, Elnasharty M, El-Magd MA. In situ hybridization and immunohistochemical localization of leptin hormone and leptin receptor in the seminal vesicle and prostate gland of rat adult. *Acta Histochem* 2012; 114: 185–91.

101 Tartaglia LA. The leptin receptor. *J Biol Chem* 1997; 272: 6093–6.

102 Davoodi-Semiromi A, Hassanzadeh A, Waterfall CH, Doney A, Atkinson M. Pyrohostin AG490 agent modestly but significantly prevents onset of type 1 in NOD mouse; implication of immunologic and metabolic effects of a Jak-Stat pathway inhibitor. *J Clin Immunol* 2012; 32: 1038–47.

103 Aquila S, Rago V, Guido C, Casaburi I, Zupo S, et al. Leptin and leptin receptor in pig spermatozoa: evidence of their involvement in sperm capacitation and survival. *Reproduction* 2008; 136: 23–32.

104 Visconti PE, Ning X, Forrós MW, Álvarez JG, Stein P, et al. Cholesterol efflux-mediated signal transduction in mammalian sperm: cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation. *Dev Biol* 1999; 214: 429–43.

105 Osheroff JE, Visconti PE, Valenzuela JP, Travis AJ, Álvarez J, et al. Regulation of human sperm capacitation by a cholesterol efflux-stimulated signal transduction pathway leading to protein kinase A-mediated up-regulation of protein tyrosine phosphorylation. *Mol Hum Reprod* 1999; 5: 1017–26.

106 Travis AJ, Kopf GS. The role of cholesterol efflux in regulating the fertilization potential of mammalian spermatozoa. *J Clin Invest* 2002; 110: 731–6.

107 Rhee SD, Sung YY, Jung WH, Cheon HG. Leptin inhibits rgsolitizane-induced adipogenesis in murine primary adipocytes. *Mol Cell Endocrinol* 2008; 294: 61–9.

108 Kim WK, Lee CY, Kang MS, Kim MH, Ryu YH, et al. Effects of leptin on lipid metabolism and gene expression of differentiation-associated growth factors and transcription factors during differentiation and maturation of 3T3-L1 preadipocytes. *Endocr J* 2008; 55: 827–37.

109 Tortorillo DV, McMinn JE, Chua SC. Increased expression of hypothalamic leptin receptor and adiponectin accompany resistance to dietary-induced obesity and infertility in female C57BL/6J mice. *J Int Obes* (Lond) 2007; 31: 395–402.

110 Tortorillo DV, McMinn J, Chua SC. Dietary-induced obesity and hypothalamic infertility in female DBA/2J mice. *Endocrinology* 2004; 145: 1238–47.

111 Landry D, Cloutier F, Martin LJ. Implications of leptin in neuroendocrine regulation of male reproduction. *Reprod Biol* 2013; 13: 1–14.